

EAVLD 2026 8th

CONGRESS OF THE

European Association of Veterinary Laboratory Diagnosticians

19-21 October 2026
Royal Olympic Hotel
Athens, Greece

ABSTRACT BOOK

Contents

Welcome Message 3

Organising & Scientific Committee 4

Invited Speakers 5

Venue / Floor Plan 6

Congress Secretariat 7

Onsite team 7

Secretariat & Information Desk during the Congress 7

Social & Cultural Events 7

Programme at a glance 10

Detailed Programme 11

Sponsors 18

Invited Speakers 21

Orals 34

ePosters 109

Welcome Message

Dear Colleagues and Friends,

It is a great pleasure to welcome you to the EAVLD Congress 2026, taking place in the vibrant and historic city of Athens, Greece. The Organising Committee looks forward to welcoming you to this important scientific event, set against the inspiring backdrop of the Acropolis.

The Congress features a rich scientific programme covering key developments in veterinary diagnostics, including new technologies, emerging diseases, and practical applications in the field. Highlights include keynote lectures by leading experts, oral and poster presentations, and opportunities for discussion and collaboration. An exhibition of laboratory equipment will also run alongside the sessions, offering a chance to explore the latest innovations from industry partners.

Our centrally located venue offers modern facilities and stunning views of one of the world's most iconic landmarks. Athens, birthplace of philosophy, science, and democracy, seamlessly blends ancient history with contemporary energy, offering a unique setting for learning and connection.

Outside the sessions, enjoy Athens' rich cultural heritage, warm hospitality, and world-renowned cuisine. From archaeological wonders to lively neighborhoods and coastal escapes, the city offers something for everyone.

We are excited to host you for what promises to be a rewarding and memorable experience.

Looking forward to meeting you in Athens.

Prof. Labrini V. Athanasiou
On behalf of the EAVLD Congress 2026 Organising Committee
Athens, Greece

Organising Committee

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DVM, MSc, PhD, Professor, Faculty of Veterinary Medicine, University of Thessaly, Greece

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Professor, Miguel Hernandez University, Alicante, Spain

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Antonio Martínez-Murcia
Professor, Miguel Hernandez University, Alicante, Spain

Jo Mayers
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George Valiakos
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Fabrizio Vitale
Director, Istituto Zooprofilattico Sperimentale della Sicilia, Italy

Els Broens
PhD, Associate Professor, University of Utrecht, The Netherlands

Invited Speakers



Marialaura Corrente
DVM, Full Professor
Director of Specialisation School in Infectious Diseases, Prophylaxis and Veterinary Policy
Department of Veterinary Medicine, University of Bari Aldo Moro, Italy



Vito Martella
Full Professor, Department of Veterinary Medicine, University of Bari Aldo Moro, Italy



Antonio Martínez-Murcia
Ph.D., Professor of Microbiology, Miguel Hernández University (since 1993), Orihuela, Alicante, Spain. Bachelor of Biochemistry and Molecular Biology, Univ. Valencia 1987. Doctorate in Microbiology, University of Reading, England



Vesna Milićević
Head of the Department of Virology, Institute of Veterinary Medicine of Serbia



Vasileios Ntafis
DVM, MSc, PhD, Head – Designated Veterinarian, Senior Research Scientist, Animal Facilities, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece



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DVM, MSc, PhD Assistant Professor Laboratory of Parasitology and Parasitic Diseases School of Veterinary Medicine Faculty of Health Sciences Aristotle University of Thessaloniki, Greece



Grigorios Tsoumakas
Professor, School of Informatics, Aristotle University of Thessaloniki, Greece



George Valiakos
DVM, MSc, PhD, Associate Professor at Faculty of Veterinary Medicine University of Thessaly, Greece



Fabrizio Vitale
Director of Molecular Biology Area, Head of National Reference Centre for Animal Leishmanioses, WOAH Reference Laboratory for Leishmaniosis, Acting Head of Entomology & Environmental Vector Control Laboratory, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy



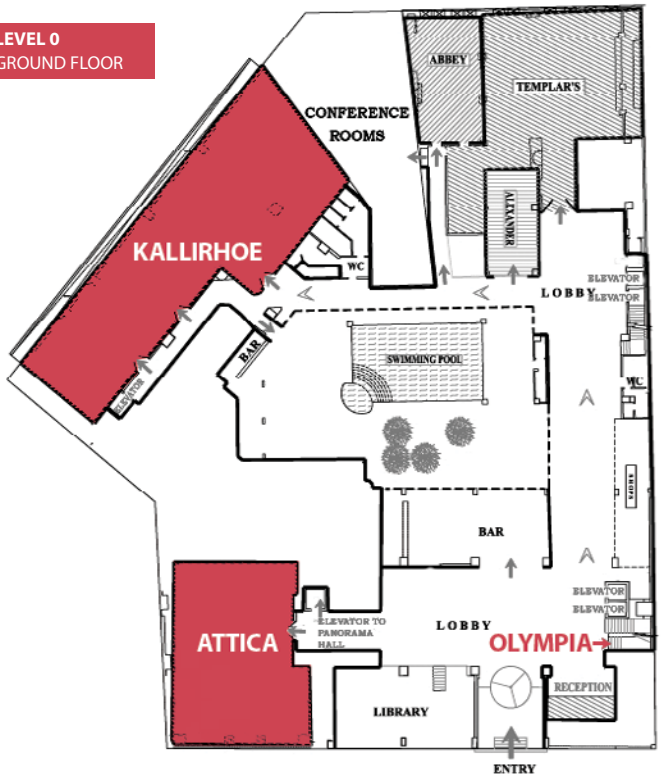
Stéphan Zientara
DVM, PhD, HDR, Director of the ANSES-Animal health Laboratory, Maisons-Alfort, France

Venue / Floor Plan

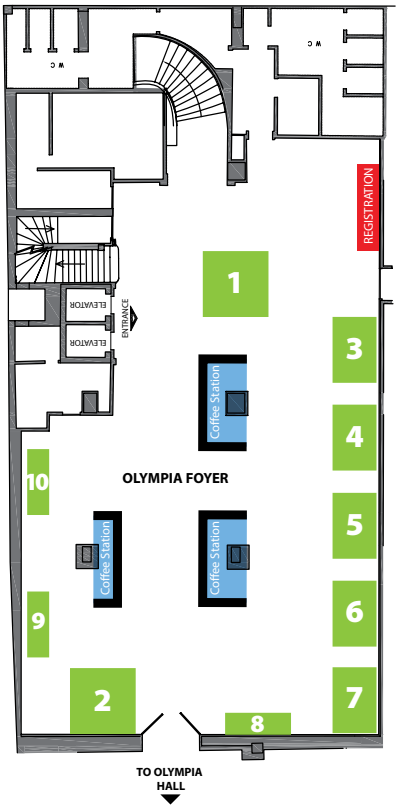
ROYAL OLYMPIC HOTEL

28 Athanasiou Diakou, 117 43 Athens, Greece

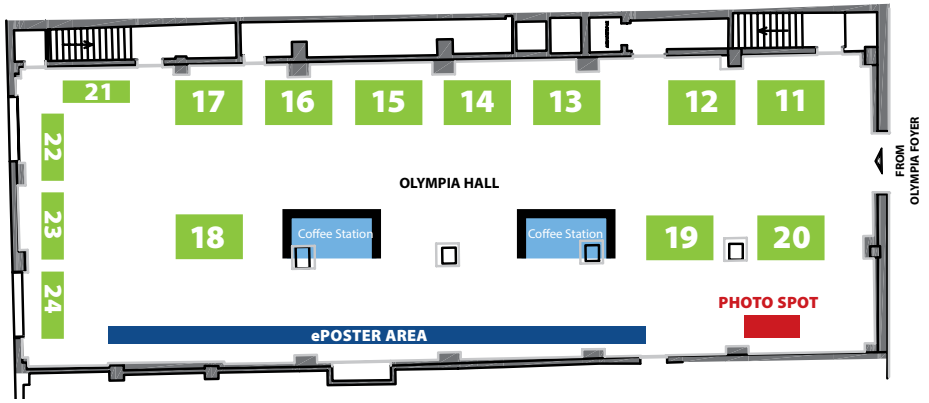
LEVEL 0
GROUND FLOOR



LEVEL -1



LEVEL -1



1	ID.VET/MINO
2	IDEXX
3	BIOSELLAL
4	MEGACOR
5	VMRD
6	CORPUS MEDICAL
7	SAN VET
8	PROMEGA
9	DIATHEVA
10	GENETIC PCRSOLUTIONS
11	INDICAL BIOSCIENCE
12	NZYTECH
13	ALPHADIA
14	AGROLABO
15	ANTISEL
16	BIOMERIEUX
17	APHA SCIENTIFIC
18	INGENETIX
19	GOLD STANDARD DIAGNOSTICS
20	LIFE DIAGNOSTICS
21	DIAGNOSTICS FOR ANIMALS
22	STAMAR
23	BRUKER
24	EXOPOL

Congress Secretariat

For any inquiries regarding your Registration, Accommodation, Social Events, or Excursions, our team will be happy to assist you!

You will find us at the **Olympia Foyer.**

ARTION
conferences & events

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Secretariat & Information Desk during the Congress

Monday 19 October 2026	08:00-18:00
Tuesday 20 October 2026	09:00-18:00
Wednesday 21 October 2026	08:30-15:30

Social & Cultural Events



WELCOME RECEPTION *open to all registered participants*

Date: Monday, 19 October 2026
Time: 18:00-19:00
Venue: Olympia Hall & foyer



CONGRESS DINNER *by ticket*

Date: Tuesday, 20 October 2026
Time: 19:30-22:30 *(19:00 pick up time from Royal Olympic Hotel)*
Venue: Agora Riviera



HALF DAY TOUR *by ticket*

Guided visit to the Acropolis & Acropolis Museum

Date: Wednesday, 21 October 2026
Time: 16:00-19:00 *(Departure from Royal Olympic Hotel)*



POST CONGRESS EXCURSION *by ticket*

Delphi Archaeological Site & Arachova

Date: Thursday, 22 October 2026
Time: 08:30-17:00 *(Departure from Royal Olympic Hotel)*







+ + + + + + CREATING CLARITY

Advancing global livestock, poultry, and dairy testing

Join IDEXX at EAVLD 2026 to learn more about our diagnostic solutions!

Date	Time	Presentation
Monday, 19 October 2026	10:00 - 10:10	Analytical and Field Validation of Multiple RT-PCR for Detection of BTV and EHDV in Ruminant Species *Presented by: Michael Angelichio*
Tuesday, 20 October 2026	10:50 - 11:00	Economic Impacts of Diagnostic Accuracy: Innovations in Livestock & Poultry Testing *Presented by: Dr. Christoph Egli*
	16:10-16:20	New IBR Serum Double-Antigen Sandwich ELISA *Presented by: Dr. Christoph Egli*
	17:10-17:20	RealPCR MilQ-ID DNA System: The New Solution for Mastitis Testing *Presented by: Dr. Christoph Egli*
Poster Session		Development and Validation of a New RT-PCR Test for Multiplex Detection of PRRSV-1 and PRRSV-2 in Europe

Help us understand your PCR workflow needs. **Take our short survey** at the IDEXX booth and **get a special giveaway!**

IDEXX

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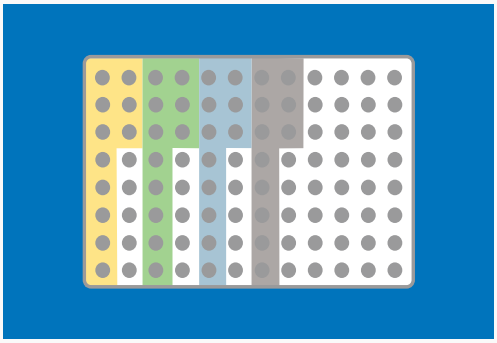


Solutions made simple.

LPD's tests span across formats: IDEXX got you covered!

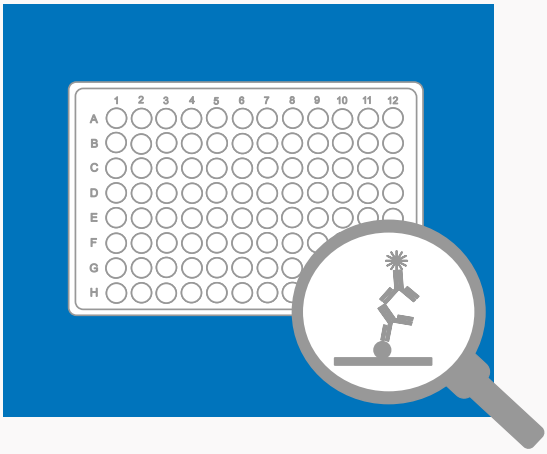
The modular RealPCR™ platform components are standardized to work together.

+ Timely and cost-effective
+ Validated on most common real-time PCR instruments
+ Internal sample control leads to confidence in accurate results



IDEXX ELISA technology has over 40 years of experience and is one of the most sensitive and reproducible diagnostic technologies available.

+ Streamline testing
+ Support decisive action
+ Get the answers you need quickly
+ Test with confidence
+ IDEXX ELISA tests are USDA licensed/permitted—many are NPIP approved[1]



References

1. *Veterinary Biological Products: Licensees and Permittees.* USDA Center for Veterinary Biologics; 2025. Accessed June 30, 2025. www.aphis.usda.gov/animal_health/vet_biologics/publications/currentprodcodebook.pdf

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Programme at a glance

Monday 19 October 2026

08:00-08:45	Registrations
08:45-09:00	Opening ceremony
09:00-09:30	State of the art opening lecture
09:30-10:00	Keynote lecture
10:00-10:50	Oral session
10:50-11:00	Corporate presentation
11:00-11:30	*Coffee break & poster session*
11:30-12:10	Indical mini seminar
12:10-13:00	Oral session
13:00-14:00	*Lunch break*
14:00-15:30	Keynote lectures
15:30-16:00	*Coffee break & poster session*
16:00–16:10	Corporate presentation
16:10–16:40	Oral Session 3
16:40–17:00	Short break
17:00-18:00	EAVLD General Meeting - HVMS - BioLD Group Meeting
18:00-19:00	Welcome reception

Tuesday 20 October 2026

09:00-09:30	Keynote lecture
09:30-10:50	Quality session
10:50-11:00	Corporate presentation
11:00-11:30	*Coffee break & poster session*
11:30-12:00	Keynote lecture
12:00-12:10	Corporate presentation
12:10-13:00	Keynote lectures
13:00-14:00	*Lunch break*
14:00-15:30	Oral sessions
15:30-16:00	*Coffee break & poster session*
16:00-18:00	Oral sessions
19:30-22:30	Congress Dinner

Wednesday 21 October 2026

09:00-09:30	Keynote lecture
09:30-10:50	AMR research groups
10:50-11:00	Corporate presentation
11:00-11:30	Coffee break & poster session
11:30-13:00	Oral session
13:00-14:00	*Light lunch*
14:00-15:00	Oral sessions
15:00-15:30	Awards - Closing ceremony
16:00-19:00	Half day tour

Detailed Programme

MONDAY 19 OCTOBER 2026

08:00-08:45 **REGISTRATIONS**

KALLIRHOE HALL

08:45-09:00 **OPENING CEREMONY**

09:00-09:30 **KEYNOTE LECTURE 1: STATE OF THE ART OPENING LECTURE**
Chairs: **Labrini Athanasiou & Zoe Polizopoulou**

09:00-09:30 Phylogenetic Taxonomy Frameworks, The Essential Basis For Microbial Genetic Diagnostics
Antonio Martínez-Murcia

09:30-10:00 **KEYNOTE LECTURE 2**
Chairs: **Labrini Athanasiou & Zoe Polizopoulou**

Preparedness Beyond Diagnostics: The Role of Veterinary Laboratories in Complex Animal Health Systems
Vesna Milićević

10:00-10:50 **ORAL SESSION 1**
EMERGING INFECTIOUS DISEASES AND RECENT OUTBREAKS IN EUROPE
Chairs: **Labrini Athanasiou & Zoe Polizopoulou**

10:00-10:10 **O01.** ANALYTICAL AND FIELD VALIDATION OF A MULTIPLEX RT-PCR FOR DETECTION OF BTV AND EHDV IN RUMINANT SPECIES
Michael Angelichio

10:10-10:20 **O02.** METATRANSCRIPTOMICS BASED APPROACH FOR VIRUS DISCOVERY IN ENCEPHALITIS CASES USING HIGH-THROUGHPUT SEQUENCING
Kara Dawson

10:20-10:30 **O03.** LABORATORY AND FIELD SOLUTIONS FOR THE RAPID DETECTION OF HIGHLY PATHOGENIC H5 AVIAN INFLUENZA VIRUSES OF CLADE 2.3.4.4B
Anna Bonomini

10:30-10:40 **O04.** RESURGENCE OF EQUINE INFECTIOUS ANEMIA IN FRANCE IN 2025
Delphine Froger

10:40-10:50 Discussion

10:50-11:00 **CORPORATE PRESENTATION**
Diagnostics for Animals & EAVLD: A shared Commitment to Veterinary Diagnostic Excellence
Christoph Egli

11:00-11:30 ***Coffee break & Poster Session***

11:30-12:10 **INDICAL MINI SEMINAR**
Chair: **Dimitrios Gougoulis**

11:30-11:50 Diagnostic Methods as a Key Component of PRRS Control Strategy
Vasileios G. Papatsiros

11:50-12:10 A New Addition to the PRRSV Diagnostic Toolbox – Fast Discrimination of PRRSV Field vs. Vaccine Strain
Henri Beulen

12:10-13:00 **ORAL SESSION 2**
THE FUTURE OF VACCINE DEVELOPMENT: INSIGHTS FROM DIAGNOSTICS
Chair: **Dimitrios Gougoulis**

12:10-12:20 **O05.** BIOCHEMICAL SAFETY PROFILE OF THE LV17/WB/RIE1-ΔCD VACCINE CANDIDATE AGAINST AFRICAN SWINE FEVER IN WILD BOAR
Mónica Sánchez-Segovia

12:20-12:30 **O06.** TRAINED IMMUNITY IN VETERINARY DIAGNOSTICS: COMPARATIVE USEFULNESS OF CYTOKINES, HISTOPATHOLOGY AND MOLECULAR TECHNIQUES ACROSS EXPERIMENTAL MODELS
Lidia Sánchez-Morales

12:30-12:40 **O08.** HEAT PRE-TREATMENT PRESERVES ANTIBODY DETECTION WHILE INACTIVATING INFLUENZA VIRUS IN RAW MILK
Ailam Lim

12:40-13:00 Discussion

13:00-14:00 ***Lunch break***

KALLIRHOE HALL

14:00-15:30 **KEYNOTE LECTURES**
Chairs: **Antonio Martínez-Murcia & Dimitrios Gougoulis**

14:00-14:30 **KEYNOTE LECTURE 3:** Overview of the Emergence of Certain Vector-borne Diseases in Animal Health in Europe since the 2000s
Stéphan Zientara

14:30-15:00 **KEYNOTE LECTURE 4:** FAST Diseases Outbreaks in Greece: Identifying and Overcoming Diagnostic Challenges for Immediate Stamping out
George Valiakos

15:00-15:30 **KEYNOTE LECTURE 5:** The Changing Landscape of Small Animal Virology: The Contribution of the School of Veterinary Medicine of Bari
Vito Martella

15:30-16:00 ***Coffee break & Poster Session***

16:00-16:10 **CORPORATE PRESENTATION**
Advancing Veterinary AST: Automated Reading and Interpretation of Disc Diffusion, MIC and Broth Microdilution
Nik Thierjung

KALLIRHOE HALL

16:10-16:40 **ORAL SESSION 3**
NEW FRONTIERS IN POINT-OF-CARE TESTING FOR VETERINARY MEDICINE
Chairs: **Marialaura Corrente & Georgios Papakonstantinou**

16:10-16:20 **O11.** ASFV MONODOSE-LAMP®: A FIELD-READY INNOVATIVE FORMAT FOR THE GENETIC DETECTION OF AFRICAN SWINE FEVER VIRUS
Caridad Miró-Pina

16:20-16:30 **O12.** LAMP TECHNOLOGY: A RAPID MULTIPLEX DIAGNOSTIC TOOL FOR THE DETECTION OF PATHOGENS INVOLVED IN BOVINE RESPIRATORY DISEASES
Fanny Laforêt

16:30-16:40 Discussion

16:40-17:00 ***Short break***

17:00-18:00 **EAVLD GENERAL MEETING - HVMS -BioLD GROUP MEETING**

18:00-19:00 **WELCOME RECEPTION**

TUESDAY 20 OCTOBER 2026

KALLIRHOE HALL

09:00-09:30 **KEYNOTE LECTURE 6**
Chair: **Serafeim Chaintoutis**

Towards Disease Control Tools to Secure Animal and Public Health in a Changing World
Fabrizio Vitale

09:30-10:50 **QUALITY SESSION**
Chair: **Serafeim Chaintoutis**
Round Table: Quality Challenges in the Private, Public, Academic and WOAH Reference Laboratories
Moderators: **Kathleen Freeman & Serafeim Chaintoutis**
Participants: **Dimitra Bitchava, Eleni Katsogiannou, Argyris Ginoudis, Stefano Reale**

ORAL PRESENTATIONS
O13. HOW VETERINARY LABORATORIES CAN LEARN FROM A HUMAN MEDICAL LABORATORY PROBLEM
Kathleen Freeman

O14. VETERINARY LABORATORY TESTING NEEDS HARMONIZATION TO ENHANCE OPTIMAL PATIENT CARE
Kathleen Freeman

10:50-11:00 **CORPORATE PRESENTATION**
Economic Impacts of Diagnostic Accuracy: Innovations in Livestock & Poultry Testing
Christoph Egli

11:00-11:30 ***Coffee break & Poster Session***

KALLIRHOE HALL

11:30-12:00 **KEYNOTE LECTURE 7**
Chairs: **Fabrizio Vitale & Labrini Athanasiou**

Beyond the Human Eye: The Role of Machine Learning in Modern Veterinary Diagnostics
Grigorios Tsoumakas

12:00-12:10 **CORPORATE PRESENTATION**
From Insight to Impact: The Exopol Diagnostic Experience Translating years of diagnostic expertise into trusted qPCR kits for animal health
Sofia Lazaro

12:10-13:00 **KEYNOTE LECTURES**
Chairs: **Fabrizio Vitale & Labrini Athanasiou**

12:10-12:35 **KEYNOTE LECTURE 8:** From Field to Lab: Challenges and Advances in Wildlife Parasitology Diagnostics
Constantina Tsokana

12:35-13:00 **KEYNOTE LECTURE 9:** Uncovering the Hidden Hurdles: Challenges in Veterinary Laboratory Diagnostics for Research Animals
Vasileios Ntafis

13:00-14:00 *Lunch break*

	KALLIRHOE HALL	ATTICA HALL
14:00-15:30	**ORAL SESSION 4** **DIAGNOSTICS FOR WILDLIFE DISEASES: CHALLENGES & EMERGING SOLUTIONS** Chair: **Constantina Tsokana**	**ORAL SESSION 5** **ZOONOSES & THE ONE HEALTH APPROACH: BRIDGING ANIMAL, HUMAN, & ENVIRONMENTAL HEALTH** Chair: **George Valiakos**
14:00-14:10	**O15.** MULTISPECIES AND MULTIMATRIX VALIDATION OF THE INGEZIM® EHDV COMPAC ASSAY **Cristina Aira**	**O22.** THE SEROLOGICAL BENCHMARK: ADVANCING ONE HEALTH THROUGH PRECISION ELISA DIAGNOSTICS FOR *Chlamydia* AND *Coxiella* SURVEILLANCE **Enrica Omiccioli**
14:10-14:20	**O16.** INTEGRATING ENVIRONMENTAL SAMPLING INTO AVIAN INFLUENZA MONITORING FRAMEWORKS **Laura Bianchessi**	**O23.** ENSURING SAFE LIVESTOCK TRADE: A COMPREHENSIVE DIAGNOSTIC PANEL FOR PREEXPORT TESTING IN SHEEP **Enrica Omiccioli**
14:20-14:30	**O17.** CIRCOVIRUS-ASSOCIATED FEATHER LESIONS IN EURASIAN GRIFFON VULTURES *(Gyps fulvus)*: CLINICAL AND LABORATORY FINDINGS FROM A CROATIAN REHABILITATION CENTRE **Siniša Faraguna**	**O24.** WILD COYPU *(Myocastor coypus)* AS A SENTINEL OF ANTIMICROBIAL RESISTANCE IN THE AQUATIC ENVIRONMENT: A PRELIMINARY ENTEROBACTERIACEAE-BASED STUDY **Elisa Massella**
14:30-14:40	**O18.** EVALUATION OF THE PERFORMANCE OF ANTIBODY-BASED TESTS FOR THE DIAGNOSIS OF TUBERCULOSIS IN CAMELIDS **Alberto Gómez Buendía**	**O25.** DIAGNOSTIC CHALLENGES AND EMERGING SOLUTIONS FOR *Encephalitozoon cuniculi* INFECTIONS IN RABBITS **Alicja Laska-Modzelewska**
14:40-14:50	**O19.** DEVELOPMENT OF A LAMP ASSAY FOR THE DETECTION OF *Borrelia* SPP. AND ITS APPLICATION TO BAT SCREENING IN NORTHERN ITALY **Martina Magliocca**	**O26.** GENOTYPIC CHARACTERIZATION OF THE *Trichophyton mentagrophytes/Trichophyton* INTERDIGITALE SPECIES COMPLEX: INSIGHTS INTO HUMAN AND ANIMAL TRANSMISSION IN A ONE HEALTH CONTEXT **Deborah Cruciani**
14:50-15:00	**O20.** FIRST MOLECULAR DETECTION OF *Bartonella capreoli* AND HEMOTHROPIC Mycoplasmas IN EUROPEAN BISON *(Bison bonasus)* **Zahra Sohani**	**O27.** SARS-COV-2 EXPOSURE IN WILD CANIDS IN SERBIA: A ONE HEALTH PERSPECTIVE ON ZOONOTIC RISK **Diana Lupulović**
15:00-15:10	**O21.** USE OF THE IR BIOTYPER SYSTEM IN A VETERINARY DIAGNOSTIC LABORATORY – CLASSIFICATION OF *Streptococcus suis* ISOLATES BY SEROTYPES **Annet Heuvelink**	**O28.** A NOVEL HT-MLST APPROACH TO TRACK TRANSMISSION DYNAMICS AND GENETIC DIVERSITY OF *Leishmania infantum* **Stefano Reale**
15:10-15:30	Discussion	Discussion

15:30-16:00 *Coffee Break & Poster Session*

	KALLIRHOE HALL	ATTICA HALL
16:00-18:00	**ORAL SESSION 6** **OTHER SUBJECTS PERTAINING TO VETERINARY LABORATORY DIAGNOSTICS** Chair: **Serafeim Chaintoutis**	**ORAL SESSION 7** **ZOONOSES & THE ONE HEALTH APPROACH: BRIDGING ANIMAL, HUMAN, & ENVIRONMENTAL HEALTH** Chair: **Vasileios Ntafis**
16:00-16:10	**O29.** NEW IBR SERUM DOUBLE-ANTIGEN SANDWICH ELISA **Christoph Egli**	**O39.** DIGITAL PCR: AN INNOVATIVE TOOL FOR IMPROVING NOROVIRUS DETECTION IN A ONE HEALTH APPROACH **Jérôme Ventosa**
16:10-16:20	**O30.** ENHANCING EIAV MOLECULAR DETECTION BY OPTIMIZED PRE-ANALYTICAL CONDITIONS FOR TARGETING VIRAL CIRCULATING PARTICLES AND PROVIRAL RESERVOIRS THROUGH RT-QPCR AND QPCR **José Carlos Valle-Casuso**	**O40.** PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS, AND RISK FACTORS FOR *Staphylococcus aureus* FROM MEAT OF ANIMAL ORIGIN IN MEKELLE, TIGRAY, NORTHERN ETHIOPIA **Haftay Abraha Tadesse**
16:20-16:30	**O31.** MOLECULAR DETECTION OF GASTROINTESTINAL PATHOGENS: EVALUATION OF A FECAL TRANSPORT SYSTEM AND NUCLEIC ACID STABILITY **Francesca Stretti**	**O41.** ORNAMENTAL FISH AS A POTENTIAL AND UNDERSTUDIED SOURCE OF CROSS-BORDER ANTIMICROBIAL RESISTANCE **Athina Papadopoulou**
16:30-16:40	**O32.** DRIED BLOOD SPOT TESTING IN DOMESTIC PIGS: FIELD APPLICABILITY AND AUTOMATED DIAGNOSTIC PROCESSING **Isabelle Mutz**	**O42.** GENOMIC CHARACTERIZATION OF RIFT VALLEY FEVER VIRUS DURING A 2025 OUTBREAK IN UGANDA REVEALS ONGOING REGIONAL TRANSMISSION OF LINEAGE C **Caroline Musubika**
16:40-16:50	**O33.** DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR THE DETECTION OF BOVINE HERPESVIRUS 2 AND 4 IN BOVINE SAMPLES **Enrica Sozzi**	**O43.** INTEGRATED ONE HEALTH SURVEILLANCE REVEALS WEST NILE VIRUS EXPANSION BEYOND ENDEMIC AREAS IN FRANCE **Gaëlle Gonzalez**
16:50-17:00	Discussion	Discussion
17:00-17:10	**O34.** ENHANCING LONG-TERM PRESERVATION OF FAECAL SAMPLES FOR BACTERIAL CULTURE **Francesca Stretti**	**O44.** BENCHMARKING THE FIELD PERFORMANCE OF BOVINE PPD, DST-F FUSION PROTEIN AND P22 PROTEIN COMPLEX IN CELL-BASED DIAGNOSIS OF *Caprine Tuberculosis* USING BAYESIAN LATENT CLASS MODELS **Carlos Velasco**
17:10-17:20	**O35.** REALPCR MILQ-ID DNA SYSTEM: THE NEW SOLUTION FOR MASTITIS TESTING **Christoph Egli**	**O45.** EVALUATION OF A TRANSPORT MEDIUM FOR MICROBIAL PRESERVATION AND DISINFECTANT NEUTRALIZATION IN ENVIRONMENTAL MONITORING **Cristiano Sabelli**
17:20-17:30	**O36.** THE AVI-FLU PROJECT: ADVANCED ENVIRONMENTAL SURVEILLANCE FOR EARLY DETECTION OF RESPIRATORY PATHOGENS IN POULTRY PRODUCTION **Aaron Navarro**	**O46.** HUMAN–ANIMAL INTERACTION SAFETY: IMPLEMENTING RELIABLE METHODS FOR DETECTING PATHOGENIC *Capnocytophaga canimorsus* IN ORAL FLORA OF COMPANION AND THERAPY ANIMALS — PRELIMINARY RESULTS **Elisa Mazzotta**
17:30-17:40	**O37.** COMPARATIVE DETECTION OF ROTAVIRUS A IN FECAL AND EXTRA-INTESTINAL SAMPLES OF NEWBORN CALVES AND LAMBS AT THE ONSET OF DIARRHEA **Evangelia Moutou**	**O47.** SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *Toxoplasma gondii* AND *Neospora caninum* INFECTION IN HORSES IN GREECE **Panagiota Tyrnenopoulou**

17:40-17:50	**O38.** COMPARATIVE ASSESSMENT OF A COMMERCIAL ELISA AND A NEWLY DEVELOPED REAL-TIME PCR FOR THE DETECTION OF SMALL RUMINANT LENTIVIRUSES IN SHEEP AND GOATS **Aphrodite Kalogianni**	**O48.** WILD BOARS AS POTENTIAL RESERVOIRS FOR *Anaplasma phagocytophilum, Toxoplasma gondii,* AND *Leishmania infantum*: ANTIBODY DETECTION IN SERUM AND MEAT JUICES **Eleni G. Katsogiannou**
17:50-18:00	Discussion	Discussion
19:30-22:30	**CONGRESS DINNER**	

WEDNESDAY 21 OCTOBER 2026

	KALLIRHOE HALL
09:00-09:30	**KEYNOTE LECTURE 10** Chairs: **George Valiakos & Maria Linou**
	Antimicrobial resistance and One Health: a lesson from the sea **Marialaura Corrente**
09:30-10:50	**AMR** Research Groups
	Activities and initiatives of the National Public Health Organization (EODY) related to antimicrobial resistance **Lida Politi**
	O49. EARS-VET: A NETWORK FOR SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN CLINICAL ISOLATES FROM ANIMALS IN THE EU/EEA **Liesbeth Allais**
	O50. PETS, PATHOGENS, POLICY: LESSONS FROM GREECE ON AMR AND COMPANION ANIMALS **George Valiakos**
	O51. EU-JAMRAI2: Developing an antimicrobial stewardship program for food-producing and companion animals within EU **Panagiotis Katsoulos**
10:50-11:00	**CORPORATE PRESENTATION** A Complete Bruker Workflow for Veterinary Microbiology: Identification, Antimicrobial Resistance and Source Tracking **Hoenings Robert**
11:00-11:30	***Coffee break & Poster Session***

	KALLIRHOE HALL	ATTICA HALL
11:30-13:00	**ORAL SESSION 8** **AMR** Chairs: **George Valiakos & Maria Linou**	**ORAL SESSION 9** **OTHER SUBJECTS PERTAINING TO VETERINARY LABORATORY DIAGNOSTICS** Chair: **Zoe Polizopoulou**
11:30-11:40	**O52.** DIVERGENT GENOMIC AND ANTIMICROBIAL RESISTANCE PROFILES OF *Escherichia coli* FROM MASTITIS CASES AND CALF FECES IN A LOW-AMR PRESSURE DAIRY DISTRICT **Maria Filippa Addis**	**O59.** BEYOND TRADITIONAL DIAGNOSIS OF *Caprine Tuberculosis*: IMMUNE AND PHYSIOPATHOLOGICAL BIOMARKERS AND GUT MICROBIOTA **Carlos Velasco**

11:40-11:50	**O53.** DEVELOPMENT AND EXPANSION OF A VETERINARY *Escherichia coli* ANTIMICROBIAL SUSCEPTIBILITY TESTING PROFICIENCY TESTING SCHEME FOR ONGOING EXTERNAL QUALITY ASSURANCE IN LMICS **Thomas Chisnall**	**O60.** REFINING HISTOPATHOLOGICAL DIFFERENTIAL DIAGNOSIS OF *Bovine Tuberculosis*: STRATEGIES TO IMPROVE THE PERFORMANCE IN ROUTINE PRACTICE **Néstor Porras González**
11:50-12:00	**O54.** ANTIMICROBIAL RESISTANCE GENES IN ACINETOBACTER ISOLATES FROM A VETERINARY TEACHING HOSPITAL IN SPAIN **Carlota Martinez Torrecilla**	**O61.** DEFINING CELLULAR IMMUNE CORRELATES OF PROTECTION AGAINST AFRICAN SWINE FEVER VIRUS IN WILD BOAR **Sandra Barroso Arévalo**
12:00-12:10	**O55.** NEW EPIDEMIOLOGICAL CUT-OFF VALUES (ECOFF) FOR SULPHONAMIDES AND TRIMETHOPRIM AND ANIMAL PATHOGENS **Gudrun Overesch**	**O62.** REVISITING DOURINE DIAGNOSIS: PHYLOGENOMIC RELATIONSHIPS WITHIN TRYPANOZOON AND PERFORMANCE OF THE COMPLEMENT FIXATION TEST **Laurent Hébert**
12:10-12:20	**O56.** COMPARATIVE GENOMICS OF NDM-PRODUCING *Klebsiella pneumoniae* HUMAN HIGH-RISK CLONE ST307 IN COMPANION ANIMALS: A ONE HEALTH PERSPECTIVE **Riccardo Polani**	**O63.** ACUTE PHASE PROTEIN RESPONSES IN LPS INDUCED INFLAMMATION IN SHEEP **Eleni-Maria Christodoulou**
12:20-12:30	**O57.** COMPARATIVE GENOMICS OF ANTIMICROBIAL RESISTANCE AND VIRULENCE DETERMINANTS IN *Candida albicans* ISOLATES OF PUBLIC HEALTH RELEVANCE **Letizia Rillo**	**O64.** COMBINED ENDOCRINE AND INFLAMMATORY BIOMARKERS FOR HEALTH STATUS ASSESSMENT IN COMMERCIAL PIGS ACROSS PRODUCTION STAGES **Raúl Caballero-Falcó**
12:30-12:40	**O58.** EVALUATION OF A LIQUID-BASED SWAB TRANSPORT SYSTEM FOR MAINTAINING MRSA AND MSSA VIABILITY UNDER DIFFERENT STORAGE CONDITIONS **Francesca Stretti**	Discussion
12:40-13:00	Discussion	
13:00-14:00	*Light lunch*	
	KALLIRHOE HALL	
14:00-15:00	**ORAL SESSION 10** **EMERGING INFECTIOUS DISEASES & RECENT OUTBREAKS IN EUROPE** Chair: **Constantina Tsokana**	
14:00-14:10	**O65.** MOLECULAR DETECTION OF PORCINE PARAINFLUENZA VIRUS 1 IN SPANISH SWINE HERDS AFFECTED BY RESPIRATORY DISEASE **María Ubieto López**	
14:10-14:20	**O66.** CONTRIBUTION OF GENOMICS TO THE CHARACTERIZATION OF THE CAUSATIVE AGENT OF CONTAGIOUS EQUINE METRITIS *(Taylorella equigenitalis)* AND *Taylorella asinigenitalis,* AND TO OUTBREAK INVESTIGATION **Sandrine Petry**	
14:20-14:30	**O67.** AUTOMATED HIGH THROUGHPUT PCR FOR HPAI A(H5N1) MILK SURVEILLANCE **Ailam Lim**	
14:30-14:40	**O68.** IMPLEMENTATION AND INTER-LABORATORY TRANSFER OF A CXCL16 GENOTYPING QPCR ASSAY FOR CHARACTERISING EQUINE ARTERITIS VIRUS PERSISTENCE IN STALLIONS. **José Carlos Valle-Casuso**	
14:40-15:00	Discussion	
15:00-15:30	**AWARDS - CLOSING CEREMONY**	

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INVITED SPEAKERS

ANTIMICROBIAL RESISTANCE AND ONE HEALTH: A LESSON FROM THE SEA

M. Corrente[1] ,V.M. Bachmann[1,2]
[1]Department of Veterinary Medicine, University of Bari Aldo Moro, 70010 Valenzano, Italy
[2]University of Pavia, Italy

Mortality attributable to antimicrobial resistance (AMR) has risen substantially over the past three decades, placing AMR among the most urgent global public health challenges. Coastal environments, often densely populated and heavily affected by anthropogenic pressures, influence long-lived marine species that mature and forage in these areas. Sea turtles may therefore serve as valuable sentinels of coastal ecosystem health. Their microbiota can include AMR Gram-negative bacteria, some with pathogenic potential for humans. In this context, the Section of Infectious Diseases of the Department of Veterinary Medicine at the University of Bari has focused on loggerhead sea turtles (*Caretta caretta*) as potential carriers of AMR bacteria, promoting prudent antimicrobial use and evaluating alternative treatments for bacterial infections in animals affected by anthropogenic stressors. Recently admitted animals at the Sea Turtle Clinic, sampled before treatment, showed a high prevalence of Enterobacteriaceae, including the genera *Citrobacter*, *Morganella Klebsiella*, and *Enterobacter,* as well as non-Enterobacteriaceae such as *Pseudomonas spp.* and *Acinetobacter spp.* Marine bacteria, including *Shewanella algae*, *Vibrio alginolyticus*, and *Aeromonas hydrophila,* were also identified. In general high levels of resistance were found, mainly to beta-lactams and particularly to third-generation cephalosporins. Metagenomic analysis detected antimicrobial resistance genes, including beta-lactamase and extended-spectrum beta-lactamase genes. Multidrug resistance involved three to seven antimicrobial classes used in human medicine, livestock, companion animals, and aquaculture. As sea turtles rely primarily on innate immune responses to bacterial pathogens, and few or none molecules are registered for the treatment of those animals, these findings position rehabilitated sea turtles as critical indicators of AMR circulation at the human–animal–environment interface and underscore the urgent need for antimicrobial stewardship. Infection-control practices, avoiding the use of Medically Important Antimicrobials and exploring innovative non-antibiotic therapies to protect animal welfare, public health, and coastal ecosystem integrity are also a priority.

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THE CHANGING LANDSCAPE OF SMALL ANIMAL VIROLOGY: THE CONTRIBUTION OF THE SCHOOL OF VETERINARY MEDICINE OF BARI

V. Martella
Department of Veterinary Medicine, University of Bari, Valenzano, Italy

Over the past few decades, the field of small animal virology has undergone significant transformations, driven by the emergence of novel pathogens, the continuous evolution of endemic strains, and rapid advancements in molecular diagnostics. This presentation explores this dynamic landscape, highlighting the role and scientific contributions of the School of Veterinary Medicine of Bari. Key milestones in veterinary infectious diseases are reviewed, including the identification and genomic characterization of novel viral agents (i.e. parvovirus, coronavirus, calicivirus) in companion animals, extensive epidemiological surveillance, and the optimization of diagnostic strategies. By tracing the evolutionary trajectories of major canine and feline viruses, research from Bari has significantly deepened the global understanding of viral pathogenesis, interspecies transmission, and host-pathogen interactions. Ultimately, this overview illustrates how the School's dedicated research initiatives continue to shape modern veterinary virology, safeguard companion animal health, and support the broader One Health paradigm.

PREPAREDNESS BEYOND DIAGNOSTICS: THE ROLE OF VETERINARY LABORATORIES IN COMPLEX ANIMAL HEALTH SYSTEMS

V. Milićević
Institute of Veterinary Medicine of Serbia, Virology Department, Belgrade, Serbia

Veterinary laboratories have never been technically stronger. Modern molecular diagnostics can detect pathogens within hours, differentiate serotypes and lineages, generate whole-genome sequences, and support near-real-time exchange of information. Yet outbreaks continue to be amplified by failures occurring outside the laboratory: delayed disease recognition and reporting, incomplete epidemiological information, unrecorded animal movements, weaknesses in biosecurity and traceability, fragmented responsibilities, and delays in translating results into action. This lecture examines preparedness as a property of the entire animal-health system rather than of the diagnostic laboratory alone. Recent foot-and-mouth disease (1) and peste des petits ruminants' events in Europe (2) illustrate the contrast between increasingly high-resolution laboratory diagnostics and the lower-resolution epidemiological systems within which pathogens spread. Molecular characterization can distinguish introductions and transmission clusters, but cannot reconstruct movements, contacts, or other epidemiological information that was never captured. In this context, veterinary laboratories should function not only as diagnostic centers, but also as sentinels, integrators, translators, coordinators, and learning systems. Their expertise should contribute before, during, and after testing: from case definitions, sampling strategies and minimum metadata requirements to result interpretation, tracing, sequencing, surge capacity, and communication with epidemiologists and competent authorities. Preparedness therefore requires testing both analytical readiness and system readiness. Emergency exercises should challenge the entire pathway from suspicion to field action, including realistic failures in reporting, transport, data exchange, staffing, and communication. Ultimately, laboratory performance should be measured not only by turnaround time, but by time from suspicion to effective decision. The fastest PCR cannot compensate for the slowest decision.

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PHYLOGENETIC TAXONOMY FRAMEWORKS, THE ESSENTIAL BASIS FOR MICROBIAL GENETIC DIAGNOSTICS

A. Martínez-Murcia
Genetic PCR Solutions® and University Miguel Hernández, Orihuela, Alicante, Spain

Infectious diagnostics is a complex term, encompassing a set of characteristics, symptoms, signs, observations, tests, and the determination of a causative agent which may be achieved by identification (in a pure, axenic culture) or taxa-specific detection (in a complex sample), only possible when supported by a reliable, stable, and robust microbial classification framework. The taxonomy of microorganisms is complex, and the currently described species are not always properly identified by phenotypic traits. Due to their broad ecological adaptability, many viruses and bacterial species represent masterful models within the One Health concept.

Genetic diagnostic strategies by both, PCR or sequencing, should be properly designed on the foundations of a clear-cut delineation of species, which seems to be only provided by phylogenetic frameworks. Whole Genome Sequencing (WGS) provides data to assess metrics such as average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) with respect to reference genomes (i.e., type strains). However, establishing a stable species boundary is not an easy task, as some genomes showed borderline ANI and is-DDH values. In addition, genome analysis is a tedious approach for standard diagnostic laboratories. Quality of sequence data repositories is a crucial issue when designing genetic methods, as it may affect specificity and diagnostic sensitivity. The “molecular clock” hypothesis should be considered the most relevant milestone. The multi-locus phylogenetic analysis (MLPA) using housekeeping protein-coding genes, supported by the hypothesis of a synchronized/concerted mode of evolution (a “mirror” of genome evolution), is considered the most reliable and pragmatic framework allowing highly related species to be discriminated with extraordinary resolution.

Strains isolated and described so far could certainly represent only a fraction of real diversity, perhaps much smaller than we imagine. Classification into taxa (i.e., species) has been established under the assumption that there is "nothing" in between. That is, species are separated from each other, containing only diversity described in the laboratory, and assuming that the strains yet to be isolated fall within the profile defined in the species descriptions. The scenario is becoming worrisome as we see data accumulating, which in many cases forces us to reorganize the schemes, and consequently the genetic diagnostic designs.

UNCOVERING THE HIDDEN HURDLES: CHALLENGES IN VETERINARY LABORATORY DIAGNOSTICS FOR RESEARCH ANIMALS

V. Ntafis
Institute for Fundamental Biomedical Research (IFBR), Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Veterinary laboratory diagnostics in research animals plays a critical role in safeguarding health and welfare, ensuring the validity and reproducibility of in vivo studies, and protecting scientific integrity. However, diagnostic investigation in research animals presents unique scientific, technical and ethical challenges that may remain overlooked. The animals are often maintained under highly controlled conditions, yet subtle clinical, subclinical, and laboratory abnormalities may significantly influence experimental outcomes. The interpretation of diagnostic findings can be complicated by species- and strain-specific characteristics, as well as age, sex, genetic background, housing and husbandry conditions, experimental procedures, and the effects of anaesthesia, analgesia, or other interventions. In addition, validated diagnostic approaches and appropriate reference ranges are not equally available for all research species, strains, or non-traditional animal models. Pre-analytical factors, including sampling methods, timing, sample volume, handling, storage, and transportation, may further affect diagnostic results. At the same time, obtaining diagnostic samples raises important ethical considerations. The choice of sampling method, required sample volume, frequency of collection, and need for repeated sampling must be carefully balanced against potential pain, distress and other effects on animal welfare. These considerations are particularly important when serial sampling is required for monitoring disease progression, treatment response, or experimental endpoints. Applying the principles of the 3Rs, particularly refinement and reduction, should therefore be an integral part of diagnostic planning. This presentation will explore the often-hidden challenges of veterinary laboratory diagnostics in research animals, with an emphasis on the need to integrate diagnostic accuracy with animal welfare and experimental requirements. It will also highlight the importance of harmonised diagnostic procedures, appropriate reference data, quality assurance, and close collaboration between veterinarians, animal-care personnel, and researchers. Recognising and addressing these challenges can improve animal welfare, reduce experimental confounding, and strengthen the reproducibility and scientific value of animal research.

FROM FIELD TO LAB: CHALLENGES AND ADVANCES IN WILDLIFE PARASITOLOGY DIAGNOSTICS

C.N. Tsokana
School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

Parasites are central to wildlife health, conservation and One Health surveillance. Yet evidence may be compromised before a specimen reaches the laboratory. As in domestic animals, results depend on aligned diagnostic question, specimen, preservation, method, quality control and interpretation. In wildlife, this diagnostic pathway is further complicated by the challenges of sampling free-ranging hosts, intermittent parasite shedding, uncertain sample origin, environmental exposure and limited assay validation1. Thus, simple questions—which parasite is present, in which host, and with what zoonotic relevance?—become complex diagnostic decisions. To this end, veterinary laboratories need workflows from field collection to final interpretation.

The process begins pre-analytically. Fitness-for-purpose selection of faeces, blood, tissues, carcass material, ectoparasites or environmental samples must be accompanied by field metadata, biosafety and appropriate preservation. Non-invasive sampling can widen surveillance coverage and support animal welfare; however, its value depends on freshness, contamination risk, reliable host assignment and sufficient material for parasite detection1,2.

Within the laboratory, classical parasitology remains the cornerstone of diagnosis. Gross examination, flotation, sedimentation and Baermann techniques, quantitative faecal methods and morphological identification provide screening and material for confirmatory testing. Their limitations—including overlapping morphological characteristics, low parasite burdens, and variable or intermittent shedding—define where molecular methods add value1. Conventional PCR and quantitative PCR assays support species-level confirmation and parasitic load quantification, whereas sequencing and metabarcoding can characterize mixed parasite communities3. These approaches cannot compensate for poor sampling or inadequate interpretation; inhibition, contamination, and host- and matrix-specific assay performance require consideration. Likewise, serological methods provide evidence of individual or population exposure but generally do not demonstrate active infection1.

A practical algorithm begins by defining the diagnostic or surveillance question; collecting and preserving aliquots for complementary testing; screening with conventional methods; molecularly confirming priority findings; and reporting uncertainty, limitations and epidemiological significance. Reliable wildlife parasitology depends on an auditable, quality-assured pathway linking field ecology, laboratory science and public-health action4.

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BEYOND THE HUMAN EYE: THE ROLE OF MACHINE LEARNING IN MODERN VETERINARY DIAGNOSTICS

G. Tsoumakas
School of Informatics, Aristotle University of Thessaloniki, Greece

This talk reviews recent collaborative work with the School of Veterinary Medicine at Aristotle University of Thessaloniki and the Faculty of Veterinary Medicine of the University of Thessaly, regarding the application of machine learning techniques on veterinary diagnostics spanning different species (chickens, canines, sheep) as well as different data types and acquisition methods (infrared thermography, lymph node smears, blood smears). I will start with our work on diagnosing subclinical mastitis in Lacaune sheep using infrared thermography. I will discuss using computer vision to identify the region of interest (udder), which can be further analyzed in a second phase using statistical methods to detect abnormalities, such as higher temperatures [1]. I will also present a new dataset and empirical results on detecting subclinical mastitis using deep learning and traditional machine learning [2]. I will continue with our work on the detection of Leishmania spp. amastigotes in canine lymph node smear images [3], where we propose an automated synthetic data generation approach to address data scarcity and imbalance issues. Our results show that incorporating synthetic data significantly enhances deep learning models' ability to detect amastigotes, offering a promising solution for veterinary diagnostics. Additionally, we introduce a new dataset that combines both original and synthetic data, contributing to further research into this important zoonotic disease. Finally, I will close with our recent work on avian blood analysis [4], where we contribute a new dataset of 4,600 blood smear images, 1,000 of which annotated with cell types (red blood cells, thrombocytes, lymphocytes, heterophils and monocytes) in order to accelerate the development of avian blood analysis systems. In addition, we show that a pipeline combining cell segmentation with self-supervised representation learning scales to low-resource settings, outperforming typical object detection.

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FAST DISEASES OUTBREAKS IN GREECE: IDENTIFYING AND OVERCOMING DIAGNOSTIC CHALLENGES FOR IMMEDIATE STAMPING OUT

G. Valiakos
Faculty of Veterinary Medicine, University of Thessaly, Greece

Greece is being challenged in the last years by a series of outbreaks of FAST diseases (Peste des Petits Ruminants/PPR, Sheep and goat pox, Foot-and-mouth disease).
First case of PPR in Greece, was confirmed on 11 July 2024, near Trikala, Thessaly region. Epidemiological tracing linked the outbreak to animal traders and importations from Romania, with 23 positive farms confirmed across seven sites in Thessaly by 1 August, rising to 41 cases in 10 sites by 30 September 2024.
Thessaly presented exceptional risk factors: approximately 2 million sheep and goats across 6,000 holdings, with high farm density, semi-nomadic husbandry, and no natural geospatial barriers. Diagnostic challenges were compounded by limited practitioner experience with this exotic disease and atypical clinical presentation: affected animals exhibited slower progression than reported in the literature, with mild early signs (nasal discharges, coughing) and low initial mortality, making early clinical diagnosis difficult.
To overcome these challenges, a Regional PPR Control Laboratory was established in Larissa within five days. The facility was equipped with robotic genomic extraction systems and dedicated qPCR platforms. Validated commercial assays (ID Gene PPR Duplex qPCR and ID Screen PPR Competition cELISA) were adopted following proficiency testing with the EURL-PPR. Field sampling teams conducted active surveillance, testing 100-200 animals per day, with intensified sampling from asymptomatic animals in high-risk zones.
During the 12-week surveillance period (5 August - 24 October 2024), approximately 3,000 field visits were conducted, 1,000 farms tested, and 7,000 animals screened. No new positive cases were detected in the final four weeks, confirming the effectiveness of the rapid diagnostic response and stamping out policy. This experience demonstrated the value of decentralized diagnostic capacity, adaptive active surveillance, and multi-stakeholder coordination in managing FAST disease incursions. The country regained the PPR free status in 18th of August 2026.

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TOWARDS DISEASE CONTROL TOOLS TO SECURE ANIMAL AND PUBLIC HEALTH IN A CHANGING WORLD

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The rapidly changing global landscape—driven by climate change, emerging and re-emerging pathogens, expanding animal mobility and trade, intensification of livestock production, and evolving human–animal interactions—poses increasing challenges to animal and public health. Addressing these challenges requires innovative, evidence-based and sustainable disease-control tools, supported by international harmonization and a One Health approach. This lecture will explore advances in disease surveillance, early-warning systems, diagnostics, vaccines, therapeutics, biosecurity, and digital technologies for the prevention and control of infectious diseases at the animal–human–environment interface. Particular emphasis will be placed on the complementary roles of the World Organisation for Animal Health (WOAH) and DISCONTOOLS in strengthening global disease-control capacity. WOAH provides internationally recognized, science-based standards that promote harmonization of disease surveillance, diagnosis, vaccination, notification, risk management, and other control measures, while supporting transparency and safe international trade. DISCONTOOLS complements this framework by identifying critical gaps and research needs in diagnostics, vaccines, pharmaceuticals, and other disease-control technologies, thereby helping to guide research priorities and innovation. The lecture will consider how these frameworks can be translated into practical solutions through genomic surveillance, rapid and field-deployable diagnostics, artificial intelligence, integrated data systems, risk-based approaches, and improved preparedness and response. Leishmania and other complex vector-borne diseases will provide illustrative examples of the challenges associated with disease emergence, surveillance, diagnosis, prevention, and control across animal and public health sectors. Ultimately, effective disease control requires scientific innovation, international harmonization, interdisciplinary collaboration, capacity building, and equitable access to technologies. All research and interventions should comply with internationally recognized principles of animal welfare, research ethics, biosafety, biosecurity, and data protection. Together, WOAH standards, DISCONTOOLS priorities, and One Health collaboration can help secure animal and public health in an increasingly interconnected and changing world.

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4. DISCONTOOLS. Disease Control Tools. European Commission-funded initiative

OVERVIEW OF THE EMERGENCE OF CERTAIN VECTOR-BORNE DISEASES IN ANIMAL HEALTH IN EUROPE SINCE THE 2000S

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Over the past thirty years, numerous diseases that were never expected to appear in Europe have emerged within the territory of the European Union: vector-borne diseases such as the orbiviruses of Bluetongue or Epizootic Hemorrhagic Disease. Before 1998, Europe was free from orbiviruses. Since that year, more than 13 serotypes of Bluetongue virus have been identified, as well as the epizootic Hemorrhagic Disease virus. Bluetongue (BT) and Epizootic Haemorrhagic Disease (EHD) are non-contagious infectious diseases that affect a large number of domestic and wild ruminants. They are transmitted to ruminants by small blood-feeding midges of the genus *Culicoides* (*Ceratopogonidae* family).
But other arboviruses have also emerged in Europe, such as the zoonotic flaviviruses West Nile and Usutu viruses. West Nile virus was first detected in the Mediterranean Basin 13 years after its discovery in Uganda (in 1937). In the 1990s, regular WNV outbreaks occurred in the MB, and the virus has expanded its area of circulation in many MB countries. The outbreaks were attributed to WNV lineages 1 and 2, and the occurrence of both lineages varied across different countries. In the last 20 years, several MB countries have witnessed large WNV outbreaks in humans and animals, particularly in 2018 and 2022 and more recently in 2025 in humans and horses. This suggests strong spatio-temporal heterogeneities in the epidemiology of WNV over the past two decades, attributed to transport and climatic changes that significantly affect the life cycle and dispersal of avian and mosquito species.
Orthobunyaviruses such as Schmallenberg virus in 2011 and Shamonda in 2026 have also emerged unexpectedly in Europe.
In 2025, the poxvirus responsible for Lumpy Skin disease has also emerged in Italy, Spain and France.
This presentation will describe these various emergences of arboviruses as well as the challenges that Europe will face in the future.



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ORALS



O01. ANALYTICAL AND FIELD VALIDATION OF A MULTIPLEX RT-PCR FOR DETECTION OF BTV AND EHDV IN RUMINANT SPECIES

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Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are closely related orbiviruses known for their co-circulation and overlapping clinical presentations across different ruminant species.
To perform analytical and field validation of the new RealPCR BTV/EHDV assay, a study was conducted. Analytical validation included *in silico* (NCBI BLAST) analysis, as well as experimental assessment of inclusivity, exclusivity, limit of detection (LOD), and diagnostic specificity.
The assay achieved a limit of detection of 10 copies per reaction for each target and demonstrated 100% diagnostic specificity.
Exclusivity testing, including *in silico* analysis and testing of various bovine pathogens, demonstrated no amplification for any of the non-target pathogens.
Inclusivity was evaluated using 39 BTV and 11 EHDV strains representing different serotypes and outbreaks. Results were 100% accurate, and consistent with the *in silico* analysis.
In a trial comparing the RealPCR BTV/EHDV assay to another commercial PCR kit, 30 samples were tested, including blood and tissue (spleen and others) from bovine (n=23), ovine (n=1), elk (n=1), mule deer (n=1), and white-tailed deer (n=4). Among these, 7 were negative, while the others were positive for BTV, EHDV, or were co-infected.
The RealPCR assay showed superior sensitivity for both BTV and EHDV:

- For BTV, 16 samples were concordant between methods, and 14 were only detected by the RealPCR assay
- For EHDV, 26 samples were concordant, and 4 were only detected by the RealPCR assay
- Ct values were consistently lower (indicating stronger signals) with the RealPCR assay *vs.* the commercial kit
- The Internal Sample Control, used to detect host RNA, was consistently positive across all species tested, demonstrating its multi-species capability.

This study demonstrates robust analytical performance and superior sensitivity of the multiplex RealPCR BTV/EHDV assay, supporting its use as a reliable tool for routine diagnostics across multiple ruminant species in diverse regions.

O02. METATRANSCRIPTOMICS BASED APPROACH FOR VIRUS DISCOVERY IN ENCEPHALITIS CASES USING HIGH-THROUGHPUT SEQUENCING

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Disseminated non-suppurative encephalitis in animals is associated with viral infections; however, the causative viral agent often remains unidentified despite established routine diagnostic methods such as PCR and immunohistochemistry. This may result from viral clearance, low viral abundance or the presence of a novel or unexpected virus. Metatranscriptomic high-throughput sequencing (HTS) combined with bioinformatic analysis provides an unbiased approach for the detection and discovery of viruses. In retrospective investigations, formalin-fixed and paraffine embedded (FFPE) tissue is often the only available material, although formalin fixation leads to degradation of nucleic acids and cross-linking. These limitations can be mitigated by optimized RNA extraction protocols and adapted HTS strategies using short read lengths (≤ 100 base pairs) and single-end sequencing. With an established bioinformatics pipeline, large sequencing datasets can be efficiently analyzed, enabling the assembly of viral sequences from FFPE material with adequate coverage. In a retrospective study, Feline Morbillivirus (FeMV) was identified as a novel virus in a two-month-old Bengal cat with encephalitis. Additionally, Tick-Borne Encephalitis Virus (TBEV) was unexpectedly detected in three Dalmatian puppies presenting with neurological signs in late autumn. Furthermore, novel neurotropic bastroviruses were identified in pigs from separate farms. Overall, HTS-based metatranscriptomics enables the detection of known unexpected viruses and the discovery of novel viruses, expanding our understanding of viral diversity in animal encephalitis.

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O03. LABORATORY AND FIELD SOLUTIONS FOR THE RAPID DETECTION OF HIGHLY PATHOGENIC H5 AVIAN INFLUENZA VIRUSES OF CLADE 2.3.4.4b

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Since 2021, the global spread of highly pathogenic H5N1 avian influenza viruses (HPAIV A(H5N1)) of clade 2.3.4.4b has reached unprecedented levels, resulting in significant economic losses in poultry production, ecosystems disruption, and sporadic zoonotic risk1. In this context, robust diagnostic tools capable of rapidly detecting 2.3.4.4b HPAIV A(H5) are essential for efficient surveillance and timely implementation of control measures. The molecular gold standard for assessing the pathogenicity of A(H5) viruses is the determination of the hemagglutinin (HA) cleavage site by RT-PCR followed by Sanger sequencing, which requires dedicated equipment and long turnaround times2.
To accelerate diagnosis and improve the capacity of laboratories equipped with standard instrumentation, we developed and validated i) a RT-qPCR assay enabling discrimination between A(H5) HPAIV and low pathogenic viruses (LPAIV) and ii) a hybrid method combining the sensitivity of RT-PCR with the specificity of CRISPR/Cas12 system. Additionally, considering the growing relevance of point-of-care (POC) tools for field detection of A(H5) viruses, we combined the CRISPR/Cas12 reaction with rapid RNA isolation and RT-RPA isothermal amplification as a proof of concept.
The analytical sensitivity of the RT-qPCR and RT-PCR/Cas12 assays was comparable to that of RT-PCR/Sanger, detecting down to 101.5 EID50/100 L of clade 2.3.4.4b A(H5) HPAIV with high repeatability. Testing of clinical specimens showed 100% diagnostic sensitivity and no cross-reactions, while enabling faster and sequencing-independent pathotyping in the laboratory. Preliminary evaluation of the RT-RPA/Cas12 isothermal assay showed promising sensitivity as a POC system for in-field diagnostics, although further optimization is required to simplify the workflow.
Overall, the proposed laboratory-based approaches proved effective in accelerating A(H5) HPAIV detection, while the isothermal assay showed potential for field applications aimed at supporting central laboratories.

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O04. RESURGENCE OF EQUINE INFECTIOUS ANEMIA IN FRANCE IN 2025

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Equine infectious anemia (EIA) is a viral disease of equids caused by the Equine Infectious Anemia Virus (EIAV), a lentivirus responsible for persistent lifelong infection, with transmission mainly occurring through hematophagous insects or iatrogenic routes (1). The objective of this study was to describe the epidemiological and molecular investigation of an EIA outbreak detected in southern France in 2025. Following the detection of an EIAV-seropositive index case in April 2025 in the Var department, an epidemiological inquiry was initiated in accordance with French regulations. In France, EIA diagnosis was based on the agar gel immunodiffusion test (Coggins test) performed by a laboratory within the national network of approved laboratories. All positive sera were confirmed by the National Reference Laboratory, and EIAV-infected equids were euthanized as part of control measures. Molecular characterization was conducted on available samples using nested PCR amplification and phylogenetic analysis of partial gag gene sequences. The investigation identified three epidemiologically linked outbreaks, leading to the detection and euthanasia of six EIAV-seropositive equids. Phylogenetic analysis of three strains showed close genetic relatedness and clustering with EIAV strains previously identified in France between 2009 and 2023, suggesting viral persistence and re-emergence rather than the introduction of a new EIAV strain. However, the exact origin of infection could not be determined due to incomplete animal movement records (2). This outbreak highlights the continued circulation of EIAV in areas with favorable vector conditions and highlights the limitations of current surveillance systems. Systematic EIA testing of horses prior to movement, introduction into new facilities, or change of ownership, and particularly for breeding purposes, in addition to official movement-related testing, would represent an effective strategy to limit the spread of EIA within the French equine population.

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02. The Future of Vaccine Development: Insights from Diagnostics

O05. BIOCHEMICAL SAFETY PROFILE OF THE Lv17/WB/Rie1-ΔCD VACCINE CANDIDATE AGAINST AFRICAN SWINE FEVER IN WILD BOAR

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African Swine Fever (ASF) is a severe, highly contagious haemorrhagic disease, causing major losses to animal health and the global swine industry. In the European Union, wild boar populations account for approximately 70% of outbreaks, making wildlife vaccination critical. Although live attenuated vaccines have shown potential, their production depends on primary cell cultures (porcine alveolar macrophages), which limits scalability and standardization. This study evaluates the biochemical safety profile of the Lv17/WB/Rie1-ΔCD vaccine candidate, produced in both PAMs (n=12) and the MA104 commercial cell line (n=9). Systemic biochemical profiling was used as a diagnostic indicator of physiological stability. Wild boars were vaccinated orally with two doses and challenged at day 28 with a highly virulent genotype II strain (Arm07). A non-vaccinated control group was included for comparative analysis (n=4). Serum biochemistry, including liver enzymes (ALT, ALP), renal markers (BUN, creatinine, urea), and total protein profiles, was monitored throughout the vaccination, revaccination, and challenge phases. Results showed that vaccinated animals maintained stable values within established physiological ranges, and hepatic and renal enzymes levels remaining largely below the threshold (Supplementary figure 1). Conversely, non-vaccinated control animals exhibited severe diagnostic shifts post-challenge, notably acute azotemia with urea and creatinine reaching 85,2 ± 19,22 mg/dL and 6,0 ± 1,31 mg/dL, respectively (Supplementary figure 1). While controls showed marked hypoglycaemia and increased hepatic enzymes, vaccinated groups remained stable ($p<0,01$). These findings demonstrate that Lv17/WB/Rie1-ΔCD does not induce systemic toxicity or metabolic impairment. The maintenance of biochemical homeostasis during the vaccination period confirms the safety of the candidate. Furthermore, the stability of biochemical parameters during the challenge period underscores its efficacy. Taken together, these findings highlight the Lv17/WB/Rie1-ΔCD candidate as a promising tool for disease control in wild boars.

Standard Abbreviations used: ASF (African Swine Fever), ALT (Alanine aminotransferase), ALP (Alkaline Phosphatase), BUN (Blood Urea Nitrogen).

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02. The Future of Vaccine Development: Insights from Diagnostics

O06. TRAINED IMMUNITY IN VETERINARY DIAGNOSTICS: COMPARATIVE USEFULNESS OF CYTOKINES, HISTOPATHOLOGY AND MOLECULAR TECHNIQUES ACROSS EXPERIMENTAL MODELS

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Trained immunity emerged as a promising strategy to enhance protection against certain pathogens relevant in veterinary medicine. In experimental models, combining trained immunity inducers with pathogen-specific vaccination, not all measured parameters provide the same diagnostic value.
Evaluate the usefulness of different diagnostic techniques applied in experimental trained immunity studies and identify those that provide best data to evaluate immune modulation and protection.
We performed a comparative analysis of experiments involving the use of live or inactivated porcine-derived BCG (dpB), alone or combined with pathogen-specific vaccination in murine and swine models challenged with *Streptococcus suis*, *Salmonella* Choleraesuis or SARS-CoV-2. The evaluated techniques were cytokine quantification, phagocytosis assays, histopathology, culture-based methods, and molecular methods for pathogen detection and quantification. Within each study, a protection score was built using direct outcome variables, including pathogen burden, lesion severity and clinical status. The association of each parameter with protection was then assessed comparatively across models.
Histopathology represented the most robust technique, showing strong associations with protection. Culture-based methods showed the highest discriminatory capacity in bacterial models, whereas molecular techniques, including PCR, also showed a strong and reproducible association with protection. By contrast, cytokines displayed variability across models, dpB format and sampling times, and their association with protection was less consistent. In some situations, increased cytokine responses were linked to worse outcomes, whereas balanced profiles were associated with lower pathogen burden and milder lesions. Phagocytosis provided functional information but showed limited discriminatory value.
In our studies, histopathology is the most robust technique for assessing protection across models, while culture and molecular detection methods provide highly informative direct measures of pathogen control. Cytokines remain useful biomarkers, but their interpretation should be done carefully, and they should not be prioritized over direct measures of lesion severity and pathogen burden in trained immunity studies.

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O08. HEAT PRE-TREATMENT PRESERVES ANTIBODY DETECTION WHILE INACTIVATING INFLUENZA VIRUS IN RAW MILK

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The spillover of highly pathogenic avian influenza A(H5N1) into dairy cattle in the United States necessitates enhanced surveillance and testing strategies. To complement PCR-based detection under the National Milk Testing Strategy, the IDEXX Avian Influenza MultiS-Screen enzyme-linked immunosorbent assay (ELISA) was implemented for antibody detection in milk. High viral loads in raw milk pose a significant occupational risk to laboratory personnel because the automated ELISA plate washer does not fit within a biosafety cabinet. This study evaluated heat inactivation methods to mitigate biosafety risk while preserving antibody integrity. A high-titer stock of a low-pathogenic influenza virus was spiked into 0.5 mL raw milk at 2.0×107 plaque-forming units (PFU)/mL in a 96-well deep-well plate. Samples were heat-treated at 63°C for 15 or 30 minutes using either a dry incubator or water bath. Following heat-treatment, 200 µL of each sample was inoculated into two 10-day-old embryonated chicken eggs to assess viral viability. Viral replication was evaluated via hemagglutination (HA) assay on allantoic fluid collected 48 hours post-inoculation, followed by a second egg passage and repeat HA assay. Minimal viral inactivation was observed following dry incubator treatment. Water bath treatment at 63°C for 15 minutes resulted in incomplete inactivation, whereas no viable virus was detected after 30 minutes. The impact of heat pre-treatment on ELISA performance was assessed using 170 known antibody positive and negative milk samples. The antibodies integrity was confirmed with ELISA optical density or sample-to-negative (S/N) values that were equivalent between untreated and heat-treated samples. These results identify a heat pre-treatment protocol that effectively inactivates influenza virus in raw milk while maintaining ELISA performance, enabling safe testing in a biosafety level 2 laboratory and supporting expanded high-throughput surveillance efforts.

O11. ASFV MONODOSE-LAMP®: A FIELD-READY INNOVATIVE FORMAT FOR THE GENETIC DETECTION OF AFRICAN SWINE FEVER VIRUS

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African swine fever is a highly contagious viral disease affecting domestic pigs and wild boars, causing severe economic losses worldwide. Although quantitative polymerase chain reaction (qPCR) remains the reference method for African swine fever virus (ASFV) detection, its implementation usually requires laboratory infrastructure and specialized equipment, limiting rapid on-site diagnosis. The objective of this study was to develop a rapid, field-ready molecular assay for ASFV detection based on loop-mediated isothermal amplification (LAMP) technology in a ready-to-use MONODOSE-LAMP® format. The assay consists of individual tubes containing dehydrated reagents stable at room temperature, requiring only diluted sample addition before amplification. Specific primer sequences transferred from the Animal Health Research Center IRTA-CReSA (Barcelona) were adapted and optimized for the commercial MONODOSE-LAMP® format. *In vitro* validation demonstrated high reproducibility, repeatability, and analytical sensitivity, with positive amplification obtained within 25 minutes. The analytical sensitivity obtained is within the range reported for qPCR assays while offering a faster and field-deployable workflow. The assay showed stability at room temperature for at least 30 days and was tested using reference samples from some ASFV genotypes provided by the national reference laboratory CISA-INIA (Madrid). In addition, the system can be combined with environmental sampling using GPSponge®, a rapid and safe sampling method, developed in collaboration with VISAVET (UCM, Madrid), that inactivates pathogens while preserving nucleic acids, enabling environmental monitoring of ASFV in farm settings. ASFV MONODOSE-LAMP® represents a promising tool for rapid field surveillance and early detection of African swine fever, facilitating faster decision-making and supporting improved biosecurity and outbreak control strategies.

O12. LAMP TECHNOLOGY: A RAPID MULTIPLEX DIAGNOSTIC TOOL FOR THE DETECTION OF PATHOGENS INVOLVED IN BOVINE RESPIRATORY DISEASES

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Bovine respiratory disease (BRD) is a polymicrobial syndrome involving viral and bacterial pathogens including *Histophilus somni*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis*, bovine coronavirus, bovine respiratory syncytial virus, and parainfluenza virus type 3. The complexity of co-infections and increasing antimicrobial resistance (AMR) limit targeted treatment, leading to empirical antimicrobial use. Rapid point-of-care diagnostics are needed to improve antimicrobial stewardship. We evaluated a multiplex isothermal loop-mediated amplification (LAMP) assay (RespiSensor) for detection of major BRD pathogens in field conditions. Nasopharyngeal swabs from 23 cattle were tested for seven respiratory targets using lyophilized reaction beads and a portable reader with ~40 min runtime. Results were compared with laboratory PCR, with Ct >35 considered negative. Agreement, sensitivity, and specificity were calculated per pathogen. Across 23 samples, 161 paired results were obtained. Overall agreement with PCR was 86.3%. Sensitivity was 71.2% and specificity 93.6%. Sensitivity was highest for bovine respiratory syncytial virus (100%) and *M. bovis* (91.7%), intermediate for bovine coronavirus (88.9%) and *P. multocida* (71.4%), and lower for *M. haemolytica* (63.6%). Specificity reached 100% for several targets including RSV, PI3, *M. bovis*, and *P. multocida*. These results indicate that multiplex LAMP provides rapid and highly specific detection of BRD pathogens at the point of care, with variable sensitivity depending on the target. Despite moderate sensitivity, the rapid turnaround time supports improved clinical decision-making and antimicrobial stewardship in cattle respiratory disease management.

Cible	NEG/NEG	POS/POS	PCR+LAMP-	PCR-LAMP+	Total	Concordance	Sensibilité (%)	Spécificité (%)
RSV	19	4	0	0	23	100%	100	100
Coronavirus	9	8	1	5	23	74%	88,9	64,3
Parainfluenza	20	1	2	0	23	91%	33,3	100
M. bovis	11	11	1	0	23	96%	91,7	100
M. haemo	11	7	4	1	23	78%	63,6	91,7
P. multo	2	15	6	0	23	74%	71,4	100
H. somni	21	0	1	1	23	91%	0	95,5

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Other

O13. HOW VETERINARY LABORATORIES CAN LEARN FROM A HUMAN MEDICAL LABORATORY PROBLEM

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Introduction: A problem with HbA1c testing in the United Kingdom resulted in an estimated 500,000 people being misdiagnosed with pre-diabetes or diabetes. Multiple contributing factors were identified by various taskforces and committees. These factors also apply to veterinary laboratories. Objectives: (1) Review the problem that occurred with HbA1c testing and the contributing factors that were identified. (2) Present the corresponding status in veterinary laboratories. (3) Discuss the actions and improvements that may be needed in many veterinary in-clinic and reference laboratories. Methods: The factors contributing to erroneous HbA1c results are reviewed. The current status of veterinary laboratory quality systems is available from the Westgard 2017 Veterinary Laboratory Quality Survey and personal conversations and experiences of the author in teaching this topic in an online course. Results: Factors contributing to erroneous results that are problematic in veterinary laboratories included (1) lack of clarity about responsibility for making decisions to suspend testing following identification of a bias problem, (2) reliance on a single test result, (3) lack of characterization of lot-to-lot variation by the manufacturer and laboratory, (4) lack of use of third-party independent quality control materials, (5) lack of key performance indicators using patient data, (6) false sense of security with accreditation, (7) insufficient information from EQA providers, (8) lack of adherence to maintenance requirements, (9) inadequate training of laboratory personnel, (10) lack of rapid response by manufacturer, (11) lack of communication between laboratories, manufacturers and clinicians, (12) lack of rerunning patient samples following QC failure, (13) lack of clarity in definition of 'patient harm.' Conclusions: Many veterinary laboratories have problematic areas that are the same as those that occurred in the human laboratories with HbA1c problems. A need exists for ongoing laboratory quality education for veterinary in-clinic and reference laboratories and better design of veterinary laboratory quality systems.

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Other

O14. VETERINARY LABORATORY TESTING NEEDS HARMONIZATION TO ENHANCE OPTIMAL PATIENT CARE

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Introduction: Many consensus-based guidelines for veterinary patients include clinical decision limits based on laboratory results. Significant bias and/or imprecision in different laboratories applying these guidelines, may result in erroneous classification of patients.
Objectives: (1) Introduce concepts of laboratory result harmonization. (2) Review the benefits and challenges associated with harmonization. (3) Review approaches to harmonization in veterinary laboratory medicine.
Results: The desired outcome of harmonization is for results produced from different clinical laboratory measurement systems to be equivalent (interpreted in the same way), within clinically meaningful limits.
Benefits of harmonization include: (1) Ethical support for accurate diagnosis and monitoring of patients, (2) Best use of clinical practice consensus guidelines, (3) Support of electronic medical records and artificial intelligence advances, (4) Support for research and meta-analyses using results across laboratories, and (5) Use of common reference intervals.
Harmonization is likely to be the best approach for veterinary medicine because: (1) Requirements are set by consensus agreement rather than traceability to a complex system of measurement, (2) It uses existing calibrators, patient sample pools with assigned values, and mathematical correction of results based on assessment studies.
Challenges for harmonization in veterinary medicine, include but not limited to the following: (1) Lack of recognition of the need for harmonization, (2) Determination of a 'will to harmonize' within the veterinary community and involved stakeholders, (3) Lack of knowledge and experience at all levels of harmonization efforts (clinicians, laboratorians, manufacturers), and (4) Determination of whether harmonization based on studies in one species is applicable for other species
Approaches to veterinary laboratory result harmonization may include harmonization of (1) In-clinic analyzers to the analyzers in their local reference laboratory, (2) Organizational networks of instruments, (3) Geographic regional instruments and (4) Global harmonization of critical measurands for important animal diseases.

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04. Diagnostics for Wildlife Diseases Challenges & Emerging Solutions

O15. MULTISPECIES AND MULTIMATRIX VALIDATION OF THE INGEZIM® EHDV COMPAC ASSAY

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Introduction. Epizootic hemorrhagic disease (EHD) is a viral infection affecting domestic and wild ruminants that has spread across Europe since 2022, mainly impacting cattle and red deer. Transmission occurs via dipterans of the Culicoides genus, which are also vectors of the bluetongue virus (BTV). Given its recent emergence and ongoing spread, reliable serological diagnosis is essential for surveillance, early detection, and the implementation of effective control and prevention strategies. Thereby, this study presents the validation of the INgezim® EHDV Compac competitive ELISA, based on the recombinant EHDV VP7 protein and a specific monoclonal antibody. The test is designed to detect antibodies against EHDV in multiple species and biological matrices.

Methodology. Assay inclusivity was evaluated using reference sera representing all virus serotypes. A total of 2,696 field samples were analyzed to determine assay performance including: 1,005 cattle, 958 sheep/goats, 370 deer, 203 fallow deer, and 160 mouflons. Samples were characterized by a commercially available competitive ELISA. Applicability across different matrices was assessed using 115 paired samples of plasma, whole blood, and dried blood spots on filter paper. Analytical specificity was evaluated using 156 bovine samples positive for other diseases: 87 for BTV, 24 for infectious bovine rhinotracheitis (IBR), 24 for bovine viral diarrhea (BVD), and 21 for tuberculosis.

Results. The ELISA showed 100% sensitivity and 99.8% specificity. Results were consistent when using whole blood or plasma, with only a slight decrease in sensitivity when using dried blood spots. No cross-reactivity was observed with samples positive for other diseases, demonstrating high exclusivity, and all viral serotypes were reliably detected.

Conclusion. These results demonstrate that INgezim® EHDV Compac is a robust, versatile, and reliable diagnostic tool, suitable for epidemiological surveillance and differential diagnosis of EHD in multi-species settings, facilitating early detection and effective disease control in Europe.

O16. INTEGRATING ENVIRONMENTAL SAMPLING INTO AVIAN INFLUENZA MONITORING FRAMEWORKS

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Highly pathogenic avian influenza (HPAI) caused by H5 viruses of clade 2.3.4.4b is a major threat to the poultry industry, the ecosystems and public health1. The detection of viral circulation in wild birds is a key component of surveillance, allowing the timely implementation of control measures at farms. Current approaches rely on testing carcasses, hunted birds, and targeted captures2. However, environmental samples (ES) are emerging as a valuable complement to animal specimens, supporting the detection of avian influenza viruses (AIVs) in high-risk wetland areas3.

In this study, we compared the detection probability of animal swab testing with that of surface water and faecal samples, and evaluated how environmental monitoring can be integrated into current surveillance frameworks.

In total of 5,614 wild birds (1,433 captured and 4,181 hunted), 1,043 faecal samples, and 452 surface water specimens collected with passive samplers were gathered from six high-risk wetlands in north-east Italy during the 2024-2025 and 2025-2026 winter seasons. Samples were screened for AIVs by RT-qPCR, followed by H5 subtyping. A binomial model was used to estimate the probability of AIVs and H5 detection across animal and environmental strategies with contrasts used for pairwise comparisons.

AIV detection probabilities were significantly higher in surface water than in captured (Odds Ratio, OR=5.72, $p<0.001$) and hunted birds (OR=3.74, $p<0.001$). Faeces showed higher detection probabilities than hunted birds (OR=2.29, $p=0.014$). Conversely, detection probabilities for H5 were higher in hunted birds than surface water (OR=3.45, $p=0.007$) and faeces (OR=2.60, $p=0.046$).

Significant differences in detection probability across surveillance strategies were observed according to the molecular target. This difference likely reflects lower viral loads in ES, which may hamper successful subtyping. Nonetheless, ES can support surveillance by detecting AIV circulation in high-risk areas and by triggering targeted sampling in wild birds, therefore providing a more robust framework for HPAI surveillance.

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O17. CIRCOVIRUS-ASSOCIATED FEATHER LESIONS IN EURASIAN GRIFFON VULTURES *(Gyps fulvus)*: CLINICAL AND LABORATORY FINDINGS FROM A CROATIAN REHABILITATION CENTRE

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Introduction: Circoviruses are important pathogens of avian species and are commonly associated with feather abnormalities and immunosuppression. The Eurasian griffon vulture (Gyps fulvus), the only remaining vulture species in Croatia, is considered regionally important for conservation. This study describes clinical and laboratory findings in juvenile griffon vultures presenting with feather lesions suggestive of circovirus infection.
Materials and Methods: Three juvenile griffon vultures undergoing rehabilitation at the Beli Visitor Centre and Rescue Centre for Griffon Vultures on Cres Island, Croatia, were examined. Birds had been rescued after falling into the sea during their first flight attempts. Blood samples were collected for complete blood count, biochemical profiling, oxidative stress assessment, and viral testing. Hematological analyses were performed manually using a Neubauer counting chamber and Wright–Giemsa-stained blood smears. Biochemical parameters were measured using automated analyzer. Antioxidant status was evaluated by determining superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in whole blood using commercial assay kits. Circovirus was detected through molecular analysis of feather samples.Total DNA was isolated from the feather calami using the NucleoSpin DNA RapidLyse kit, followed by PCR using both BFDV-specific primers and broad-spectrum/nested circovirus primers.Positive PCR products were visualized on a 1.5% agarose gel, purified, sequenced by the Sanger method, and then confirmed as BFDV/circovirus using BLAST and phylogenetic analysis.
Results: Affected birds exhibited extensive feather abnormalities, including feather breakage, retained feather sheaths, shaft discoloration, circumferential constrictions, keratin deposits, spontaneous feather loss, and ectoparasite egg deposits. Hematological examination revealed anemia in two birds and leukocyte alterations in all examined individuals. One bird showed leukocytosis, heterophilia, severe lymphopenia, and monocytosis, findings compatible with a viral infection. Biochemical analysis demonstrated hypoalbuminemia and decreased creatinine concentrations as the most consistent abnormalities, while increased aspartate aminotransferase activity was observed in affected birds. Antioxidant enzyme analysis showed reduced SOD activity in one bird and elevated SOD activity in two birds; GPx activity was moderately increased in the two birds for which measurements were available. All birds tested positive for circovirus on molecular analyses.
Conclusions: Birds with severe feather lesions exhibited hematological, biochemical, and oxidative stress alterations consistent with systemic disease and a possible viral etiology. These findings highlight the value of integrated clinical and laboratory assessments in the investigation of feather disorders in threatened avian species.

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O18. EVALUATION OF THE PERFORMANCE OF ANTIBODY-BASED TESTS FOR THE DIAGNOSIS OF TUBERCULOSIS IN CAMELIDS

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Tuberculosis (TB) caused by *Mycobacterium bovis* is an emerging concern in camelids, particularly as New World camelids (NWC) are increasingly kept as pets in European countries where bovine TB is endemic in certain areas (EURL for Bovine Tuberculosis, 2013). The intradermal comparative tuberculin test, the official antemortem screening method for TB in camelids, has demonstrated limited sensitivity in this species, highlighting the need for reliable alternative or complementary diagnostic tools (Infantes-Lorenzo et al., 2018; Wernery and Kinne, 2012).

This study evaluated the diagnostic performance of three antibody-based assays: two commercial ELISAs, the INgezim® TUBERCULOSIS DR (Ingezim) and IDEXX *M. bovis* Ab Test (IDEXX) modified, and one rapid lateral-flow immunochromatographic test, the Lionex® Rapid Test (Lionex). The assays were applied in alpacas from four populations with distinct epidemiological status: *M. bovis*-infected herds from Spain (n=16) and Peru (n=11), and two TB-free populations from Chile (n=400) and Norway (n=6). A total of 443 samples were tested with both ELISAs and 73 with all three assays simultaneously.

Given the lack of a perfect reference test, Bayesian Latent Class Models, which do not rely on a gold standard, were use to estimate their diagnostic performance. Sensitivity estimates were 74.2% (95% PPI: 54.2–93.0%), 83.8% (73.6–92.4%) and 79.3% (61.2–96.0%) for Ingezim, IDEXX and Lionex, respectively. Specificity estimates were 99.8% (99.3–100%), 98.5% (97.4–99.4%) and 64.4% (51.6–77.2%), respectively.

ELISA-based assays showed variable performance, with the IDEXX kit demonstrating the highest sensitivity and specificity for TB diagnosis in NWC. Despite its lower specificity, the Lionex rapid test represents a practical field tool given its ease of use and portability. Given the increasing relevance of *M. bovis* infections in NWC, the official validation of serological tests under different epidemiological conditions is necessary.

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O19. DEVELOPMENT OF A LAMP ASSAY FOR THE DETECTION OF BORRELIA SPP. AND ITS APPLICATION TO BAT SCREENING IN NORTHERN ITALY

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The genus Borrelia includes two major groups of spirochetes: the Borrelia burgdorferi sensu lato complex, responsible for Lyme disease (LD), and species associated with relapsing fever (RF). Borrelia infections have also been reported in bats, suggesting a possible role in the circulation and persistence of these bacteria [1-2]; however, it is unclear whether bats act as true reservoirs or incidental hosts [3]. Currently, diagnostic approaches rely mainly on serology, while molecular methods such as PCR require well-equipped laboratories and are not easily applicable in field conditions. In this context, this study aimed to develop a loop-mediated isothermal amplification (LAMP) assay for the detection of Borrelia spp. DNA. The assay was applied to spleen samples from 75 bats found dead in Northern Italy (Emilia-Romagna region) between June 2023 and November 2025, and detected bacteria were genetically analysed. Sensitivity and limit of detection (LOD) of the LAMP assay were evaluated using ten-fold serial dilutions of a recombinant plasmid containing a fragment of B. afzelii DNA, while specificity was assessed using different Borrelia isolates. The assay showed a LOD of 10^2 DNA copies/µL, with high specificity for Borrelia species belonging to both LD and RF groups. Two out of 75 bats tested positive. Sequencing of 16S rRNA gene fragments revealed a higher nucleotide identity with RF Borrelia species available in GenBank. These preliminary findings provide the first evidence of Borrelia in bats in Italy, highlighting the need for further studies to clarify their epidemiological role. This research was funded by the European Union–NextGeneration EU, Mission 4 Component 2 (CUP H53D23000740006, PRIN 2022 COMBAT 2022YERXWX) and the European Union–NextGeneration EU-MUR NRRP Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

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O20. FIRST MOLECULAR DETECTION OF Bartonella capreoli AND HEMOTHROPIC MYCOPLASMAS IN EUROPEAN BISON *(Bison bonasus)*

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Hemotropic vector-borne pathogens such as Bartonella spp. and hemoplasmas (Mycoplasma spp.) are recognized causes of subclinical and clinical infections in a variety of wild and domestic ruminants (1, 2). Although these pathogens are well-documented in cattle and other wild ungulates, their presence in free-living European bison (Bison bonasus)—a near-threatened species integral to European ecosystem restoration—remains poorly characterized (3). This study aimed to investigate the prevalence and molecular diversity of Bartonella spp. and hemoplasmas in the Polish population of European bison. A cross-sectional survey was conducted between 2021 and 2023, involving 85 European bison sampled from four regions in Poland. Spleen tissues (n=81) and whole blood (n=74) were collected. Pathogen detection was performed using conventional PCR assays targeting the 16–23S ITS region for Bartonella spp. and the 16S rRNA gene for hemoplasmas, followed by Sanger sequencing for species identification. Prevalence and associations with demographic variables were analyzed using binomial statistics and chi-squared or Fisher's exact tests. At least one vector-borne pathogen was detected in 27.06% (23/85) of the animals. Bartonella spp. DNA was identified in 11.76% (10/85) of individuals, with positive results in 11.11% of spleen and 2.47% of blood samples. Hemoplasmas were detected in 15.29% (13/85) of animals. No co-infections were observed. There was no significant association between infection status and sex, age, or region, although regional sample sizes were imbalanced. The study identified Bartonella capreoli, Mycoplasma wenyonii, and M. ovis as circulating species. This study provides novel insights into the epidemiology of hemotropic bacteria in European bison, revealing a moderate prevalence of Bartonella and hemoplasma infections. These findings underscore the role of European bison as a reservoir for pathogens with potential relevance at the wildlife–livestock interface and highlight the need for ongoing surveillance to inform conservation and livestock health strategies.

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O21. USE OF THE IR BIOTYPER SYSTEM IN A VETERINARY DIAGNOSTIC LABORATORY – CLASSIFICATION OF *Streptococcus* SUIS ISOLATES BY SEROTYPES

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The IR Biotyper (IRBT) is a new system for routine bacterial typing based on Fourier-Transform Infrared Spectroscopy. Resulting from the interaction of all the different (bio)molecules in the cells with the infrared light applied, an infrared light absorption spectrum is being generated, which reflects the biochemical composition of the bacterial cells. For many different bacterial species, FTIR analysis has been shown to have a high discriminatory power, enabling differentiation of bacteria beyond species level.

Some years ago, Royal GD implemented the IRBT system in their laboratories. It was first introduced in the R&D laboratory for validation, exploration of new applications, and research projects. Since 2023, it has been used in our routine diagnostics as well. Isolates are subjected to clusteranalysis to group together isolates with similar IR spectra and to distinguish isolates with different IR spectra, e.g. during outbreak investigations, longitudinal studies at specific farms or for composing farm-specific autovaccines. Additionally, classification of isolates is being performed to assign isolates to different classes, each representing isolates with a specific (set of) characteristic(s).

By applying the artificial intelligence capabilities of the IRBT software, we generated models for classifying Streptococcus suis isolates by serotype using different machine learning algorithms. Almost 900 spectra from 122 isolates belonging to 7 serotypes (1 , 2, 3, 4, 5, 7, and 9) were included in a training set. Another circa 800 spectra from 153 isolates (serotypes 1, 2, 3, 4, 5, 7, 9, 10, and 21) were used as validation set. Then, the classifier with the highest accuracy was selected for the final external validation with around 1400 spectra from 263 isolates.

Nowadays, the final serotyping classifier built has replaced routine serotyping by traditional agglutination and PCR. Typing of S. suis isolates at serotype level by using the IRBT system proves to be user-friendly and cost-effective.

05. Zoonoses & the one Health Approach: Bridging Animal, Human, & Environmental Health

O22. THE SEROLOGICAL BENCHMARK: ADVANCING ONE HEALTH THROUGH PRECISION ELISA DIAGNOSTICS FOR CHLAMYDIA AND COXIELLA SURVEILLANCE

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Small ruminant abortion, primarily driven by Chlamydia abortus and Coxiella burnetii, represents a critical challenge for livestock productivity and public health. These zoonotic agents are the most significant infectious pathogens associated with reproductive disorders and circulate in approximately 75% of Iberian flocks, with regional seroprevalence reaching up to 44%. Given the risk of human spillover, standardized serological tools are essential for One Health surveillance. This study describes the validation of two novel ELISA kits (DIA-check Q Fever ELISA Test and DIA-check Chlamydia abortus ELISA Tests by Diatheva). By ensuring diagnostic consistency and minimizing cross-reactivity, these assays establish a benchmark for the large-scale management of infectious abortions in sheep, goats, and cattle.

The validation followed WOAH principles. Analytical performance was assessed using international reference materials (ANSES/WOAH) and testing serum panels for the exclusivity against other abortifacient agents. Repeatability was tested through intra- and inter-assay tests across multiple days, operators, and instruments. A total of 753 field samples for C. burnetii and 657 for C. abortus were analyzed to determine Diagnostic Parameters.

Both assays demonstrated high analytical specificity with no cross-reactions observed. Q Fever Kit showed a diagnostic sensitivity (DSe) of 98.23% and specificity (DSp) of 97.50%. Chlamydia Kit achieved a DSe of 76.32% and a DSp of 98.71%. Comparative testing against established commercial kits showed high correlation for both kits (Spearman's r = 0.58 for C. burnetii and r = 0.86 for C. abortus).

The implementation of DIA-check ELISA platforms provides a reliable "Serological Benchmark" for ruminant surveillance. The technical validation confirms these tools' ability to accurately map the prevalence of Chlamydia abortus and Coxiella burnetii, enabling the identification of active clusters and the differentiation of infectious abortion causes within the flock, effectively reducing the economic impact of reproductive failures and mitigating zoonotic risks.

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O23. ENSURING SAFE LIVESTOCK TRADE: A COMPREHENSIVE DIAGNOSTIC PANEL FOR PRE-EXPORT TESTING IN SHEEP

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The international trade of small ruminants requires stringent diagnostic protocols to prevent the transboundary spread of infectious diseases. Pathogens such as Chlamydia abortus, Brucella ovis, Coxiella burnetii, Small Ruminant Lentiviruses (SRLVs), and Salmonella abortusovis pose significant threats to animal health and economic stability. Efficient pre-export testing relies on diagnostic tools that combine high accuracy with rapid turnaround times to meet global shipping demands.

This study evaluates a comprehensive panel of five optimized ELISA kits (Diatheva) designed as a specialized bundle for pre-export testing in sheep. The objective was to validate the analytical and diagnostic performance, focusing on sensitivity, specificity, and workflow optimization. Validation was conducted on extensive serum cohorts (n > 2,500), including field samples, naturally infected subjects, and vaccinated groups to assess potential interference. Performance was rigorously assessed through repeatability (CV%), analytical sensitivity (LOD), and diagnostic parameters (DSe, DSp) compared to reference methods or established commercial gold standards.

The diagnostic bundle offers a 20% reduction in execution time compared to standard market alternatives, significantly streamlining high-throughput laboratory workflows. All assays feature optimized cut-offs that eliminate "grey zones," providing clear-cut interpretation of results for simplified decision-making. Precision was high, with CV% consistently ≤15%. Diagnostic specificity reached 100% for B. ovis and S. abortusovis, while Q Fever testing demonstrated 99.73% specificity in ovine populations (See Tab 1).

This validated ELISA bundle represents a significant advancement in veterinary diagnostics, providing a standardized and efficient solution for pre-export screening. By combining an accelerated workflow with the elimination of clinical uncertainty, the panel facilitates health clearance processes. These tools are essential for ensuring the health status of livestock in international trade, supporting high-throughput demands while mitigating the risk of transboundary disease transmission.

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O24. WILD COYPU (*Myocastor coypus*) AS A SENTINEL OF ANTIMICROBIAL RESISTANCE IN THE AQUATIC ENVIRONMENT: A PRELIMINARY ENTEROBACTERIACEAE-BASED STUDY

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The aquatic environment acts as an effective disseminator of antimicrobial resistance (AMR), which can be monitored using animal sentinel species. This study investigates the role of wild coypu (Myocastor coypus), a semiaquatic species, as AMR sentinels and reservoirs in the aquatic environment, focusing on resistance to critical antimicrobials (third-generation cephalosporins–3GCs, carbapenems and colistin) relevant to humans.
Between December 2024 and November 2025, 235 wild coypu faecal samples and carcasses were collected along the Reno River, Italy, within the wild coypu population control plan (1). Standard microbiological analyses were performed to isolate Enterobacterales, identified by MALDI-TOF and tested by the broth microdilution method. AMR results were interpreted using epidemiological cut-offs or, when unavailable, clinical breakpoints. ESBL production was assessed by the combination disk test. ESBL-, carbapenem- and colistin-resistant isolates were screened for resistance genes by multiplex PCR (2,3,4).
A total of 232 Enterobacterales, belonging to 21 species, were isolated, with Escherichia coli being the most common (119/232, 51.29%). Interestingly, 50/232 (21.55%) isolates were multidrug-resistant (MDR). The most frequent resistances were to ampicillin (114/232, 49.14%), sulphonamides (54/232, 23.28%) and trimethoprim (45/232, 19.40%), while resistance to other antimicrobials was below 11%.
Critical resistances were detected in 31/232 (13.36%) strains. In particular, 21/24 3GC-resistant isolates, belonging to Escherichia coli, Klebsiella spp. and Enterobacter spp., showed ESBL profiles, harboring blaSHV, blaTEM and blaCTX-M genes, alone or in combination. Colistin resistance was found in two Enterobacter cloacae isolates and was likely chromosomal, as mcr genes were not detected.
Notably, carbapenem resistance was detected in five blaKPC-positive Enterobacter cloacae isolates (Figure 1).
Our findings suggest that wild coypu could serve as promising AMR sentinels, with potential application in future aquatic AMR surveillance programs. Moreover, they may contribute to the persistence and spread of MDR pathogens to humans and other animals, representing a potential public health concern.

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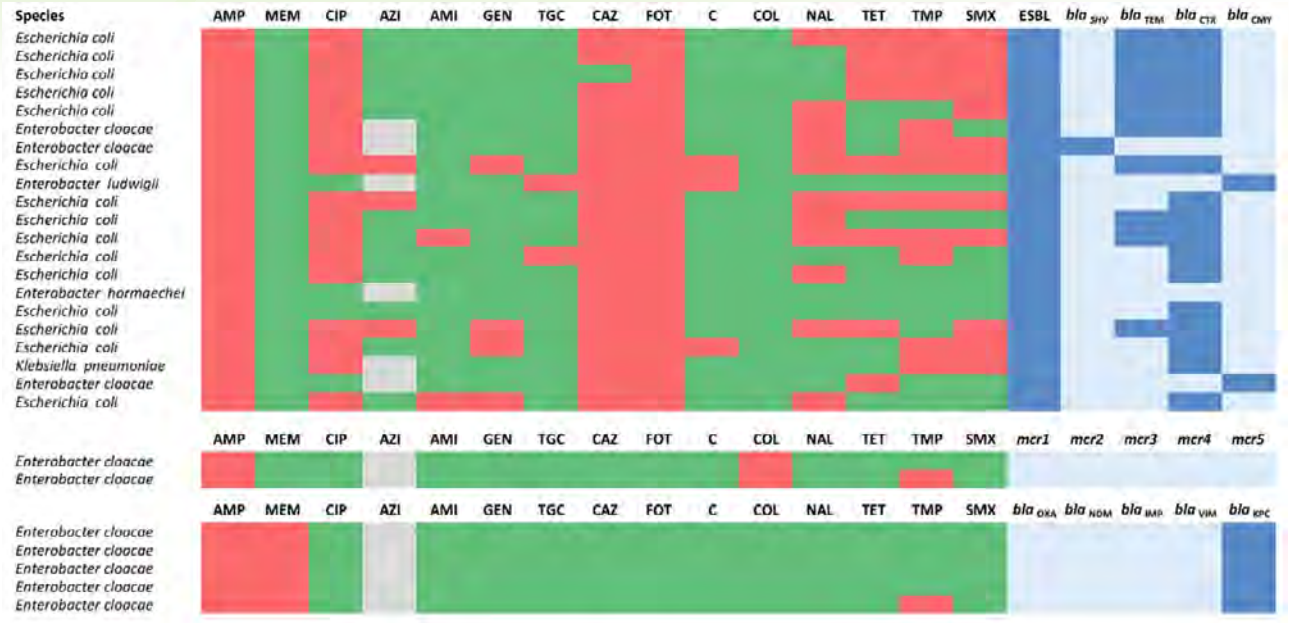


Figure 1

The three heatmaps illustrate the complete antimicrobial resistance profiles of ESBL-producing, colistin-resistant and meropenem-resistant isolates, presented in the order listed above. In the first section of each heatmap, red and green squares indicate phenotypic resistance and susceptibility, respectively, to each specific antimicrobial. Grey squares indicate that the epidemiological cut-off value or breakpoint for azithromycin was not available for the given bacterial species. In the second section of the heatmap, blue and grey squares represent the presence or absence, respectively, of the corresponding antimicrobial resistance genes. Bacterial species are listed on the left side of the heatmap.
AMP: ampicillin, MEM: meropenem, CIP: ciprofloxacin, AZI: azithromycin, AMI: amikacin, GEN: gentamicin, TGC: tigecycline, CAZ: ceftazidime, FOT: cefotaxime, C: chloramphenicol, COL: colistin, NAL: nalidixic acid, TET: tetracycline, TMP: trimethoprim; SMX: sulfamethoxazole.

025. DIAGNOSTIC CHALLENGES AND EMERGING SOLUTIONS FOR *Encephalitozoon cuniculi* INFECTIONS IN RABBITS

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Encephalitozoon cuniculi is an obligate intracellular microsporidian parasite causing neurological, renal, and ocular disease in rabbits. Reliable ante-mortem diagnosis remains challenging because current PCR- and serology-based methods have limitations in sensitivity, specificity, and routine applicability. This study aimed to support the development of rapid diagnostic tools through the identification of suitable molecular and immunological targets.

Molecular investigations were performed using urine, feces, and blood samples from rabbits to evaluate parasite detection in different biological matrices. In parallel, candidate antigens for future immunochromatographic assay development were selected based on bioinformatic analyses, predicted immunogenicity, and parasite specificity. Among the evaluated targets, protein tyrosine phosphatases PTP1, PTP2, and PTP3 were identified as promising candidates for further characterization.

PCR analyses demonstrated successful detection of E. cuniculi DNA in urine samples, with sequence confirmation of selected amplicons. Although conventional PCR showed limited sensitivity in blood-derived samples, next-generation sequencing (NGS) detected parasite genetic material in blood, indicating that blood-based molecular diagnosis may be feasible when highly sensitive methods are applied. These findings warrant further optimization of analytical workflows.

The confirmation of E. cuniculi nucleic acids in blood-derived samples also provides indirect evidence that parasite-derived molecules may circulate during infection. Although this hypothesis requires experimental validation, it supports ongoing investigations of blood-circulating parasite antigens, including PTP1–PTP3, as targets for rapid immunodiagnostic assays.

The combined identification of sensitive molecular markers and parasite-specific immunological targets provides a basis for the development of rapid diagnostic tools capable of detecting active infection, improving epidemiological surveillance, facilitating earlier diagnosis and disease management in rabbit populations, and supporting One Health approaches addressing the zoonotic potential of E. cuniculi.

This research was co-financed by the European Union under the European Funds for Modern Economy (FENG) programme, implemented by the Polish Agency for Enterprise Development (PARP), project no. FENG.01.01-IP.02-0126/24.

O26. GENOTYPIC CHARACTERIZATION OF THE *Trichophyton Mentagrophytes/Trichophyton* INTERDIGITALE SPECIES COMPLEX: INSIGHTS INTO HUMAN AND ANIMAL TRANSMISSION IN A ONE HEALTH CONTEXT

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From January 2016 to April 2026 a total of 131 dermatophyte strains were isolated and identified by PCR and Sanger sequencing as Trichophyton mentagrophytes/Trichophyton interdigitale species complex (TMTISC). Most isolates were of human origin (n=117), while a smaller proportion derived from animals (n=14). A subset of 63 human, one rabbit, and one dog strains, collected between 2023 and 2026, were further characterized at the genotype level (1) and also analyzed by a qPCR to assess terbinafine behavior (2).
Genotyping analysis revealed five distinct epidemiological patterns (Figure 1). Genotypes I and II T. interdigitale, consistent with anthropophilic profile, were detected in 20 and 26 human samples, respectively. Genotype III* T. mentagrophytes, associated with a zoophilic source of infection, was identified in five humans and in the two animal strains, suggesting a potential zoonotic transmission to humans. Genotype VII T. mentagrophytes, an anthropophilic genotype potentially transmitted through sexual contact, was isolated from one human patient. Finally, zoophilic and anthropophilic mixed-type genotype II* T. interdigitale/mentagrophytes, was found in 11 human strains. All the isolates were susceptible to terbinafine.
In Italy, genotypes I, II, III*, and VII have already been reported, although only in a limited number of studies (3,4). To the best of our knowledge, genotype II* has not yet been described in Italy, despite having been previously reported in other European countries. Data on terbinafine susceptibility were encouraging, particularly considering the global emergence of antifungal resistance.
This report highlights the genetic diversity within the TMTISC and the complex interplay between human and animal reservoirs in the spread of dermatophyte infections. Molecular investigations provide critical insights into transmission pathways, enabling more accurate differentiation between anthropophilic and zoophilic strains, in a One Health scenario.
Ongoing studies are focused on genotyping the remaining 66 fungal isolates to further elucidate dermatophyte circulation and transmission dynamics.

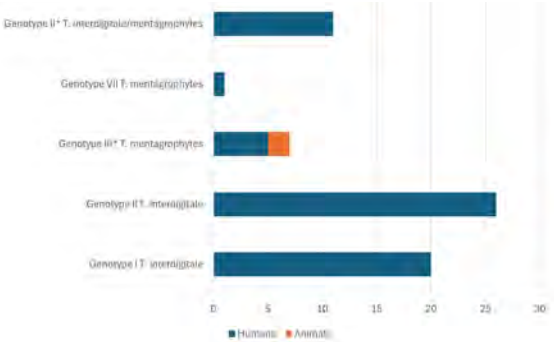


Figure 1: Results of genotypic characterization of Trichophyton mentagrophytes/Trichophyton interdigitale strains isolated in humans and animals.

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O27. SARS-COV-2 EXPOSURE IN WILD CANIDS IN SERBIA: A ONE HEALTH PERSPECTIVE ON ZOONOTIC RISK

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Following the COVID-19 pandemic, increasing attention has been directed toward the role of wildlife in the epidemiology of SARS-CoV-2 and its potential zoonotic and reverse zoonotic transmission pathways [1,2].
This study investigated SARS-CoV-2 exposure in free-ranging wild canids, red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*), in Serbia within a One Health framework. A total of 165 serum samples collected across ten districts between August 2024 and March 2025. All the sampled animals were older than one year, while data on animal sex were not available. The sera samples were tested for anti-SARS-CoV-2 IgG antibodies using a commercial multi-species ELISA (IDVet, France) and a validated in-house ELISA [3] developed at the Institute for the Application of Nuclear Energy (INEP), Serbia. Agreement between assays was assessed using Cohen's kappa coefficient.
Serological evidence of exposure was detected in 17/165 animals (10.3%) using the commercial ELISA, including 11/83 red foxes (13.3%) and 6/82 golden jackals (7.3%). The in-house ELISA identified 12 seropositive animals (7.3%), indicating substantial agreement between methods (κ = 0.66). Seroprevalence varied across districts, with the highest proportion observed in Južni Banat (64.7%), and followed by Mačva (17.6%), Kolubara (11.8%), and Braničevo (5.9%), while no seropositive animals were detected in the remaining districts (Figure 1).

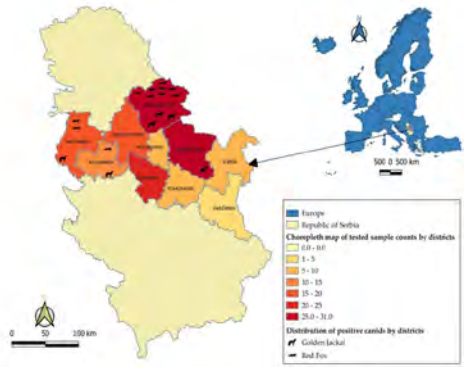


Figure 1. Spatial distribution of tested samples by IDVet ELISA and seropositive wild canids in Serbian districts.

These findings provide the first evidence of SARS-CoV-2 exposure in wild canids in Serbia and support the hypothesis that wild carnivores may act as sentinels of viral circulation at the human–animal interface. The results highlight the importance of integrated wildlife surveillance for early detection of emerging zoonotic threats within a One Health context [4].
The authors acknowledge the support provided by Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contracts No: 451-03-33/2026-03/ 200019 and 451-03-33/2026-03/ 200030) and journal Veterinary Sciences (MDPI) for attendance at the conference.

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O28. A NOVEL HT-MLST APPROACH TO TRACK TRANSMISSION DYNAMICS AND GENETIC DIVERSITY OF *Leishmania infantum*

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Leishmaniasis is an endemic zoonosis in the Mediterranean basin, with Leishmania infantum as the primary etiological agent (1). Although enzyme electrophoresis (MLEE) has long been the gold standard, its low resolution and difficulties in standardization limits its epidemiological utility (2). This study presents the development of an Ht-MLST (High-throughput Multilocus Sequence Typing) system designed to overcome the limitations of traditional zymo-systematic. The system is based on high-throughput sequencing of seven housekeeping genes (HD, RK39, MP-M32, GDP, HSP70, MTF, ACC) distributed across different chromosomes. It was validated on 77 L. infantum and L. donovani strains isolated from human, canine, feline, and wildlife hosts, covering various clinical manifestations and geographical regions.
The results showed a high discriminatory power (DP = 0.946), resolving 57 Sequence Types (STs). Phylogenetic reconstructions identified three main lineages: Lineage A, characterized by moderate variability, it includes the majority of canine isolates and the MON-1 zymodeme; Lineage B, composed predominantly of non-MON-1 human isolates (MON-24, MON-29, MON-187), associated with visceral leishmaniasis forms; Lineage C, exclusively composed of human isolates linked to cutaneous forms and associated with the MON-24 zymodeme. The identification of these groups is aligned to previous studies (3). High multilocus heterozygosity and the presence of putative hybrid genotypes (HYB1, HYB2, HYB3) were also observed, indicating natural genetic recombination events (4).
The Ht-MLST system proved to be a robust and scalable tool for molecular epidemiology, capable of tracking parallel transmission cycles (zoonotic vs. human-centric) and monitoring the emergence of hybrid variants. This makes it essential for integrated surveillance strategies within a One Health framework.

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O29. NEW IBR SERUM DOUBLE-ANTIGEN SANDWICH ELISA

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While IBR eradication is progressing in Europe, the incidence remains a concern in some areas (1). To address the challenges of IBR eradication in the final phase of programs, the sensitivity and specificity of diagnostic tools must be maximized. With this in mind, a new ELISA has been developed: the IBR Serum Ab test. This test uses a new single-step double antigen sandwich ELISA and employs a recombinant protein as the antigen.
The IBR Serum Ab test allows the testing of individual serum, plasma and meat juice samples, as well as pools of up to 10 serum or plasma samples, with the same kit.
Sensitivity, specificity, and reproducibility parameters were evaluated with a view to submitting a validation dossier to the French and German regulatory authorities, including the use of reference samples from these countries.
The results showed improved analytical sensitivity for approximately a third of the samples analyzed (a gain of up to two 2-fold dilution steps) compared to other tests available on the market, as well as significantly improved specificity, particularly for challenging samples from animals positive for antibodies against BoHV2.
A sensitivity of 100% (95% CI: 99.1% - 100%) and a specificity of 99.9% (95% CI: 99.2% - 100%) were obtained with a panel of positive (n=522) and negative (n=847) field samples, respectively.
Finally, field trials involving three laboratories in France and one in Austria demonstrated the good reproducibility and repeatability of the test (CV < 15%).
Thanks to its superior specificity and ease of use, the IBR Serum Ab ELISA should contribute to strengthening the effectiveness of IBR control programs in the field.

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06. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O30. ENHANCING EIAV MOLECULAR DETECTION BY OPTIMIZED PRE-ANALYTICAL CONDITIONS FOR TARGETING VIRAL CIRCULATING PARTICLES AND PROVIRAL RESERVOIRS THROUGH RT-QPCR AND QPCR

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Equine infectious anemia virus (EIAV) remains a diagnostic challenge due to its high genetic variability and the limitations of current serological and molecular tools. Serological assays may fail to detect infected animals during early stages of infection, highlighting the need for complementary approaches targeting viral genetic material.
In this study, we developed and optimized RT-qPCR and qPCR assays for EIAV detection based on a comprehensive genomic dataset generated from our strain collection using Next-Generation Sequencing (NGS) (1). Comparative genomic analyses enabled the identification of semi-conserved regions suitable for primer and probe design, aiming to improve detection across genetically diverse strains.
A panel of RT-qPCR and qPCR assays was evaluated on >50 spleen tissue samples from naturally infected horses, selected to represent a broad diversity of EIAV strains. Initial assay designs achieved a detection rate of up to 95% when combining two optimized primer–probe sets, demonstrating improved performance compared to previously described molecular approaches on this sample set 70%.
In parallel, we investigated the impact of pre-analytical process on detection sensitivity, including DNA extraction procedures and input quantity. Increasing DNA input and optimizing extraction conditions significantly improved the detection of low-copy proviral DNA, highlighting the importance of pre-analytical steps in the detection of integrated viral genomes.
Importantly, the detection of proviral DNA in tissue samples provides molecular evidence of infected cellular reservoirs, contributing to the characterization of persistent infection. These findings support the relevance of targeting proviral DNA as a complementary virological marker alongside serological approaches.
Overall, this work demonstrates that combining genome-informed assay design with optimized pre-analytical conditions enhances EIAV molecular detection and provides a robust framework for improving direct diagnostic strategies.

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O31. MOLECULAR DETECTION OF GASTROINTESTINAL PATHOGENS: EVALUATION OF A FECAL TRANSPORT SYSTEM AND NUCLEIC ACID STABILITY

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Gastrointestinal (GI) infections are common in veterinary medicine, affecting both companion animals1 and livestock2. Rapid and accurate pathogen detection is essential for appropriate clinical management and treatment decisions. Molecular multiplex assays have improved diagnostic speed and sensitivity compared to traditional culture methods3; however, limited data exists on nucleic acid stability during sample transport and storage in veterinary settings. This study evaluates the suitability of Copan FecalSwab™ for molecular detection of GI pathogens, assessing its applicability. A panel of enteric pathogens (7 bacteria, 3 viruses, 3 parasites) was tested using four commercial molecular assays. Strain dilutions were prepared to obtain Ct values of 25-35 when spiked on FecalSwab™. From a set of samples, acid nucleic was extracted (T0). The remaining inoculated samples were stored at 5°C ± 3°C and 22.5°C ± 2.5°C to mimic transport conditions for up to 15 days, or at −80°C for up to 6 months. After that, DNA extraction and RT-PCR were performed. In addition, a subset of inoculated FecalSwab™ tubes was kept at −20°C to assess the effect of repeated freeze-thaw cycles on PCR performance. The absence of molecular interference with any of the evaluated kits was confirmed by performing extraction and amplification on non-inoculated FecalSwab™ tubes. Molecular performances were evaluated as acceptable considering a maximum increase of 3.5 Cts between T0 and the stability time under evaluation (ΔCt). For all the tested pathogens and molecular assays, FecalSwabTM performances resulted in compliance with the acceptance criterium after storage at refrigerated and room temperatures, including 3 freeze thaw cycles, for up to 15 days of storage. No interferences with the molecular assays were detected. Copan FecalSwabTM showed reliable molecular performance for gastrointestinal pathogen detection, maintaining stability for up to 15 days at tested temperatures and 3 freeze-thaw cycles, with no interference across multiple diagnostic kits.

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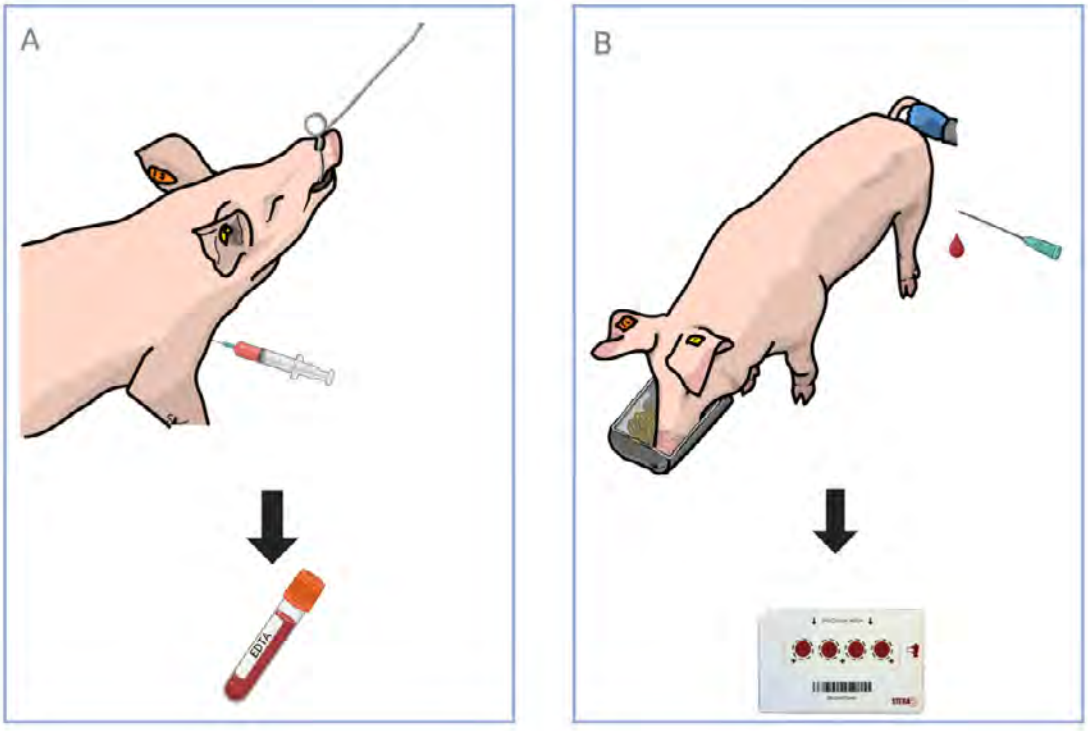
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06. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O32. DRIED BLOOD SPOT TESTING IN DOMESTIC PIGS: FIELD APPLICABILITY AND AUTOMATED DIAGNOSTIC PROCESSING

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Dried blood spots (DBS) are widely used as sample matrix in human medicine and offer several advantages over liquid blood, including minimally invasive sampling and smaller sample volumes. DBS are obtained after a short prick with a lancet and absorbing blood drops onto filter paper (Figure 1B). In pigs, standard liquid blood collection requires restraining of the animals with a maxillary sling (Figure 1A), which can cause considerable stress.
In our laboratory, DBS have been successfully evaluated as an alternative sample matrix to liquid blood in domestic pigs for use in commercial ELISAs to detect antibodies against Hepatitis E virus, African swine fever virus and Aujeszky's disease virus. This study aims to evaluate the applicability of DBS sampling under field conditions and to advance automation of DBS processing.
In a controlled paired cross-over study 34 fattening pigs underwent both DBS and liquid blood sampling at a one-week interval. Saliva samples were collected before and 20 minutes after liquid blood/DBS sampling to assess the stress response via salivary cortisol ELISA.
Salivary cortisol levels showed a greater increase after liquid blood sampling than after DBS collection. HEV antibody testing showed comparable diagnostic performance between plasma and manually extracted DBS samples.
To evaluate semi-automated DBS processing, DBS were prepared from porcine EDTA blood samples with or without spiking of antibodies. After automated (Hamilton easyPunch, Figure 1D) and manual extraction (Figure 1C), the samples were analyzed in commercial ELISA assays for various porcine infectious agents. Comparison of the ELISA results shows that the semi-automated method performs with similar accuracy as the manual extraction. Further efforts are being made to extend automation to the entire workflow, including antibody detection by ELISA (Figure 1E).
DBS sampling represents a reliable and animal-friendly alternative to conventional blood collection in pigs. Combined with automated processing, DBS have strong potential to improve animal welfare and routine diagnostics.



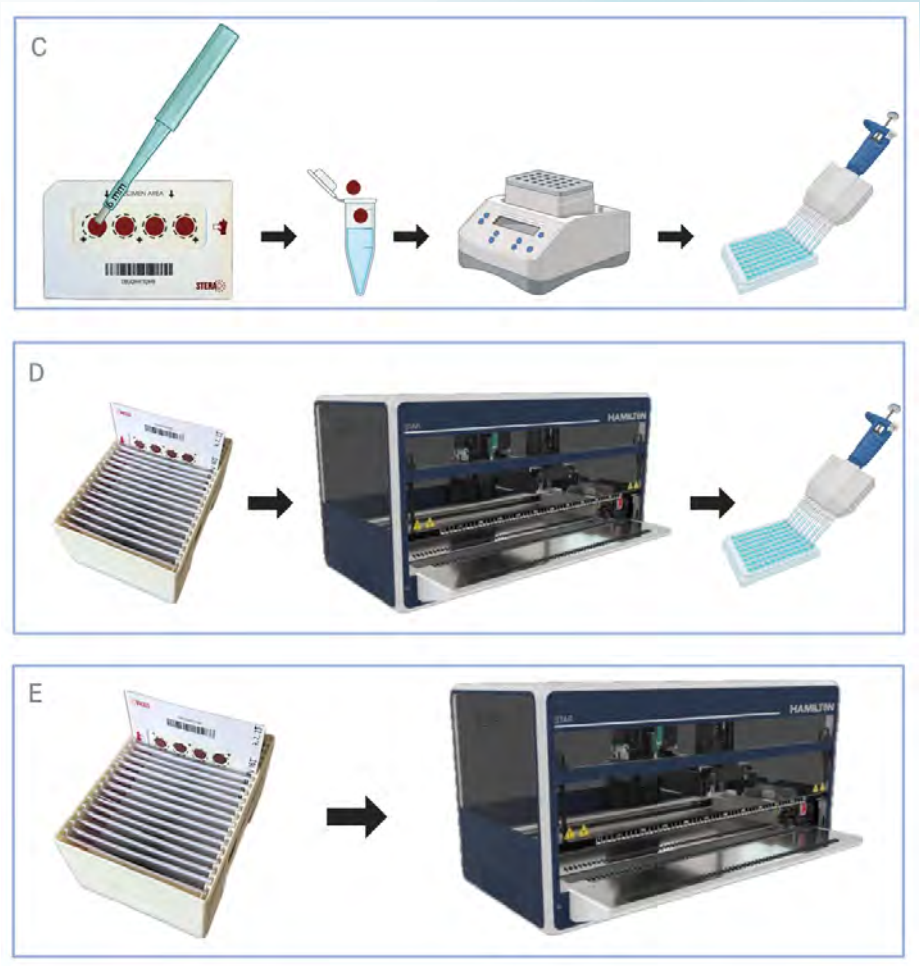


Figure 1: A) Conventional blood sampling in pigs, B) DBS sampling in pigs, C) Manual DBS extraction workflow, D) Semi-automated DBS processing (extraction) on a Hamilton easyPunch, E) Fully automated DBS processing (extraction and ELISA) (Created in BioRender.com).

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06. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O33. DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR THE DETECTION OF BOVINE HERPESVIRUS 2 AND 4 IN BOVINE SAMPLES

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Bovine infectious diseases represent a major challenge for livestock production worldwide, causing significant economic losses and negatively impacting animal health. Bovine herpesvirus 4 (BoHV-4) is associated with reproductive disorders, mastitis, and immunosuppression, while bovine herpesvirus 2 (BoHV-2) is the etiological agent of bovine mammillitis and pseudo-lumpy skin disease. Current diagnostic methods are often time-consuming and unable to simultaneously detect multiple pathogens. This study aimed to develop a multiplex real-time PCR assay for the detection of BoHV-2 and BoHV-4 in bovine samples.
Complete BoHV-2 and BoHV-4 genomic sequences available in GenBank were retrieved and aligned using MAFFT to identify highly conserved target regions. Primers and probes were designed to maximize intra-species conservation while minimizing inter-species homology. In silico specificity was evaluated using Primer-BLAST, and probes were labeled with distinct fluorophores to enable multiplex detection.
The assay was evaluated for analytical performance using a panel of non-target bovine pathogens. No cross-reactivity or non-specific amplification was observed, confirming high analytical specificity. Sensitivity testing using serial viral dilutions demonstrated reliable detection down to low target copy numbers, with a limit of detection (LOD) of 2 genomic copies per reaction, defined as a ≥95% positive detection rate across replicates. Diagnostic applicability was further validated using field samples collected from cattle from 2007 to the present, allowing assessment of assay performance across genetically diverse viral populations. These samples had been previously characterized by end-point PCR and restriction enzyme analysis and confirmed by successful viral isolation. The assay consistently detected all positive samples, demonstrating robustness against viral genetic variability.
The multiplex TaqMan-based real-time PCR assay developed in this study enables simultaneous detection of BoHV-2 and BoHV-4 with high specificity, sensitivity, and reproducibility. This assay represents a rapid, accurate, and reliable diagnostic tool that may support improved surveillance, disease control, and herd health management in cattle production systems.

O34. ENHANCING LONG-TERM PRESERVATION OF FAECAL SAMPLES FOR BACTERIAL CULTURE

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Reliable bacterial culture of faecal samples in veterinary medicine depends on appropriate storage conditions, as temperature and time can significantly affect microbial viability and cultivability1-2. Standardized protocols for long-term preservation remain poorly defined, potentially impacting diagnostic accuracy and reproducibility3. This study assessed the use of Copan FecalSwabTM with 20% glycerol to determine whether bacterial viability is preserved after long-term storage of faecal samples. ATCC strains dilutions of Escherichia coli, Bacteroides fragilis, Enterococcus faecalis, Salmonella Typhimurium, and Shigella flexneri were prepared (1:8000 for all strains, except E. coli at 1:10,000) and used to inoculate 100 µL into FecalSwab™ tubes supplemented with 20% glycerol. Bacterial viability during storage at −80 °C was evaluated over a 6-month period with monthly time points (T1–T6), using as baseline control samples processed immediately after inoculation (T0). Two experimental setups were employed:
Set 1: repeated freeze–thaw cycles applied to the same triplicate tubes at each time point
Set 2: independent triplicates prepared for each time point and discarded after analysis.
At each storage interval, 100 µL from each replicate was plated onto Tryptic Soy Agar, while B. fragilis was cultured on Chocolate Agar. Colony-forming units were quantified to assess bacterial survival over time. Mean colony counts (± SD), obtained from triplicate measurements, remained generally stable up to T2 for all tested microorganisms in both sets. The percentage recovery relative to T0 ranged from 86.64% to 155.22%, indicating that microbial viability was overall maintained over time, with some variability. No statistically significant differences were observed between Set 1 and Set 2, demonstrating comparable performance across the different conditions tested. Combining FecalSwab™ with 20% glycerol effectively preserves microbial viability for up to two months at −80 °C, with stable colony counts across all tested microorganisms. Analyses through six months are currently being finalized to confirm long-term stability.

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06. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O35. REALPCR MILQ-ID DNA SYSTEM: THE NEW SOLUTION FOR MASTITIS TESTING

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Mastitis is the most frequent infectious disease with the most important economic consequences in dairy cattle (1). Causative pathogens were historically identified by culture (2), but today, the RealPCR* MilQ-ID DNA System, for use with milk samples, allows for identification of 4–16 targets in a single test run in 3 hours.
Analytical evaluation using synthetic DNA and clinical samples demonstrated high sensitivity, with limits of detection of 1–10 DNA copies per reaction and PCR efficiencies ranging from approximately 98% to 108% across targets.

Target	LDPCR (copies/reaction)	PCR % Efficiency
MilQ-ID DNA Mix 1		
M. bovis	1 copy	104.5%
S. aureus	1 copy	108.9%
S. uberis	1 copy	103.0%
S. agalactiae	1 copy	105.6%
MilQ-ID DNA Mix 2		
S. dysgalactiae	1 copy	100.5%
ß-lactamase	10 copies	99.4%
E. coli	10 copies	98.6%
Staphylococcus spp.	10 copies	106.3%
MilQ-ID DNA Mix 3		
T. pyogenes	10 copies	98.0%
Enterococcus spp.	10 copies	98.7%
Prototheca spp.	1 copy	97.8%
Klebsiella spp.	1 copy	99.7%
MilQ-ID DNA Mix 4		
Mycoplasma spp.	10 copies	99.8%
Pseudomonas aeruginosa	1 copy	101.6%
Yeast	10 copies	90.2%
Corynebacterium bovis	1 copy	106.2%

The practical relevance of such high-performance PCR diagnostics is supported by national-scale experience from Finland, where multiplex real-time PCR has been implemented for routine mastitis diagnostics since 2010 (3). This system processes over 130,000 samples annually with same-day turnaround times of approximately 4 hours, compared with ≥48 hours for culture, and achieves pathogen detection rates of 83–92%. Integration of PCR diagnostics with herd-level monitoring tools,
such as somatic cell count data, has contributed to improved udder health and a reduction in antimicrobial usage. Rapid and accurate diagnostics support more targeted and responsible treatment decisions. Overall, the RealPCR* MilQ-ID DNA System combines high analytical performance with demonstrated field relevance, supporting improved mastitis management and antimicrobial stewardship in modern dairy production.

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O36. THE AVI-FLU PROJECT: ADVANCED ENVIRONMENTAL SURVEILLANCE FOR EARLY DETECTION OF RESPIRATORY PATHOGENS IN POULTRY PRODUCTION

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Respiratory pathogens, particularly avian influenza viruses, represent a major challenge for poultry production due to their impact on animal health, productivity, and public health. Most current surveillance strategies in poultry production are mainly based on direct animal sampling, which may limit early detection at flock level. Recent evidence indicates that air and environmental surfaces can act as reservoirs and transmission routes for respiratory pathogens and antimicrobial resistance determinants. Previous environmental studies performed by our group in hospital settings have demonstrated the feasibility of combining air and surface sampling with quantitative polymerase chain reaction (qPCR) analysis for early pathogen detection [1]. Using this approach, pathogens and antimicrobial resistance genes were successfully detected in environmental samples, suggesting a potential of molecular environmental surveillance as an early warning strategy. Based on these findings, the AVI-FLU project explores the applicability of environmental molecular surveillance for early detection of respiratory pathogens in poultry farms through air and surface sampling combined with qPCR analysis. The project aims to optimize sampling strategies adapted to poultry production conditions and to explore modular qPCR assays for the detection of avian influenza viruses and other relevant respiratory pathogens and standardized workflows suitable for routine implementation in veterinary diagnostic laboratories. Validation studies will be performed under real farm conditions to evaluate analytical sensitivity, robustness, and operational applicability. AVI-FLU proposes a proactive and non-invasive surveillance model for poultry production with potential applications in biosecurity, outbreak preparedness, and One Health monitoring of emerging zoonotic respiratory pathogens.

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06. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O37. COMPARATIVE DETECTION OF ROTAVIRUS A IN FECAL AND EXTRA-INTESTINAL SAMPLES OF NEWBORN CALVES AND LAMBS AT THE ONSET OF DIARRHEA

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Rotavirus A (RVA; species Rotavirus alphagastroenteritidis) is a major pathogen associated with neonatal diarrhea syndrome in calves and lambs1,2 leading to substantial economic losses in livestock production. Clinical manifestations include diarrhea, dehydration, and occasionally high mortality rates. Growing evidence indicates viremia and extra-intestinal viral detection3,4 in both species; however, data on shedding rates across different specimen types remain limited. This study aimed to investigate fecal shedding patterns of RVA in newborn ruminants, to assess viral detection in blood and nasal swabs at diarrhea onset, and to compare positivity rates in calves and lambs for each specimen type. The study was conducted on two RVA-positive farms. On the sheep farm, blood, nasal swabs, and fecal samples were collected from 31 newborn lambs at 2 days of age, and on the first day of diarrhea. On the cattle farm, the same samples were obtained from 20 calves at diarrhea onset. Fecal samples were examined parasitologically and all specimens were tested by real-time RT-PCR for RVA and coronavirus, and positivity rates were calculated. Samples with a Ct value <40 were considered positive. All samples tested negative for coronavirus and Cryptosporidium spp. At the onset of diarrhea, RVA was detected in the nasal swabs of all calves but in only 32.3% of lambs' swabs. Fecal and plasma positivity rates were 25.8% and 19.3%, respectively, in diarrheic lambs, compared with 85% and 65% in calves. Viral load was significantly higher in all sample types from calves than from lambs. The study confirmed the occurrence of viremia and extra-intestinal dissemination of RVA in newborn ruminants, and therefore, feces, blood, and nasal swabs all provide useful information for viral detection when screening a herd. Notably, viral excretion rates differed markedly between species, with consistently higher viral loads and positivity rates in calves than in lambs.

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O38. COMPARATIVE ASSESSMENT OF A COMMERCIAL ELISA AND A NEWLY DEVELOPED REAL-TIME PCR FOR THE DETECTION OF SMALL RUMINANT LENTIVIRUSES IN SHEEP AND GOATS

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Small ruminant lentiviruses (SRLV), the causative agents of maedi-visna in sheep and caprine arthritis-encephalitis in goats, are widely distributed globally, yet accurate detection of infected animals remains challenging. The present study comparatively assessed the diagnostic performance of serological and molecular assays for the detection of SRLV in naturally infected dairy sheep and goats in Greece. A total of 2,406 and 390 blood samples were collected from five purebred Chios and Lacaune sheep farms and four purebred Saanen and Skopelos goat farms, respectively. All samples were tested using a commercial indirect ELISA (CAEV/MVV Total Ab Test, IDEXX) and a nested real-time PCR assay targeting a 125 bp fragment of gag gene. The PCR protocol was adapted from a previously described nested conventional PCR method [1] by modifying the second amplification round to incorporate a degenerate reverse primer targeting conserved SRLV regions. Overall, 57.3% (1,602/2,796) of samples tested positive by ELISA, while 58.9% (1,648/2,796) were positive by PCR. Infection prevalence varied markedly across farms, ranging from 8.1% in one sheep farm to 100% in one goat farm. Concordance between two methods was 81.3% in sheep and 86.9% in goats. Agreement was substantial, with k-values of 0.62 and 0.69 for sheep and goats, respectively ($p < 0.001$). ELISA identified 80.9% of PCR-positive sheep and 97.6% of PCR-positive goats, whereas PCR assay detected 86.1% of ELISA-positive sheep and 84.5% of ELISA-positive goats. These findings demonstrate substantial agreement between serological and molecular assays and support their complementary use for improving SRLV detection accuracy. The lower PCR sensitivity in goats likely suggests the circulation of genetically diverse SRLV strains, underscoring the need for further molecular characterization to optimize the assay performance.
The research project is implemented in the framework of H.F.R.I call "4th Call for H.F.R.I.'s
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O39. DIGITAL PCR: AN INNOVATIVE TOOL FOR IMPROVING NOROVIRUS DETECTION IN A ONE HEALTH APPROACH

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Noroviruses of genogroups GI and GII are the leading cause of acute viral gastroenteritis worldwide and represent a major challenge for food safety and environmental monitoring. Their high genetic diversity, low infectious dose, and persistence in aquatic environments complicate their detection, particularly in complex matrices such as seashell and wastewater. In Europe, most reference methods, based on RT-qPCR, are validated according to the EN ISO 15216-2:2019 standard. These methods have limitations in terms of sensitivity, absolute quantification, and robustness against inhibitors.

To address these limitations, Biosellal has developed and validated two new tools using digital PCR (dPCR) to better meet field requirements. These kits enable the detection and quantification of GI and GII noroviruses in a single reaction. Each kit also includes a process control virus as well as an exogenous internal positive control (IPC) within the same PCR well.

Biosellal collaborated with a coastal departmental laboratory to evaluate analytical performance using field samples (oyster digestive glands, wastewater from sewage treatment plant outfalls, and water from seashell farming ponds). A comparison with the reference method was done. The results demonstrate improved sensitivity, precision, and tolerance to inhibitors.

These new dPCR tools represent a significant advancement for norovirus surveillance and health risk assessment within a One Health framework.

O40. PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS, AND RISK FACTORS FOR *Staphylococcus aureus* FROM MEAT OF ANIMAL ORIGIN IN MEKELLE, TIGRAY, NORTHERN ETHIOPIA

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Background: Staphylococcus aureus (S. aureus) is well known for causing human and animal diseases, primarily through the consumption of contaminated meat.
Objective: This study aimed to determine the presence of S. aureus, its antimicrobial susceptibility test, and risk factors from meat of animal origin in Mekelle, Northern Ethiopia.
Method: Socio-demographic data were collected using a pre-designed questionnaire. Meat samples were collected sterilely from butchers and abattoir facilities and transported in an icebox to the microbiology laboratory. An isolation, characterization, and antimicrobial susceptibility test was performed using the disc diffusion method. The data were cleaned and entered into STATA 20.0. Logistic regression models with odds ratios were used to assess risk factors and bacterial contamination. A p-value of < 0.05 was considered statistically significant.
Results: During the current investigation, we collected 250 samples. The analysis revealed the presence of contamination by S. aureus in 88 (35.2%) of the samples. The prevalence of S. aureus in samples from the butcher and abattoir houses was 37.6% (n=47) and 32.8% (n=41), respectively. Significant factors included, use of gloves (COR = 0.523; 95% CI: 0.308-0.889, P-value: 0.017, AOR = 0.222; 95% CI: 0.104-0.473, P-value: 0.001), strict separation between clean and dirty areas (COR=2.48; 95% CI: 1.45-4.24), and poor handwashing practice (COR=2.93; 95% CI: 1.41-6.06), with all associated with S. aureus contamination. The presence of resistance to cefotaxime, ampicillin, and penicillin G was observed in all isolates of S. aureus from butcher and abattoir house sources. In contrast, the in vitro antimicrobial sensitivity pattern of amikacin, cephalexin, gentamicin, trimethoprim-sulfamethoxazole, and nitrofurantoin was tested.
Conclusion: This study highlights the widespread presence of S. aureus in meat products. Consequently, early identification of the pathogen and antimicrobial susceptibility testing for S. aureus are crucial to improve treatment outcomes and prevent the development of drug resistance.
Keywords: abattoir houses, antimicrobial resistance, butchery houses, Ethiopia, S. aureus

O41. ORNAMENTAL FISH AS A POTENTIAL AND UNDERSTUDIED SOURCE OF CROSS-BORDER ANTIMICROBIAL RESISTANCE

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The global trade of ornamental fish is significant and expanding rapidly, yet this sector remains largely excluded from structured antimicrobial resistance (AMR) surveillance despite evidence that ornamental fish and their transport water can harbour antimicrobial resistant bacteria, resistance genes (ARGs), and antibiotic residues with potential relevance to human and environmental health. This study aimed to pilot an active AMR surveillance programme for freshwater ornamental fish imported into the United Kingdom, identifying suitable sampling matrices, culture media, indicator bacterial species, and an antibiotic residues method to support routine surveillance. Seventeen import consignments of goldfish (n = 16) and koi carp (n = 1) were sampled during routine border inspections. Samples collected included head kidney swabs and associated transport water, followed by culture-based bacterial isolation, species identification using matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) and whole genome sequencing, phenotypic antimicrobial susceptibility testing, genomic detection of resistance determinants, and targeted liquid chromatography tandem mass spectrometry screening for antibiotic residues. A total of 316 bacterial isolates representing 12 genera were recovered, with Aeromonas and Shewanella predominating. Six bacterial species were identified as suitable AMR indicators based on prevalence, zoonotic potential, and capacity to harbour mobile ARGs. Reduced susceptibility to multiple antimicrobial classes, including quinolones, tetracyclines, β-lactams, and carbapenems, was widespread, with multidrug resistance detected across all indicator species and phenotypic resistance frequently supported by corresponding ARGs, including plasmid-mediated determinants. Antibiotic residues, including fluoroquinolones and amphenicols, were detected in transport water and aligned with observed resistance phenotypes. This pilot surveillance demonstrates that ornamental fish and their transport water represent an under-recognized pathway for AMR dissemination between countries and provides an evidence-based framework to inform national surveillance, policy development, and risk mitigation strategies within a One Health context.

O42. GENOMIC CHARACTERIZATION OF RIFT VALLEY FEVER VIRUS DURING A 2025 OUTBREAK IN UGANDA REVEALS ONGOING REGIONAL TRANSMISSION OF LINEAGE C

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Rift Valley Fever (RVF) is a neglected zoonotic disease caused by Rift Valley Fever Virus (RVFV) and is endemic in East African countries. Although transmission can remain dormant for extended periods, the virus frequently re-emerges, posing significant public health and economic risks. Timely investigation of suspected cases is essential for effective outbreak control. We investigated suspected RVF outbreaks in Nakasongola and Sironko districts to determine whether the cases represented isolated spill over events or ongoing local or regional transmission.

Whole-genome sequencing was performed on viral RNA obtained from three confirmed human RVF cases using next-generation sequencing. Viral lineages were identified using the Genome Detective tool. Phylogenetic analysis was conducted for all three genome segments (Large (L), Medium (M), and Small (S)) and compared with human and animal RVFV sequences from Uganda, Kenya and Egypt available in the National Center for Biotechnology Information database (2016–2023). Phylogenetic trees were constructed using generalised time-reversible and gamma distribution distance metric with 1000 bootstrap replicates.

All positive RVF cases (n=3) belonged to lineage C. The S segment clustered closely with human isolates from the 2016¬–2017 Uganda and 2018 Kenya outbreaks. The M and L segments were close to recent Uganda human isolates obtained between 2022–2023. All three genomic segments showed phylogenetic relatedness to Kenya livestock isolates from 2021, with bootstrap support values greater than 70%. These findings indicate sustained circulation of genetically related RVFV strains across time, geography, and host species.

This investigation provides genomic evidence of sustained regional circulation of 2025 RVFV and a cross-border transmission between livestock and humans. Our findings underscore the benefits of strengthening and maintaining coordinated regional surveillance and control strategies such as real time sequencing and a One health approach across East Africa.

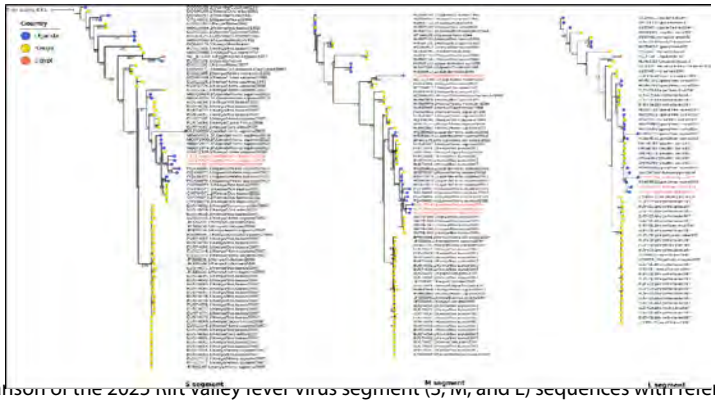


Figure 1: Phylogenetic comparison of the 2025 Rift Valley fever virus segment (S, M, and L) sequences with reference sequences stored in the National Center for Biotechnology Information database between 2016 and 2023

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07. Zoonoses & the one Health Approach: Bridging Animal, Human, & Environmental Health

O43. INTEGRATED ONE HEALTH SURVEILLANCE REVEALS WEST NILE VIRUS EXPANSION BEYOND ENDEMIC AREAS IN FRANCE

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Introduction: The emergence of zoonotic arboviruses in France represents a major public and animal health challenge. West Nile virus (WNV), maintained in an enzootic bird–mosquito cycle, can cause severe neurological disease in humans and horses in 1–10% of infections. Since 2022, WNV circulation has expanded beyond its historical Mediterranean endemic area into regions such as Nouvelle-Aquitaine[1] and Île-de-France.
Objectives: This study aimed to strengthen WNV surveillance in newly affected regions through a One Health approach and to improve understanding of viral circulation dynamics in animal and environmental compartments.
Methods: Regional One Health surveillance networks integrating human and animal health, wildlife, environmental stakeholders, and national authorities were established to support coordinated monitoring and near real-time data sharing. The National Reference Laboratory for WNV conducted operational surveillance activities, including mosquito xenomonitoring through excreta analysis by RT-qPCR, viral genome detection in wild birds admitted to wildlife care centers (n = 744), and equine seroprevalence surveys in Nouvelle-Aquitaine and Île-de-France (n = 484 and 499, respectively).
Results: The findings demonstrated active WNV circulation in Nouvelle-Aquitaine and a more moderate and localized circulation in Île-de-France, with equine seroprevalence rates of 9% and 2.8%, respectively[2]. The detection of WNV-positive mosquito pools[3] and acutely infected wild birds (n=72) in several departments of both regions further supported these observations. This integrated surveillance framework enabled rapid identification of areas and periods of active viral circulation, facilitated timely alerts to competent authorities, and improved data interpretation through multidisciplinary collaboration.
Conclusion: This study highlights the added value of a One Health approach for emerging arboviruses surveillance. Integrating animal, environmental, and public health surveillance improved understanding of WNV circulation dynamics[4], strengthened early warning capacities, and supported the development of locally adapted early detection strategies. This framework could serve as a model for other territories and for the surveillance of arboviruses with significant public health impact.

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O44. BENCHMARKING THE FIELD PERFORMANCE OF BOVINE PPD, DST-F FUSION PROTEIN AND P22 PROTEIN COMPLEX IN CELL-BASED DIAGNOSIS OF CAPRINE TUBERCULOSIS USING BAYESIAN LATENT CLASS MODELS

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Ante-mortem diagnosis of caprine tuberculosis (cTB) relies on cell-based techniques including the comparative cervical test (CCT) and occasionally the interferon-gamma release assay (IGRA). These techniques could have limited performance due to the use of the bovine Purified Protein Derivative (PPDb) as reagent for antigenic stimulation (1). In this sense, experimental antigens (DST-F and P22) were evaluated as potential alternatives using Bayesian modelling to estimate their sensitivity and specificity in the skin test/ST and IGRA (using the Bovigam and IDScreen kits) in the absence of a gold standard test (2).

An infected (n=167 goats - from two herds with history of cTB) and non-infected population (n=192 - from two herds with no history of cTB) were enrolled in the study. Seven three-test-two-population models were built: Model 1 included PPDb tuberculin based tests (CCT, Bovigam-PPDb, IDScreen-PPDb) and served as reference whereas models 2-7 each replaced one PPDb test with a DST-F or P22-based equivalent, retaining the other two (Table 1). Severe criteria were applied as follows: CCT (PPDb≥3mm plus PPDb>avian PPD/PPDa), ST-DST-F (DST-F≥3mm), ST-P22 (P22≥3mm), Bovigam (PPDb/DST-F/P22≥0.1) and IDScreen (S/P%≥16). For IGRAs using PPDb and P22, correction with PPDa was considered, whereas DST-F responses were corrected using a nil antigen.

Median posterior estimates for the sensitivity and specificity of the CCT (74.2-82.0% and 99.4-99.6%, respectively) were slightly higher than those of the ST-DST-F (70.6 and 98.0%) and ST-P22 (75.2 and 95.7%; Figure 1). Using the Bovigam kit, the median sensitivity of the PPDb (58.9-61.7%) exceeded that of DST-F (50.0%) and P22 (52.2%). With the IDScreen kit, DST-F sensitivity (58.6%) closely aligned with PPDb estimates (54.7-63.2%), both exceeding P22 (51.4%). Regarding specificity of IGRAs, all tests showed nearly perfect estimates across models, regardless of the antigen used (median>98.0%) (Figure 1). Overall, DST-F and P22 did not appear to improve PPDb tuberculin based CCT.

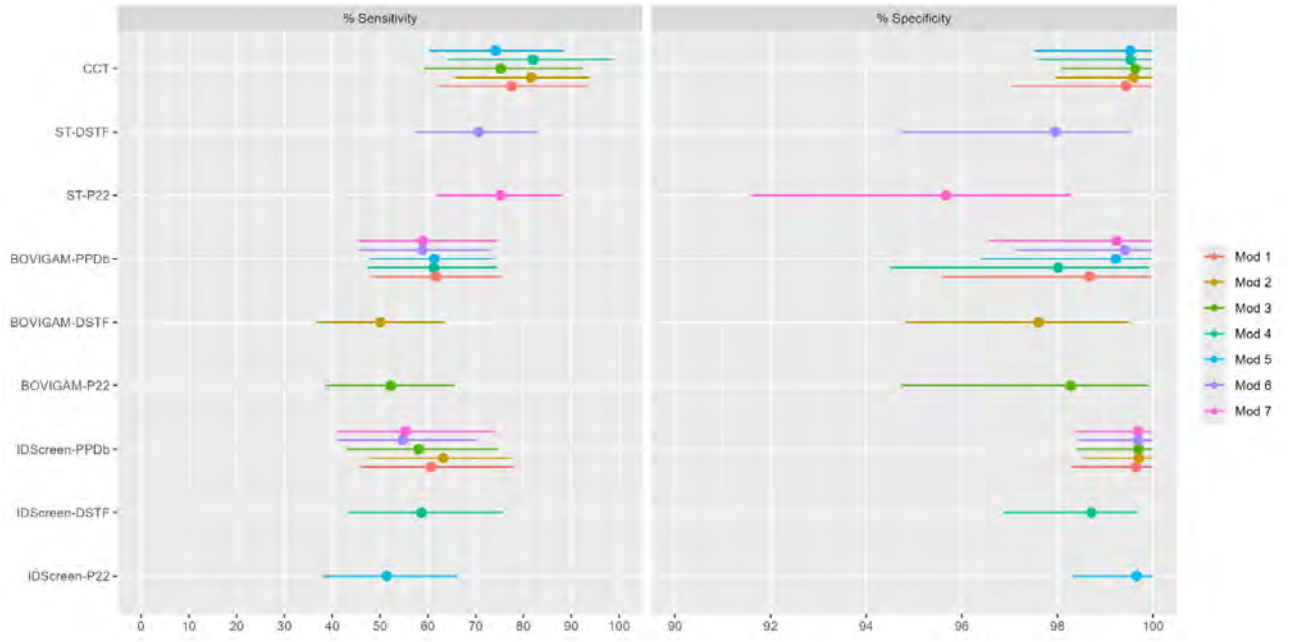
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Table 1: Combination of tests introduced in each model in the study.

Model	Test 1	Test 2	Test 3
1	CCT	Bovigam-PPDb	IDScreen-PPDb
2	CCT	Bovigam-DST-F	IDScreen-PPDb
3	CCT	Bovigam-P22	IDScreen-PPDb
4	CCT	Bovigam-PPDb	IDScreen-DST-F
5	CCT	Bovigam-PPDb	IDScreen-P22
6	ST-DST-F	Bovigam-PPDb	IDScreen-PPDb
7	ST-P22	Bovigam-PPDb	IDScreen-PPDb

Figure 1: Posterior estimates of test sensitivity and specificity by model. Lines represent the 95% of posterior credible intervals and dots indicates the median estimates.



O45. EVALUATION OF A TRANSPORT MEDIUM FOR MICROBIAL PRESERVATION AND DISINFECTANT NEUTRALIZATION IN ENVIRONMENTAL MONITORING

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Copan Italia S.p.A

Environmental monitoring of microbial contamination is critical across healthcare, food production, and veterinary settings to ensure biosecurity, outbreak control, and hygiene validation. Reliable sample collection and transport systems that preserve microbial viability while neutralizing residual disinfectants are essential to avoid false-negative results and ensure accurate bioburden assessment. This study evaluated the performance of Copan SRK®, an isotonic transport medium designed for environmental sampling, focusing on its ability to preserve a diverse panel of microorganisms and neutralize disinfectant activity. A total of 27 microbial targets, including bacteria and fungi, were inoculated into the medium at concentrations ranging from 10 to 120 CFU. Recovery was assessed immediately (T0) and after storage under different conditions: 24 hours at room temperature (22–25°C), and 24 and 48 hours at 5°C. Viability was considered acceptable when recovery remained within ±30% of the initial inoculum. To assess disinfectant neutralization, serial dilutions (1:1 to 1:10,000) of disinfectants from five antimicrobial classes (oxidizing compounds, quaternary ammonium compounds, biguanides, alcohols, and phenolic compounds) were tested. Microbial suspensions exposed to disinfectants in the transport medium and in water (control) were incubated for 24 hours, and growth was then evaluated. All tested microorganisms were successfully recovered under all experimental conditions within predefined acceptance limits. The medium effectively preserved microbial viability for up to 48 hours. Furthermore, it demonstrated consistent inactivation of residual disinfectants, whereas control samples without the medium showed complete growth inhibition. These findings support the use of this transport system for standardized environmental monitoring, including veterinary applications, by enhancing microbial recovery and minimizing the risk of false-negative results due to disinfectant interference.

O46. HUMAN–ANIMAL INTERACTION SAFETY: IMPLEMENTING RELIABLE METHODS FOR DETECTING PATHOGENIC *Capnocytophaga canimorsus* IN ORAL FLORA OF COMPANION AND THERAPY ANIMALS — PRELIMINARY RESULTS

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Capnocytophaga spp. (Csp) are common components of the oral microbiota of dogs and cats, but only selected species and serotypes have zoonotic relevance. Capnocytophaga canimorsus (Cc) serotypes A-D are responsible for the most severe human infections. The project supports the protection of immunocompromised people and Animal Assisted Interventions (AAI) participants, by addressing the limitations of current diagnostic tools: culture is slow and technically demanding, MALDI TOF libraries lack adequate spectral representation, and available molecular assays do not reliably identify species or pathogenic serotypes directly from oral swabs. The aim is to develop a rapid method to screen companion animals for pathogenic Cc serotypes, strengthening One Health risk mitigation strategies. Archived oral swabs (n=505) and isolates (n=43) from our previous research were used to optimize microbiological, MALDI TOF and molecular workflows. Real time PCR assays based on Suzuki et al. primers were adapted and tested, while a duplex PCR for serotypes A–D was evaluated using reference strains. Field sampling has so far enrolled 24 dogs and 10 cats, including AAI animals, each sampled at three oral sites for both cultural and molecular analyses (Figure 1). MALDI TOF libraries were enriched with Main Spectrum Profile from 2 Csp strain.
Preliminary results show cross reactivity of published methods, confirming the absence of species specific assays for detecting Cc. MALDI TOF correctly identified newly acquired isolates but showed limitations for strains from earlier collections. Diagnostic yield across the three oral sampling sites was comparable, supporting the use of a single multi site swab to reduce animal stress and analytical costs. Microbiological challenges included slow growth, pleomorphic colonies and reduced survival of some strains during freezing and biobanking procedures.
The integrated workflow will enable rapid, reliable detection of pathogenic Csp serotypes, strengthening diagnostic capacity across sectors.
Funding: Italian MoH, Ricerca Corrente IZSVE 09/2024, CUP B23C24000910001



Figure 1. Sampling sites tested in both dogs and cats
Sampling was performed at three oral sites: oral mucosa at the upper third premolar (PM3), aboral portion of the oral mucosa and/or oropharyngeal mucosa (OF), and oral mucosa at the lower molars (MI)

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O47. SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *Toxoplasma gondii* AND *Neospora caninum* INFECTION IN HORSES IN GREECE

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Toxoplasma gondii is a significant zoonotic pathogen responsible for abortion and congenital infections in humans and animals, while Neospora caninum is a protozoan associated with reproductive losses. Epidemiological data regarding equine exposure to these parasites in Greece remain limited. This cross-sectional study aimed to determine the seroprevalence of antibodies against T. gondii and N. caninum and identify associated epidemiological risk factors in horses from different regions of Greece. Serum samples from 473 randomly selected horses originating from Thessaly, Crete, Epirus, and Lesvos were examined using an indirect immunofluorescence antibody test (IFAT), while interpretation criteria were applied to minimize potential cross-reactivity between the two closely related coccidian parasites. Horses were classified into three age groups: <5 years, 5–12 years, and >12 years. Statistical analysis was performed using MedCalc Statistical Software, and variables with $p < 0.05$ in univariate analysis were included in multivariate logistic regression models. For T. gondii, a total seropositivity of 28.4 % was observed while significant risk factors included the presence of cats (OR = 2.68; $p < 0.001$), humid climate (OR = 1.69; $p = 0.026$), and origin from Lesvos (OR = 2.12; $p = 0.020$), while horses <5 years had lower odds of seropositivity (OR = 0.37; $p = 0.002$). For N. caninum, a total seropositivity of 26.2% was observed and the presence of dogs (OR = 4.04; $p < 0.001$), cattle farming (OR = 1.65; $p = 0.033$), and humid climate (OR = 1.83; $p = 0.010$) were independently associated with seropositivity. Co-seropositivity was associated with farms hosting both cats and dogs and with humid climate. These findings highlight the importance of environmental and management-related factors in equine exposure to protozoal infections and emphasize the need for improved farm biosecurity measures.

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07. Zoonoses & the one Health Approach: Bridging Animal, Human, & Environmental Health

O48. WILD BOARS AS POTENTIAL RESERVOIRS FOR *Anaplasma phagocytophilum, Toxoplasma gondii,* AND *Leishmania infantum:* ANTIBODY DETECTION IN SERUM AND MEAT JUICES

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The expansion of wild boar (Sus scrofa) populations due to climate change, land-use changes, and human activities has increased contacts at the human-wildlife interface and has raised concerns about their role in the maintenance of zoonotic pathogens. This study investigated the presence of antibodies against Anaplasma phagocytophilum, Toxoplasma gondii, and Leishmania infantum in serum and meat juices of tongue and diaphragm retrieved from wild boars. Samples were collected from 60 extensively farmed wild boars at the slaughterhouse. Indirect Immunofluorescence Antibody Assay was performed by using a cut-off of 1/40. Anti-A. phagocytophilum, anti-T. gondii, and anti-L. infantum antibodies were identified in 10.00%, 8.30%, and 5.00% of serum samples, in 8.30%, 6.60%, and 3.30% of tongue meat juice, and in 8.30%, 5.00%, and 3.30% of diaphragm meat juice. A very good agreement was observed between antibodies against A. phagocytophilum detected in serum and tongue meat juice (κ=0.81). A good agreement was observed between antibodies against T. gondii (κ=0.64) and L. infantum detected in the same materials (κ=0.79), and A. phagocytophilum in serum and diaphragm juice (κ=0.70). A moderate agreement was observed against T. gondii detected in serum and diaphragm juice (κ=0.47), and A. phagocytophilum (κ=0.50) and L. infantum (κ=0.48) detected in tongue and diaphragm juice. The specificity of antibody detection in tongue and diaphragm juices, compared with serum, was over 98% in all cases. Furthermore, the highest sensitivity was observed for anti-A. phagocytophilum antibodies in tongue juice. The detection of antibodies in wild boar suggests exposure to these infectious agents and indicates that wild boar may contribute to the maintenance and circulation of these pathogens in the environment, potentially by facilitating their transmission among animal populations and humans. Finally, tongue and diaphragm meat juices appeared to be valuable alternative samples for serological surveillance of hunted/harvested wild boars, where blood sampling is unfeasible.

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Other

O49. EARS-VET: A NETWORK FOR SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN CLINICAL ISOLATES FROM ANIMALS IN THE EU/EEA

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The EU strategy for surveillance of antimicrobial resistance (AMR) in animals has been focusing on healthy food-producing animals at slaughter and their derived meat. While this approach is relevant to evaluate foodborne exposure, it is of limited value to guide antimicrobial stewardship in veterinary medicine. Bringing together 36 partners from 17 EU/EEA countries working in collaboration with several EU and international agencies, the EARS-Vet network aims to close a gap on surveillance of AMR in clinical isolates from animals in Europe.
A comprehensive review of existing systems showed that, in 2020, only approximately half of the EU/EEA countries had established a national surveillance system of AMR in clinical isolates of animals. Existing systems were highly diverse, both in terms of methods used by veterinary diagnostic laboratories and volumes/coverage of data being generated. Building on this review, a collaborative work was conducted to define the EARS-Vet objectives, scope of animal species/bacteria of interest, and standards for antimicrobial susceptibility testing (AST) and interpretation criteria (i.e. EUCAST epidemiological cut-offs, where available). In addition, cross-country analyses were performed as part of a pilot study (9 countries, 140,110 bacterial isolates included) and demonstrated substantial variations of resistance levels, both among and within countries (Fig. 2).
Key challenges at this stage include the lack of harmonization of AST methods, the absence of interpretation criteria for many bacteria-antibiotic combinations of interest, and the shortage of data from many European countries. These challenges will be addressed as part of the EU Joint Action on AMR and Healthcare associated infections (EU-JAMRAI2) funded over 2024-2027. Planned activities include i) the development of tools for routine data collection (e.g. EARS-Vet database), ii) improved harmonisation (writing of an EARS-Vet manual, inter-laboratory proficiency testing, design of EARS-Vet customized MIC plates), and iii) whole genome sequencing of selected isolates.

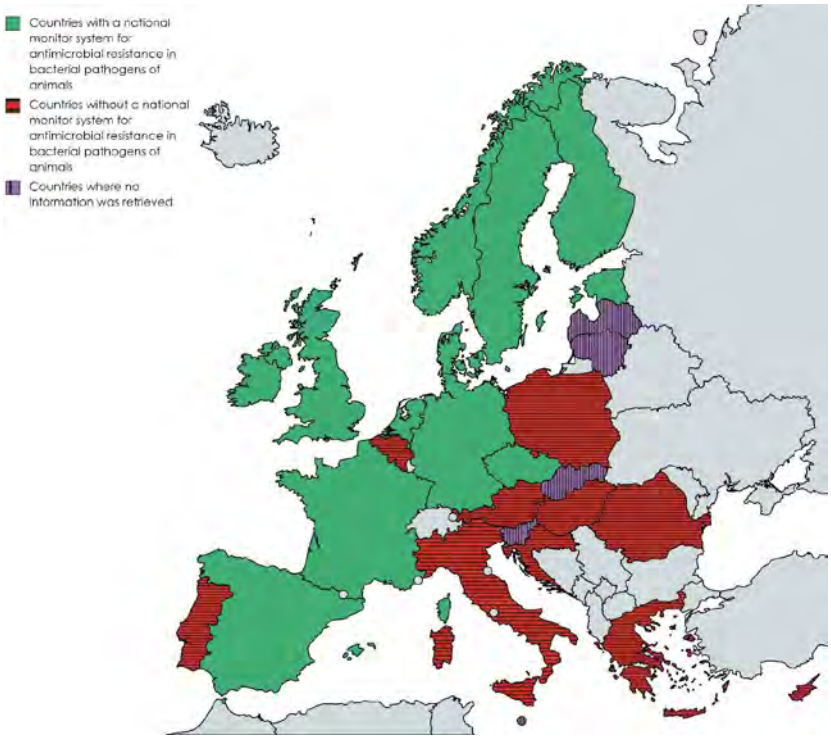


Figure 1. Map of Europe showing the countries that have at least one national monitoring system for antimicrobial resistance in bacterial pathogens of animals, do not have no such system, or where information was missing, among countries affiliated to the European Union Joint Action on Antimicrobial Resistance and Healthcare Associated Infections (EU-JAMRAI) and as of 2020

Other

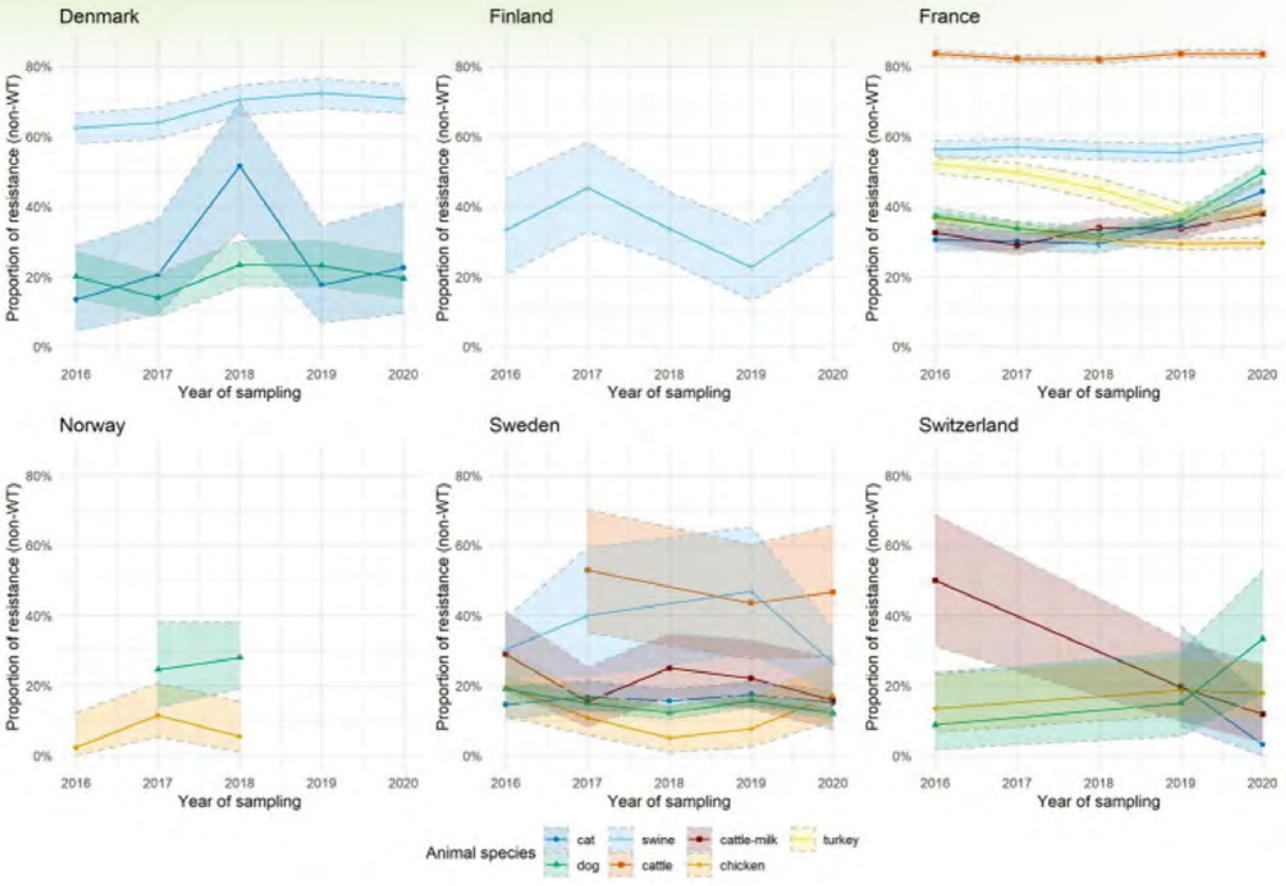


Figure 2. Trends of resistance (non-wild-type) in E. coli to aminopenicillins over 2016–2020. Only countries and animal species with sufficient data (at least 30 isolates per animal species and per year) are displayed here. Coloured areas around the curves represent 95% confidence intervals.

Other

O50. PETS, PATHOGENS, POLICY: LESSONS FROM GREECE ON AMR AND COMPANION ANIMALS

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Antimicrobial resistance (AMR) in companion animals is an emerging public health challenge demanding urgent attention within a One Health framework. Relevant studies conducted in Greece between 2020 and 2026 have demonstrated important findings.

A total of 41 *Acinetobacter baumannii* isolates were obtained from animal clinical samples across veterinary clinics in Greece over 2.5 years. High resistance rates were documented for nearly all tested agents, with 48.8% of isolates classified as carbapenem-resistant and 46.3% as XDR. A subsequent investigation of the same Ab collection examined biofilm formation. All 41 isolates produced biofilms, with 56.1% classified as strong producers. Notably, a statistically significant inverse correlation was identified between MDR/XDR phenotypes and biofilm strength, suggesting adaptive strategies among resistant strains.

Most recently, an XDR, NDM-1-producing *Pseudomonas aeruginosa* strain was isolated from an ear sample of a stray cat. Whole-genome sequencing revealed 67 antibiotic resistance genes, 46 efflux pump genes, 23 biocide tolerance genes, and 221 virulence factor-related genes. The strain showed close genomic similarity to human clinical isolates.

Extending beyond direct clinical specimens, an evidence synthesis of 96 global studies demonstrated veterinary healthcare settings as critical amplification hubs for MDR pathogens. Environmental contamination was notably concentrated in high-contact and critical care niches, with MDR bacteria reported in more than 60% of studies evaluating intensive care units, animal cages and sinks/drains. Furthermore, significant MDR carriage was identified across veterinary personnel—reaching 81.8% in nasal swabs and 52.0% on hands—demonstrating complex transmission loops between patients, contact surfaces, plumbing biofilms, and staff.

Collectively, these findings highlight companion animals and their clinical environments as interconnected reservoirs of high-priority AMR pathogens. Establishing standardized environmental monitoring protocols, curbing biofilm reservoirs, and implementing rigorous infection prevention and control (IPC) standards in veterinary facilities are urgent, indispensable priorities within a comprehensive One Health policy framework.

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Other

O51. EU-JAMRAI2: DEVELOPING AN ANTIMICROBIAL STEWARDSHIP PROGRAM FOR FOOD-PRODUCING AND COMPANION ANIMALS WITHIN EU

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Antimicrobial resistance (AMR) threatens human, animal and environmental health, requiring coordinated action across sectors. The European Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI2) supports countries in implementing measures to combat AMR through a One Health approach. A key objective of work package 6.2 is to develop antimicrobial stewardship (AMS) frameworks for food-producing and companion animals and address implementation barriers through behavioral change strategies. Expert working groups are developing the frameworks by integrating professional expertise, findings from a workshop on AMS and infection prevention and control in animal health held in 2025, and a comprehensive literature review conducted in collaboration with the Federation of Veterinarians of Europe. The frameworks provide core elements and guiding principles to support the development, implementation and evaluation of AMS programs, aiming to optimize antimicrobial use, improve treatment outcomes and preserve antimicrobial effectiveness. Draft frameworks have been developed for food-producing animals, focusing on cattle, poultry and swine, and for companion animals. They provide an overview of AMS programs, define core elements and required professional competencies, and outline indicators for monitoring and assessing implementation. They are intended to support adaptation to national and sectoral contexts while facilitating harmonization and comparison of AMS programs across countries. The EU-JAMRAI2 frameworks aim to provide practical tools for strengthening veterinary AMS and supporting National Action Plans on AMR. For veterinary diagnostic laboratories, their implementation offers an opportunity to strengthen collaboration with clinicians and promote diagnostic stewardship, including appropriate test selection, reliable microbiological diagnosis and clinically relevant interpretation of antimicrobial susceptibility results. Integrating laboratory evidence into clinical decision-making can support responsible antimicrobial prescribing in both food-producing and companion animals.

08. AMR

O52. DIVERGENT GENOMIC AND ANTIMICROBIAL RESISTANCE PROFILES OF Escherichia coli FROM MASTITIS CASES AND CALF FECES IN A LOW-AMR-PRESSURE DAIRY DISTRICT

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The Arborea dairy district in Sardinia, Italy, is a bovine dairy farming enclave with minimal external AMR pressure from other livestock sectors, urbanisation, or industry. Farms operate under continuous veterinary supervision and structured risk-based monitoring (ClassyFarm); antimicrobial use is tightly regulated with rapid on-farm diagnostics guiding calf diarrhoea management. This setting constitutes a 'natural laboratory' to investigate the ecological roles of Escherichia coli across bovine mastitis cases, pre-weaned calves, and the farm environment. Twelve dairy farms with mastitis history were monitored over six months; six showed recurrent E. coli mastitis and were enrolled. Quarter milk from mastitis cases, faeces from pre-weaned calves (10/farm), and environmental samples (buckets, huts, water) were analyzed. All mastitis-associated E. coli (MAEC) and calf/environmental isolates underwent antimicrobial susceptibility testing1, PCR for ESBL genes (blaTEM, blaCTX-M groups, blaSHV)2,3, PFGE, and whole-genome sequencing (WGS) via Nanopore long-read technology. Clonal relatedness, virulence, and AMR profiles were resolved via core-SNP analysis and ABRicate against ecoli_vf and ResFinder. Of 622 quarter milk samples, 46 MAEC were recovered; isolates were genetically heterogeneous, consistent with environmental origin. All were non-MDR and mostly antibiotic-susceptible; few MAEC carried a plasmid encoding trimethoprim-sulfamethoxazole resistance. By contrast, third-generation cephalosporin resistant (3GCR) E. coli were isolated from 60-90% of calf fecal samples, depending on the farm; 97.67% carried blaCTX-M, 76.74% co-harboured blaTEM, and 97.5% were MDR. WGS uncovered chromosomal integration of blaCTX-M-14/15. Plasmidic blaTEM-176, an inhibitor-resistant variant4, predominated among calf isolates (52.5%). In this modern dairy setting, largely susceptible, genetically diverse strains were associated with mastitis, whereas calves and their environment formed a stable reservoir of clonal ESBL/MDR E. coli. MAEC and calf strains belonged to different ST, supporting ecological segregation between mastitis-associated and calf-colonizing populations. However, presence of plasmid-mediated resistance to antibiotics used only in calves, such as trimethoprim-sulfamethoxazole, requires attention concerning potential AMR spillover events.

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08. AMR

O53. DEVELOPMENT AND EXPANSION OF A VETERINARY ESCHERICHIA COLI ANTIMICROBIAL SUSCEPTIBILITY TESTING PROFICIENCY TESTING SCHEME FOR ONGOING EXTERNAL QUALITY ASSURANCE IN LMICS

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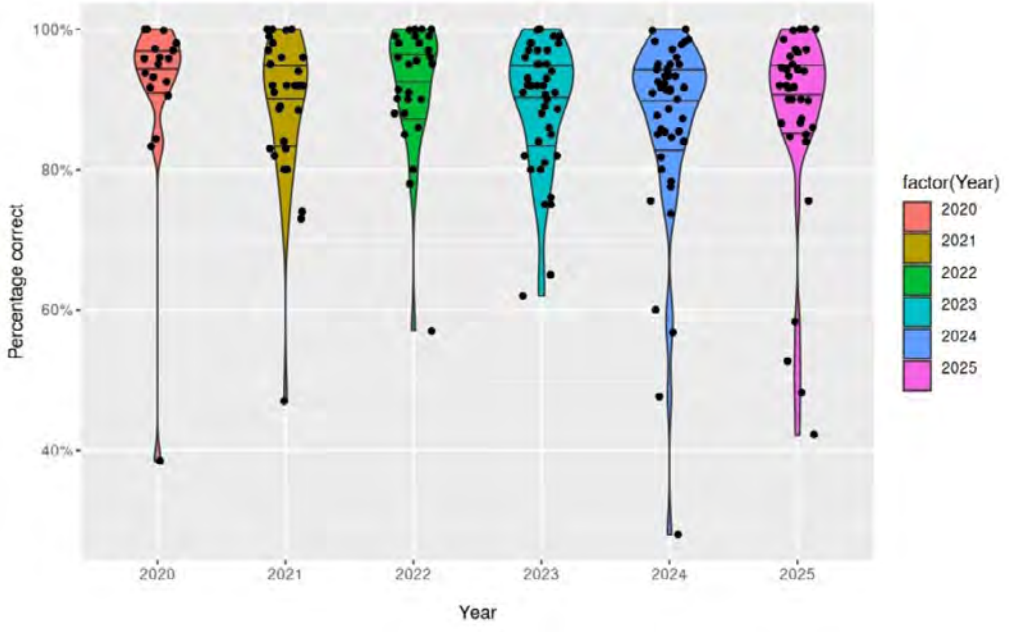
Ensuring the quality and accuracy of antimicrobial susceptibility testing (AST) results is critical. Inaccurate results can lead to inappropriate antibiotic prescribing or unreliable antimicrobial resistance (AMR) surveillance, both of which may contribute to increased AMR prevalence. Participation in external quality assurance (EQA) schemes, such as proficiency testing (PT), helps validate laboratory performance and ensures the production of reliable, quality assured data.

As part of the UK National Action Plan on AMR, we developed and distributed an E. coli AST PT scheme in 2020, primarily targeting veterinary laboratories in low and middle income countries (LMICs). Here we describe the development and outcomes of this scheme.

The PT scheme evaluates AST performance using both disc diffusion and minimum inhibitory concentration (MIC) methods, and accepts CLSI and EUCAST methodologies and interpretive criteria to reflect the diversity of laboratory practices. Since 2020, annual distributions have been provided, each comprising five E. coli isolates. Laboratory performance was assessed by comparing reported interpretations with expected results. Percentage correct scores were calculated to avoid bias against laboratories unable to test all assessed antibiotics.

Participation increased from 13 laboratories in 2020 to 40 in 2024. Median percentage correct fluctuated from 96 to 91%, primarily due to the inclusion of new participants. The 75th quartile range was above 80% across all years. Most laboratories displayed improvement in subsequent distributions following feedback of results and technical guidance. Notably, laboratories reporting correct control strain results had significantly lower median correct rates (94.3% correct in 2024) than those that did not (78.3% correct in 2024), highlighting the importance of appropriate quality control.

Overall, the PT scheme has improved AST accuracy in participating LMIC veterinary laboratories. Importantly, participation has contributed to two laboratories achieving ISO 17025 accreditation for AST, demonstrating the value of EQA in strengthening and sustaining laboratory quality.



O54. ANTIMICROBIAL RESISTANCE GENES IN ACINETOBACTER ISOLATES FROM A VETERINARY TEACHING HOSPITAL IN SPAIN

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Acinetobacter is a bacteria associated with infections in hospitalised patients. However, its current importance in veterinary centres is unclear. The main objective of this study was to characterise Acinetobacter isolates analysed at the Complutense Veterinary Teaching Hospital of the Complutense University of Madrid, with special emphasis on detecting antimicrobial resistance. A total of 23 isolates obtained from different animal species and samples over a 25-year period were included in the study. Identification was made by using MALDITOF, VITEK-2, whole-genome sequencing and a chromogenic medium. Antimicrobial susceptibility was interpreted according to CLSI guidelines using the Kirby-Bauer disk diffusion and broth microdilution method. The proportion of clinical isolates identified as Acinetobacter spp. at the HCV-UCM was 0.3% (13 isolates). 15.4% of the isolates were classified as multidrug-resistant. Certain genes, such as blaOXA and blaADC variants, were found in Acinetobacter as expected. While a large variety of antimicrobial resistance mechanisms was detected in one to seven of the 13 sequenced Acinetobacter isolates, some of them were expected (such as beta-lactamase encoding genes). Still, finding the tet(X) gene, even at a low frequency (2/13 isolates), is a more concerning result, particularly given the increased MIC observed especially in one of the two tet(X3)-carrying isolates. This highlighting the risk of horizontal spread of such MGE in one health settings. Similarly, the presence of QRDR mutations in Ciprofloxacin and Nalidix Acid A. baumannii resistance isolates. The results of this study suggest that, in our Complutense Veterinary Teaching Hospital, antimicrobial resistance in Acinetobacter isolates of veterinary origin might not be yet widespread. Nevertheless, as animals can act as reservoirs of resistant strains, continuous monitoring of antimicrobial susceptibility by validated methods is recommended.

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08. AMR

O55. NEW EPIDEMIOLOGICAL CUT-OFF VALUES (ECOFF) FOR SULPHONAMIDES AND TRIMETHOPRIM AND ANIMAL PATHOGENS

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Targeted interpretation of antimicrobial susceptibility testing (AST) raw data is highly dependent on the availability of appropriate interpretative criteria, such as clinical breakpoints (CBP) or at least epidemiological cut-off values (ECOFF), which are prerequisite for the setting of CBPs according to the veterinary subcommittee (VetCAST) of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Treatment options for animals, especially the use of critically important antimicrobials, are nowadays limited by legislation, and therefore maintaining the effectivity of first-line antibiotics such as sulphonamides and trimethoprim is of utmost importance. Unfortunately, for veterinary pathogens interpretative criteria for AST related to sulphonamides alone and in combination with trimethoprim and are based solely on historical human data or entirely lacking. ECOFFs are required for the setting of CBPs. Therefore, we performed an interlaboratory study including five European laboratories to produce minimum inhibitory concentration (MIC) distributions, that meet the EUCAST acceptance criteria for establishing of ECOFFs. The following bacterial pathogens were tested against trimethoprim - sulfamethoxazole in a 1:19 ratio and sulfamethoxazole and trimethoprim alone: Actinobacillus pleuropneumoniae, Aeromonas salmonicida, Escherichia coli, Mannheimia haemolytica, Pasteurella multocida, Staphylococcus pseudintermedius, Streptococcus (S.) equi subsp. equi, and S. equi subsp. zooepidemicus. Based on these aggregated MIC distributions ECOFFs could be realised, which are now publicly available on the EUCAST homepage (https://mic.eucast.org/).

08. AMR

056. COMPARATIVE GENOMICS OF NDM-PRODUCING *Klebsiella pneumoniae* HUMAN HIGH-RISK CLONE ST307 IN COMPANION ANIMALS: A ONEHEALTH PERSPECTIVE

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Although carbapenems have never been authorized in the veterinary sector, and recently prohibited (EU Regulation 2022/1255), carbapenemase-producing Enterobacterales have been increasingly reported in Europe in food-producing and companion animals (1,2). Twelve carbapenem-resistant K. pneumoniae (KP) were identified between 2021 and 2024 during routine diagnostics of clinical samples and organs from post-mortem examination of cats and dogs from Northern and Central Italy and sequenced with an Illumina platform. Eight of them were also sequenced by ONT long-read sequencing. Plasmid replicons, AMR and virulence-associated genes were identified on hybrid assemblies using PlasmidFinder, ResFinder, and Kleborate. Plasmid analysis revealed that all isolates carried blaNDM-5 and blaCTX-M-15 and belonged to ST307, a high-risk clone largely widespread in human clinical settings (3). The isolates were compared with: 10 K.P. ST307 genomes from the NRL-AR, IZSLT internal database including sequences from animals and food samples from different sampling contexts and 1620 ST307 genomes downloaded from the BIGSdb-Pasteur and NCBI. In all the 8 hybrid assemblies, blaNDM-5 was carried by an identical IncX3 plasmid, while blaCTX-M-15 was located on a pKpN, IncFIB(K) plasmid. Compared with contemporary Italian human-associated pKpN plasmids, companion animal-associated plasmids were larger, approximately 235-246 kb versus 133 kb, and carried additional putative virulence-associated clusters, including the fec iron-acquisition system glutathione transport systems and the lacZYI operon. Additionally, an E. coli ST410 carrying the same IncX3-blaNDM-5 plasmid was isolated from the same dog found positive for NDM-5-producing K. pneumoniae. Similar plasmids were detected in human samples from Italy and China (4). Interestingly, the IncX3 plasmid was identified, in only 2.43% of all 1642 ST307 isolates. These findings represent the first report of IncX3-blaNDM-5 in high-risk clone KP ST307 in Italy and suggest that companion animals may act as accidental hosts of human clinically relevant pathogens, resistances and virulence determinants, and possibly as secondary spill-back reservoirs.

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08. AMR

O57. COMPARATIVE GENOMICS OF ANTIMICROBIAL RESISTANCE AND VIRULENCE DETERMINANTS IN *Candida albicans* ISOLATES OF PUBLIC HEALTH RELEVANCE

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This study presents a comparative genomic analysis to identify genetic determinants of virulence and antimicrobial resistance (AMR) in two Candida albicans isolates: one acting as pathogen, isolated from a vaginal swab of a symptomatic human, and one acting as commensal, isolated from a blowhole swab of a captive bottlenose dolphin (Tursiops truncatus) of an Italian marine park. Available epidemiological data are limited, however, C. albicans was historically detected within the park tanks, raising concerns about the risk of exposure to contaminated water for both the animals living in the park and human staff and visitors.
Both C. albicans genomes underwent Illumina high-throughput sequencing. Genomic analysis and identification of AMR-related genes were performed using the ChroQueTas bioinformatics command-line tool [1]. After genes variant calling, variant impact was assessed by Variant Effect Predictor (VEP) [2-3]. Finally, virulence genes were identified by genomes alignment against the NCBI database. Interestingly in both Candida albicans genomes, ChroQueTas analysis revealed identical missense mutations in the Mrr2 protein (L144V, T145A) associated to fluconazole resistance. Complementary phenotypic characterization was performed, but MIC values for both C. albicans isolates indicated no phenotypic fluconazole resistance. Therefore, the isolates virulence profiles were evaluated using an in silico-only approach and the als3 gene was identified as a common virulence genetic determinant. Finally VEP analysis further revealed a genomic correlation with 4,452 shared missense variants between the two genomes.
In conclusion, the genomic overlapping between the C. albicans genomes suggests that the C. albicans animal derived could represent an environmentally acquired strain and that may have adapted to a shared human-animal environment [4]. While the analysis of only two C. albicans isolates prevents definitive epidemiological conclusions, the obtained results highlight the value of a One Health approach and the importance of monitoring C. albicans strains across different sources to better study potential inter-species transmission pathways.

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O58. EVALUATION OF A LIQUID-BASED SWAB TRANSPORT SYSTEM FOR MAINTAINING MRSA AND MSSA VIABILITY UNDER DIFFERENT STORAGE CONDITIONS

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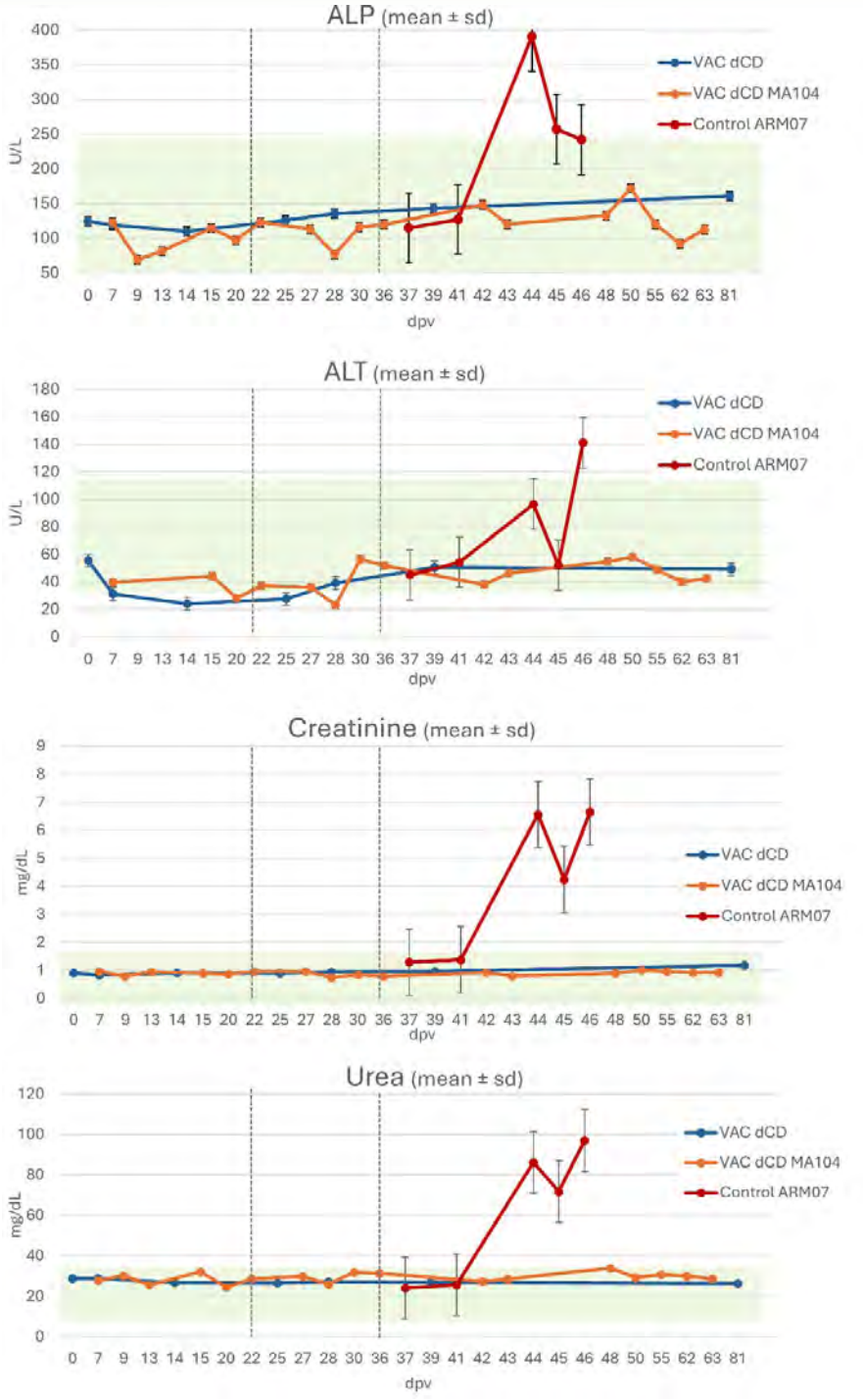
Staphylococcus aureus (SA), including both Methicillin-Resistant (MRSA) and Methicillin-Susceptible (MSSA), is a major One Health concern due to its ability to colonize and infect humans and animals, with companion animals acting as potential reservoirs for household transmission1-2. In addition, the extensive antimicrobial use has further promoted its spread across different hosts and environment 1-2. Ensuring bacterial viability during transport is therefore essential for accurate diagnostics. This study evaluated Copan eSwabtm liquid-based transport system for maintaining MRSA and MSSA viability over time under room temperature and refrigerated conditions. A panel of two MSSA (ATCC 25923, ATCC 6538) and one MRSA (ATCC 43300) strains were tested. Strain dilutions were prepared to obtain 25-250 CFU when spiked into Flocked Swabs (Copan Italia, n=25). After the inoculation, swabs were transferred to their transport medium tube (eSwabTM, Copan) and stored at room temperature (RT, 20–25 °C) and 4°C for 24 (n=10) and 48 (n=10) hours before plating. A set of swabs (n=5) were analyzed at time 0 (T0), maintained for 5-10 minutes in the transport tube before plating. Results were obtained counting CFU/plate (average of 5 plates) withing acceptable criteria: T0 25-250 CFU/plate; T24-48h ≥5 CFU/plate. All tested MSSA and MRSA strains showed robust recovery under both storage conditions, meeting acceptance criteria in all experimental conditions. While at RT bacterial counts increased to >500 CFU/plate at 24 and 48 hours, storage at 4 °C better preserved the stability of colony counts over time across all strains, indicating a more controlled maintenance of viability. These results confirm that the Copan eSWABtm ensures both maintenance and recovery of MSSA and MRSA under different storage conditions, fully complying with criteria. Notably, storage at 4 °C provides greater stability in colony counts across all strains compared with RT, supporting its suitability and reliability for clinical transport applications.

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08. AMR

Supplementary material



Supplementary figure 1: Comparative analysis of key serum biochemical parameters (ALP, ALT, creatinine, and urea) across experimental groups. Profiles show non-vaccinated control animals challenged with Armenia 2007 (Control ARM07, red line), animals vaccinated with PAM-produced Lv17/WB/Rie1-ΔCD (VAC dCD, blue line), and animals vaccinated with MA104-produced Lv17/WB/Rie1-ΔCD (VAC dCD MA104, orange line). Data are plotted as mean ± SD. The green shaded area represents the established baseline physiological range for wild boars.

O59. BEYOND TRADITIONAL DIAGNOSIS OF CAPRINE TUBERCULOSIS: IMMUNE AND PHYSIOPATHOLOGICAL BIOMARKERS AND GUT MICROBIOTA

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Traditional diagnosis of caprine tuberculosis (cTB) relies on the detection of the cell-mediated immune response, using the single intradermal tuberculin test/SITT and the interferon-gamma release assay/IGRA (1). However, the immune response is complex and can be influenced by many factors, which highlight the need for improving the detection of the disease (2). Here, we aimed to characterize the immune and physiopathological response against cTB and to assess its potential impact on gut microbiota, thus identifying potential biomarkers associated with infection. The study was conducted in a naturally M. bovis infected herd. Differences between reactor (positives in the SITT or IGRA) and non-reactor (negatives in both tests) goats in the levels of: [i] increase in plasma cytokines in PPDb-stimulated samples minus baseline concentrations (Δ-cytokine; 10 positives vs. 9 negatives), [ii] haptoglobin/Hp, serum amyloid A/SAA, total antioxidant status/TAS and malondialdehyde/MDA in serum (34 vs. 56), [iii] haematological parameters (21 vs. 23) and [iv] gut microbiota composition via 16S rRNA sequencing of fecal samples (7 vs. 8) were evaluated.
Reactor animals showed significantly higher ($p<0.05$) levels of Δ-IFN-γ, Δ-IP-10, Δ-IL-1α and TAS compared with non-reactor (Figure 1), while no significant differences were observed for the other markers. Microbiota analysis revealed a dysbiosis trend in reactor animals as previously reported (3), characterized by increased relative abundance of Bacteroidetes, Verrucomicrobia, and Proteobacteria, and decreased Firmicutes. However, these differences between groups were not statistically significant (Figure 2).
Beyond IFN-γ, our data demonstrate the association between the expression of IP-10, IL-1α and TAS and the reactivity to cTB tests and suggest their potential as biomarkers of infection. In addition, this study firstly describes the gut microbiome in goats from a cTB infected herd, although no significant differences in phylum relative abundance as regards their reactivity status to cTB tests.

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09. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

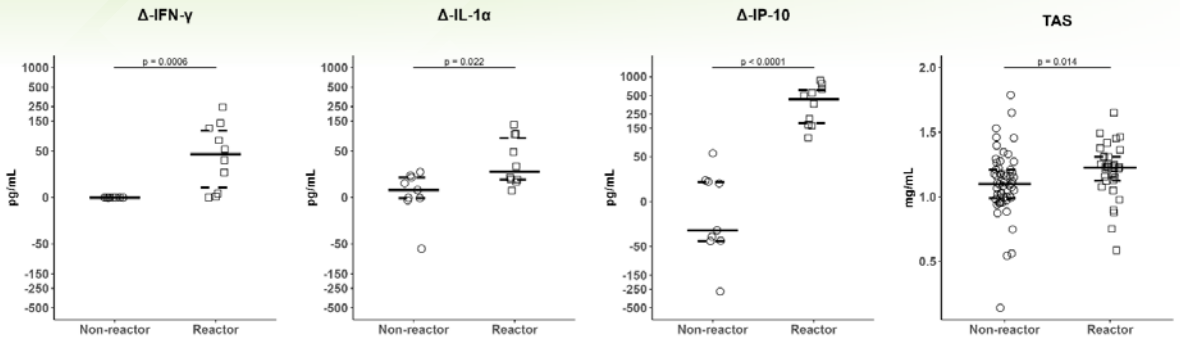


Figure 1: Plasma levels of Δ-IFN-γ, Δ-IL-1α and Δ-IP-10, and serum TAS in non-reactor (circles) and reactor goats (squares). Solid line represents the median and dashed lines represent the 25th (lower) and 75th (upper) percentiles.

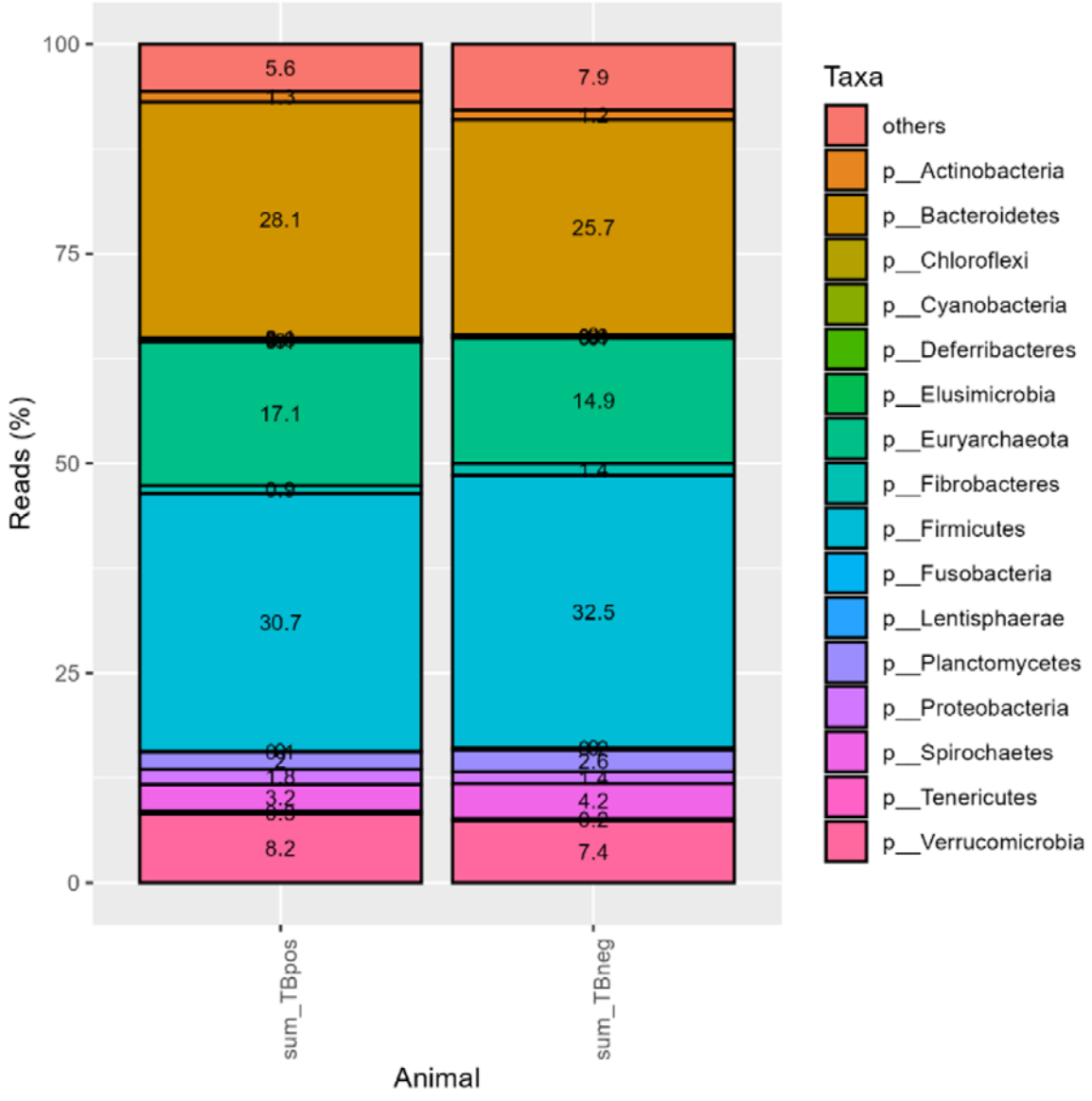


Figure 2: Relative abundance of the main phylum in the gut microbiota of reactor (left) and non-reactor (right) groups.

09. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O60. REFINING HISTOPATHOLOGICAL DIFFERENTIAL DIAGNOSIS OF BOVINE TUBERCULOSIS: STRATEGIES TO IMPROVE THE PERFORMANCE IN ROUTINE PRACTICE

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Bovine tuberculosis, caused by members of the *Mycobacterium tuberculosis* complex (MTC), remains a major animal health and economic concern, highlighting the need for rapid, reliable, and routine-applicable diagnostic tools. In this context, pathological diagnosis plays an important role not only in identifying lesions compatible with tuberculosis, but also in providing essential differential diagnostic information that allows discrimination from other infectious or neoplastic processes in routine surveillance settings. This study aimed to evaluate the sensitivity and specificity of histological (hematoxylin & eosin, H&E; Ziehl–Neelsen, ZN) and immunohistochemical (IHC) techniques, in combination and individually, using direct PCR and microbiological culture from tissue samples as reference methods. The study included 756 bovine tissue samples suspected of tuberculosis, collected through passive surveillance at slaughterhouses and active surveillance programs in Spain. The combined histopathological diagnosis (H&E, ZN, and IHC) showed a sensitivity of 97.4% and a specificity of 88.5% compared with PCR. Among discordant cases (PCR-negative but histopathology-positive), culture confirmed the presence of MTC in 33 of 39 cases, increasing the specificity of the histopathological approach to 98% when culture was considered as a complementary reference standard. Individually, H&E showed high sensitivity (96%) and specificity (88.8%). ZN staining demonstrated high specificity (90.5%) and sensitivity (90.2%). IHC exhibited high sensitivity (97%) but reduced specificity (63.3%), partly due to cross-reactivity with non-target pathogens. To address this limitation, samples showing strong immunoreactivity, but negative ZN results were reclassified as non-compatible, reducing false positives and improving overall specificity. All these cases were PCR-negative for MTC, and in some instances *Rhodococcus equi* was detected by PCR. Overall, refining interpretation criteria improved diagnostic performance. The combined use of H&E, ZN, and IHC represents a robust and highly sensitive strategy for bovine tuberculosis diagnosis, effectively complementing molecular techniques in surveillance and control programs.

Acknowledgements
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09. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O61. DEFINING CELLULAR IMMUNE CORRELATES OF PROTECTION AGAINST AFRICAN SWINE FEVER VIRUS IN WILD BOAR

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African swine fever (ASF) is a devastating viral disease affecting domestic and wild suids, with major global economic consequences. The development of effective vaccines remains challenging due to the ability of African swine fever virus (ASFV) to evade and modulate host immune responses, interfering with both innate and adaptive immunity. In addition, the high genetic diversity of ASFV complicates the identification of reliable immunological correlates of protection. As virus-specific antibodies alone do not necessarily confer protection, it is essential to define cellular immune response profiles, particularly T-cell responses, associated with protection in vaccinated animals. A better understanding of these mechanisms may help optimize vaccination strategies and improve vaccine evaluation.

An experimental study was conducted in wild boar using the vaccine candidate Lv17/WB/Rie1-ΔCD (patent PCT/EP2023/082518). Animals were orally vaccinated and later challenged with the virulent genotype II Arm07 isolate. Serum and blood samples were collected at baseline, during vaccination, and after challenge. Serum levels of immunomodulatory cytokines were measured using commercial Luminex assays. Peripheral blood mononuclear cells (PBMCs) were isolated to evaluate antigen-specific T-cell responses. IFN-γ-producing T cells were quantified by ELISpot, and T-lymphocyte populations, including γδ T cells and CD4+ and CD8+ T-cell subsets, were phenotypically characterized by flow cytometry.

Vaccinated animals showed significant differences in the frequency and activation status of effector T-cell populations compared with controls, together with specific cytokine modulation associated with protection and survival. These findings contribute to the identification of cellular correlates of protection against ASFV and may support the development of in vitro cross-protection studies, as well as improved assessment of vaccine immunogenicity and efficacy in wild boar.

Keywords

African swine fever, live attenuated vaccines, cellular immunity, ELISpot, flow cytometry

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O62. REVISITING DOURINE DIAGNOSIS: PHYLOGENOMIC RELATIONSHIPS WITHIN TRYPANOZOON AND PERFORMANCE OF THE COMPLEMENT FIXATION TEST

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Dourine is an equine parasitic disease caused by *Trypanosoma equiperdum*. This parasite belongs to the subgenus *Trypanozoon*, which also includes the causative agents of surra (*T. evansi*) and nagana (*T. brucei*). These diseases infect equids and represent a major threat due to high mortality and the lack of approved treatments. To limit their spread, the World Organisation for Animal Health (WOAH) recommends the complement fixation test (CFT) for dourine [1]. Although this test has been used for more than a century, concerns remain regarding its sensitivity, specificity, and potential cross-reactions with surra and nagana, which complicate the control of international equine movements [2]. The first objective of this study was to clarify the genetic relationships among the causative agents of dourine, surra, and nagana. The second objective was to establish controlled reference methods to evaluate and monitor the specificity and sensitivity of the dourine CFT. Relationships among *Trypanozoon* isolates were investigated through comparative phylogenomic analyses based on whole-genome sequencing data from representative isolates of worldwide origin. The sensitivity and specificity of the CFT were assessed using a combination of serum from an experimentally infected horse and serum from a horse likely to induce false-positive CFT reactions, together with a comparative titration method. Phylogenomic analyses demonstrated the polyphyletic nature of the causative agents of dourine, surra, and nagana, and showed that clinical presentation alone is insufficient to assign a *Trypanozoon* isolate to a specific taxon. The comparative titration method enabled the establishment of a control procedure capable of accurately assessing antigen sensitivity and specificity. In conclusion, a revised nomenclature is needed to reconcile Trypanozoon phylogeny with the historical disease names dourine, surra, and nagana, and reference-based control procedures could improve the standardization and quality control of the long-established dourine CFT.

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09. Other Subjects Pertaining to Veterinary Laboratory Diagnostics

O63. ACUTE PHASE PROTEIN RESPONSES IN LPS-INDUCED INFLAMMATION IN SHEEP

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Acute phase proteins (APPs) are recognised as markers of inflammation in animals. Experimental models using lipopolysaccharide (LPS) to induce systemic inflammation are well-established. However, the inflammatory response in such studies is typically assessed by clinical evaluation, and limited data are available regarding changes in APP levels. This study aimed to evaluate alterations in APP blood levels and explore their association with clinical status during an LPS-induced inflammation model in sheep. Sixteen healthy sheep (34.5±4.8 kg b.w.) received LPS (Escherichia coli O55:B5) intravenously (dose: 0.5 μg/kg) at time 0 and 8 hours later. Clinical score, rectal temperature, and blood levels of selected APPs (i.e., serum amyloid A, haptoglobin, albumin, and paraoxonase-1) were assessed before and after LPS administrations. A sustained inflammatory response was induced in all animals, characterised by a sharp increase in rectal temperature and the manifestation of additional clinical signs, including cough, respiratory distress, tremor, and lethargy. A composite score was utilised to evaluate their overall intensity. Serum amyloid A levels exhibited a statistically significant ($p<0.005$) increase. Baseline levels were below the LOD (5 mg/L). Following LPS administration, they increased approximately 2- to 80-fold. Haptoglobin concentrations also increased, although not significantly. The drop of albumin and paraoxonase-1 levels (as negative APPs) did not reach statistical significance, as they displayed high interindividual variability. Significant positive correlations were observed between serum amyloid A, the only substantially altered APP, and both rectal temperature and composite score within individual animals. These findings indicate that, among the investigated APPs, serum amyloid A is the most suitable marker for verifying and monitoring LPS-induced inflammation in sheep. Its measurement can provide greater sensitivity and objectivity compared with clinical assessment alone. This highlights its practical value for confirming and quantifying, in a diagnostic laboratory context, ongoing inflammatory processes, especially (albeit not solely) the ones associated with endotoxemia.

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O64. COMBINED ENDOCRINE AND INFLAMMATORY BIOMARKERS FOR HEALTH STATUS ASSESSMENT IN COMMERCIAL PIGS ACROSS PRODUCTION STAGES

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Accurate laboratory-based detection of systemic inflammation and stress in commercial swine demands multi-parameter approaches, yet the diagnostic validation of combined endocrine and inflammatory biomarker panels under field conditions remains limited. The integration of serum acute-phase proteins and stress indicators into a single diagnostic framework represents a promising, though insufficiently evaluated, approach to swine health surveillance.

This study evaluated the diagnostic utility of a biomarker panel combining C-reactive protein (CRP), pig major acute phase protein (Pig-MAP) and salivary cortisol to distinguish health status in commercial pigs throughout their production cycles, characterizing the influence of age, sex and disease etiology on biomarker performance.

A total of 1,000 (Landrace×Large White)×Pietrain pigs (2.5–30 weeks) from 24 commercial farms in Aragón (Spain) were included. Serum CRP and Pig-MAP were quantified individually, while salivary cortisol was measured in 200 pooled oral fluid samples (5 animals per pool). Animals were classified as healthy or clinically affected based on digestive, respiratory, or cutaneous signs. A univariate general linear model with robust estimators (HC3) was applied following log-transformation, with health status, age, sex, and disease type as fixed effects.

All three biomarkers showed significantly higher concentrations in clinically affected animals ($p < 0.001$). Stage-specific analysis revealed that cortisol discriminated between groups in all stages, reflecting activation of the HPA axis by pro-inflammatory cytokines except Transition (2.5–5 weeks), likely reflecting elevated baseline HPA activation associated with weaning[1,2]. Pig-MAP discriminated across all stages except Growth I (8–13.5 weeks), while CRP only reached significance during Growth II (14–20 weeks)[3]. Neither sex nor disease etiology significantly influenced any biomarker, confirming their independent diagnostic performance[4].

These findings demonstrate stage-dependent diagnostic sensitivity across the panel, supporting biomarker-specific interpretation windows in swine laboratory diagnostics and highlighting the complementary value of combining acute-phase proteins with salivary cortisol for comprehensive health characterization.

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10. Emerging Infectious Diseases & Recent Outbreaks In Europe

O65. MOLECULAR DETECTION OF PORCINE PARAINFLUENZA VIRUS 1 IN SPANISH SWINE HERDS AFFECTED BY RESPIRATORY DISEASE

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Porcine parainfluenza virus 1 (PPIV-1), species *Respirovirus suis*, has been reported in several European countries since its first detection in 2020, but data from Spanish swine herds remain limited [1–3]. PPIV-1 has been included within the porcine respiratory disease complex (PRDC), and the available evidence suggests that it acts primarily as a secondary pathogen, although it may also contribute to the development of lesions [4]. The aim of this study was to evaluate the detection of PPIV-1 in different sample types from Spanish farms affected by respiratory disease.
Between July 2025 and February 2026, 934 clinical cases suspected of PRDC were submitted to Exopol Laboratory (Zaragoza, Spain) from different provinces and production stages, including finishing pigs, nursery pigs, breeding sows, and suckling piglets. A real-time PCR (qPCR) panel targeting 13 respiratory pathogens, including an in-house assay for PPIV-1, was performed. A preliminary validation of the PPIV-1 qPCR assay was confirmed through the partial sequencing of 15 positive samples. Sample types mainly included oral fluids, lungs, and respiratory swabs. PPIV-1 was detected in 67 cases (7.2%). The highest detection rate was observed in nursery pigs (11.7%, $p = 0.004$) and in oral fluid samples (57.7%, $p < 0.001$). PPIV-1-positive farms were identified in 20 of the 39 provinces represented in the dataset. Among PPIV-1-positive cases, 10/67 (14.9%) showed PPIV-1 as the only virus detected, with Cq (quantification cycle) values ranging from 22.8 to 37.8. The remaining cases showed viral coinfections, with porcine reproductive and respiratory syndrome virus (PRRSV) being the most frequently co-detected virus (36/67; 53.7%), followed by porcine circovirus type 2 (PCV2) (25/67; 37.3%). Overall, these findings confirm the circulation of PPIV-1 in Spanish swine herds and highlight the suitability of oral fluids for farm-level screening, supporting its surveillance within the multifactorial PRDC.

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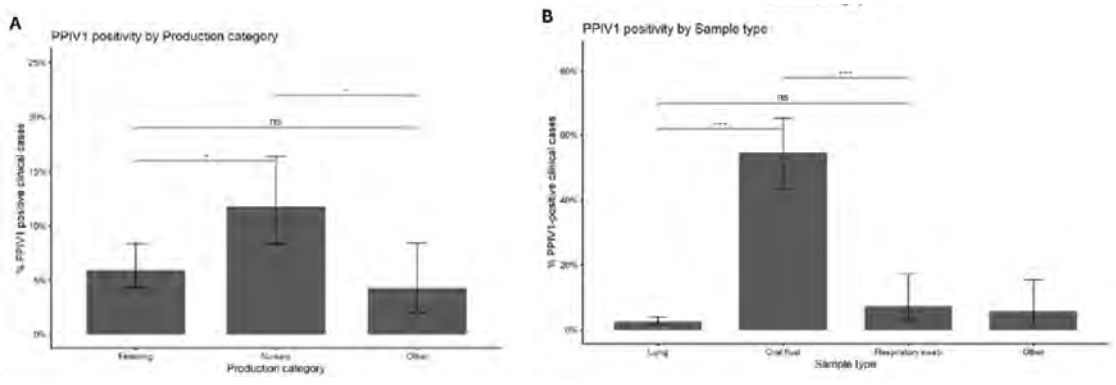


Figure 1. Proportion of PPIV-1–positive clinical cases by production category (A) and sample type (B). Bars represent observed proportions and error bars indicate 95% confidence intervals. Significant pairwise differences are indicated (* $p < 0.05$; *** $p < 0.001$; ns, not significant).

066. CONTRIBUTION OF GENOMICS TO THE CHARACTERIZATION OF THE CAUSATIVE AGENT OF CONTAGIOUS EQUINE METRITIS (*Taylorella equigenitalis*) AND *Taylorella asinigenitalis*, AND TO OUTBREAK INVESTIGATION

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Contagious equine metritis (CEM) is a venereal disease of equids caused by Taylorella equigenitalis, with significant impact on breeding activities and international trade [1]. Taylorella asinigenitalis, closely related species primarily isolated from donkeys, is considered non-pathogenic despite i) vaginal/cervical discharges, cervicitis and shortened estrus cycles induced in mares after experimental intrauterine inoculation [2], and ii) severe purulent endometritis observed in mares inseminated with contaminated donkey semen in the United Arab Emirates [3]. Advances in molecular epidemiology provide powerful tools for high-resolution strain characterization and outbreak investigation. This study aimed to characterize Taylorella spp. strains from two distinct epidemiological contexts in France. The first dataset included 26 T. equigenitalis from five stallions and one mare in 2025, while the second involved 39 T. asinigenitalis from six jacks during the establishment of a Grand Noir du Berry (GNB) breeding herd between 2024 and 2025. Following whole-genome sequencing using the Illumina technology, strains were subjected to multilocus sequence typing (MLST) and core genome single nucleotide polymorphism (cgSNP) analyses to assess genetic diversity, relatedness and potential transmission patterns. For the CEM agent, combined genomic and epidemiological data were consistent with a single introduction event followed by fomite transmission among stallions during semen collection and subsequent transmission to the mare via artificial insemination. In the GNB breeding herd comprising six jacks and 22 jennies, five of the six jacks carried distinct T. asinigenitalis strains upon arrival, supporting independent acquisitions. Genomic data from subsequent isolates revealed that recurrent detections resulted both from within-group horizontal transmissions (potentially via fomites during semen collection) and from incomplete clearance of the initial strains following treatment. These findings demonstrate that the integration of genomic and epidemiological data provides enhanced resolution for investigating Taylorella outbreaks, improving understanding of pathogen circulation and supporting more targeted control strategies in equine and asinine populations.

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O67. AUTOMATED HIGH-THROUGHPUT PCR FOR HPAI A(H5N1) MILK SURVEILLANCE

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The spillover of highly pathogenic avian influenza (HPAI) A(H5N1) into U.S. dairy cattle in March 2024 created significant challenges for disease surveillance and diagnostic capacity. Concurrent outbreaks in poultry and cattle sharply increased testing demand within National Animal Health Laboratory Network (NAHLN) laboratories. To support the United States Department of Agriculture's National Milk Testing Strategy (NMTS), Wisconsin Veterinary Diagnostic Laboratory implemented monthly testing of approximately 5,500 dairy farms, necessitating a highly scalable, high throughput workflow. An automated molecular workflow to maximize efficiency and testing capacity was implemented. Video in presentation illustrates sample processing was performed using Biomek Span 8 platform to dispense diluent and transfer milk samples from collection tubes into 96 well prefilled extraction plates and RNA extraction utilized prefilled IndiMag Pathogen KF96 cartridges on the KingFisher Flex system. PCR testing was initially conducted in a 96 well format using the NAHLN approved AgPath ID One step RT PCR master mix. This integrated extraction workflow reduced hands on time by approximately 60 minutes per 96 samples, translating to an estimated $49 savings in technician labor per run. To further increase throughput, alternative PCR workflows using Biomek i5 multichannel automation to prepare reactions in 384 well formats. NAHLN PCR assay was successfully optimized for 20 µL reaction volumes on the QuantStudio 7 Pro system. These adaptations reduced runtime by up to 4.5 hours per batch and yielded approximately 20% reagent cost savings, while maintaining 100% diagnostic sensitivity, specificity, precision, and limits of detection comparable to standard NAHLN approved methods. Following implementation of the NMTS in May 2025, up to 1,400 samples were processed within an 8 hour shift using one technician and two bench microbiologists. This scalable, automation driven workflow establishes a flexible framework readily adaptable for high throughput molecular testing of emerging disease such as HPAI infected dairy herds lacking overt clinical signs or other surveillance of regulated animal diseases.

O68. IMPLEMENTATION AND INTER-LABORATORY TRANSFER OF A CXCL16 GENOTYPING QPCR ASSAY FOR CHARACTERISING EQUINE ARTERITIS VIRUS PERSISTENCE IN STALLIONS

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The establishment of a long-term Equine arteritis virus (EAV) carrier state in stallions is a key determinant of viral persistence and transmission. Host genetic factors, particularly polymorphisms in the CXCL16 gene, are known to influence susceptibility to persistent infection, making their integration into diagnostic workflows highly relevant for refining disease control strategies.

In this context, a published TaqMan® allelic discrimination qPCR assay targeting CXCL16 was implemented and optimised in our European Reference Laboratory for Equine Infectious Diseases, with a focus on robustness and routine diagnostic applicability. Its transferability was subsequently assessed through implementation in a partner diagnostic laboratory (LABÉO), including protocol standardisation, training, and inter-laboratory comparison, in view of future dissemination to National Reference Laboratories across the European Union.

The assay was applied during an EAV outbreak in a breeding centre in Normandy (France, 2025), in combination with targeted virological analyses of blood, respiratory and semen samples. Viral RNA detection was largely restricted to semen, consistent with persistent infection, and revealed prolonged shedding in a subset of stallions 18.

CXCL16 genotyping enabled clear discrimination between resistant (r/r) and susceptible (r/s, s/s) profiles, in agreement with virological findings. Eight stallions were classified as low risk (short-term shedding), whereas ten were identified as high risk for long-term persistence. Notably, one CXCL16 s/s stallion remained semen-positive eight months after infection, while resistant r/r animals cleared viral shedding over the same period. High concordance between laboratories results confirmed both assay robustness and successful transfer.

Overall, CXCL16 genotyping provides a powerful diagnostic tool to characterise EAV persistence in stallions, bridging host genetics and virology, and supporting more targeted, risk-informed management of infected populations.

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ePOSTERS

P001. DEVELOPMENT AND VALIDATION OF A TRIPLEX QPCR KIT FOR AFRICAN SWINE FEVER VIRUS DETECTION IN HUNGARY

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African swine fever (ASF) remains one of the most significant transboundary animal diseases affecting the global swine industry. Since its first detection in wild boar populations in Hungary in 2018, ASF has posed a continuous challenge to national surveillance and disease control programs. The increasing number of ASF-positive cases in wildlife has emphasized the need for rapid, sensitive, and reliable diagnostic tools capable of supporting large-scale monitoring efforts. This study describes the development and analytical validation of a triplex real-time PCR (qPCR) assay for the simultaneous detection of African swine fever virus (ASFV) and two internal quality controls. The assay targets a highly conserved region of the ASFV B646L (p72) gene while incorporating an endogenous porcine control to assess sample quality and an exogenous internal amplification control to monitor PCR performance. Primers and hydrolysis probes were optimized for multiplex detection using three fluorescent channels within a single reaction. Analytical performance was evaluated using synthetic DNA standards, reference ASFV-positive materials, and a panel of common swine pathogens. The assay demonstrated high specificity, with no cross-reactivity observed for non-target organisms. The limit of detection is around 20 target copies per reaction, enabling reliable identification of low-level infections. Repeatability and reproducibility studies confirmed robust performance under routine laboratory conditions. Our triplex qPCR kit is validated by the WOAH, FAO and European Union reference laboratory for ASF (EURL-ASF). The kit provides a sensitive, specific, and cost-effective solution for ASFV detection. Its multiplex design improves testing efficiency while maintaining high diagnostic confidence, making it suitable for veterinary diagnostic laboratories and international monitoring programs.

P002. INTRAFAMILIAL SPREAD OF *Trichophyton benhamiae* IN A HOUSEHOLD WITH EXOTIC PETS: A ONE HEALTH PERSPECTIVE

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A case of tinea capitis, tinea faciei, and tinea corporis was investigated in a 3-year-old Italian child who presented with multiple itchy, erythematous, and scaly lesions. Skin manifestations consistent with tinea corporis in the mother and with combined tinea corporis and tinea barbae in the father were subsequently observed, suggesting secondary intra-familial transmission. The family lived with a dog, a hazel dormouse, and six guinea pigs, all of which showed no visible lesions.
Direct microscopy and fungal culture were performed on human samples, followed by MALDI-TOF, PCR, and Sanger sequencing for species identification. In a One health framework, samples were also collected from cohabiting animals and household objects, such as dog's bed, carpet, sofa and cushions. Additionally, to assess antifungal susceptibility, a qPCR assay targeting mutations associated with terbinafine resistance was performed.
Through this diagnostic approach, terbinafine-sensitive Trichophyton benhamiae was identified in the child, both parents, and the guinea pigs.
Trichophyton benhamiae is a zoonotic dermatophyte, often associated with small animals like rodents, primarily guinea pigs, and causing dermatophytosis in humans and animals (1).
The absence of clinical signs in the animals could have suggested contamination in an extra-domestic setting. Indeed, a detailed medical history revealed a recent travel by ship approximately two weeks before symptoms onset. In particular, the child had contact with the carpeted floor potentially acting as a contaminated fomite due to the usual presence of pets. Nonetheless, the recovery of T. benhamiae in guinea pigs underscores the importance of a One Health approach, particularly in the context of cohabitation with exotic pets.
The integration of conventional culture with advanced molecular diagnostics enabled reliable species identification and rapid assessment of antifungal susceptibility, contributing to better understanding of emerging dermatophytes' behavior, such as T. benhamiae, recently identified in Italy also in dogs and cats (2,3).

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P003. HIGH SEROPREVALENCE AND SILENT VIRUS SHEDDING DURING AN EQUINE VIRAL ARTERITIS OUTBREAK IN BREEDING STALLIONS

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Equine viral arteritis (EVA) is a worldwide infectious disease of major concern for the equine breeding industry due to its impact on reproduction and international trade. In 2025, an EVA outbreak was declared in the department of Calvados, Normandy (France). As the affected premises was an equine semen collection facility, stallions intended for breeding were routinely monitored for their EVA serological status. During this surveillance, seroconversion was unexpectedly detected in horses that had been initially tested as seronegative. The objective of this study was to describe the epidemiological investigation and laboratory findings associated with this outbreak. Serological screening was performed using the virus neutralization test in accordance with AFNOR standard NF U47-035. Virological investigations were carried out by RT-qPCR targeting ORF7 (1) on EDTA blood samples, nasopharyngeal swabs, and semen samples. A total of 282 equine sera from several premises representing 17 breeds were tested serologically, revealing a high seroprevalence, with 174 animals positive for EAV-neutralizing antibodies, particularly among breeding stallions. Virological investigations showed that no EAV genome was detected in blood samples (n = 151) or nasopharyngeal swabs (n = 67). In contrast, EAV RNA was detected in semen samples, leading to the identification of 24 stallions of 29 classified as virus shedders and therefore prohibited from breeding until EAV is no longer detected in their semen. These results confirm the critical importance of EVA monitoring in breeding stallions, which play a central role as reservoirs of EAV, and highlight the limited diagnostic value of blood or respiratory testing outside the acute phase of infection. This outbreak emphasizes the need for systematic EVA screening when new horses enter breeding facilities, combined with quarantine measures until serological and virological results are available. Furthermore, routine semen testing in breeding facilities is essential to prevent silent circulation and international dissemination of EAV.

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P004. SEROLOGICAL EVIDENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN WILD UNGULATES IN SPAIN

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by a *Nairovirus* and transmitted by ticks of the genus *Hyalomma*. Since 2002, the virus has circulated in Spain in a tick–vertebrate cycle in which animals contribute to maintaining both tick populations and virus transmission. Wild ungulates, particularly red deer, are key reservoirs and useful models for understanding CCHFV ecology and assessing human infection risks in Iberia (1). This study aimed to determine the presence of antibodies against CCHFV in wild ungulates and to evaluate their potential role in the maintenance of the virus in natural ecosystems. Between 2024 and 2026, 184 wild ungulates from central and southern Spain were sampled. Blood samples were collected from red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), and fallow deer (*Dama dama*). Serum antibodies were detected using a commercial ELISA kit (ID Screen CCHF Double Antigen Multispecies, IDvet, France), following the manufacturer's instructions. Antibodies against CCHFV were detected in 58.15% of the animals analyzed, including red deer (87/124, 70.16%; 95% CI: 62.1–78.2%), wild boar (12/49, 24.49%; 95% CI: 12.4–36.5%), and fallow deer (8/11, 72.73%; 95% CI: 46.4–99.1%). Positive results were obtained in all localities where samples were collected. These results are consistent with previous studies demonstrating that cervids may play a key role in the epidemiology and circulation of CCHFV (1,2). In contrast, wild boar populations showed lower seroprevalence, consistent with previous reports from Spain (3). Additionally, ungulates are important hosts for *Hyalomma* spp. ticks in the Iberian Peninsula (4), thus contributing to the manteinance and circulation of the virus in natural ecosystems. These findings highlight the value of wild ungulates as useful sentinels for CCHFV surveillance and reinforce the importance of active monitoring programs in high-risk areas within a One Health framework.

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P005. SPORADIC DETECTION OF *Salmonella enterica subsp. enterica serovar Typhimurium* IN WHITE-WINGED SNOWFINCHES *(Montifringilla nivalis subsp. nivalis)* IN THE SWISS ALPS

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Following mortality events associated with salmonellosis in White-winged Snowfinches (*Montifringilla nivalis* subsp. *nivalis*, hereafter snowfinches) in 2018 and 2019, a large-scale study was initiated in Switzerland to assess the potential risk posed by *Salmonella* spp. to this alpine species, already under pressure from climate change, as well as to evaluate possible public health implications. Between 2019 and 2025, 288 faecal swabs were collected from snowfinches at nine winter feeding sites in the Swiss Alps and analysed for the presence of *Salmonella* spp. according to international standard protocols. Seven samples (2.4%), all obtained during a single sampling event at one feeder in 2024, tested positive. Serotyping identified *Salmonella enterica* subsp. *enterica* 4,12:-:1,2 (monophasic *S.* Typhimurium). Whole genome sequencing of this isolate and *S.* Typhimurium strains obtained from post-mortem analyses of Swiss songbird mortality events (2010 – 2024, [Table 1]) revealed close clustering of the strains from the 2018 and 2019 outbreaks in snowfinches, suggesting recirculation, whereas all other strains were genetically distinct. Monophasic *S.* Typhimurium 1,4,[5],12:i:-ranked among the most frequently isolated serovars in human infections in Europe in 2024. In contrast, strains monophasic in the first phase (e.g. 4,12:-:1,2) are rarely described. The clinical relevance of this monophasic strain remains unclear, as all sampled birds were apparently healthy. However, diseased and dead birds had been observed at the same sampling site in 2024. Notably, one of these birds tested positive for *Salmonella* Typhimurium (Table 1). All analysed strains carried chromosomally encoded resistance genes to tetracycline, polymyxin B and aminoglycoside. This study underlines the importance of continued public education on bird-feeding practices as a potential source of infection, which remains an important preventive measure to reduce disease transmission among songbirds and potential spillover to humans.

Table 1 Salmonella enterica subsp. enterica serovar Typhimurium isolated from songbirds linked to mortality events in Switzerland 2010 – 2024 with comparison to the monophasic strain isolated in the monitoring research

Year	Submitted	Bird species	Post-mortem findings	Sample	Salmonella Serovar	MLST2
2010	Two birds	Eurasian siskin	Necrotizing esophagitis/ingluvitis	Oesophagus	Typhimurium	ST19
2018	One bird	Snowfinch1	Autolysis	Oesophagus	Typhimurium	ST5683
2019	One bird	Snowfinch1	Necrotizing esophagitis/ingluvitis	Oesophagus	Typhimurium	ST5683
2020	One bird	House sparrow	Multifocal esophagitis	Oesophagus	Typhimurium	ST568
2021	One bird	House sparrow	Necrotizing hepatitis	Oesophagus	Typhimurium	ST19
2024	One bird	Snowfinch1	Necrotizing esophagitis/ingluvitis	Liver, kidney	Typhimurium	ST19
2024	288 swabs	Snowfinch1	Alive and clinically healthy	7 positive swabs	4,12: - : 1,24	ST568

[1]White-winged snowfinch, [2]ST19 corresponds to phage type DT40; ST568 corresponds to DT56,
[3]2018 and 2019 strains are closely clustering, [4]Monophasic strain of S. Typhimurium

P006. WHOLE GENOME SEQUENCING REVEALS THE SOURCE OF A FOODBORNE OUTBREAK CAUSED BY MONOPHASIC *Salmonella enterica* SEROVAR TYPHIMURIUM ST34

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Whole genome sequencing (WGS) has become a key tool in investigating foodborne outbreaks, enabling high-resolution pathogen characterization and improving source attribution[1]. Under a One Health framework, integrating epidemiological, microbiological, and genomic data from human, food, and animal sources enhances outbreak detection and supports timely risk management[2]. This study describes the use of WGS during the investigation of a foodborne outbreak caused by monophasic *Salmonella enterica* serovar Typhimurium ST34 linked to pork products. In August 2024, an increase in human salmonellosis cases was detected in two municipalities in eastern Spain. Epidemiological investigations identified locally produced dry-cured pork sausage as the suspected vehicle. Official inspections and food sampling were conducted according to European regulations[3,4], together with sampling of pig carcasses at the supplying slaughterhouse. *Salmonella* isolates from human cases, food samples and slaughterhouse investigations underwent serotyping, antimicrobial susceptibility testing, and WGS. Genomic relatedness was assessed using core genome multilocus sequence typing (cgMLST) within the EnteroBase HierCC scheme, while antimicrobial resistance genes were identified using ResFinder. A total of 32 human cases were confirmed, with a peak in mid-August. Two dry-cured sausage batches from the same retail establishment tested positive for monophasic *S.* Typhimurium ST34, matching the outbreak strain detected in patients. WGS identified a shared HC5 cluster between human and food isolates, differing by ≤3 alleles, confirming a common outbreak source. Although ST34 isolates were detected at the supplying slaughterhouse, they showed 55–60 allelic differences, excluding them as the direct source. Outbreak-associated isolates displayed multidrug-resistant, including the presence of bla_{TEM-1B}, aac(6')-Iaa and tet(B) genes. These findings highlight the value of WGS within a One Health framework for confirming epidemiological links, distinguishing related from unrelated strains, and supporting source attribution. Integration of genomic surveillance into official control systems strengthens food safety and improves outbreak management.

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P007. ONLINE AFRICAN SWINE FEVER INFORMATION FOR FARMERS: PILOT VALIDATION OF A WEB QUALITY ASSESSMENT TOOL

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African swine fever (ASF) is a major transboundary animal disease affecting domestic pigs and wild boar, with severe economic, sanitary and environmental consequences in Europe. In the absence of effective treatment and widely available vaccination strategies, early detection remains a cornerstone of surveillance and outbreak control. However, laboratory diagnosis can only be timely if farmers rapidly recognise risk situations, access reliable operational information and correctly activate veterinary services. This pilot study aimed to develop and validate a farmer-oriented tool to assess whether online ASF information supports preparedness, biosecurity and early detection. Fifteen websites potentially consulted by pig farmers were selected and classified as institutional, commercial or farmers' association websites. A structured scoring grid was developed including eight criteria: disease description, prevention, on-farm management, reference legislation, practical measures to comply with regulations, procedures in case of outbreak, information on disease distribution, and ease of consultation. Each criterion was scored from 1, absent, to 5, excellent. The tool was reviewed by three experts in communication and animal infectious diseases and tested with five farmers using face-to-face, synchronous remote and asynchronous self-completion approaches. Most websites were judged incomplete or only partially adequate. Institutional websites generally achieved higher scores, followed by commercial websites, whereas farmers' association websites were less informative. The weakest areas were those most directly linked to early detection and diagnostic preparedness: biosecurity measures, outbreak procedures, legislation and updated information on disease distribution. Face-to-face administration was the most effective approach, allowing clarification of doubts and collection of qualitative comments. This pilot study suggests that assessing the accessibility and operational quality of digital information may strengthen ASF surveillance by improving farmers' ability to recognise warning signs, apply biosecurity measures and promptly involve veterinary diagnostic services.

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P008. DEVELOPMENT AND VALIDATION OF A MULTIPLEX RT-qPCR ASSAY WITH DUAL INTERNAL CONTROLS FOR BROAD DETECTION OF AVIAN INFLUENZA A VIRUS ACROSS DIVERSE SAMPLE MATRICES

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Avian Influenza A virus (AIV) remains a major threat to animal and public health, highlighting the importance of a One Health approach for surveillance across animal populations and environmental interfaces. Robust molecular diagnostic tools are required to support early detection and outbreak control. We describe the development and validation of a multiplex RT-qPCR assay (VetMAX™ Influenza A Virus Detection Kit 2.0) for detection of AIV in avian species.
The assay targets a conserved region of the Influenza A matrix gene and incorporates endogenous and exogenous RNA internal positive controls (IPC). This dual IPC design enables monitoring of sample integrity, nucleic acid extraction, and PCR inhibition, supporting reliable detection across diverse matrices. The assay was validated on multiple sample types including oropharyngeal, tracheal and cloacal swabs, organs, fecal samples, environmental samples (wipes, boot covers), and FTA cards.
Analytical validation demonstrated high inclusivity, with >99% in silico coverage across more than 45,000 AIV sequences from 224 avian species, and detected all 103 strains tested, representing 29 subtypes. No cross-reactivity was observed with the panel of 65 non-target pathogens tested. The assay showed high efficiency (97%), a limit of detection of 10 copies per reaction, and strong repeatability and reproducibility (CV <1%).
Diagnostic validation across 482 avian samples demonstrated 100% concordance with expected results, including complex matrices. Comparative evaluation with established RT-qPCR assays showed equivalent qualitative performance and strong correlation (r ≥0.90), with robust performance in the presence of inhibitors. The assay is compatible with multiple real-time PCR platforms, supporting consistent qualitative results.
In conclusion, the VetMAX™ Influenza A Virus Detection Kit 2.0 demonstrates high analytical sensitivity and robust performance for AIV detection. Dual internal controls support reliable performance across heterogeneous sample types, supporting surveillance and rapid outbreak response within a One Health framework.

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P009. OCCURRENCE OF SWINE CORONAVIRUSES IN HUNGARIAN FARMS

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Swine coronaviruses (SCoVs) are economically important pathogens, causing predominantly enteric, respiratory or neurological diseases with substantial impacts on animal health and production efficiency. Currently, six major porcine CoVs are recognized, including transmissible gastroenteritis virus (TGEV), porcine respiratory CoV (PRCV), porcine epidemic diarrhea virus (PEDV), swine acute diarrhea syndrome CoV (SADS-CoV), porcine deltacoronavirus (PDCoV), and porcine hemagglutinating encephalomyelitis virus (PHEV). CoVs are prone to frequent mutation and recombination. PRCV, a deletion mutant of TGEV, is a great example of the emergence of a new viral strain following a deletion on the spike protein coding region. The novel variants, recombinants remain a continuous epidemiological concern. Therefore, molecular surveillance and genomic characterization of circulating SCoVs are essential for understanding viral evolution and establishing effective prevention strategies. The aim of the present study was to compare variable pan-CoV detection systems using swine samples collected from eleven pig herds in Hungary. Two broad-spectrum, CoV-specific RT-PCR assays were used to surveil 121 (84 oral fluid, 34 pooled nasal swab, and 3 processing fluid) swine samples originated from clinically healthy pigs. Using a novel system, 24 samples from five farms have shown CoV nucleic acid presence. Based on sequence analysis, twelve amplicon sequences showed high nucleotide identity with PRCV/TGEV, while the remaining twelve corresponded to PHEV sequences. Using another PCR assay described in the literature, CoV was detected in eight cases. The detection of multiple SCoVs in asymptomatic animals highlights the potential role of subclinically infected swine in viral maintenance and transmission within herds. To further investigate the genetic variability, shotgun next-generation sequencing is planned to reveal recombination patterns and evolutionary relationships of the detected viruses through complete genome characterization.

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P010. ROTAVIRUS H DETECTED IN A WEANED PIGLET WITH DIARRHEA FOR THE FIRST TIME IN THE CZECH REPUBLIC

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Rotavirus H (RVH) was first described in humans in China in the 1980s, where it caused widespread outbreaks of gastroenteritis. It was subsequently detected in pigs on all continents; its prevalence in pigs with diarrhea is reported to be 9–14%. Moreover, the RVH was detected in bats in South Korea. In pigs, RVH is usually detected in co-infections with other rotavirus species; its epidemiological significance currently remains unclear. In our ongoing project dealing with swine rotaviruses of all known species, RVH was detected for the first time in Czechia. Previously published primers were used targeting the gene for the species-specific surface protein VP6 across its entire coding sequence.[1] To increase the sensitivity of the reaction, amplification was repeated with the same primers in the second round (reamplification). All samples in which a band of the corresponding length of 1240 bp was observed during electrophoretic evaluation were sent for sequencing (Eurofins Genomics, Germany). A product with a sequence corresponding to rotavirus group H has so far been found in one sample, which was collected from a weaned piglet with signs of diarrhea. Phylogenetic analysis of the newly detected RVH assigned this strain P9/25 to genotypes G13, P3, I7 and E4 clade I. The genes coding VP7 and NSP4 showed the highest similarity to Italian strains of poRVH detected in 2018.[2] Other rotavirus groups (RVA, RVB, and RVC) were also detected in this sample. Co-infections have been reported to increase the severity of diarrhea, however, the clinical impact of co-infections is not known.

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P011. DEVELOPMENT AND VALIDATION OF THE VIROTYPE BTV PAN/3 2.0 RT-PCR KIT FOR RAPID DETECTION OF BLUETONGUE VIRUS AND SEROTYPE 3 TYPING

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Bluetongue virus serotype 3 (BTV-3) emerged in the Netherlands in September 2023 and subsequently spread rapidly across Europe, causing severe disease and substantial mortality in affected ruminants. Reported impacts have been especially significant in sheep, while cattle have also shown increased mortality and reproductive losses. These outbreaks highlight the need for rapid, sensitive diagnostics to support effective disease management and containment strategies. INDICAL Bioscience developed the virotype® BTV pan/3 2.0 RT-PCR kit, a triplex assay, enabling simultaneous detection of all BTV serotypes (pan-BTV) and specific identification of BTV-3 in a single reaction. The assay utilizes FAM channel for pan-BTV detection, Cy5 for BTV-3 identification, and JOE for the endogenous internal control. Analytical sensitivity was evaluated using in vitro RNA dilutions across four thermocycler platforms. Diagnostic performance was assessed using 318 field samples (292 blood, 26 tissue) from cattle, sheep, goats, deer, and alpacas, compared against reference methods including virotype BTV pan/8 2.0 kit and competitor assays. The assay demonstrated exceptional analytical sensitivity, detecting single in vitro RNA copies for both pan-BTV and BTV-3 targets across all tested platforms. Limit of detection studies confirmed reliable detection of 5 copies per reaction with 100% frequency. Diagnostic validation yielded 100% sensitivity, specificity, and efficiency for the corresponding sample panel. The assay successfully detected all 32 tested BTV reference serotypes (1-28, 30, 33, 35, 36) with specific BTV-3 identification. Pooled sample analysis demonstrated maintained sensitivity in pools up to 20 samples. International validation using 648 field samples from Netherlands and Belgium confirmed robust performance. Repeatability studies showed coefficients of variation <5% for intra-assay, inter-assay, and batch-to-batch variability. The virotype BTV pan/3 2.0 RT-PCR kit provides a rapid (<70 minutes), highly sensitive diagnostic solution for BTV surveillance and outbreak management, enabling simultaneous pan-BTV screening and BTV-3 specific identification crucial for the current European epidemic response.

P012. NOVEL DOUBLE-ANTIGEN ELISA FOR DETECTION OF ANTIBODIES AGAINST CAPRIPOXVIRUSES IN RUMINANT SERUM SAMPLES

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Accurate detection of antibodies against *Capripoxviruses* (CaPV), including *Lumpy Skin Disease Virus* (LSDV), *Sheeppox Virus* (SPPV) and *Goatpox Virus* (GTPV), is essential for surveillance and control of lumpy skin disease (LSD) and related infections. To tackle the increasing incidence of CaPV in Europe and worldwide INDICAL BIOSCIENCE have developed and evaluated the performance of a double-antigen ELISA in comparison to the Immunoperoxidase Monolayer Assay (IPMA) (1), as a reference method. A total of 254 sera samples were analysed, including negative field sera (n = 46), experimental samples from cattle infected with classical (genotype 1.2) (n = 9) and recombinant (genotype 2.5) (n = 7) LSDV strains, as well as vaccinated cattle (homologous and heterologous vaccination) (n = 9) and sheep vaccinated against SPPV (n = 3). The cattletype CaPV Ab Multispecies ELISA demonstrated excellent relative specificity (100 %) when tested with the negative sera sample panel, with low background reactivity. In infected animals, the assay detected antibodies early and showed strong agreement with IPMA results. Depending on the borderline IPMA result interpretation, relative sensitivity of cattletype CaPV Ab Multispecies ELISA kit to IPMA was calculated as 96.8 % or 103.7 % in experimentally infected and vaccinated sample panels. Overall, the cattletype CaPV Ab Multispecies ELISA kit demonstrated performance comparable to IPMA, supporting its suitability as a reliable tool for detection of antibodies against *Capripoxviruses*.

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P013. RETROSPECTIVE WHOLE-GENOME SEQUENCING OF HISTORICAL ITALIAN ASFV GENOTYPE I ISOLATES CLARIFIES THE ORIGIN OF THE 1960s ASFV OUTBREAKS

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African swine fever virus (ASFV) genotype I circulated widely across Europe from the 1960s to the 1990s, causing multiple outbreaks in several mainland Italian regions; in Sardinia, the disease became endemic from 1978 to 2024 [1]. Despite the historical significance of these events, the genomic diversity and evolutionary relationships of early Italian ASFV isolates remain poorly characterized, largely due to the scarcity of whole-genome data from archival strains [2, 3].
We sequenced spleen and kidney samples collected from domestic pigs in mainland Italy during the 1967-1968 epidemic wave using Illumina technology. Reads were assembled using de novo and reference-guided approaches based on Lisbon 60 genome (KM262844). The complete ASFV genomes ranged from 182,360 to 182,374 nucleotides in length, with 99.9-100% horizontal coverage. Phylogenetic analyses demonstrated that the Italian ASFV isolates collected during the 1967-1968 epidemic clustered closely (99.98-99.99% nucleotide identity) with the historical Lisbon 60 strain, one of the earliest and best-characterized genotype I isolates linked to the Iberian epidemic following the reintroduction of ASFV into Portugal in 1960. These findings support a close genomic relationship between the mainland Italian strains and the Iberian lineage circulating contemporaneously in Portugal and Spain, consistent with historical evidence of transboundary dissemination events across southwestern Europe during the mid-20th century. Comparative genomic analyses further revealed a high degree of conservation among the mainland Italian isolates, reflecting the overall genetic stability of ASFV genotype I. Similar evolutionary dynamics have been described for Sardinian strains, likely associated with the large double-stranded DNA genome of ASFV and its relatively slow evolutionary rate. Despite this genomic stability, distinct evolutionary trajectories emerged between the early mainland Italian isolates and the later Sardinian lineage, highlighting divergent epidemiological histories and prolonged geographic isolation. These findings provide new insights into the historical dissemination and molecular epidemiology of ASFV genotype I in Italy.

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P014. BORDER DISEASE VIRUS IN ITALIAN SMALL RUMINANTS: AN UPDATE ON GENOMIC VARIABILITY AND EMERGING STRAINS

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Within the genus *Pestivirus* (family *Flaviviridae*), Pestivirus ovis (Border disease virus, BDV) is a globally distributed pathogen of small ruminants associated with reproductive disorders, particularly abortion, leading to substantial economic losses in sheep and goat production. Recent advances in molecular diagnostics and phylogenetic analyses have greatly improved the detection and genetic characterization of BDV strains. Traditionally, BDV has been classified into eight genotypes (BDV-1 to BDV-8) [1,2,3] based on the sequence analysis of conserved genomic regions, such as 5' untranslated region (*5'-UTR*) and N-terminal protease gene (*Npro*). Recent studies in Italy have highlighted considerable genetic heterogeneity, with several BDV genotypes, atypical strains [4], and novel phylogenetic clusters circulating. This suggests ongoing viral evolution and limitations in the current classification system.

This study aimed to characterize BDV-positive samples collected from abortion cases in small ruminants in Italy between 2015 and 2026. Molecular and phylogenetic analyses confirmed the circulation of genotypes BDV-3, BDV-6, and BDV-8, previously described [3]. Notably, BDV-3 showed marked genetic variability, indicating possible ongoing evolutionary dynamics and emphasizing the need for further epidemiological investigation. Ovine Pestiviruses were detected in Tuscany, whereas North African Pestivirus strains, including Tunisian and Tunisian-like variants, were detected in Friuli-Venezia Giulia (Northern Italy), representing their first identification in this region, and their presence was also confirmed in Calabria and Lazio, suggesting multiple viral introductions and possible long-term, uncontrolled circulation within the Italian small-ruminant population. No evidence of BDV-2 and BDV-4 was detected, in agreement with previous studies [3].

Overall, these findings demonstrate the high genetic diversity of Pestiviruses currently circulating in Italian sheep and goats. The data support evidence of interspecies transmission, continuous viral introductions, and dynamic epidemiological patterns. Continuous molecular surveillance is therefore essential to monitor emerging strains, improve understanding of Pestivirus evolution, and support the development of effective prevention and control strategies.

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P015. ADDRESSING THE RE-EMERGENCE OF NEWCASTLE DISEASE VIRUS GENOTYPE VII.1.1: RAPID REAL-TIME RT-PCR DIFFERENTIATION FROM VACCINE-ASSOCIATED GENOTYPES I/II

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Newcastle disease virus (NDV; avian paramyxovirus 1, PMV-1) remains a major threat to poultry health, production and international animal health regulation. Recent European reports of genotype VII.1.1-related NDV highlight the need for rapid molecular tools that support differentiation of emerging genotype VII field strains from classical vaccine-associated genotype I/II-related strains (Berihulay et al., 2025; Bortolami & Monne, 2025; World Organisation for Animal Health, n.d.).
KYLT developed and validated a triplex real-time RT-PCR assay for simultaneous detection and differentiation of PMV-1 genotype VII, PMV-1 genotype I/II-related strains and an exogenous internal control. Analytical sensitivity was assessed using defined serial dilutions of genotype VII and genotype II targets. Diagnostic performance was evaluated using 148 veterinary field samples, including FTA cards, swabs and poultry tissues. Sanger sequencing and phylogenetic analysis were used as reference methods for phylogenetic genotype assignment according to the current unified NDV classification framework (Dimitrov et al., 2019; SAN Group Biotech Germany GmbH, 2026).
The assay detected genotype VII and genotype II targets down to 0.5 copies/µl, corresponding to 2 copies per reaction, with a 100% detection rate. At 0.25 copies/µl, corresponding to 1 copy per reaction, both targets were detected with a 66.7% detection rate. PCR efficiencies were 97.0% for genotype VII and 102.2% for genotype II. In field-sample evaluation, genotype VII-positive samples were reliably detected in the genotype VII channel, while genotype I/II-related vaccine-associated strains were detected in the Cy5 channel. Reactivity of genotype I strains in the genotype I/II channel was consistent with genetic relatedness and the intended diagnostic scope of distinguishing genotype VII field strains from vaccine-associated genotypes (SAN Group Biotech Germany GmbH, 2026).
This triplex real-time RT-PCR provides a rapid, laboratory-compatible approach for NDV genotype differentiation and supports outbreak investigation, surveillance and vaccine-strain discrimination in veterinary diagnostic laboratories.

Keywords
Newcastle disease virus; PMV-1; genotype VII.1.1; real-time RT-PCR; molecular diagnostics; vaccine differentiation; veterinary laboratory diagnostics

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01. Emerging Infectious Diseases and Recent Outbreaks in Europe

P016. METABOLIC FINGERPRINTING OF EPIZOOTIC HAEMORRHAGIC DISEASE REVEALED BY HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Epizootic Haemorrhagic Disease (EHD) of ruminants represents an emerging viral vector-borne disease, with a significant impact on animal health and economics (1). Routine diagnosis of the EHD relies on the detection of specific antibodies against the V7 viral protein (2). However, despite a nearly perfect specificity has been attributed to humoral diagnosis, detection of infected animals remains challenging in some cases, such as in the early stages of infection (3). In this study, we aimed to characterize metabolic disturbances underlying EHE in cattle and to evaluate diagnostic performance of high resolution nuclear magnetic resonance (HR-NMR)-based metabolomics.

Positive (n=20) and negative (n=20) serum samples in an ELISA test (IDScreen EHDV Competition, Innovative Diagnostics, Grabels, France) were analyzed trough HR-NMR. Samples were filtered to remove macromolecules such as proteins and lipids that may alter metabolic profile, marked with TSP for calibration and analyzed with HR-NMR spectroscopy (Bruker AVANCE III 500 MHz, Bruker, Massachusetts, USA). Spectral data were processed with MestReNova software and multivariate statistical analyses were performed using MetaboAnalyst 6.0.

Partial Least Squares Discriminant Analysis (PLS-DA) of HR-NMR spectra enabled discrimination between positive and negative animals (Figure 1), achieving an accuracy of 66%. Some of those 15 key discriminant metabolites were glucose or glutathione, whose levels were significantly increased (p<0.05; Student´s t-test) in positive animals. Also, the model based on all identified metabolites fulfilled an AUC-ROC of 0.991 (95% CI: 0.939-1; Figure 2), with 100% sensitivity and 95% specificity.

In conclusion, HR-NMR analysis revealed a distinct metabolic signature between animals as regards their ELISA positivity status, likely due to the response against EHDV infection. In addition, robust operating characteristics of HR-NMR observed here highlight their potential use for EHD diagnosis.

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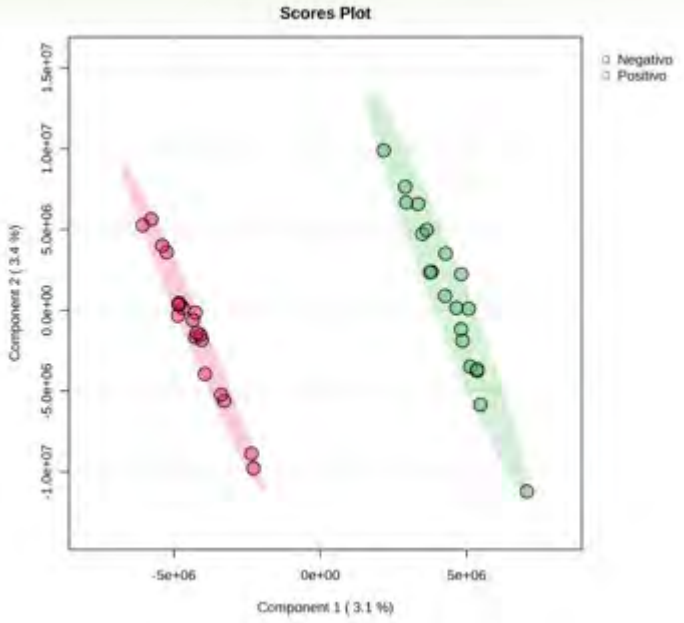


Figure 1: Partial Least Squares Discriminant Analysis (PLS-DA) score plots of serum spectra in negative (red) vs. positive (green) cattle.

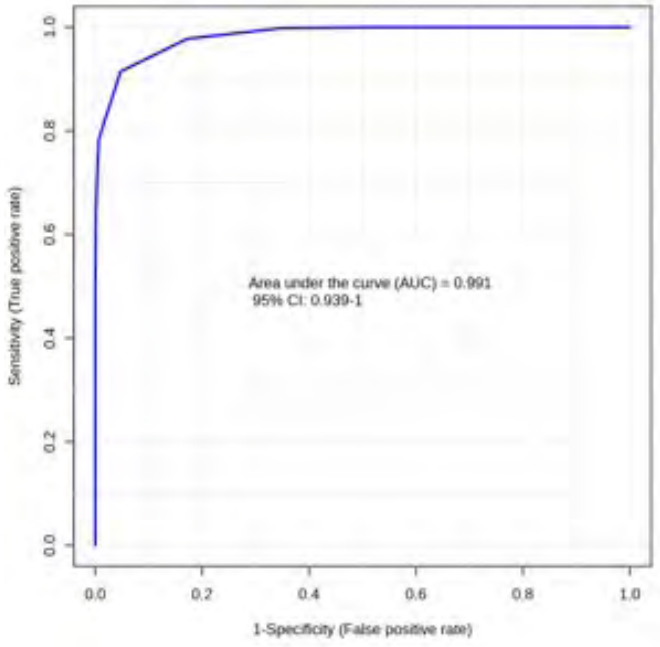


Figure 2: Area Under the Receiver Operating Characteristics Curve (AUC-ROC) based on the metabolites identified.

P017. RAPID MOLECULAR SEROTYPING OF *Salmonella:* VALIDATION OF THE CTS 2.0 QUANTSTUDIO 5 WORKFLOW FOR VETERINARY AND FOOD SAFETY SURVEILLANCE

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Salmonella enterica remains a leading foodborne pathogen worldwide and is commonly associated with poultry, beef and other animal-derived products. Rapid and accurate serotype identification is essential for surveillance, outbreak detection, and regulatory action. Traditional Kauffmann-White (KW) serotyping is limited by lengthy turnaround times, labor-intensive workflows, dependence on extensive antisera panels, and specialized expertise. Rare or atypical isolates often require referral to reference laboratories, extending reporting times to 4-6 weeks. Although whole-genome sequencing offers high-resolution characterization, routine implementation remains limited by cost, complexity, and bioinformatics requirements. The Check & Trace Salmonella (CTS) 2.0 assay addresses these limitations through a multiplex qPCR platform for rapid serovar identification. The assay targets 27 genetic loci across six reaction wells using TaqMan chemistry. Automated interpretation using a machine-learning algorithm, enabling detection of up to 106 epidemiologically important serovars. The assay generates either a direct serovar call, or a standardized six-digit "genovar" code representing a unique genetic fingerprint. Genovar assignments allow consistent tracking of genetically related isolates across laboratories and over time, supporting outbreak investigations and source tracing. CTS 2.0 requires minimal sample preparation from a pure colony and provides results within 2-2.5 hours. A machine-learning algorithm interprets multiplex PCR outputs to generate automated, standardized results, supports high-throughput testing of up to 16 isolates per run. We evaluated CTS 2.0 on the QuantStudio 5 platform using 70 archived isolates serotyped by KW. Sixty of the isolates were approved serovars, while 10 were non-typable. Of the 60 approved serovars, 52 were correctly identified, five generated genovar codes, and three discrepancies were ultimately confirmed by sequencing to be misclassified by KW. Genovar codes were generated for all ten non-typable isolates. These findings support CTS 2.0 as a rapid and reliable alternative for Salmonella characterization in veterinary and food safety laboratories, enabling more timely surveillance and outbreak response.

P018. DEEP LEARNING-BASED AUTOMATED COLONY COUNTING FOR TOTAL MESOPHILIC COUNT DETERMINATION IN RAW SHEEP MILK

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Total Mesophilic Count (TMC) is a widely accepted indicator of the microbiological quality of raw ovine milk, reflecting farm hygiene and animal health status[1]. However, conventional colony enumeration is labor-intensive, time-consuming, and subject to variability. Recent advances in artificial intelligence offer opportunities to automate microbiological analyses and improve efficiency[2]. This study aimed to develop and evaluate a deep learning-based approach for automated colony enumeration for TMC determination in sheep milk.
Bulk tank milk was collected from eight sheep farms in Greece. Following decimal dilutions, 1 mL aliquots were plated on Plate Count Agar. Images of Petri dishes displaying a range of colony densities were used to create a TMC dataset. A YOLO-based object detection model was developed for colony detection. Two training strategies were evaluated: (i) a zero-shot approach, where the model was trained exclusively on the publicly available AGAR dataset[3] and tested on 41 TMC images, and (ii) a fine-tuning approach, where the AGAR-pretrained model was further trained on TMC images. Model performance was assessed using 10-fold cross-validation and standard object detection metrics.
The YOLO model achieved excellent performance on the AGAR dataset (10,789 training and 1,352 testing images), reaching an F1-score of 0.957 and mAP50 of 0.968. However, when applied to TMC images in a zero-shot setting, performance decreased substantially (F1-score = 0.698, mAP50 = 0.696), indicating limited cross-dataset generalization. Fine-tuning on TMC images significantly improved performance, increasing the F1-score to 0.793 and mAP50 to 0.787. These findings suggest that TMC-specific colony characteristics and imaging conditions were underrepresented in the AGAR dataset.
Although pre-training on AGAR dataset provided strong transferable features for colony detection, substantial performance degradation was observed when the model was applied to TMC images without adaptation. Fine-tuning on TMC-specific data improved detection accuracy, demonstrating the importance of method- and matrix-specific adaptation for automated microbiological analysis.

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P020. IDENTIFICATION OF MORPHOLOGICAL CHARACTERISTICS IN FELINE INTESTINAL PARASITE EGGS USING A FACTOR VARIATIONAL AUTOENCODER

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Microscopic examination of fecal samples is used for detecting parasitic elements and identifying them at genus or species level in animal and human medicine. However, it highly depends on observer expertise[1]. A better understanding of morphological characteristics that differentiate parasitic elements could support the objectivity of diagnosis. The present study developed and evaluated a Factor Variational Autoencoder (FVAE) as an unsupervised artificial intelligence framework for extracting and disentangling morphological characteristics of feline gastrointestinal parasites, establishing a methodological foundation for application to more complex problems.

A publicly available dataset comprising microscopy images of feline gastrointestinal parasites was used[2]. Following image preprocessing, a final dataset of 1,086 samples representing three parasitic genera (*Cystoisospora, Ancylostoma,* and *Platynosomum*) was assembled. Data were partitioned into training/validation/test sets. The FVAE architecture comprised of a convolutional encoder, a transpose-convolutional decoder, and a discriminator network for total correlation minimization, with a 32-dimensional latent space. Latent representations were investigated using k-means clustering, UMAP visualization, principal component analysis, mutual information analysis, Spearman correlation, pairwise ROC-AUC, Cohen's *d* statistics, and generative latent traversals combined with residual attention mapping.

The FVAE exhibited stable convergence throughout training and produced highly informative latent representations. Unsupervised clustering achieved strong agreement with biological labels (ARI = 0.957, NMI = 0.927, V-measure = 0.927), including near perfect separation of *Cystoisospora* spp. samples. Seven latent dimensions were identified as diagnostically informative and largely non-redundant. Visualization analyses demonstrated that three of these dimensions encoded interpretable morphological traits, including egg content brightness, luminosity distribution, peripheral zone intensity, and internal content clarity. Pairwise ROC-AUC values exceeded 0.80, supported by large effect sizes.

FVAE-based disentangled representation learning can successfully separate interpretable morphological features of parasitic elements in a controlled setting and could serve as a reproducible adjunct to parasitological diagnosis, and as a foundation for complex multi-species classification and morphological analysis.

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P021. ARTIFICIAL INTELLIGENCE-ASSISTED DETECTION OF CANINE AND FELINE GASTROINTESTINAL PARASITIC ELEMENTS IN FECAL MICROSCOPY IMAGES

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Gastrointestinal parasites are a major cause of disease in both animals and humans worldwide [1]. Companion animals may serve as reservoirs for zoonotic parasites representing an important public health concern [1]. Routine microscopic fecal examination constitutes the standard diagnostic method to identify parasitic elements at genus level. However, microscopic examination is labor-intensive, time-consuming and subject to inter-observer variability [2]. The aim of the present study was to evaluate the applicability of an artificial intelligence-assisted image analysis system for the detection of common canine and feline gastrointestinal parasitic elements in microscopy images obtained following fecal examination.

A dataset of 310 annotated microscopy images (×40 magnification, acquired via smartphone and dedicated digital cameras) was developed from samples examined by zinc sulfate flotation method. Four parasitic elements were included: *Toxocara* spp. eggs (248 instances), hookworm eggs (72 instances), *Giardia* spp. cysts (361 instances), and *Cystoisospora* spp. oocysts (315 instances), totalling 996 annotated instances. A YOLOv11-nano object detection model was fine-tuned on this dataset, enabling localization and classification of all parasitic elements from microscopy images. Model performance was evaluated using independent validation and test datasets.

The model demonstrated high detection performance, achieving an overall mAP of 98.9% at IoU=0.5, precision of 97.8%, recall of 98.8%, and F1-score of 98.3% while maintaining satisfactory performance under stricter overlap thresholds. Confusion matrix analysis showed limited interclass misclassification, supporting reliable differential detection across parasite categories. Lower detection performance was observed for *Giardia* spp., consistent with their small size and morphological variability. Visualization methods indicated that predictions were driven by morphologically relevant parasitic structures.

These findings support the utility of artificial intelligence-assisted systems as complementary tools in veterinary diagnostic parasitology[3,4], particularly for rapid screening, reduced operator dependency, and improved standardization of fecal microscopy. Validation on larger, more diverse datasets is warranted before routine implementation.

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P022. IN VITRO GASTRIC-MIMICKING CULTURE OF ANISAKIS LARVAE: EXPLORING INTERSPECIFIC VARIABILITY IN PERSISTENCE

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Anisakis spp. are foodborne zoonotic nematodes with a pathogenic potential depending on species identity, larval integrity and the ability to withstand to host-like conditions. This study investigated the larval persistence of Anisakis larvae by an in vitro gastric-mimicking system providing preliminary evidence of a possible association with molecular profile. Twenty-one viable third-stage larvae (L3), recovered from fresh European hake (*Merluccius merluccius*), were individually cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, pepsin, antibiotics and reduced glutathione, adjusted to pH 4.0 and maintained at 37±1°C. The larvae were monitored for spontaneous motility, survival and morphological changes compatible with developmental progression. All larvae were initially viable and morphologically consistent with the L3 stage. During early incubation, most retained high motility and developed increased cephalic differentiation, lip-like structures and progressive cuticular ridging, compatible with transition to L4 stage. These changes occurred without appreciable overall body growth. Subsequently, motility progressively declined and mortality increased, indicating a dissociation between morphological activation and sustained fitness. Three larvae showed greater persistence than the remaining individuals, and one survived until the final experimental phase after 50 days. The PCR-RFLP analysis a restriction pattern compatible with a putative *A. pegreffii* × *A. simplex sensu stricto* hybrid profile for the longest-persisting larva. Although based on a limited number of individuals, these findings support the use of gastric-mimicking culture as a possible phenotyping model for studying larval persistence under host-like conditions. Integration with higher-resolution multilocus genotyping may help determine whether genetic background, including hybrid or introgressed profiles, contributes to differences in Anisakis fitness and potentially to zoonotic risk.

P023. MOLECULAR AND SEROLOGICAL INVESTIGATION OF HEPATITIS E VIRUS IN LIVERS AND MUSCLE TISSUES OF WILD BOARS AND DOMESTIC PIGS SLAUGHTERED IN SOUTHERN ITALY

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Hepatitis E is an emerging zoonotic disease in industrialized countries. Food-borne transmission of hepatitis E virus (HEV) is mainly associated with consumption of uncooked or undercooked meat products from domestic pig, wild boar and deer. The aims of this study were to evaluate the prevalence of HEV in wild boars intended for domestic consumption and in slaughtered domestic pigs from Apulia and Basilicata regions and to investigate HEV circulation through a serological survey in both populations to provide additional epidemiological data.
A total of 280 wild boar and domestic pig samples (230 muscles and 50 livers) were collected from March 2024 to December 2025. The samples were subjected to RNA extraction and tested to detect the presence of HEV by RT-qPCR. Serological tests were performed on 70 meat juice samples from muscle to investigate the presence of total antibodies (IgG, IgM, IgA) against HEV, by commercial enzyme-linked immunosorbent assay (ELISA) test.
Overall, HEV RNA was detected in 18 (6.4%) out of 280 samples. HEV loads ranged from 1.42×102 g.c./µl to 8.14×107 g.c./µl. Thirty (30) meat juice samples revealed HEV antibodies, corresponding to a seroprevalence of 42.8%. Among these samples, 6 were also found to be positive for viral RNA.
This study has provided insight into the status of HEV infection in wild boars and domestic pigs
from Apulia and Basilicata regions. The detection of HEV RNA and serological data confirmed an active circulation of HEV in both populations. Our findings showed the urgent need to implement surveillance in wild boars and biosafety measures and management practices during the swine farming and slaughtering process. In order to minimise the risk of an HEV infection, consumers should thoroughly cook meat and offal, especially pork and wild boar meat products. This work was supported by the Italian Ministry of Health (IZSPB 04/23 RC).

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P024. ANIMALS AS PUBLIC HEALTH MIRROR: CASE-CONTROL STUDY OF NEONATAL BOVINE DEATH IN THE EXPOSURE AREA OF THE CONTAMINATED SITE OF FALCONARA MARITTIMA (ITALY)

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The SINTESI project is a three-year project (2024–2026), included in National Plan for Complementary Investments, aimed at evaluating the effectiveness of primary and secondary prevention interventions to reduce the health impact of diseases, associated with Sites of National Interest (SIN), attributable to environmental factors and social inequalities. The main objectives are: i) a case-control study of mortality rates in calves within the Exposure Area (EA) and the Non-Exposure Area (NEA); ii) a description of the health profile of the resident population; iii) an investigation of population cancers, childhood cancers, congenital malformations, and other rare diseases. The SIN of Falconara Marittima (FM) within Ancona province extends over 108 ha on land and 1,200 ha at sea being one of the most complex industrial hubs on the Adriatic side. The area shows contamination referred to heavy metals, polycyclic aromatic hydrocarbons (PAHs), PCBs, and tetraethyl lead. The EA was defined using QGIS by creating a polygon extending within a 10 km radius around the boundary of the municipality of FM, while the NEA the entire Ancona province. Cases were identified as calves died within 30-day postnatal due to non-infectious causes, while controls those surviving beyond 30-day. Data on farms within the study area were extracted from the Official National Database and subsequently processed, defining as inclusion criteria farms registered from 01/01/2015 to 31/12/2024. Cattle farms in the EA were identified and georeferenced: 32 farms were found, of which 30 were oriented toward meat production and 2 toward dairy production. In the NEA, 215 breeding were recorded during the study period (1 wildlife collection, 9 dairy, 10 mixed, and 195 meat). Data analysis is still ongoing. Animals, sharing the environment with humans, can act as sentinels, providing early signals of risks to exposure to environmental pollutants.

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03. Zoonoses and the One Health Approach Bridging Animal, Human, and Environmental Health

P025. DERMATOPHYTE TRANSMISSION ACROSS HUMANS, ANIMALS, AND THE ENVIRONMENT

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Dermatomycoses are superficial fungal infections affecting both humans and animals, caused by dermatophytes traditionally classified as anthropophilic, zoophilic, or geophilic according to their ecological niche. This study applied a One Health approach to investigate eight cases of dermatomycosis identified from 2018 onwards in the Umbria region (Central Italy), involving five human patients, two cats, and one dog as "index cases". A two-step sampling protocol was implemented, including sampling of index patients followed by extensive investigation of cohabiting humans, animals, and domestic environments to identify the source of infection (SOI). Overall, a total of 14 human, 20 animal, and 41 environmental samples were collected and analyzed using mycological techniques followed by PCR and Sanger sequencing on positive cultures (1,2).
Microsporum canis was the most frequently zoophilic species detected in this study. In three human cases it was associated with infected animals and contaminated environments. In one case it was isolated from a kitten identified as the SOI and subsequently detected in its owners as well as on multiple household samples. In one human index patient, *Trichophyton mentagrophytes* genotype III* was detected, but it was not identified in cohabiting animals or environmental samples, despite its typical zoophilic behavior. Finally, the geophilic *Nannizzia gypsea* was detected in two animal cases and one human case, although the second sampling was negative (3).
These findings highlight the importance of integrated surveillance involving humans, animals, and environments to effectively identify infection sources, preventing recurrence, limiting pathogen spread, and reducing antifungal use, thereby contributing to the control of emerging dermatophyte infections.
Through this work, the authors hope that this operational procedure—already endorsed by the CDC (4)—should be integrated into routine diagnostic practice for dermatomycoses, rather than being confined to pilot initiatives or sporadic applications.

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03. Zoonoses and the One Health Approach Bridging Animal, Human, and Environmental Health

P026. ENVIRONMENTAL CONTAMINANTS AND ANIMAL VIRUSES: AN INTRICATE RELATIONSHIP

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Canine coronavirus (CCoV) and feline coronavirus (FCoV) are both *Alphacoronaviruses*. CCoV is a highly contagious, widespread pathogen that typically targets the epithelial cells of the canine enteric tract, resulting in mild enteritis, especially in puppies. FCoV is ubiquitous in global cat populations and includes two biotypes: feline enteric coronavirus (FECV), which generally induces moderate enteritis or chronic asymptomatic infection in cats, and feline infectious peritonitis virus, which, in a low percentage of cats, can develop severe feline infectious peritonitis. Environmental contaminants like 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), known as dioxin, can suppress host immunity, making humans and animals more susceptible to infectious agents, like viruses. Thus, 2,3,7,8-TCDD may increase the risk assessment of infectious diseases. Indeed, ex vivo analyses in multiple organs (liver, lungs, spleen, intestine) on cats and dogs from Campania region, reveal the presence of CCoV in 23.5% of canine samples, and feline coronavirus (FCoV) in 16.3% of feline samples. Chemical analyses in the livers of the above subjects show higher levels of congener 2,3,7,8-TCDD in CoVs-infected dogs and cats than in non-infected animals, suggesting a positive correlation between CoVs infection and dioxin exposure. Intriguingly, experimental investigations *in vitro* show that 2,3,7,8-TCDD interferes with the replication of CCoV in a canine fibrosarcoma (A72) cell line, where virus titer and the expression of nucleocapsid N protein are significantly increased by exposure to dioxin. Hence, herein we have explored the influence of 2,3,7,8-TCDD during FECV (München strain) replication in Crandell Feline Kidney. Very low doses of 2,3,7,8-TCDD reduced virus yield and provoked alterations in morphology of infected cells. Moreover, during infection, 2,3,7,8-TCDD induced a modulation in lysosome acidity of infected cells.
Overall, our results suggest that exposure to dioxin might interfere with the replication of CoVs, acting as an additional risk factor for progression of infections in dogs and in cats.

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P027. LEISHMANIOSIS IN STRAY AND SHELTER CATS IN THE COMMUNITY OF MADRID, 2025–2026

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Leishmaniosis, caused by *Leishmania infantum*, remains a major zoonotic concern in Mediterranean regions. Although dogs are the primary reservoir, increasing evidence suggests that cats may contribute to parasite maintenance or act as sentinels of environmental transmission. Stray and shelter cats in urban and peri-urban colonies are frequently exposed to sandflies, yet their epidemiological role remains poorly defined. In the Community of Madrid, an endemic area, updated data on feline infection and exposure are essential for surveillance and risk assessment.
Ear-tip samples collected from stray and shelter cats during 2025–2026 were analysed by PCR as previously described by Francino *et al*. (2006). Antibody detection was performed using indirect immunofluorescence assay (IFI). Statistical analyses were conducted using WINPEPI 11.65 and IBM SPSS Statistics 29.0.2.0. Prevalence estimates were stratified by sex, age and location, and annual differences were evaluated.
L. infantum DNA was detected by PCR in 16.8% (95% CI: 11.5–23.9) of the samples analyzed. Stratified values ranged from 12.1% to 19.1% by sex and age, and from 9.5% to 27.3% by location. PCR positivity was significantly higher in 2025 than in 2026 ($p = 0.020$). IFI revealed antibodies against *L. infantum* in 26% of the animals (95% CI: 13.7–46.1), a prevalence significantly higher than PCR results ($p = 0.004$), indicating broader exposure than active infection.
Notably, the prevalence observed in this study is higher than that reported in previous studies conducted in Spain, suggesting an increased level of exposure and/or transmission within the studied population. These findings confirm the active circulation of *L. infantum* in the Community of Madrid and support sustained environmental transmission. Overall, the results highlight the relevance of stray and shelter cats in leishmaniosis surveillance and the need for continued monitoring in endemic urban and peri-urban settings.

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P028. APPLICATION OF TWO CULTURE METHODS FOR THE DETECTION OF *Candidozyma (Candida) auris* IN BOVINE MILK: PRELIMINARY FINDINGS

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Background: *Candidozyma auris* is an emerging multidrug-resistant yeast of public health concern due to its environmental persistence and ability to colonize multiple ecological habitat. Milk may warrant investigation as a possible niche for *C. auris*, particularly given opportunities for environmental and human-associated contamination during milking procedures. This preliminary study aimed to investigate the presence of *C. auris* in bovine milk using two selective culture protocols.
Materials and Methods: A total of 170 milk samples submitted for routine mastitis diagnostics were processed using two enrichment strategies. In protocol A, 1 mL of milk was inoculated into Sabouraud broth +10% NaCl and incubated at 41°C for 7days, followed by subculture onto Selective Auris Medium (SAM). In protocol B, samples were inoculated into CABroth™ (Copan Italia) and incubated at 41°C for 48h before subculture onto SAM. All samples were incubated at 41°C for 7days on SAM. A *C. auris* positive control was included. Yeast-like colonies were identified by MALDI-TOF mass spectrometry (Bruker Daltonics).
Results: No *C. auris* was isolated from any sample using either protocol. Protocol A yielded five yeast-positive samples, recovering (n.2 *Pichia kudriavzevii*, n.1 *Candida glabrata*, and n.1 *Meyerozyma farinosa*). Protocol B yielded only one positive sample (*M. farinosa*). The positive control was successfully recovered with both methods.
Conclusions: *C. auris* was not detected in any bovine milk sample in this preliminary study. Since the analyzed samples were naturally contaminated field specimens, whereas the positive control was artificially spiked, the results should be interpreted with caution and do not allow a formal evaluation of the analytical sensitivity of the methods. Both protocols suppressed background contamination, though protocol A recovered non-target yeasts (*C. glabrata, P. kudriavzevii*, and *M. farinosa*), indicating incomplete selectivity, while protocol B yielded only M. farinosa.Variations in enrichment media and incubation times likely account for these recovery profiles.

P029. ANTIBIOTIC RESISTANCE AND GENOMIC CHARACTERISTICS OF MONOPHASIC AND BIPHASIC *Salmonella* Typhimurium ISOLATED FROM POULTRY IN SOUTH KOREA BETWEEN 2022 AND 2024

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Salmonella (*S.*) *enterica* serovar Typhimurium and its monophasic variant (*Salmonella* 4,[5],12:i:-) are among the most important zoonotic foodborne serovars with a wide range of food-producing animals worldwide. The emergence of monophasic variant of *S.* Typhimurium (MVST), particularly multidrug-resistant (MDR) ST34 lineages, has become a major public health concern because of their rapid global dissemination and increasing antimicrobial resistance. This study aimed to investigate the antibiotic resistance and genomic characteristics of *S.* Typhimurium and MVST isolates recovered from poultry and its environments in South Korea between 2022 and 2024. Antimicrobial susceptibility testing and PFGE were performed for all 78 isolates, and 29 representative isolates were subjected to whole-genome sequencing (WGS), followed by whole-genome single nucleotide polymorphism (wgSNP) phylogenetic analysis together with 157 publicly available genomes. In addition, antimicrobial resistance genes, plasmid replicons, and *Salmonella* pathogenicity islands (SPIs) were analyzed using AMRFinderPlus, MOB-suite, and SPIFinder, respectively. MVST isolates showed significantly higher resistance rates to ampicillin, ciprofloxacin, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole than *S.* Typhimurium isolates ($p<0.05$). PFGE classified the isolates into three major clusters, with MVST showing a progressive increase and becoming predominant in 2024. wgSNP analysis revealed that most Korean MVST isolates belonged to the global ST34-associated MDR lineage and clustered closely with isolates from Asia, Europe, and the Americas. ST34-MVST isolates commonly carried *qnrS1*, $bla_{\text{TEM-1}}$, *sul2*, *tet(A)*, *aph(6)-Id*, and *aph(3")-Ib* and harbored plasmid replicons including IncHI1B, IncQ1, and Col156, while the overall SPI profiles remained largely conserved among the isolates. These findings are consistent with the expansion of MDR ST34-MVST in Korean poultry and its phylogenetic relatedness to internationally circulating lineages, supporting the need for continued genomic surveillance and antimicrobial resistance control strategies.

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03. Zoonoses and the One Health Approach Bridging Animal, Human, and Environmental Health

P030. FIRST MOLECULAR GENETIC DETECTION OF PORCINE NOROVIRUS IN SLOVAKIA

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Noroviruses, belonging to the family *Caliciviridae*, represents the most common non-bacterial cause of foodborne gastroenteritis worldwide, can infect vertebrates including humans and swine. Genome of noroviruses contain a positive-sense, single-stranded RNA, consists of at least three open reading frames (ORFs). Currently, noroviruses are classified into at least ten genogroups, further divided into 48 VP1 capsid-based (VP1) and more than 60 RdRp/pol-based genotypes (1). Porcine noroviruses (PoNoVs) are genetically most similar to human noroviruses and have been classified among a diverse range of human norovirus genotypes in GII as GII.11, GII.18, and GII.19 (2). The aim of this study was to reveal the status of norovirus infection in pigs from Slovakia. A total of 229 samples (25 rectal swabs, and 204 stool samples) of various age (2 weeks to 8 months old) were examined from 13 localities of Slovakia. Total RNA was extracted from 10 % (w/v) fecal homogenates. A conventional nested RT-PCR assay targeting the 344 bp fragment in VP1 was carried out using PCR primers and protocols previously described (3, 4). From 229 tested samples, 29 pigs in fattening production stage from 4 localities were found to be positive (the prevalence of 12.66%). PCR products for sequencing of VP1 (n = 17) were used for the phylogenetic analysis. The phylogenetic analysis of the VP1 fragment (292 bp) revealed that all isolates obtained in this study clustered within the same lineage as GII.18 swine isolates from Hungary and the USA. This study confirms the presence of GII.18 PoNoVs in domestic pigs and represents the first detection of norovirus in domestic pigs from Slovakia.

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P031. WHOLE GENOME SEQUENCING FOR PUBLIC HEALTH PROTECTION WITHIN A ONE HEALTH FRAMEWORK: IMPLEMENTATION IN THE VALENCIAN COMMUNITY (SPAIN)

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Whole genome sequencing (WGS) has emerged as a powerful tool for the surveillance and control of foodborne pathogens, providing high-resolution data that enhance outbreak detection, source attribution and risk management. Within a One Health (OH) framework, the integration of genomic, epidemiological and microbiological information from human, animal, food and environmental sources is essential to effectively protect public health[1]. This study describes the implementation of a regional OH genomic surveillance system based on WGS for the detection and investigation of genomic clusters, foodborne outbreaks and persistent strains along the food chain. A coordinated OH surveillance programme has been implemented in the Valencian Community (Spain) since January 2026, integrating all isolates of *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella* obtained from official food controls, slaughterhouses, primary production and public health investigations. These priority foodborne pathogens have been subjected to WGS, and genomic analyses have been conducted using standardized bioinformatic pipelines. Genomic relatedness has been assessed using core genome multilocus sequence typing (cgMLST) and hierarchical clustering approaches, allowing the identification of clusters, persistence events and potential cross-sector transmission. Epidemiological metadata have been linked to genomic data to support traceability investigations, root-cause analysis and the adoption of official control measures. The application of WGS has enabled the early detection of genomic clusters associated with events of public health relevance, including foodborne outbreaks and persistent contamination in food production environments. High-resolution genomic analysis has allowed the discrimination between epidemiologically related and unrelated isolates, supporting accurate source attribution and excluding suspected operators when genetic distances exceeded established thresholds. The integration of antimicrobial resistance and virulence gene profiling further has improved risk characterization. Overall, these results demonstrate that the implementation of WGS-based surveillance within a OH framework strengthens food safety and public health protection and supports timely, evidence-based official control actions.

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P032. CANINE LEPTOSPIROSIS IN EUROPE: A GROWING CLINICAL AND DIAGNOSTIC CHALLENGE

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Canine leptospirosis is a significant zoonotic disease caused by pathogenic spirochetes of the genus Leptospira. In recent years, an increase in reported cases has been observed across Europe, including Poland, potentially associated with environmental changes such as rising temperatures and increased humidity, which promote pathogen persistence in the environment. The disease is widely maintained and disseminated by reservoir hosts, particularly rodents, which chronically shed bacteria in urine, leading to contamination of water sources and soil. Dogs are typically infected through contact with contaminated water or urine from infected animals, while transmission to humans may occur through direct or indirect exposure, emphasizing the importance of this disease in both veterinary and public health contexts. The clinical presentation in dogs is highly variable, ranging from subclinical infection to severe, life-threatening disease. Common clinical signs include acute kidney injury, hepatic dysfunction, fever, lethargy, and hemorrhagic disorders. Due to the nonspecific nature of these symptoms, leptospirosis can be challenging to differentiate from other conditions such as toxic nephropathies, liver diseases, babesiosis, or infection with canine adenovirus type 1. Diagnosis relies on a combination of clinical findings and laboratory testing. Serological methods, including the microscopic agglutination test and ELISA, are commonly used to detect antibodies, although their diagnostic value may be limited in the early phase of infection. Molecular techniques, such as PCR performed on blood or urine samples, enable detection of bacterial DNA at earlier stages but require specialized laboratory infrastructure and are not always readily accessible in routine practice. Given these limitations, rapid point-of-care diagnostic methods, such as lateral flow immunoassays, may represent a useful complementary tool in clinical settings, supporting faster decision-making and facilitating differential diagnosis. Continued improvement in diagnostic approaches remains essential for effective disease management and control, particularly in the context of the increasing incidence of leptospirosis observed across Europe.

P033. DIA-CHECK Q FEVER ELISA TEST: SPECIES-SPECIFIC OPTIMAL CUT-OFF VALUES

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The DIA-check Q Fever ELISA Test is an indirect immunoassay specifically developed to detect antibodies against Coxiella burnetii in serum samples from cattle, sheep, and goats. Q fever remains a significant worldwide zoonosis that poses a dual threat: it causes substantial economic losses in the global livestock industry and represents a serious risk to public health. Despite its impact, the diagnostic landscape is challenged by the absence of a universally accepted "gold standard". Consequently, the precise calibration of cut-off values during the validation of new diagnostic kits is paramount to ensure clinical reliability.
To determine the cut-off, 113 Q fever-positive and 604 Q fever-negative samples (bovine, ovine, and caprine sera), previously confirmed by commercial ELISA kits, were tested. The cut-off was determined using the sample S/P values, the S/P cut-off value identified was 0.5 with a sensitivity value (Se) of 98.23% and a specificity value (Sp) of 97.03%. The area under the ROC curve (AUC) was 0.938 (P value <0.0001). Subsequent species-specific analysis revealed slightly different optimal thresholds: 0.65 for cattle (Se: 98.04%, Sp: 98.04%), 0.55 for sheep (Se: 100%, Sp: 99.73%), and 0.45 for goats (Se: 100%, Sp: 92.42%). These findings align with recent literature (Rivière et al., 2025) suggesting that species-specific differentiation can refine diagnostic accuracy.
However, although these variations exist, the overall cut-off of 0.5 maintains high diagnostic performance across all groups. Considering that the use of different cut-offs for each species may be operationally restrictive for veterinary laboratories, which are accustomed to interpreting results uniformly for all three species, the implementation of a single, validated cut-off remains a reliable and practical solution for routine diagnostics.

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P034. DIAGNOSIS OF CANINE BRUCELLOSIS – DON'T FORGET *B. SUIS*

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Canine brucellosis caused by *Brucella* (*B.*) *canis* has been reported worldwide with an increasing trend in Europe. The disease is associated typically with reproductive and osteo-articular disorders, and subclinical infections occur in a significant proportion of infected dogs. Besides being responsible for economic losses in commercial kennels, it is a serious public health issue due to the zoonotic potential of the pathogen. Although the gold standard for diagnosis is culture, it is not widely available due to its time-consuming nature and biosafety restrictions for the laboratory. Molecular detection is fast; however, accuracy is highly variable due to the pathogenesis of the disease and biosafety risks remain. Therefore, serology is commonly applied in diagnosis of canine brucellosis. Serological tests for *B. canis* differ in the methodology, but they all detect antibodies only for *B. canis,* exhibiting rough cell wall lipopolysaccharides (rLPS). However, canine brucellosis can also be caused by *B. suis* and may pose an even higher public health threat, as *B. suis* biovar 1 is known to be more virulent for humans than *B. canis*. For serological detection of this smooth LPS (sLPS) producing pathogen, methods based on sLPS antigen such as rose bengal test (RBT) or enzyme-linked immunosorbent assays (ELISA), which are prescribed for surveillance of brucellosis in livestock, must be used. Firstly, we carried out a retrospective analysis by RBT and sLPS based ELISA of 88 canine sera from 2020 to 2025 which had tested negative with an rLPS based immunochromatographic test. In RBT and sLPS based ELISA 87 sera were negative, but one serum reacted positively in both tests. These results underscore the need to include both rLPS- and sLPS-based serological methods to ensure a reliable diagnosis of canine brucellosis. Moreover, performance of rLPS based immunochromatographic and indirect fluorescence tests for *B. canis* were compared.

P035. INSIGHTS INTO THE BACTERIAL MICROBIOME OF *Pulex irritans* AND *Ctenocephalides felis* FLEAS FROM ITALY

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Knowledge on flea-associated bacterial communities remains limited, despite the veterinary and public-health relevance of fleas as hematophagous ectoparasites and potential vectors of bacterial pathogens. To provide a first snapshot of the flea microbiota and explore its relevance for veterinary diagnostic surveillance in the Italian context, we analysed five pooled flea samples environmentally collected from three sites in two geographic areas, two in Piedmont (Northern Italy) and one in Basilicata (Southern Italy). The samples were obtained from households with domestic animals and livestock that had previously been infested by fleas. Fleas were assigned to *Pulex irritans* and *Ctenocephalides felis* by combined morphological examination and molecular confirmation. Bacterial community profiling was performed using next-generation sequencing on an Illumina platform, and results were interpreted in terms of relative read abundance. Across pools, *Wolbachia* was overwhelmingly dominant, accounting for more than 90% of total reads, consistent with its role as a common flea-associated endosymbiont frequently reported as a major component of flea microbiomes (Manvell et al., 2022; Cutillas et al., 2025). Additional taxa were detected at substantially lower relative abundance, including *Romboutsia*, *Clostridium* and *Staphylococcus*. Although based on a limited number of pools and locations, these data represent the first preliminary evidence on the bacterial microbiota of *P. irritans* and *C. felis* collected in Italy. The strong predominance of *Wolbachia* suggests that endosymbiont-associated reads may substantially influence the overall bacterial community profile detected in fleas and should be considered when designing future surveys (Gin et al., 2026). Expanded sampling at individual-flea level and across additional Italian regions will be needed to assess geographic and host-related variability and to support vector-borne pathogen surveillance within a One Health framework. This study was supported by the projects "Ricerca corrente" (grant number: IZSPLV RC 09/24 CUP J19I24001470001) funded by the Italian Ministry of Health.

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03. Zoonoses and the One Health Approach Bridging Animal, Human, and Environmental Health

P036. THE INTEGRATED WNV SURVEILLANCE PROGRAM IN SERBIA: CHANGES IN THE METHODOLOGY OVER TIME

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The integrated WNV surveillance program in Serbia established by the Veterinary Directorate was active since 2014. The main aim of the program was an early detection of WNV presence in the environment and the timely information of local and public health authorities - to control the mosquitoes and to prevent the epidemics in humans and animals WNV surveillance was done by serological testing of sentinel animals and it was changed during the time (testing by ELISA of WNV IgG antibodies in horses and poultry in 2014; only WNV IgM antibodies in horses from 2015 to 2021; and from 2022 IgM Ab in horses and IgG Ab in calves), and by testing of virus presence by RT-qPCR in vector mosquitoes (*Culex pipiens*), and in samples of found dead / live captured susceptible wild birds as virus natural hosts. By surveillance all municipalities in Serbia were covered, and the distribution of sampling points were determined based on risk assessment.
The changes in WNV surveillance system overtime and the outcome on the obtained data are presented and discussed. The results of the established integrated WNV surveillance system from the last 12 years shows that the veterinary service together with ornithologist and entomologist successfully managed to implement highly sensitive WNV surveillance system, and to inform the public health authorities on time.
It can be reasonably assumed that WNV, now endemic infection, will continually present a significant problem for the veterinary and public health in Serbia. Consequently, it is necessary to establish an effective surveillance system for WNV, but also for the other flaviviruses (TBEV and USUV) of public health concern. However, the establishment of the sensitive and effective surveillance system is never finished job - has to be changed accordingly due to the new situations, changing environment and influences, new needs and expectations etc.

Key words
WNV, integrated WNV surveillance system, sentinel animals, changing in methodology

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P037. MAINTAINING DISEASE FREEDOM: AN EVALUATION OF SEROSURVEILLANCE FOR THE DETECTION OF *Brucellosis* IN SMALL RUMINANTS IN GREAT BRITAIN

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Brucellosis is a globally widespread zoonosis and is a reportable or notifiable disease in many countries. A limited number of countries hold 'Officially Brucellosis Free (OBF)' status. Great Britain (GB) was awarded OBF status in 1985 following the successful eradication of *Brucella abortus* in cattle. *Brucella melitensis* (*B. melitensis*) has never been recorded in livestock in GB, and the United Kingdom has been recognised as officially free from *B. melitensis* by the European Union (EU) since 1993. An annual serological survey for *B. melitensis* in sheep and goats is carried out, alongside other measures, to provide evidence to maintain officially-free status. For this annual survey a target of 800 flocks/herds were sampled annually across GB (England, Scotland & Wales). Serum samples were collected and tested using a competitive ELISA (cELISA). Samples non-negative by cELISA were tested in series by the complement fixation test (CFT). Where non-negative results could not be resolved by serological testing, necropsy and bacterial culture was performed. Here we present data arising from this annual survey over a 10-year period (2016- 2025). Initial serological testing resulted in seven disease investigations across the period, requiring veterinary assessment of clinical signs, repeat sampling and one instance of culling to permit postmortem examination and bacterial culture from tissues. We use these data to estimate the diagnostic performance (specificity) of the cELISA as a screening tool for *B. melitensis* and investigate temporal, geographic and host species variation in test performance. Assessment of the performance of on-going surveillance activities provides confidence in the systems which generate evidence for the maintenance of GB's officially brucellosis free status.

P038. THE EOLO MEDIUM (ENHANCED OPTIMAL *Leishmania* OUTGROWTH) ENHANCES IN VITRO PROLIFERATION OF *Leishmania* PROMASTIGOTES

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Leishmania spp. are obligate intracellular protozoan parasites of humans and other mammals, transmitted by phlebotomine sand flies. Parasite isolation on specific media, such as Evans' modified Tobie medium (EMTM) supplemented with defibrinated rabbit blood, remains the reference standard for culture-based diagnosis and laboratory maintenance of the parasite [1,2]. However, conventional media may be labor-intensive, costly, and dependent on animal-derived components. This study evaluated the ability of the novel blood-free monophasic EOLO medium (Enhanced Optimal Leishmania Outgrowth) to support the in vitro growth of *Leishmania* promastigotes. The EOLO medium consisted of 21.25 mL RPMI 1640 and 64.25 mL of a custom-formulated basal medium, supplemented with 10% heat-inactivated serum, 1% L-glutamine, and 4% antibiotic solution. The basal medium contained peptone, yeast extract, inorganic salts, proline, and human urine; it was autoclaved, adjusted to pH 7.18, and sterilized by filtration before use. Promastigotes were seeded at 4×10^6 parasites/mL in 25 cm^2 flasks containing 10 mL of medium. Cultures were monitored daily up to 192 hours, and parasite density was determined using a Bürker chamber under light microscopy. Six independent replicates were performed. The EOLO medium showed a strong capacity to sustain promastigote proliferation. Compared with the initial inoculum, parasite density increased 72.5-fold at 24 hours, 122.5-fold at 48 hours, 182.5-fold at 72 hours, and reached a peak of 275-fold at 96 hours, indicating robust logarithmic growth. Thereafter, parasite density gradually declined to 177.5-fold at 120 hours, 112.5-fold at 144 hours, 47.5-fold at 168 hours, and 32.5-fold at 192 hours, consistent with transition to stationary and decline phases. These results suggest that this blood-free monophasic EOLO medium may represent a practical and economical alternative for Leishmania cultivation in diagnostic and research settings, potentially supporting more reproducible workflows across laboratories.

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P039. ANTIMICROBIAL RESISTANCE IN ESKAPE PATHOGENS AND ESCHERICHIA COLI ISOLATED FROM HEDGEHOGS (*Erinaceus europaeus*): A ONE HEALTH PERSPECTIVE

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Antimicrobial resistance (AMR) represents a major global health challenge, with wildlife increasingly recognized as both a reservoir and a transmission interface for clinically relevant resistant bacteria. Among these, ESKAPE pathogens are high-priority multidrug-resistant organisms in clinical settings, while *Escherichia coli* is widely used as an indicator species for tracking AMR dissemination across ecosystems. The objective of this study was to investigate the occurrence and characteristics of AMR in hedgehogs from the Lombardy region (Italy) within a One Health framework. A total of 103 animals were sampled, yielding 188 bacterial isolates obtained through standard microbiological methods and identified by MALDI-TOF. A subset of 92 priority isolates was selected for detailed analysis, including *E. coli* (n = 66) and isolates belonging to bacterial species classically included among the ESKAPE pathogens, regardless of their resistance phenotype, namely *Enterobacter spp.* (n = 12), *Enterococcus faecium* (n = 8), *Klebsiella pneumoniae* (n = 4), and *Pseudomonas aeruginosa* (n = 2). Antimicrobial susceptibility testing, performed using the disk diffusion method according to international guidelines, revealed that 78% of isolates exhibited phenotypic resistance, particularly to β-lactams (e.g., ampicillin, cefotaxime, and ceftazidime), while multidrug resistance was observed in 48% of isolates. Notably, high-risk phenotypes included one metallo-β-lactamase-producing *Enterobacter hormaechei*. PCR-based genotypic analysis confirmed the presence of clinically relevant resistance genes, including *bla*CTX-M, *bla*TEM, and *bla*SHV, showing strong concordance with phenotypic resistance patterns and suggesting the potential involvement of mobile genetic elements. These findings highlight hedgehogs as carriers and sentinels of AMR, reflecting anthropogenic selective pressures at the human–animal–environment interface. The isolation of ESBL-producing Enterobacterales, together with isolates from ESKAPE-associated species exhibiting clinically relevant resistance profiles, suggests that wildlife may contribute to the environmental dissemination of high-priority resistant bacteria and supports integrating wildlife more systematically into One Health surveillance frameworks (Silva et al. 2020; Russo et al. 2022; Grilli et al. 2025).

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P040. MONITORING OF MTBC AND MAC IN POLISH FOXES AS INDICATORS OF ENVIRONMENTAL MYCOBACTERIA

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Bacteria belonging to *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium avium* complex (MAC) comprise environmental mycobacteria affecting a wide range of hosts, including wildlife. Foxes are predators at the top of the food chain and can act as an indicator species – their health status may reflect the epidemic situation in the environment. Data on MTBC and MAC occurrence in wild carnivores remain limited. Free-ranging foxes can act as potential environmental sentinels for *Mycobacterium* spp. in Poland. The aim of the study was to monitor the Polish free-ranging fox population for the presence of MTBC and MAC as an environmental pathogens with zoonotic potential what is crucial in the One Health approach. Tissue samples of lungs, liver, spleen, and/or lymph nodes from a total of 210 foxes from the Małopolskie, Lubelskie, Podlaskie, Świętokrzyskie, Podkarpackie, and Mazowieckie Voivodships were tested for MTBC and MAC presence in 2025. Homogenates of tissue samples from each animal were examined using culture method. Specifically, after treatment with oxalic acid it was inoculated onto Stonebrink and Petragnani solid media. Next, bacterial isolates were identified with the GenoType MTBC and CM assays (Hain Lifescience, Germany). Bacteria belonging to MTBC was not detected in the examined animals. Single mycobacterium species - *M. avium* was isolated from 14 animals (6.7%). The relatively large number of animals detected with a positive test result proves its occurrence in the ecosystem and is in accordance with previously published data (Orłowska et al. 2023). The results indicate that foxes may serve as environmental sentinels for *M. avium*. It reflects its potential risk factor for the environment and public health in the One Health framework.

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P041. LABORATORY DETECTION OF BOVINE TUBERCULOSIS IN CATTLE IN POLAND BASED ON ISOLATION AND IDENTIFICATION OF *Mycobacterium tuberculosis* COMPLEX (MTBC)

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Bovine tuberculosis (BTB) is a chronic disease caused by the *Mycobacterium tuberculosis* complex (MTBC), with *M. bovis/caprae* being the most common in cattle. As zoonosis it can also affect people. The disease primarily affects cattle, but it can also be detected in other domestic animals and wildlife. According to the latest EFSA surveillance data, the overall prevalence of MTBC-infected cattle herds across the European Union was as low as 0.61%. In Poland, the primary screening method for BTB in cattle is the tuberculin skin test (TST). Next, confirmatory laboratory diagnostics are performed, consisting of the isolation of bacteria and identification using molecular methods. This method enables the differentiation of bacteria belonging to the MTBC complex into species. The objective of this study was to estimate the prevalence of MTBC in cattle herds based on tissue samples from BTB-infected cattle. In 2025, 302 cattle were eliminated in Poland due to a positive TST. Lymph nodes and lung or liver tissue samples were collected, and mycobacterial isolation was performed. Specially prepared tissue homogenates from each animal were inoculated onto Stonebrink and Petragnani solid media. The samples were incubated for 6 weeks at 37°C. Next, identification of bacterial colonies was performed using GenoType MTBC (Hain Lifescience, Germany). The presence of MTBC was detected in 107 of 302 animals (35.4%), including 97 animals infected with *M. caprae* and 10 with *M. bovis*. The number of outbreaks was 17. MTBC-positive animals were derived from Mazowieckie, Wielkopolskie, Lubuskie, and Warmińsko-Mazurskie Provinces. The results indicate that *M. caprae* was the predominant species responsible for BTB cases in the examined cattle, suggesting that this pathogen currently plays a major role in the epidemiology of BTB in Poland. The results proves relatively low national prevalence of MTBC infections. Lowering the number of infections in animal herds reduces threat to public health.

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P042. PANDEMIC PREPAREDNESS BY PATHOGEN DETECTION (P3DX): A HIGH-THROUGHPUT TESTING WORKFLOW FOR POPULATION-LEVEL PATHOGEN DETECTION

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Large-scale population-level testing during a pandemic places fundamentally different demands on laboratory systems than routine clinical diagnostics. The COVID-19 pandemic demonstrated that reliance on repurposed clinical laboratory infrastructures leads to competition for personnel, consumables and logistics, highlighting the absence of dedicated, parallel infrastructure for scalable population-level pathogen testing.
Within the P3Dx programme, which integrates complementary diagnostic, operational and governance activities to strengthen pandemic preparedness from a One Health perspective, we develop and validate a high throughput testing workflow designed for the pandemic transition phase, when widespread testing is required but validated self-tests are not yet available. This workflow supports scalable deployment in a federated laboratory network and is positioned as complementary to clinical diagnostics. It prioritises scalability, speed, robustness and accessibility over maximal analytical sensitivity.
The workflow integrates low-threshold home-based self-sampling, extraction-free processing, and alternative molecular methods to reduce dependence on PCR-specific consumables, combined with automation to achieve high sample throughput. Highly pathogenic avian influenza (HPAI) H5N1 was selected as a primary use case, as recent outbreaks in the Netherlands enabled access to outbreak-derived samples for large-scale evaluation and because avian influenza has well-recognised zoonotic and pandemic potential.
An extraction-free LAMP-CRISPR-based molecular assay for HPAI H5N1 detection (scopeDx®) was developed and integrated into the automated workflow. The assay and workflow are being evaluated for analytical performance, matrix tolerance, robustness under automated handling, achievable throughput, and operational bottlenecks, with benchmarking against an ISO 17025-accredited multiplex PCR. A real-world outbreak case study comprising approximately 1,500 swabs is used to assess system-level performance under outbreak conditions. Together, these activities provide a framework to assess the feasibility, scalability, and operational boundaries of an automated, extraction-free testing workflow for pandemic preparedness, contributing to the definition of the role of population-level testing infrastructures in future pandemic response strategies.

P043. VETERINARY CYTOLOGY REPORTING NEEDS MORE STANDARDIZATION

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Introduction: Human cytology reporting systems are organ-system based and have undergone inspection over decades, with periodic updates based on evolving knowledge about various diseases. The updates are aimed at enhancing report reproducibility and alignment across institutions. Veterinary cytology reporting has undergone limited scrutiny during its development and has not addressed these issues.
Objectives: (1) Briefly review the evolution of cytologic reporting in human medicine. (2) Review differences of cytologic reports in veterinary medicine compared to human medicine. (3) Present suggested actions for a global task force to provide a consensus guideline for standardization of veterinary cytology reporting.
Results: Human cytology reporting has evolved based on the site of origin, method of collection and method of sample processing. These efforts have emphasized the need for standardization to provide more effective communication from the laboratory to the clinician; more effective communication between pathologists; facilitation of cytologic-histologic correlation; and, facilitation of research into the epidemiology, biology, and pathology of various conditions, by providing reproducible and reliable data for national and international statistical analysis comparisons across organizations and cytopathologists. Veterinary cytology reporting lacks standardization that promotes similar reproducibility. Previous published studies in veterinary cytology identified a need to reduce the number of interpretation confidence modifying terms used by clinical pathologists and to explain the significance of these terms. Previously published surveys by two of the authors (MR, KF) of 466 clinicians and 210 clinical pathologists and residents identified 7 personal confidence ranges and reduced 19 possible modifying terms to 7. A survey of over 1014 veterinarians showed that they preferred a template style report with limited modifying terms and personal confident ranges over the traditional narrative format. Further refinement of cytologic standardization is required. A consensus taskforce is proposed to further make recommendations for providing more standardization in veterinary cytology reports.

P044. LABORATORY MARKERS OF INFLAMMATION IN CATTLE: AN EXPLORATORY CORRELATION STUDY WITH FOCUS ON HAPTOGLOBIN ASSAY COMPARABILITY

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Haptoglobin (Hp), leukocyte count, neutrophil percentage, total protein, and albumin are routinely used to assess inflammatory conditions in bovine clinical pathology [1,4]. Hp, a positive acute phase protein (APP), is of diagnostic interest, while albumin, a negative APP, and leukocyte dynamics reflect distinct, potentially interrelated pathomechanisms [2,3]. This exploratory, retrospective study investigated associations among these parameters and evaluated two Hp quantification methods. Samples from patients at the Clinic for Cattle, University of Veterinary Medicine Hannover (August 2025 – March 2026; n = 48 patients, n = 67 samples, 11 with repeated measurements) were analyzed. Spearman rank correlations were calculated with false discovery rate (FDR) correction. Method comparison of an immunoturbidimetric assay (OSR6165, Beckman Coulter) versus an ELISA (Bovine Haptoglobin ELISA, Immunology Consultant Laboratory) was assessed by three-category contingency table, including descriptive statistics of quantifiable results (Table 1). After FDR correction, significant within-pair correlations were identified: leukocyte count and neutrophil percentage ($r = 0.562$, $p < 0.001$) and total protein and albumin ($r = 0.470$, $p < 0.001$). Results were stable in sensitivity analysis. Overall censoring rates were 69.8% (37/53) for Method A and 75.5% (40/53) for Method B; of 53 samples with results from both methods, none was simultaneously quantifiable, as the immunoturbidimetric assay (10–400 mg/dL) and ELISA (0.156–9.85 mg/dL) cover adjacent but non-overlapping concentration ranges. Although a Spearman correlation was calculable ($r = 0.362$, $p = 0.008$), this reflects rank ordering of censored values only. Above-range ELISA results remained non-quantifiable despite serial dilution, consistent with the expectedly high Hp concentrations in affected cattle, while the immunoturbidimetric assay paradoxically showed a high proportion of below-detection-limit results. Limitations include incomplete patient data and diagnoses. Known within-pair associations were confirmed with no cross-pathomechanism correlations. Hp quantification proved challenging, underscoring the need for careful assay selection and validation in veterinary diagnostic laboratories.

Table 1. Contingency table of haptoglobin measurement results by method category (n = 53)

	Method B (ELISA)			
Method A (immuno turbidimetric assay)	**Below detection limit**	**Quantifiable**	**Above measurement range**	**Total**
Below detection limit	0	13	24	**37**
Quantifiable	0	0	16	**16**
Above measurement range	0	0	0	**0**
Total	**0**	**13**	**40**	**53**

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P045. RABIES FAVN TESTING AT A NATIONAL REFERENCE LABORATORY: WORKLOAD TRENDS, OPERATIONAL CHALLENGES AND CAPACITY MANAGEMENT

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Rabies virus neutralising antibody testing is a key requirement for international movement of dogs and cats and an essential component of rabies prevention. Wageningen Bioveterinary Research (WBVR) serves as the Dutch national reference laboratory for veterinary rabies and performs the fluorescent antibody virus neutralisation (FAVN) assay for this purpose, in accordance with the WOAH manual.
To characterise workload trends, species distribution of submitted samples and their operational implications for a national reference laboratory, we retrospectively analysed rabies FAVN testing performed at WBVR between 2012 and 2025. During this period, annual testing volumes increased substantially, rising from fewer than 600 samples in 2012 to more than 3000 samples in 2025. Sera from companion animals (dogs and cats) constituted the majority of submissions throughout the study period, while human sera and samples from other mammalian species contributed consistently and episodically to the overall workload. In addition to testing for routine pet travel diagnostics, WBVR regularly accommodated non-routine testing demands. For example, WBVR temporarily tested samples on behalf of other (national) laboratories. In addition, large-scale testing was performed for companion animals accompanying refugees from Ukraine in 2022–2023, as well as for human vaccine studies and experimental animal research. These episodic sample streams contributed to marked fluctuations in annual workload and increased operational complexity. Our findings illustrate the evolving role of a national reference laboratory in rabies serology testing, where increasing routine testing volumes coincide with unpredictable demand. Given the labour-intensive nature of the FAVN assay, particularly the manual microscopic readout by trained personnel, this combination highlights growing pressure on laboratory capacity. In response, partial automation of the FAVN workflow is currently under development and being prepared for validation. This represents a key step towards ensuring long-term capacity, resilience and sustainability while maintaining analytical quality in an increasingly dynamic testing environment.

P046. CULTURE-ENRICHED NANOPORE METAGENOMICS IMPROVES GENOME RECOVERY AND RESISTOME DETECTION IN LOW-BIOMASS ANIMAL MICROBIOMES

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Wildlife microbiomes are relevant for One Health surveillance and biotechnological mining[1,2], but low microbial biomass and high host DNA often limit the performance of direct metagenomics[3]. We evaluated a 6-hour non-selective culture-enrichment step[4] before Oxford Nanopore sequencing to improve genome-resolved and resistome-focused characterization of bat intestinal and fecal samples. Three paired sample sets from *Pipistrellus* spp. were analysed by direct metagenomics and culture-enriched metagenomics (CEM), with downstream taxonomic profiling, assembly, binning, AMR gene detection, and MALDI-TOF MS-based culturomics. CEM consistently reduced host-derived DNA (15.7% to 5.7%, and 25.7% to 3.6% in two intestinal samples; 7.2% to 0.1% in pooled feces) with 2.8-fold, 7.1-fold, and 72-fold reductions, respectively. Only CEM yielded high-quality metagenome-assembled genomes (HQ-MAGs), including four complete single-contig genomes. These included *Enterococcus faecalis*, *Enterococcus faecium*, *Serratia marcescens*, and *Hafnia paralvei*, with the *S. marcescens* genome carrying 12 biosynthetic gene clusters (BGCs) and other MAGs harbouring multiple BGCs, indicating notable biosynthetic potential for natural-product discovery. Resistome detection was markedly enhanced, with 37 read hits in direct metagenomes versus 1969 in CEM and a broader set of acquired determinants, including *erm(B)*, *msr(C)*, *oqxB*, and multiple tetracycline resistance genes. Culturomics confirmed the viability of dominant enriched taxa, including *E. faecalis*, *E. faecium*, *S. marcescens*, *Lactococcus garvieae*, and *Klebsiella oxytoca*. Short-term culture enrichment substantially improves long-read metagenomic resolution in host-rich, low-biomass wildlife samples. However, CEM should be considered an enrichment strategy rather than an unbiased taxonomic census, and Nanopore sequencing remains constrained by input DNA requirements. The 6-hour enrichment was chosen in line with the literature, but protocol optimization across sample matrices and target outcomes will be necessary to tailor and maximize performance. Overall, the CEM workflow supports One Health surveillance and biotechnological mining by enhancing recovery of viable bacteria, complete genomes, clinically relevant AMR determinants, and biosynthetic potential from bat microbiomes.

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P047. ANTIMICROBIAL RESISTANCE IN E. coli FROM FINNISH BROILER PRODUCTION: INDICATOR AND PATHOGENIC ISOLATES

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Antimicrobial resistance (AMR) in *Escherichia coli* in Finnish broilers is monitored both on isolates from diseased flocks and in commensal indicator *E. coli* collected at slaughterhouses. In Finland, broilers are not treated with antibiotics and breeder flocks are treated only very rarely (1), yet antimicrobial resistance is still detected. The aim of this study was to describe the level, annual variation and population-specific differences in AMR in *E. coli* from broiler flocks.
Pathogenic *E. coli* isolates were collected between 2016 and 2024 from broiler flocks with colibacillosis (16–252 isolates/year). From 2016–2020, only breeder flock isolates were included; thereafter, also isolates from production broilers were tested. Indicator *E. coli* were studied every two years (170–184 isolates per sampling year). Antimicrobial susceptibility testing was performed using Sensititre™ panels and EUCAST epidemiological cut-off values when available (2).
Resistance levels varied markedly between years, but no increasing or decreasing trends were observed. Resistance was detected annually against ampicillin, tetracycline, sulfamethoxazole and trimethoprim. Among indicator *E. coli*, resistance against these antimicrobials was typically below 10%. In pathogenic isolates, resistance levels were also mostly low, but ampicillin resistance occasionally increased substantially, reaching up to >30% in some years. The highest overall resistance levels were observed for ampicillin, followed by tetracycline and sulfamethoxazole. Resistance to trimethoprim generally remained below 5%. Compared with indicator isolates, pathogenic *E. coli* showed greater variation in resistance levels and higher peak values, reflecting the impact of sporadic dissemination of individual virulent strains. Resistance to fluoroquinolones, cefotaxime and gentamicin was rare, and no resistance was detected against other antimicrobial classes.
AMR in *E. coli* from Finnish broiler production remains low and limited to a small number of antimicrobials. These findings highlight the importance of continuous surveillance and isolate-based susceptibility testing, even in production systems with negligible antibiotic use.

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P048. HORIZONTAL GENE TRANSFER IN A MULTIDRUG-RESISTANT (MDR) *Escherichia coli* STRAIN ISOLATED FROM A CATTLE EGRET (*Bubulcus ibis*) IN ITALY

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Antimicrobial resistance (AMR) is a serious global threat, and the real challenge comes from horizontal transfer mediated by mobile genetic elements. From a One Health perspective, the determinants of resistance spread freely among human, livestock, and environmental ecosystems, with migratory birds acting as a crucial biological indicator and a long-range vector.

A multidrug-resistant (MDR) strain of Escherichia coli, ARAS115, was isolated from a Cattle Egret (*Bubulcus ibis*) in Apulia in 2024 and characterized using a combination of classical microbiological techniques and genomic analysis. Specifically, *in vitro* conjugation, transformation, and RFLP assays were performed, while in silico sequencing was conducted using short-reads (Illumina) and long-reads (Oxford Nanopore). ARAS115 carries Plasmid 1 (IncI1), which lacks resistance genes, and Plasmid 2 (IncFIB/FIC), which harbors all resistance determinants, transferred at baseline frequencies of 10^{-4}.

The in vitro conjugation assays yielded a transconjugant, Tra407, characterized by an intact IncI1 and a macro-deletion of approximately 26 kb on Plasmid 2 (IncFIB/FIC). This rearrangement removed the IncFIC origin of replication, nearly all resistance genes—sparing only the tet(A) gene (Figure 1)—and partially truncated the finO repressor gene. Tra407 exhibits a hyper-conjugant phenotype capable of transferring tetracycline resistance at a frequency of 10^{-1}, which is approximately 1000 times higher. Through transformation, the Tra413 clone was obtained from Tra407; it has only Plasmid 2 with the deletion but has lost Plasmid 1; in this case, the frequency drops again to 10^{-4}. This finding supports the evolutionary hypothesis of inter-plasmid cooperation or synergy: when the IncI1 and IncF plasmids are separated, the high capacity to transfer resistance declines. Spontaneous mobilome rearrangements greatly amplify the spread of AMR, confirming wildlife as a dynamic biological sentinel.

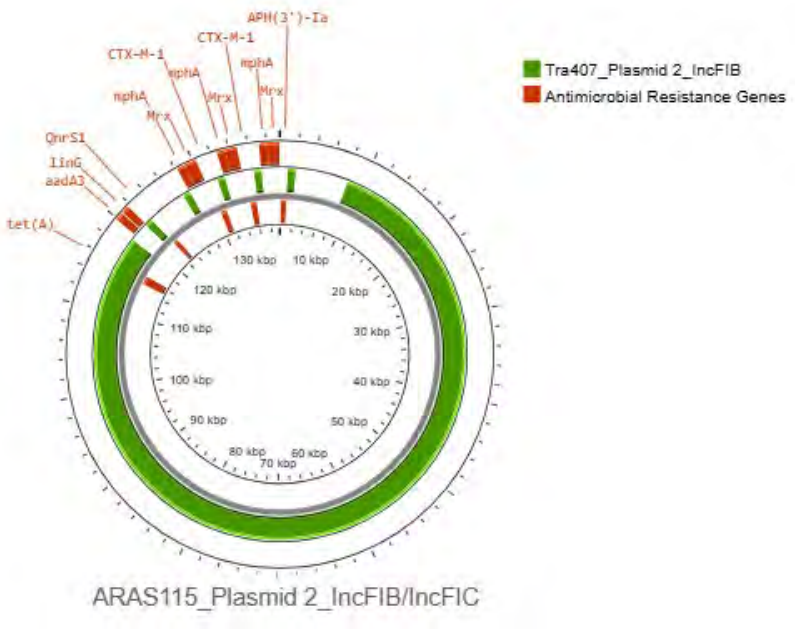


Figure 1. Alignment of the nucleotide sequences of plasmid 2 (IncFIB/IncFIC) from strain ARAS 115 (in gray) and the same plasmid with the IncFIB deletion from the transconjugant Tra407. The alignment was performed using Proksee, and the resistance genes were annotated using the CARD database.

P049. WHOLE GENOME CHARACTERIZATION OF *Klebsiella* STRAINS IN EUROPEAN HEDGEHOGS AND HUMAN NOSOCOMIAL SETTINGS

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Klebsiella pneumoniae is a major pathogen associated with healthcare-acquired infections and antimicrobial resistance (AMR), particularly to β-lactams and carbapenems. Although wildlife is rarely exposed to antimicrobial treatments, wild animals may act as reservoirs of resistant bacteria. The European hedgehog (Erinaceus europaeus) increasingly inhabits urban environments, where interactions with humans, domestic animals, and anthropogenic sources may facilitate the circulation of AMR bacteria. This study characterized 24 *Klebsiella* spp. isolates from urban hedgehogs using whole-genome sequencing (WGS). Hedgehog isolates were compared with publicly available human clinical isolates (n=49 *Klebsiella pneumoniae* genomes) from the same geographic region to investigate sequence types (STs), antimicrobial resistance genes, and plasmid profiles, aiming to better understand resistance dissemination between environmental and human compartments. A high prevalence of AMR determinants was detected among hedgehog isolates, including the carbapenemase gene blaOXA-48. Internationally disseminated nosocomial lineages, including ST11, ST15, ST307, and ST392, were identified in both hedgehog and human populations. Conjugative plasmids, particularly IncFIB(K) and IncL/M types, were shared between wildlife and human isolates, highlighting plasmid-mediated transmission as a key mechanism driving AMR spread. Although direct transmission between wildlife and healthcare settings cannot be demonstrated, the detection of clinically relevant clones and resistance elements in hedgehogs suggests potential ecological connectivity between environmental reservoirs and human-associated bacteria. These findings support the importance of wildlife surveillance within a One Health framework and underscore the need for further investigation of multidrug-resistant Klebsiella spp. and other resistant bacteria in wildlife to better understand their contribution to the dissemination of antimicrobial resistance across ecosystems.

P050. URBAN WILD BIRDS ARE RESERVOIRS OF NOSOCOMIAL MULTI-DRUG-RESISTANT BACTERIA IN CATALONIA, BY WHOLE GENOME SEQUENCING

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Urban birds are increasingly recognized as effective sentinels of antimicrobial resistance (AMR), reflecting the impact of human activity on the environment through their ecological plasticity and frequent contact with anthropogenic sources of bacteria. This study assessed the prevalence and genomic characteristics of AMR bacteria in urban birds from Catalonia, by whole-genome sequencing (WGS) for monitoring antimicrobial resistance genes (ARGs) of public and animal health relevance. Cloacal swabs from 119 wild birds across multiple urban areas were analyzed using ceftriaxone-selective culture, antimicrobial susceptibility testing, and WGS. Overall, 60 samples (50.4%; 95% CI: 41.5–59.4) were positive, with the highest prevalence in Apodiformes (80%), followed by Passeriformes (47%) and Columbiformes (41%). Insectivorous birds (swifts and common house martins), showed the greatest colonization rates (75–100%), followed by corvids (Eurasian jays and magpies, 67%), and sparrows (52%). Enterobacterales represented 53% of isolates, dominated by *Escherichia coli* (17%), *Klebsiella pneumoniae* (15%), and *Enterobacter* spp. (12%), while *Pseudomonas aeruginosa* was the most prevalent single species (35%). Insectivorous birds harbored the greatest bacterial diversity, whereas sparrows carried most of *K. pneumoniae* and *P. aeruginosa* isolates. Phenotypic resistance was most pronounced in *Pseudomonas* spp. and *Klebsiella/Enterobacter* spp., particularly to β-lactams, lincosamides, and phenicols; *K. pneumoniae* showed up to 70% resistance to fluoroquinolones. WGS revealed chromosomal ARGs in *Pseudomonas* spp. and predominantly plasmid-mediated resistance in Enterobacterales. Notably, *K. pneumoniae* harbored ARGs conferring resistance to up to seven antimicrobial classes and included nosocomial clinically relevant sequence types (ST307, ST392). *E.coli* ST117, ST127 and ST135 were associated with extraintestinal pathogenic clones and nosocomial infections. In conclusion, sparrows emerged as key reservoirs of multidrug-resistant bacteria, carrying high burdens of clinically relevant *K. pneumoniae* clones. Detection of high-risk clones in urban birds highlights possible bidirectional transmission between human environments and wildlife, supporting the need for integrated phenotypic–genomic One Health surveillance programs.

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05. Antimicrobial Resistance Challenges and Innovations in Diagnostics

P051. INTEGRATED ONE HEALTH SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN FOODBORNE BACTERIA WITHIN OFFICIAL FOOD CONTROL IN THE VALENCIAN COMMUNITY (SPAIN)

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Antimicrobial resistance (AMR) represents a major global public health threat, partly driven by antimicrobials use in food-producing animals and the dissemination of resistant bacteria along the food chain. Within a One Health framework, harmonised AMR surveillance in zoonotic and commensal bacteria is essential to monitor trends, assess risks and support evidence based-control measures[1]. This study describes the implementation and main outcomes of AMR surveillance activities conducted during 2024 in the Valencian Community, (Spain) within official food control. Mandatory surveillance under Commission Implementing Decision (EU) 2020/1729[2] focused on extended-spectrum beta-lactamase- and AmpC-producing *Escherichia coli* (ESBL/AmpC) isolated from fresh poultry meat at retail level[3]. Additionally, a voluntary regional programme extended surveillance to *Salmonella spp. Campylobacter spp and indicator E. coli.* isolates collected along the food chain. Broth microdilution and ECOFF values has been used as interpretative criteria according to EU-harmonised criteria. In 2024, 35 chicken meat samples and 17 turkey meat samples were analysed for ESBL/AmpC producing *E. coli*, together with 145 *Salmonella* 50 *Campylobacter* isolates and 10 indicator *E. coli*. ESBL-producing *E. coli* were detected in 28.6% of chicken meat and 23.5% of turkey meat samples with prevalences comparable to EU-level data[4]. *Salmonella* from broiler showed very high resistance to quinolones, while resistance to ampicillin, sulfamethoxazole and tetracycline decreased. Multidrug resistance (MDR) was 17.1%, and fully susceptible isolates remained low (7.3%). In pigs, *Salmonella* exhibited high resistance to ampicillin, sulfamethoxazole and tetracycline, with MDR reaching 50%, above EU reference values[4], whereas cattle isolates were fully susceptible. *Campylobacter jejuni* and *Campylobacter coli* from broiler carcasses showed extremely high resistance to ciprofloxacin, exceeding EU levels. These findings highlight the value of integrating mandatory and voluntary AMR surveillance within official food control systems. The One Health based approach provides robust evidence to support risk management actions and prudent antimicrobial use along the food chain.

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P052. TRENDS IN FLUOROQUINOLONE RESISTANCE IN ENTEROTOXIGENIC *Escherichia coli* FROM 2016 TO 2024 AND RESTRICTIONS ON THE USE OF FLUOROQUINOLONES IN FINNISH PORK PRODUCTION

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Fluoroquinolones are classified as critically important antimicrobials in both human (1) and veterinary medicine (2), and their use plays a significant role in the development of antimicrobial resistance. Under Finnish legislation, the use of fluoroquinolones in animals is permitted only when no other effective treatment option is available (3). Fluoroquinolone use in Finnish piggeries was further restricted through sector-led organization rules. Restricted case-by-case use was applied from June 2019, followed by a complete ban in July 2021. The aim of this study was to examine fluoroquinolone resistance trends in enterotoxigenic *Escherichia coli* (ETEC) isolated from piglets with post-weaning diarrhea in Finland during 2016–2024. ETEC isolates were cultivated from piglet fecal samples and their pathogenicity i.e. presence of fimbria and toxins was assessed by PCR. Antimicrobial susceptibility testing was performed using Sensititre™ panels and EUCAST epidemiological cut-off values when available (4). Decreasing trend in fluoroquinolone resistance was observed within tested ETEC isolates; resistance against ciprofloxacin, enrofloxacin, and nalidixic acid decreased between 2019 and 2024, from 22% to 2.6%, 14% to 2.6%, and 22% to 13.2%, respectively. The number of tested isolates varied between 20 and 98 annually. The decline in fluoroquinolone resistance is consistent with the hypothesis that strict restriction and complete discontinuation of fluoroquinolone use in Finnish pig production may be associated with reduced resistance. However, causal inference remains limited due to the small and variable number of isolates analyzed annually and the relatively short observation period after the sector-led restrictions. Continued surveillance will be essential to determine whether fluoroquinolone resistance in ETEC remains low and whether the downward trend observed in recent years is sustained over time.

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P053. CHALLENGES IN VETERINARY MYCOLOGY: FROM PATHOGENESIS TO ANTIFUNGAL RESISTANCE

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Fungal infections are being diagnosed with higher frequency in both human and veterinary medicine. However, there is still multiple gaps in understanding fungal diseases in veterinary medicine. Therefore, our objective is to highlight the challenges faced by veterinary mycology and to propose potential solutions.
Unlike human medicine, many fungal diseases affect animals that apparently show no immunosuppression status. This is the first major challenge we face: understanding the true pathogenesis of fungal infections in animals. In human medicine, certain fungi are considered primary pathogens (e.g. coccidioidomycosis, blastomycosis, and cryptococcosis) because they are capable of infecting healthy individuals. Also, there are opportunistic fungi, which infect individuals with prolonged antimicrobial or immunosuppressive treatments (e.g. aspergillosis and candidemia). As previously mentioned, this pattern does not occur in the same way in animals. Therefore, we need to understand how the fungus infects and developed itself.
Treatment in animals is more complex than in humans, as the pharmacokinetics and toxicity profiles of antifungal agents remain poorly characterised across animal species. In addition, treatment courses are often prolonged, and owners are frequently unable to commit the necessary time and financial resources.
Undoubtedly, one of the biggest challenges is antifungal resistance. Awareness of this issue is increasing in human medicine, whereas it remains limited in veterinary medicine. It is important to consider that animals may act as carriers of antifungal-resistant fungi, therefore, this problem must be deal from a One Health perspective. Another important limitation is the absence of species-specific clinical breakpoints for animals, which complicates the classification of resistant and susceptible strains.
Our research group is currently leading studies on antifungal resistance in fungi isolated from clinical samples at the Complutense Veterinary Teaching Hospital in Madrid, Spain. We hope that this work will help solving some of these challenges.

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P055. EPIDEMIOLOGICAL CUT OFF (ECOFF) VALUES FOR *Mycoplasma (Mycoplasmopsis) Bovis* – FEASIBLE OR NOT?

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Mycoplasma (*M.*) *bovis* is a leading pathogen in the context of various clinical manifestations in cattle. Antimicrobials are the only option for treatment. Due to intrinsic resistance against several important antimicrobial classes, e.g. beta-lactam antibiotics and sulfonamides, antimicrobial susceptibility testing (AST) plays a key role as the rational basis of targeted antimicrobial therapy, especially in mollicutes. In contrast to human medicine, currently no harmonised methods for assessing minimum inhibitory concentrations (MIC), nor clinical breakpoints for the reliable interpretation of MIC are available for *M. bovis*. Even epidemiological cut-off (ECOFF) values, which serve as one essential basis for clinical breakpoints, are not defined. Therefore, the MyMIC consortium aggregated non-published and published MIC data based on different methods for *M. bovis* to amphenicols, tetracyclines, macrolides, pleuromutilins and fluoroquinolones. The resulting MIC distributions were analysed whether they meet the European committee for antimicrobial susceptibility testing (EUCAST) acceptance criteria for establishing ECOFFs or not. For several antimicrobials non-truncated MIC distributions with only a potential weak effect from the different broths used by the different laboratories demonstrated the potential for setting an ECOFF for *M. bovis*. In the case of macrolides in particular, it was found that methodological effects are more pronounced, leading to more dispersed and widespread MIC distributions. On the basis of a harmonised protocol for determining MIC values for *M. bovis*, initial ECOFF values can be established for certain antimicrobials.

P056. ANTIMICROBIAL RESISTANCE OF *Yersinia ruckeri* ISOLATED FROM FARMED RAINBOW TROUT (*Oncorhynchus mykiss*)

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Yersinia ruckeri is the causative agent of yersiniosis, a bacterial disease of salmonid fish, also known as Enteric Redmouth Disease (ERM). Due to the increasing number of *Y. ruckeri* cases, the aim of this study was to determine the bacterial susceptibility to selected antimicrobial agents and link them with the occurrence of antibiotic resistance genes. A total of 20 strains from farmed rainbow trout (*Oncorhynchus mykiss*) were used. Identification to the species level was confirmed using MALDI-TOF biotyping and serological tests. The Minimum Inhibitory Concentration (MIC) of strains was determined against a panel of 18 antibiotics using Sensititre AVIAN1F Plate. Whole genome sequencing was done on MiSeq platform (Illumina, San Diego, CA, USA) and the sequences were screened for antimicrobial resistance genes using ResFinder 4.7.2 and NCBI Bacterial Antimicrobial Resistance Reference Gene Database. The majority of strains were inhibited by the lowest concentrations of tested antibiotics. Individual strains showed increased MICs for sulphadimethoxine, sulphathiazole, trimetoprim/sulfamethoxazol, phenicol, streptomycin, and resistance genes sul2, dfrA14, aph(6)-Id, aph(3'')-Ib were detected. Our studies showed the occurrence of antimicrobial resistance genes in *Y. ruckeri*. Increased MICs correlated with the presence of the respective resistance genes. However, further studies based on a larger number of strains are needed to confirm this correlation. The study was funded from subsidy provided by the Ministry of Science and Higher Education under the grant no. S/631 performed in 2025-2027 at the National Veterinary Research Institute, Puławy, Poland.

P057. EXTENDED-SPECTRUM B-LACTAMASE- AND AMPC-PRODUCING *Escherichia coli* IN WILD BIRDS AND EUROPEAN HEDGEHOGS ADMITTED TO A WILDLIFE RESCUE CENTRE IN NORTHWESTERN ITALY

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Wildlife rescue centres (WRCs) may contribute to the dissemination of antimicrobial resistance (AMR) by housing different species in close contact with humans and animals. This study aimed to characterize extended-spectrum β-lactamase (ESBL)- and AmpC-producing *Escherichia coli* isolated from wild birds and European hedgehogs (*Erinaceus europaeus*) admitted to the "Centro Animali Non Convenzionali" (C.A.N.C.) WRC, University of Turin, Northwestern Italy. The isolates originated from fecal samples previously collected from hospitalized wild birds (n = 172) and hedgehogs (n = 121) (Prandi et al., 2023; 2025) and were screened for ESBL/AmpC-producing *E. coli* using MacConkey agar supplemented with cefotaxime. Presumptive ESBL/AmpC colonies were phenotypically characterized by Combination Disk Test and AmpC detection assays, followed by PCR targeting ESBL- and AmpC-encoding genes (Dallenne et al., 2010). Antimicrobial susceptibility testing was performed, and phylogroups were determined according to the Clermont et al. (2013).

A total of 43 representative isolates were genetically characterized: 24 strains were identified as ESBL producers, including 22/24 belonging to CTX-M group 1 and 1/24 to CTX-M group 9, whereas 19 isolates were classified as AmpC producers and 2 as putative ESBL/AmpC co-producers. The *bla*TEM gene was detected in 17/43 isolates (39.5%), *bla*SHV in 1/43 (2.3%), and *bla*CMY-2 in all 19 AmpC-positive isolates (100%). The occurrence of ESBL/AmpC co-carriage and the characterization of *bla*TEM variants will be further investigated by sequencing selected isolates.

All isolates exhibited multidrug-resistant profiles but remained susceptible to imipenem. The number of antimicrobial resistances was significantly higher in wild birds (median = 5, IQR = 1.25) than in hedgehogs (median = 4, IQR = 1.00) (p = 0.003), and significant differences were detected among phylogroups (p = 0.008). The predominance of phylogroups F and D, frequently associated with extraintestinal pathogenic *E. coli* (ExPEC), suggests a potential zoonotic relevance of AMR strains circulating in wildlife admitted to WRCs.

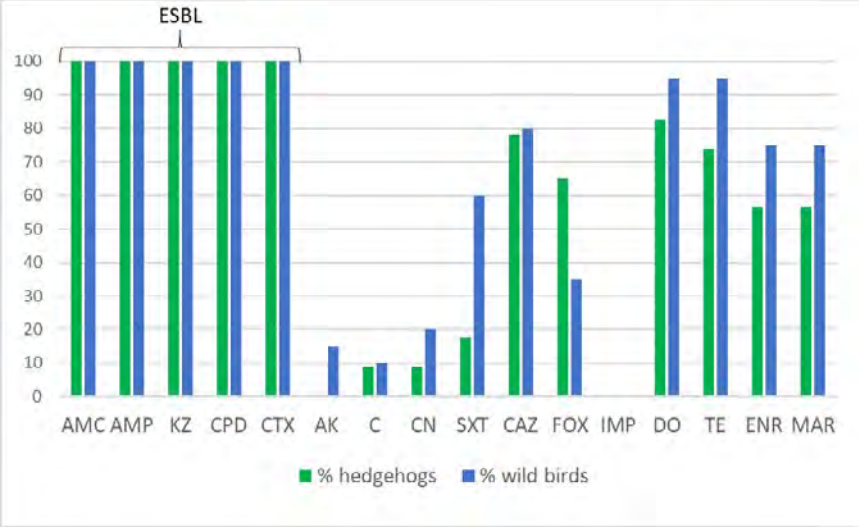


Figure 1 - Distribution of antimicrobial resistance rates (%) among ESBL/AmpC-producing *Escherichia coli* isolates from wild birds and European hedgehogs admitted to a wildlife rescue centre in Northwestern Italy.

Phylogroup	Sum of class resistance (median ± IQR)
A	3.00 ± 1.50
B1	6.00 ± 0.50
B2	5.00 ± 2.25
C	3.00 ± 0.00
D	4.00 ± 1.00
F	4.00 ± 0.00

Table 2 - Median number of antimicrobial resistance classes according to *Escherichia coli* phylogroups isolated from wild birds and European hedgehogs admitted to a wildlife rescue centre in Northwestern Italy.

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P058. SELECTIVE ENRICHMENT OF *Candida auris* TO IMPROVE MOLECULAR DETECTION

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Diagnosing *Candida auris* remains challenging using conventional methods, potentially delaying outbreak detection and control measures. Rapid and accurate identification is therefore essential, driving the adoption of molecular approaches to improve sensitivity and turnaround time[1]. In a routine setting where C. auris samples are usually tested using chromogenic media, this study investigated the use of CABroth (Copan), a *Candida auris* enrichement broth, to support organism recovery within a qPCR-based workflow for *C. auris* identification. The *Candida auris* CDCB11-903 strain was tested in CABroth alone and in combination with eSwab® (Copan Italia) using a 0.5 McFarland suspension diluted 1:10. A 30 µL aliquot of the eSwab® sample was added to 2 mL of CABroth. Samples were then incubated at 37°C and analyzed after 24 h (T24) and 48 h (T48). DNA extraction was performed at T0, T24, and T48, followed by qPCR quantification according to the manufacturer's instructions (Applied Biosystems). Additional *C. auris* strains (ATCC* B11103, B11220, B11221, B11244) were tested under CABroth conditions only, following the same protocol. In CABroth™, all *C. auris* strains showed a progressive decrease in Ct values over time, indicating effective enrichment. For the CDC*B11-903 strain, differences in Ct (ΔCt) values, compared with T0, were −2.68 at T24 and −4.83 at T48, while eSwab® samples exhibited a more pronounced reduction (ΔCt −4.93 at T24 and −7.36 at T48), supporting enhanced recovery. Consistent Ct reductions observed across all tested strains confirmed the ability of CABroth™ to promote selective growth and improve molecular detection sensitivity through increased detectable target DNA. CABroth™ showed a marked reduction in Ct values after 24 h and 48 h across all tested *C. auris* strains, consistent with its enrichment capability and its potential to enhance the sensitivity of molecular detection. In addition, CABroth™ was shown to be compatible with eSwab® samples, potentially supporting patient surveillance workflows.

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05. Antimicrobial Resistance Challenges and Innovations in Diagnostics

P059. ANTIBIOTIC-RESISTANCE PATTERNS OF *Escherichia coli* AND *Salmonella* SPP. FROM BROILER FARMS AND COMPARATIVE ASSESSMENT OF ANTIMICROBIAL STEWARDSHIP AMONG FARMERS IN THREE REGIONS OF GHANA

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Antimicrobial resistance (AMR) is an increasing public health and food safety concern, particularly in poultry production systems where inappropriate antibiotic use promotes the emergence of resistant bacteria. This study assessed the prevalence and antimicrobial resistance profiles of *Escherichia coli* and *Salmonella* spp. isolated from broiler chickens in the Bono, Bono East, and Western Regions of Ghana. A total of 200 pooled faecal samples from 100 broiler farms between January – April 2025 was analysed using conventional microbiological techniques, MALDI-ToF mass spectrometry for bacterial identification, the Kirby–Bauer disk diffusion method for antimicrobial susceptibility testing, and inhibition zone interpreted using the CLSI guidelines 2025. *E. coli* was detected in 92.5% of samples, while *Salmonella* spp. was identified in 5.5%. Multidrug resistance (MDR) was observed in 74.6% of *E. coli* isolates, with 24.3% classified as extensively drug-resistant (XDR). Extended-spectrum beta-lactamase (ESBL) production occurred in 41.6% of *E. coli* isolates, and 90.9% of *Salmonella* isolates showed resistance to fluoroquinolones. Farms participating in Farmer Field School (FFS) training demonstrated comparatively lower resistance rates and fewer XDR isolates, suggesting that structured structed education in antimicrobial stewardship and biosecurity can reduce resistance prevalence. Market-ready broilers exhibited higher resistance levels compared with day-old chicks, indicating notable differences across production stages.Overall, the findings highlight the need for strengthened antimicrobial stewardship, improved regulatory enforcement, and expanded farmer education to reduce AMR risks in poultry systems. The study provides important evidence supporting One Health–based AMR surveillance and integration into Ghana's national food safety strategies.

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P060. ANTIBIOTIC RESISTANCE GENES DETECTED IN INSECTS FROM THE DOMESTIC TRADE NETWORK

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Antibiotic resistance represents a major global public health concern. Antibiotic resistance (AR) genes are widely distributed across diverse environmental compartments such as surface water, wastewater, soil, livestock, as well as foods of both animal and plant origin. Significant reservoirs of AR genes include hospitals and agricultural production systems. Here, we investigated the potential role of edible insects/ farmed insects used as animal feed in the occurrence and dissemination of AR genes. Current literature indicates that insects in general can serve as reservoirs of antibiotic resistance genes. The most frequently identified AR genes in both types of insects are associated with resistance to tetracyclines, macrolides, β-lactams, and other antibiotic classes and the primary sources of these genes are identified as the rearing substrate, feed and the breeding system.

In our project, we focused on the detection of AR genes in homogenates from insects from the domestic trade network. We analyzed 66 samples (e.g. *Alphitobius diaperinus* - larvae, *Zophobas morio* - larvae, *Grylus assimilis* - adult, *Locusta migratoria* - adult, *Blaptica dubia* - adult) using conventional molecular methods (PCR) targeting 8 AR genes (*tetK*, *tetM*, *tetS*, *tetO*, *ermB*, *blaZ*, *vanB* and *metA*). Tetracycline AR genes (*tetM* and *tetS*) were detected in 8 (12,1%) and 10 (15,2%) samples, respectively. Vancomycin AR gene was detected in 5 (7,6%) samples. We were able to find AR genes in at least one sample of each species.

So far, we continue to screen edible/farmed insects for AR genes. Moreover, we aim to focus on the detection of AR genes in individual parts of the breeding process (feed, rearing conditions, different stages of insects, waste) to determine the potential risk to animals, public health and food/feed safety.

The work was financially supported by the NAZV project QL24010336.

P061. A NOVEL SELECTIVE ENRICHMENT BROTH (CAB) FOR ENHANCED *IN-VITRO* DETECTION OF *Candida auris*

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Candida auris is an emerging multidrug-resistant yeast of global concern, associated with healthcare-associated outbreaks, high mortality rates, and frequent misidentification. *Candida auris* has recently been detected in environmental and animal reservoirs, raising One Health concerns regarding potential zoonotic transmission[1]. This study evaluated the performance of a novel selective enrichment medium, Candida auris Broth (CABroth™, Copan Italia), for the isolation of *Candida auris* under in vitro conditions. The *Candida auris* ATCC® B11-903 strain was evaluated alongside other clinically relevant Candida species (*C. parapsilosis, C. albicans, C. krusei, C. tropicalis, C. glabrata, C. dubliniensis, and C. lusitaniae*). All strains were inoculated into 2 mL of CABroth™ using suspensions prepared in PBS at different concentrations (1×10^3 CFU/mL for Candida auris and 1×10^5 CFU/mL for the other species). Cultures were incubated at 40°C for 24 and 48 hours. Subsequently, 100 µL aliquots were plated onto Sabouraud Dextrose Agar (SDA) and Chromogenic Candida Agar (BioLife), incubated for an additional 24 - 48 hours, and evaluated by colony counting. Enrichment of *Candida auris* cultures after 24 and 48 hours showed progressive growth, with colony counts increasing from approximately 150 - 200 CFU/plate at time zero (T0) to 10^3 CFU/plate after 24 hours and 10^6 CFU/plate after 48 hours. In contrast, the growth of the other Candida species, starting from an initial inoculum of 1×10^5 CFU/mL, was markedly reduced, with approximately 50 - 100 CFU/plate observed after 24 hours and 0 - 30 CFU/plate after 48 hours. Overall, CABroth™ demonstrated a strong enrichment effect for *Candida auris* while effectively inhibiting the growth of non-auris Candida species. Additional studies will be conducted to further explore its potential applications in the veterinary field.

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P062. TEN-YEAR MONITORING OF *Bovine mastitis*-CAUSING BACTERIA IN NORTHERN ITALY AND EVALUATION OF ANTIMICROBIAL RESISTANCE IN RAW MILK

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Bovine mastitis is a prominent pathology in dairy farms worldwide, its etiology is multifactorial and it consists of inflammation of the udder responsible for loss of milk production, culling of animals, and treatment costs [1].
Mastitis can originate from physical damage or from infections due to *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma spp*, and environmental pathogens like *E. coli* and other *Enterobacteriaceae*. Pathogenic bacteria usually infect the healthy cows during milking procedures or come from the stable environment, litter, soil, stagnant water, and manure [2], [3].
The aim of this study is to analyze a dataset of 240,000 observations from the National Reference Centre for Bovine Milk Quality (IZSLER) from 2015 to 2024 to evaluate the pathogen prevalence in Lombardy and Emilia Romagna and provide an overview of the antimicrobial resistance in the area.
Raw milk samples from single udder quarter or bulk milk underwent bacteriological test. A total of 240,210 samples were analyzed on Bile Esculin Agar (BEA), Tallium Kristalviolette Tossin (TKT), Gassner, Baird Parker (BP) media.
Media were incubated at +37 °C for 48 h, for Gassner and BP media a further 24 h incubation at 4 °C (Gassner) and 5 °C (BP) was performed, and colonies identified.
After isolation, 2848 samples from 2022-2024, were tested for antibiotic-resistance for several antibiotics: minimum Inhibitory Concentration (MIC), after an incubation at 37 °C for 18-24 h on 96 well-plate were evaluated.
The study revealed that Streptococcus uberis (13.66%), E. coli (12.23%), S. aureus (8.72%), and Enterococcus spp. (10.85%) are the most relevant pathogens. MIC evaluation shows an increased resistance in S. aureus to erythromycin, penicillin, oxacillin, pirlimycin, and trimethoprim-sulfonamides; E. coli to kanamycin and trimethoprim; and S. uberis to rifampicin. These findings highlight the need for a targeted therapy based on accurate diagnosis to limit resistance spread in dairy farms.

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P063. AN INNOVATIVE DIAGNOSTIC TEST FOR THE FIELD DIAGNOSIS OF TAPEWORM INFECTION IN HORSES

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Anoplocephala perfoliata infection is an important health concern in horses, particularly in temperate climates. The parasite colonizes the intestine, predominantly targeting the ileocecal junction, where it can induce localized inflammation, impaired intestinal motility, gastrointestinal obstruction, colic, and, in severe cases, intestinal perforation. Diagnosis of the infection remains a challenge, as conventional coproscopic examinations exhibit limited sensitivity and parasite eggs are shed seasonally. Conversely, molecular and serological methods require specialized laboratory infrastructure, which restricts their utility in routine field diagnostics. Consequently, horses are frequently dewormed prophylactically without confirmation of an active infection, potentially promoting the unjustified use of antiparasitic drugs and contributing to the growing issue of drug resistance.

The objective of this project is to develop a rapid lateral flow immunochromatographic assay for the detection of *Anoplocephala perfoliata* antigens directly in equine fecal samples, designed for field use.

The obtained results confirmed the presence of numerous proteins with a molecular weight of approximately 12-13 kDa within the *A. perfoliata* excretory-secretory fraction and demonstrated their immunoreactivity, indicating their potential utility in the development of diagnostic tools. The LC-MS/MS analysis enabled the identification of additional parasite proteins present in equine fecal samples, which provides a basis for selecting additional antigens used in the active infection detection assay. The conducted studies establish a foundation for developing a rapid immunochromatographic assay capable of detecting *A. perfoliata* antigens directly in equine feces. Such a solution could enhance field diagnostics and reduce unnecessary prophylactic use of antiparasitic drugs.

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P064. TOWARDS THE FIRST POINT-OF-CARE SEROLOGICAL TEST FOR CANINE *Dirofilaria repens* INFECTION

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Subcutaneous dirofilariosis is a mosquito-borne disease of carnivores, primarily dogs, caused by the filarial nematode *Dirofilaria repens*. Owing to climate change and increasing animal movement, *D. repens* has become one of the fastest-spreading vector-borne pathogens in Europe and is also an emerging zoonotic agent [1]. Despite its growing importance, no commercial serological test is currently available. Therefore, this project aims to develop the first prototype point-of-care lateral flow assay for the serological detection of *D. repens* infection in dogs. Recombinant *D. repens* antigens expressed in *Escherichia coli* and synthetic peptides representing selected immunoreactive epitopes were evaluated by ELISA using sera from 95 naturally infected dogs and 117 dogs negative by the Knott test and qPCR [2]. ROC analysis was performed to evaluate diagnostic performance, and recombinant antigens and synthetic peptides were compared with the reference DrSA (whole-worm homogenate) ELISA to identify candidates for lateral flow assay development. One of the most promising synthetic peptides demonstrated diagnostic performance comparable to DrSA (AUC = 0.783 vs. 0.727), with higher specificity (68.4% vs. 53.8%) and only a slight reduction in sensitivity (81.1% vs. 84.2%). ELISA reactivity correlated significantly with DrSA (Spearman's $\rho = 0.469$, $p < 0.0001$). Diagnostic specificity should be interpreted with caution, as all dogs originated from endemic regions with unknown infection histories. Consequently, seropositive animals classified as negative by Knott test and qPCR may reflect previous parasite exposure or occult infection rather than non-specific antibody binding. The selected peptide candidates were successfully incorporated into prototype lateral flow assays and recognized by antibodies from infected dogs. Further validation using well-characterized serum panels, including *D. immitis*-positive sera, is required to evaluate cross-reactivity and identify species-specific epitopes. These findings provide proof-of-concept for the development of the first prototype point-of-care serological assay for *D. repens* infection in dogs.

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P065. FROM DROP TO DATA: PRELIMINARY STUDIES ON NOVEL QUANTITATIVE ASSAYS FOR PRL, TSH AND T4 IN RAT SERUM

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Accurate determination of prolactin (PRL), thyrotropin (TSH), and thyroxine (T4) levels is essential for assessing endocrine function, metabolic status, and neuroendocrine regulation in laboratory animals. These hormones serve as key biomarkers in biomedical and veterinary research. However, their routine measurement remains limited in practice. Currently, hormone determination relies mainly on enzyme-linked immunosorbent assays (ELISA) or automated biochemical analyzers. Despite their high sensitivity, these methods are associated with high costs, the need for specialized laboratory infrastructure, and prolonged turnaround times. Therefore, there is a growing demand for rapid, cost-effective, and easy-to-use diagnostic tools that enable quantitative measurements directly at the point of care. The aim of this study was to develop a panel of rapid assays for quantitative determination of PRL, TSH, and T4 in rat serum. Antigenic fragments of PRL and TSH were selected, synthesized, and cloned into the vector, followed by optimization of protein expression and purification. T4 conjugates with BSA and OVA were also obtained. Monoclonal antibodies were generated using hybridoma technology and screened for specificity. Selected high-affinity antibody pairs will be used to develop quantitative lateral flow assays, representing a promising point-of-care diagnostic solution for rapid hormone assessment.

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P066. DEVELOPMENT OF A POINT-OF-CARE MULTIPLEX qPCR ASSAY FOR *Dirofilaria repens*, *Dirofilaria immitis* AND *Leishmania* spp.

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Vector-borne parasitic diseases are an emerging challenge in Europe. Over recent decades, canine dirofilariosis has undergone substantial geographic expansion across the continent, becoming one of the fastest-spreading infectious diseases affecting dogs. Depending on the causative agent, dirofilariosis occurs as cardiopulmonary disease caused *by Dirofilaria immitis* or subcutaneous disease caused by *Dirofilaria repens* [1]. Canine leishmaniosis caused by *Leishmania* spp. is also increasingly reported beyond its traditional Mediterranean range [2]. As co-circulation of these zoonotic pathogens becomes more common, veterinarians require rapid diagnostic tools capable of detecting multiple infections from a single sample. The aim of this project is to develop and validate a multiplex qPCR assay for simultaneous detection and differentiation of *D. repens*, *D. immitis*, and *Leishmania* spp., followed by implementation on the QubeMDx point-of-care platform. Species-specific primers and MGB-ECLIPSE probes targeting mitochondrial DNA of *D. repens* and *D. immitis* were designed and validated using recombinant plasmids and DNA isolated from naturally infected and non-infected dogs. Diagnostic performance was evaluated against conventional PCR and the Knott test. Selection and validation of *Leishmania*-specific molecular targets are currently ongoing. Preliminary validation included 50 naturally infected and 50 non-infected dogs. The assay enabled reliable differentiation between *D. repens* and *D. immitis*, with no cross-reactivity observed for *Acanthocheilonema reconditum* or *Brugia pahangi*. It demonstrated 100% concordance with reference methods and correctly identified both single and mixed *Dirofilaria* infections. Amplification efficiencies reached 89.56% (*D. repens*) and 96.68% (*D. immitis*), with with R^2 values of 0.9996 and 0.9991, respectively. The analytical LOD95 for both targets was 1,000 recombinant plasmid copies per reaction, while the biological LOD95 for *D. repens* was established at a single microfilaria per DNA isolate. These findings support further completion of the multiplex assay and its implementation on the QubeMDx platform for rapid point-of-care molecular diagnosis of canine vector-borne diseases.

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07. New Frontiers in Point-of-Care Testing for Veterinary Medicine

P067. SPOTTING *Giardia* IN MINUTES: A NOVEL LATERAL FLOW ASSAY FOR DETECTION OF *Giardia* CYSTS IN CANINE AND FELINE FECAL SAMPLES

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Giardia duodenalis is one of the most common intestinal parasites affecting dogs and cats worldwide, posing a diagnostic challenge in routine veterinary practice. Although several diagnostic methods are available, rapid and reliable point-of-care detection of *Giardia* remains essential for accurate diagnosis and effective infection control. In this study, we present a novel lateral flow assay designed for detection of *Giardia* cysts in canine and feline fecal samples and compare its performance with commercially available diagnostic tests. The assay was evaluated using fecal samples from 71 dogs and 46 cats and validated against established diagnostic methods. When compared with commercially available immunochromatographic assays, the newly developed test demonstrated excellent performance, achieving a diagnostic sensitivity of 98%, specificity of 100%, and overall accuracy of 100%. In comparison with enzyme-linked immunosorbent assay (ELISA) methods, the test achieved a sensitivity of 87%, specificity of 62%, and overall accuracy of 78%. The newly developed test demonstrated high diagnostic sensitivity, specificity, and overall accuracy, providing reliable detection of *Giardia* cysts across diverse clinical fecal samples. The results highlight the assay's potential as a practical and robust diagnostic tool for routine veterinary use, offering a rapid and convenient solution for the diagnosis of giardiasis in companion animals and supporting effective parasite control strategies.

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P068. ASSOCIATION BETWEEN COMPLETE BLOOD COUNT PARAMETERS AND PCR POSITIVITY IN HORSES TESTED FOR EQUINE *Piroplasmosis*

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This study aimed to determine whether routine complete blood count (CBC) parameters can discriminate between horses infected and non-infected with equine piroplasmosis, regardless of clinical status. Molecular testing was performed using the VETFIRE™ panel, a sample-to-answer diagnostic test combining automated nucleic acid extraction with real-time multiplex PCR for the detection of *Theileria equi* and *Babesia caballi*. A retrospective analysis was conducted on 56 equine blood samples collected in France for which CBC data, clinical status, and PCR results were available. Nineteen horses were classified as symptomatic of which ten tested positive for at least one piroplasmosis agent (*T. equi*, n = 7; *B. caballi*, n = 3), including three co-infected horses, while six tested PCR-negative. Among the 37 asymptomatic horses, ten were positive for *T. equi*, two were co-infected with both pathogens, and 25 tested negative. PCR positivity was defined as the detection of at least one pathogen, including both mono-infected and co-infected animals. Hematological parameters were analyzed as continuous variables. Comparisons between PCR-positive and PCR-negative horses were performed using Kruskal–Wallis and chi-square tests, after stratification by clinical status. Among symptomatic horses, PCR-positive animals showed lower median erythrocytic parameters compared with PCR-negative horses, suggesting an anemia-related trend; and, among asymptomatic horses, PCR-positive animals showed higher median white blood cells parameters compared with PCR-negative horses. However, these observed differences did not reach statistical significance ($p > 0.05$). Overall, these findings indicate that although hematological alterations may accompany equine piroplasmosis in clinically affected horses, CBC parameters alone are insufficient to reliably distinguish infected from non-infected animals. CBC findings may support clinical suspicion but cannot replace pathogen-specific testing. Importantly, CBC analysis also fails to identify asymptomatic carrier horses, which are reported to represent a substantial proportion of the equine population in France[1], highlighting the essential role of molecular diagnostics.

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P069. DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR *Chlamydia abortus* DETECTION

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Ovine enzootic abortion (OEA), caused by *Chlamydia abortus*, remains a major infectious cause of abortion in sheep despite available vaccines and control measures. Annual losses are estimated at up to £20 million, and surveillance data suggest that *C. abortus* is associated with approximately 35% of confirmed ovine abortion diagnoses. Abortions occur in the final 2 to 3 weeks of pregnancy, with transmission linked to contaminated placenta, foetal tissues and parturition fluids. Rapid identification supports veterinary decision-making, biosecurity and the reduction of onward transmission. This study aimed to develop a loop-mediated isothermal amplification (LAMP) assay for the detection of *C. abortus* as a rapid test for laboratory use and future point-of-care application. A conserved *C. abortus* surface-protein gene target was selected. Candidate primer sets were designed, screened using positive-control material, and optimised in fluorescence and colourimetric-based formats using commercial LAMP reagent systems. Simplified sample preparation and a direct extraction approach compatible with placental material were evaluated. Analytical sensitivity was assessed using serial dilutions, while specificity was evaluated using 69 chlamydial isolates, including mammalian and avian *C. abortus* strains, and 20 unrelated bacterial species. Candidate designs showed variable amplification performance, and a lead assay was selected. The optimised MOMP LAMP assay detected *C. abortus* DNA under isothermal conditions, with an analytical sensitivity of 10^4 genomic copies/µL and LAMP time-to-positivity of 30 minutes. Combined with direct extraction, the extraction-to-result time was 1 hour 15 minutes. The assay generated interpretable fluorescence-based and colourimetric results. Specificity testing supported selective detection of mammalian *C. abortus* strains, with no amplification of avian *C. abortus*, related *Chlamydia* species or unrelated bacteria. Blind scoring of qPCR-defined samples by scientific and non-scientific observers demonstrated good inter-observer agreement. These findings support LAMP as a rapid platform for *C. abortus* detection, with potential to strengthen diagnostic access and inform flock-level control decisions.

P070. EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE VCHECK C10 IN-CLINIC CHEMISTRY ANALYZER FOR CANINE BIOCHEMICAL TESTING

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With the rapid expansion of point-of-care testing in veterinary diagnostic medicine, the need for rigorous analytical validation of newly developed in-clinic diagnostic analyzers has become increasingly important. This study evaluated the analytical performance of the Vcheck C10 dry chemistry analyzer in canine blood samples. Analytical precision was assessed using pooled canine serum, and method comparison was performed using 85 canine serum samples measured for 16 biochemical analytes, with AU480 wet chemistry analyzer serving as the reference method. Passing–Bablok regression and Bland–Altman analysis were used to assess correlation and agreement, and percent bias were calculated. Serum–plasma agreement was evaluated using 11 paired samples. The Vcheck C10 demonstrated acceptable precision for all analytes (CV <10%) except LIPA. Strong correlations were observed for all 16 analytes (r = 0.85–0.99), although several demonstrated constant (AST, CREA, LIPA, PHOS, TP, TRIG) or proportional errors (ALP, ALT, AST, CA, LIPA, TRIG). Bland–Altman analysis indicated small mean biases for most analytes, whereas 6 analytes (ALP, AST, CREA, GGT, TBIL, and TP) exceeded TE_a thresholds. Serum–plasma results showed high concordance for most analytes, with two showing weaker correlations. Overall, the Vcheck C10 demonstrated clinically acceptable analytical performance for most routine analytes, though analyzer-specific interpretation may be required for some showing substantial bias, which can be addressed by establishing instrument-specific reference intervals.

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P072. A SALT-TOLERANT, RAPID, AND LYOPHILIZATION-COMPATIBLE Bst POLYMERASE FOR NEXT-GENERATION LAMP DIAGNOSTICS

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Quantitative PCR (qPCR) remains the gold standard in molecular diagnostics due to its high sensitivity and specificity; however, its dependence on thermocycling, sophisticated instrumentation, and trained personnel limits its use in decentralized and point-of-care (PoC) settings. Loop-mediated isothermal amplification (LAMP) provides a powerful alternative by enabling nucleic acid amplification at a constant temperature (60–65 °C), reducing equipment requirements and delivering rapid results while maintaining sensitivity and specificity comparable to qPCR in many applications. LAMP also exhibits increased tolerance to inhibitors present in crude or minimally processed samples and supports multiple detection formats, including fluorescence- and colorimetric-based readouts, making it well suited for field-deployable veterinary diagnostics and outbreak response.
The performance of LAMP assays is strongly dependent on the underlying strand-displacing DNA polymerase. So, we developed our Robust Bst Polymerase, a proprietary recombinant Bst exo(-) enzyme engineered for enhanced speed, robustness, and versatility. Produced through optimized recombinant expression and purification processes, the enzyme achieves >95% purity and is free of contaminating nuclease activities. Unlike conventional Bst enzymes, it demonstrates exceptional salt tolerance, showing increased activity between 70–110 mM KCl and maintaining functionality at concentrations up to 150 mM, thereby enabling reliable amplification from challenging sample matrices. The enzyme supports amplification with time-to-result in under 10 minutes and possesses intrinsic reverse transcriptase activity, enabling both DNA and RNA detection within a simplified single-enzyme workflow. Combined with compatibility for lyophilized formulations, these features support next-generation, portable molecular diagnostic platforms for rapid and reliable testing in resource-limited and decentralized environments.

P073. A NEW METHOD FOR DETECTING TRICHINELLOSIS INFECTION

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Trichinellosis remains one of the most important foodborne parasitic zoonoses worldwide and represents a persistent One Health challenge at the interface of wildlife, domestic animals, food safety, and human health. Infection occurs through consumption of raw or undercooked meat containing Trichinella larvae, primarily pork and game. Due to the lack of specific clinical signs in infected animals, reliable meat testing remains essential.

The currently recommended magnetic stirrer method, based on artificial digestion followed by microscopic detection of larvae, requires specialized equipment, trained personnel, and time-consuming sample preparation. Therefore, there is a need for rapid and accessible diagnostic tools that could support Trichinella surveillance, particularly in settings where laboratory facilities are limited.

The aim of this study is to develop a rapid antigen-based lateral flow assay for the detection of Trichinella spp. occurring in Europe (T. spiralis, T. britovi, T. nativa and T. pseudospiralis). The study involves optimization of the parasite recovery procedure and identification of suitable antigenic targets for immunochromatographic detection. To accelerate larval recovery, the conventional sedimentation step was replaced with a filtration-based approach. In parallel, bioinformatic analyses combined with a literature review were performed to identify conserved and diagnostically relevant antigen candidates suitable as targets for Trichinella detection.

The preliminary results indicate that filtration-based larval recovery significantly shorten the sample preparation stage compared with the conventional sedimentation procedure. Moreover, the selected antigen candidate showed promising characteristics for application in a lateral flow assay designed for detection of Trichinella infection. Further validation studies are required to determine diagnostic sensitivity and specificity.

The proposed assay is intended as a rapid screening tool supporting official diagnostics, particularly in situations requiring fast preliminary assessment outside specialized laboratory settings (survival/war conditions). Such a method could improve accessibility of Trichinella screening and strengthen surveillance capacity within veterinary public health and food safety systems.

P074. OPTIMIZATION OF SEROLOGICAL ASSAYS FOR ENHANCED DETECTION OF INFECTIOUS *Bovine Rhinotracheitis*

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Introduction. Infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus type 1 (BoHV-1), is a significant cattle pathogen with major economic impact. Control relies on DIVA (Differentiating Infected from Vaccinated Animals) strategies using marker vaccines lacking glycoprotein E (gE). Vaccinated animals remain gE-seronegative, whereas infected animals develop anti-gE antibodies. Serological diagnosis combines gE-specific, gB, and total antibody assays, enabling accurate determination of infection status and vaccine response. This integrated approach supports effective herd-health management, targeted control, and eradication programs, facilitating improved monitoring of IBR in cattle worldwide. For this reason, in this work we aimed to improve Gold Standard Diagnostics' serological tools for IBR diagnosis.
Methodology. Commercially available assays were updated to better match diagnostic needs. After optimizing the three ELISAs, the new INgezim IBR gE (anti-gE antibody detection), INgezim IBR Compac 3.0 (anti-gB antibody detection), and INgezim IBR 3.0 (total antibody detection) were evaluated using new panels of milk, tank milk, and serum samples.
Results. The new version of the INgezim IBR gE showed an increased specificity when compared to previous versions of the kit, exhibiting a 99.6% specificity in a group of 488 interfering sera, and 92% specificity in a group of 386 interfering milk and tank milk samples, while conserving its good sensitivity. The INgezim IBR Compac 3.0 exhibited a specificity of 98% in a panel of 223 interfering sera and 97% specificity in a panel of 170 interfering milk samples, maintaining good sensitivity. Last, the INgezim IBR 3.0, which is still under final validations, showed an overall improvement in its diagnostic performance in sera and milk samples.
Conclusion. The enhanced serological tools described demonstrated significantly improved specificity, accurately distinguishing infected from healthy and vaccinated cattle. This precision strengthens IBR monitoring, informs targeted control measures, and provides a reliable, practical advancement for herd health management and eradication efforts.

P075. APPLICATION OF 3RS IN VITRO METHODS FOR VETERINARY AUTOGENOUS VACCINES QUALITY CONTROL: THE TEN-YEAR EXPERIENCE OF ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'UMBRIA E DELLE MARCHE "TOGO ROSATI"

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Veterinary autogenous vaccines (AVs) play an important role in control & prevention of animal diseases are an efficient tool against antibiotic resistance. AVs, as all injectable products, undergo to quality control process (QC) to ensure their safety and suitability. The acceptance of the 3Rs principle is leading to the adoption of *in vitro* methods (i.e. Limulus amoebocyte lysate (LAL) test, Monocyte Activation Test (MAT) and cytotoxicity assay MTS) in several QC processes.
In this work we report a ten-year activity on the application of three different *in vitro* methods as QC assays for AVs produced at IZSUM.
LAL test, the gold standard assay for Lipopolysaccharide detection in injectable molecules, was adopted for endotoxin load in gram-negative Avs matrices. MAT was performed on immortalized murine monocyte–macrophage cell line as animal-based alternative method for pyrogens detection.
Finally, MTS cell-based assay, was adopted to analyze changes in cell life/metabolism after exposition to different injectable mixtures.
At our unit, since 2020, all gram negative Avs lots were exclusively submitted to LAL test, while MAT and MTS were adopted in parallel to *in vivo* assay for gram positive and viral Avs.
All Avs tested showed different concentration of endotoxin among different strains. MAT assay highlighted increasing in pro-inflammatory cytokines production: among the biomarkers investigated TNF-α showed higher levels, followed by IL-6, and IL-1β, respectively.
Finally, a safe AVS dilution (70% cell viability) was defined by cell-based MTS after Avs exposition.
Application of *in vitro* methods for AVs QC instead of *in vivo* pyrogen test could determine a substantial replacement in the use of animals. Data collected during this 10-year activity should be updated, implementing collaboration with other laboratories performing ring test.
The final perspective is the application of different harmonized and validated alternative tests for QC. Funded by Italian Ministry of Health, IZSUM PALTEX22-PMETAL25.

P076. SAFE TRANSPORT, STABLE RESULTS: VIRAL INACTIVATION AND NUCLEIC ACID PRESERVATION FOR VETERINARY DIAGNOSTICS

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In veterinary molecular diagnostics, ensuring biosafety and nucleic acid stability during the preanalytical phase is critical, especially when samples are collected in field conditions and transported under variable environments. Transport media that combine pathogen inactivation with molecular compatibility are increasingly needed. This study evaluates Copan UBM™, a novel guanidine-free inactivating medium, in comparison with conventional non-inactivating viral transport media for molecular detection and biosafety. The inactivation performance of Copan UBM™ was assessed using a panel of enveloped and non-enveloped viruses, including SARS-CoV-2, Influenza A, RSV, Adenovirus, HSV1, and Rotavirus, following 10-minute incubation and infectivity evaluation in cell culture. In parallel, molecular performance was compared against conventional viral transport media using standardized viral suspensions (Influenza A, RSV, SARS-CoV-2), tested at baseline (T0) and after 48 hours at room temperature using Xpert Xpress Flu/RSV/SARS-CoV-2 assays (Cepheid). Additional compatibility testing was performed using PrepSEQ™ Express Kit (Thermo Fisher) and RIDAGENE / QIAGEN assays. Copan UBM™ demonstrated effective viral inactivation, reducing viral titer by >3 $\log_{10}$ for enveloped viruses and >2.5 $\log_{10}$ for non-enveloped viruses, indicating a strong reduction in infectivity. Molecular analysis showed high stability of nucleic acids over time, with minimal variation in Ct values. Compared to conventional media, Copan UBM™ maintained stable Ct values after 48 hours (ΔCt close to zero), while other media showed higher variability, up to ΔCt ~1.5–1.7 depending on the target. Across extended storage conditions, ΔCt remained below 3, indicating no significant degradation and full compatibility with molecular platforms. Copan UBM™ ensures effective viral inactivation and preservation of nucleic acids, supporting safe handling and reliable molecular diagnostics in veterinary settings. Its stability under variable conditions makes it suitable for decentralized sample collection and transport workflows.

P077. MOLECULAR SURVEILLANCE OF ORTHOAVULAVIRUS JAVAENSE AND *Chlamydia psittaci* IN FERAL PIGEONS UNDER DIFFERENT MANAGEMENT SYSTEMS IN SWITZERLAND

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Orthoavulavirus javaense (OAVJ) poses a significant threat to domestic poultry, where infection can result in Newcastle disease, classified as highly contagious animal disease in Switzerland requiring flock culling upon detection. Occasional human infections have been reported, indicating zoonotic potential. *Chlamydia psittaci* is the causative agent of avian chlamydiosis, also notifiable, and can lead to psittacosis/ ornithosis in humans following inhalation of contaminated aerosols from infected birds.
This study investigates the prevalence of pigeon strains of OAVJ (also known as pigeon paramyxovirus-1) and *C. psittaci* in feral pigeons (*Columba livia forma domestica*) across 11 Swiss cities implementing different pigeon management strategies (managed lofts vs. culling). Choanal and cloacal swabs were collected from live pigeons in lofts and from birds culled in the context of population control. Samples were analyzed using real-time PCR (rt-PCR). A novel rt-PCR assay for the detection of the F gene [1] was developed to improve the detection of currently circulating OAVJ strains. The study aims to (i) provide an evidence-based assessment of the risk of OAVJ transmission to domestic poultry [2] and (ii) evaluate associations between pigeon management systems and pathogen shedding, particularly of zoonotic relevance.
Preliminary results indicate a geographically widespread distribution of *C. psittaci*, with an average prevalence of 8.7 % in sampled pigeons. In contrast, OAVJ detection appears sporadic in cities with managed pigeon lofts but suggests more consistent circulation in cities lacking such management. Furthermore, there are clear differences between cities with managed pigeon lofts and those without. In cities with pigeon lofts, *C. psittaci* was detected twice as often and OAVJ 16 times more frequently than in cities without them.
These findings highlight the importance of targeted molecular diagnostics in surveillance programs and contribute to risk assessment for both animal and public health.

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09. Diagnostics for Wildlife Diseases Challenges and Emerging Solutions

P078. WILDLIFE AS SENTINELS OF DERMATOPHYTOSES: A 15-YEAR MOLECULAR SURVEY IN ONE HEALTH PERSPECTIVE

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Dermatophytes are among the most common causes of mycoses in both humans and animals, and their epidemiology is strongly influenced by changing patterns of human–animal interaction. Transmission may occur through direct contact or indirectly via contaminated environments, as fungal spores can persist for extended periods. The progressive expansion of urbanized areas increases interactions between humans and wildlife, leading to the emergence of novel ecosystems and raising concerns about the transmission of zoonotic pathogens, including dermatophytes (1). In this context, wild animals play a dual role as reservoirs of infectious agents and as sentinels of ecosystem health within a One Health context (2). This study aimed to investigate the occurrence and molecular characterization of dermatophyte species in wild fauna collected between 2010 and 2024 through passive surveillance and targeted monitoring programs. A total of 704 samples were analyzed using conventional mycological methods and molecular assays, such as PCR and Sanger sequencing (Figure 1). Dermatophytes were detected in 77 animals (10.94%). Molecular identification revealed *Paraphyton mirabile* (5.96%) as the most prevalent species, followed by the *Trichophyton mentagrophytes* complex (2.41%), and *Microsporum canis* (0.71%), along with *Arthroderma curreyi* and *A. thuringiense* (0.57% each), and *A. uncinatum* and *A. eboreum* (0.43% each). A co-infection involving *T. mentagrophytes* and *M. canis* was identified in a red squirrel (Figure 2). Positive cases were primarily observed in squirrels (n=66), but also in red foxes (n=8), in one European badger, one European hedgehog, and one crested porcupine (3). These findings underscore the importance of continuous surveillance, particularly for individuals in close contact with wildlife, as well as the citizens. Monitoring wildlife health is therefore essential to prevent zoonotic outbreaks and to better understand the dynamics of dermatophyte infections in increasingly anthropized environments.

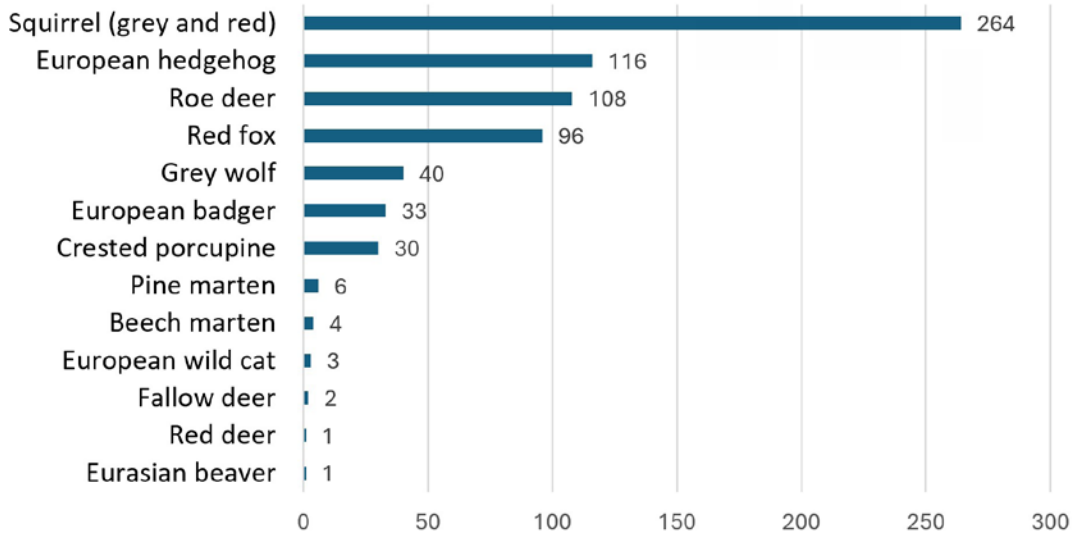


Figure 1: Distribution of wildlife species sampled from 2010 to 2024.

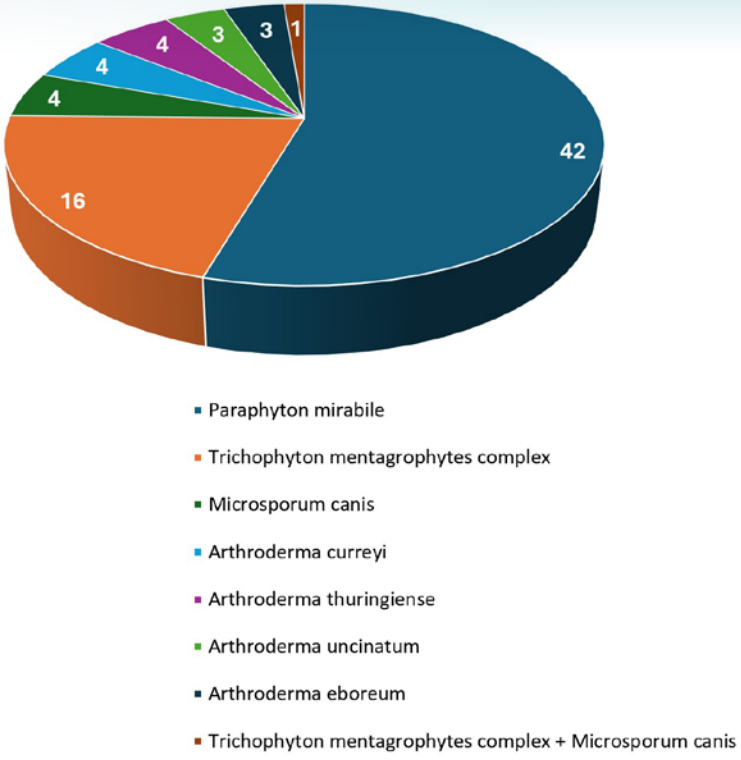


Figure 2: Dermatophyte species isolated from samples.

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P079. SEQUENCE TYPE DIVERSITY OF *Ornithobacterium rhinotracheale* ISOLATES REVEALED BY WHOLE GENOME SEQUENCING

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Ornithobacterium rhinotracheale (ORT) is an important poultry pathogen associated with respiratory diseases, decreased production performance, and significant economic losses. Despite the growing importance of this microorganism, knowledge regarding the genomic diversity of ORT isolates and the pathogenic potential of individual sequence types (STs) remains limited.
The aim of this study was to characterize ORT isolates using whole genome sequencing (WGS), with particular emphasis on sequence type diversity and the association between STs and selected virulence- and antimicrobial resistance-related genes.
ORT isolates obtained from poultry flocks were subjected to WGS followed by phylogenomic and comparative genomic analyses. Sequence typing revealed the presence of distinct ST groups clustering separately within the phylogenetic tree. Based on WGS data, it was possible to assign characteristic genomic profiles to individual STs, including genes associated with adhesion, biofilm formation, iron acquisition, and toxin-related determinants. Additionally, antimicrobial resistance-associated genes and mutations were identified and compared between sequence types.
The analysis demonstrated clear genomic diversity among ORT isolates, with selected virulence-associated genes being unevenly distributed between ST groups. Some sequence types were characterized by broader virulence-associated gene repertoires, including genes potentially involved in colonization and persistence within the respiratory tract. Variability in antimicrobial resistance determinants was also observed between phylogenetic clusters, suggesting ST-dependent adaptation and evolution of ORT strains circulating in poultry production systems.
The study demonstrates that WGS-based sequence typing provides valuable epidemiological insight into the population structure of ORT and enables detailed characterization of strain-specific virulence and resistance profiles. Integration of phylogenomic analysis with molecular characterization may improve understanding of ORT epidemiology and support the development of more effective diagnostic and control strategies in poultry flocks.

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P080. INVESTIGATION OF CANINE DISTEMPER VIRUS IN GOLDEN JACKAL *(Canis aureus)* AND RED FOX *(Vulpes vulpes)* IN HUNGARY

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Canine distemper virus (CDV) is a globally distributed pathogen of considerable veterinary and conservation relevance. The infection affects numerous domestic and wild carnivore species, and due to its broad host range and diverse clinical manifestations, it is of major epidemiological and genomic importance. In domestic dogs, the disease is well characterized, and effective vaccines are available, however, viral circulation in wildlife populations may constitute a continuous source of infection. The spread of the pathogen is facilitated by the natural movement and population dynamics of wild animals, enabling transboundary transmission. The present study aimed to investigate the occurrence and genetic characteristics of CDV in the golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*) populations in Hungary. A total of n=234 spleen samples were collected and analyzed from Southern Transdanubia, Hungary, between 2021 and 2025. Viral RNA detection was performed using real-time RT-PCR. Positive samples were sequenced using an amplicon-based complete-genome approach with Oxford Nanopore technology (1), followed by phylogenetic analysis to determine CDV lineages. CDV infection was confirmed in three cases, comprising one red fox sampled in 2021 and two golden jackals sampled in 2021 and 2022, respectively. All detected strains belonged to the Europe lineage based on phylogenetic analysis. The Europe lineage is considered endemic in the region, with sustained circulation among wild carnivore populations (2). The results provide novel genomic data on CDV strains circulating in Hungarian carnivores and contribute to a more precise understanding of regional epidemiological patterns. These findings highlight the role of wildlife as a reservoir and underline the importance of integrated surveillance strategies, supporting both conservation efforts and the prevention of spillover to domestic animal populations.

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09. Diagnostics for Wildlife Diseases Challenges and Emerging Solutions

P081. NON-INVASIVE AND CONVENTIONAL PATHOGEN SURVEILLANCE IN IBERIAN IBEX *(Capra pyrenaica)*: A COMPARATIVE APPROACH

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Since 2022, health surveillance programs for Iberian ibex (*Capra pyrenaica*) in the Sierra de Guadarrama (Community of Madrid) have been implemented to monitor infectious diseases within the framework of long-term population monitoring. Following initial reintroductions, the population experienced exponential growth, reaching maximum density and expansion between 2017 and 2019, after which growth stabilized and changed towards a declining trend. This recent decrease, faster than expected under management plans, highlights the importance of continued health surveillance.

This study represents the results of a 2024-2025 monitoring program combining conventional sampling (necropsy and biological samples of captured animals) with non-invasive environmental sampling based on environmental nucleic acid detection (ENAD) in the framework of the PID2024-159558OB-I00 project (financed by MICIU/AEI /10.13039/501100011033 and FEDER). The aim was to evaluate the usefulness of environmental sampling to detect microorganisms of epidemiological relevance (e.g. *Brucella* spp., *Mycobacterium tuberculosis* complex, *Mycobacterium avium* subsp. *paratuberculosis*, *Coxiella burnetii*, *Salmonella enterica*, and pathogenic *Escherichia coli*) and compare it with direct diagnostic approaches in this epidemiological context.

Environmental DNA was analyzed using a real time PCR targeting specific molecular markers depending on the pathogen. Results from both approaches were consistent as no evidence of *Brucella* spp., *Coxiella burnetii*, *Mycobacterium avium* subsp. paratuberculosis, or *Salmonella enterica* was detected. Two environmental samples tested positive for *E. coli* strains producing toxins with pathogenic potential, agreeing with the findings from direct sampling. Results for *Mycobacterium tuberculosis* complex were inconclusive, highlighting the diagnostic limitations based on molecular detection on environmental samples.

These preliminary findings support the use of non-invasive environmental sampling as a complementary tool for pathogen surveillance in *Iberian ibex*. The agreement observed between both methods suggests that ENAD-based approaches can provide interesting results highlighting their combination with traditional methods, which remains essential, particularly for confirmatory diagnosis.

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P082. THE RETURN OF *Mycobacterium caprae* TUBERCULOSIS TO THE BIESZCZADY BISON POPULATION (SOUTHERN POLAND)

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Tuberculosis is highly contagious chronic disease of humans and animals. Although Poland has held bovine tuberculosis (BTB)-free status for 17 years, several to a dozen cases are reported annually in cattle. Some areas in Poland have endemic BTB in wild animals. Between 1996 and 2013, 45 cases of bovine tuberculosis were confirmed in European bison (*Bison bonasus caucasicus*) in the Bieszczady Mountains. In 2025, tissue samples from 33 European bison were tested for BTB in Poland. The disease has now reappeared for the third time, affecting a herd in a demonstration enclosure in Muczne. Here we report a severe case of multisystem TB in an affected animal from this enclosure, for which comprehensive examinations were performed. By decision of the General Director for Environmental Protection, 12 infected bison were euthanized to prevent the spread of zoonotic bacteria. Histopathological examination, bacterial culture with identification (GenoType MTBC, Hain Lifescience), and spoligotyping (Gentaur molecular products, Kampenhout, Belgium) of obtained isolate were performed. Tests confirmed multisystem tuberculosis due to *Mycobacterium caprae*, in the animal described in the case. Histopathology revealed multiple granulomas in different tissues, characterised by caseous necrosis surrounded by epithelioid macrophages with occasional giant cells, rimmed by lymphocytes and fibroblasts. Bacilli presence in the tissues was confirmed with Ziehl – Nielsen staining. SB2391 spoligotype was assessed, the same as previously reported in the area. Tuberculosis poses a serious threat to the Bieszczady bison population, as well as to other susceptible forest game. According to the March 2026 inventory, a record number of 880 bison already live in the Polish part of the Bieszczady Mountains. Near the border areas, the total number in the region exceeds 900 individuals, and population is constantly growing, which pleases naturalists, but also raises concerns about overcrowding of the herd and spread of diseases to which bison are susceptible.

11. The Future of Vaccine Development Insights from Diagnostics

P083. USE OF VIABLE BACTERIA DETECTION TO ASSESS LONG TERM EFFICACY OF *Brucella abortus* RB51 VACCINATION IN PREGNANT WATER BUFFALOES EXPOSED TO CHALLENGE

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RB51 vaccine is live attenuated rough mutant derived from virulent *Brucella abortus* 2308 strain. As a DIVA-like vaccine, RB51 distinguishes vaccinated from naturally infected animals, supporting vaccination alongside test-and-slaughter programs (1). The study's aim was to assess long-term efficacy of RB51 vaccination protocol and applied to young (6-9 months) buffaloes (Italian Ministry of Health Authorization n° 524/2024-PR) (Funded by Italian Ministry of Health project grant IZSAM 09/19 RC and IZSAM 04/22 RC). The protocol followed WOAH Manual, with modifications including the administration of a triple vaccine dose followed by a booster 30 days apart (1,2). 10 vaccinated and 6 unvaccinated pregnant water buffaloes were included (3). Following experimental infection with *B. abortus biovar 1* (10^8 CFU), both dams and their aborted foetuses or live born calves were examined. Bacterial load was assessed to determine viable bacteria presence and distribution. Isolation and enumeration were conducted on different organs (brain, spleen, lung, abomasum), lymph nodes, foetal tissues. Over a two-year period were analyzed: 20 foetal, 54 lymph node, and 29 organ samples. Three additional animals are pending slaughter. 47.57% vaccinated and 33.98% unvaccinated samples tested negative, while 17.48% unvaccinated and 0.97% vaccinated tested positive (figure 1). 36.84% of positive cases originated from foetuses, 21.05% from lymph nodes, and 42.11% from organs (figure 2). Among positive samples, 73.68% showed bacterial loads exceeding 100,000 CFU/g, 10.53% around 1,000 CFU/g, and 15.79% around 100 CFU/g (figure 3). Notably, vaccinated animals exhibited no clinical signs of disease, apart from measurable immunological responses. In the only vaccinated animal with bacterial detection, *B. abortus* biovar 1 was found only in the retropharyngeal lymph nodes, suggesting effective containment near the entry site.
RB51 vaccination in water buffaloes markedly reduces bacterial burden and systemic dissemination, thereby supporting its effectiveness as a key component of integrated brucellosis control and eradication programs.

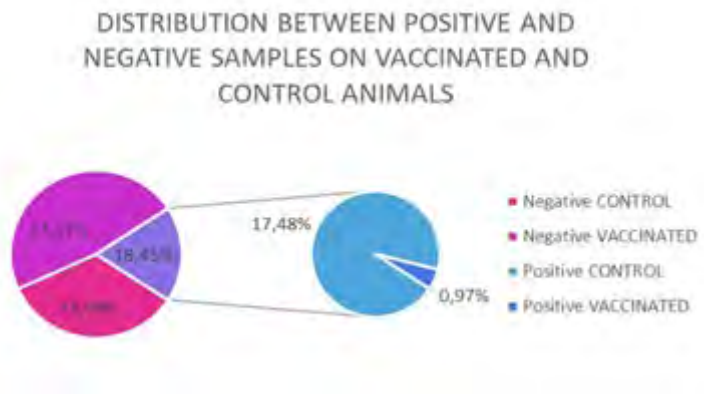


Figure 1: distribution between positive and negative samples on vaccinated and unvaccinated animals.

11. The Future of Vaccine Development Insights from Diagnostics

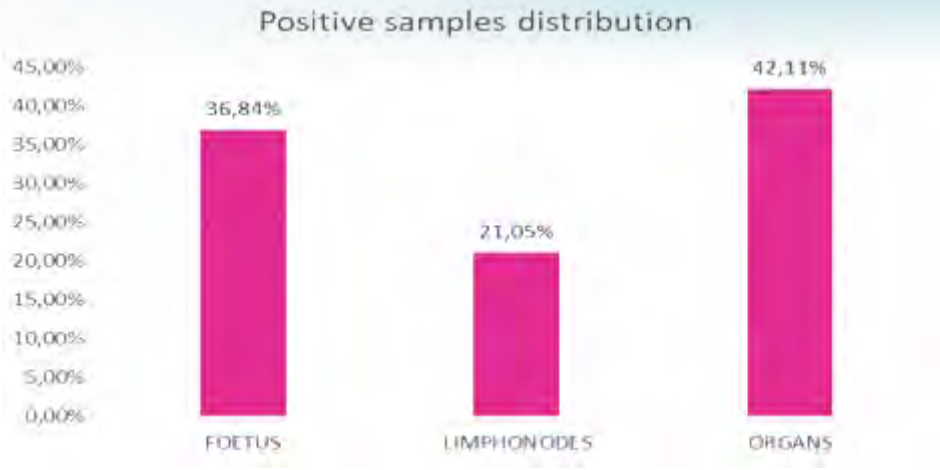


Figure 2: Distribution of positive samples across the various examined matrices.



Figure 3: number of CFU/g detected in the positive samples.

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13. Other

P084. LONGITUDINAL PHYLOGENOMICS OF Non-*aureus Staphylococci* IN DAIRY GOATS: STRAIN PERSISTENCE, TRANSMISSION DYNAMICS, ANTIMICROBIAL RESISTANCE, AND VIRULENCE CHARACTERISTICS

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Non-aureus staphylococci (NAS) are the main bacteria isolated from dairy goat milk[1,2,3]. We applied longitudinal genomics to assess whether NAS colonizing primiparous goats establish persistent, strain-specific intramammary infections (IMI) throughout lactation. *Staphylococcus caprae*, *S. simulans*, and *S. xylosus* were selected for their high prevalence, repetitive isolation, and association with increased milk somatic cell counts[3]. Half-udder milk isolates were obtained at early-mid lactation (T0) and dry-off (TF) across three commercial dairy herds. A total of 48 isolates identified by MALDI-TOF MS, representing 24 T0–TF pairs, were subjected to long-read Nanopore whole genome sequencing (WGS), core-genome phylogenomic reconstruction, virulome profiling, and resistome characterization. Biofilm production was quantified *in vitro* by microtiter plate assay. Longitudinal phylogenomics demonstrated remarkable clonal persistence across lactation for all species, with paired T0–TF isolates consistently clustering as identical sister taxa. *S. caprae* showed tight genetic conservation (scale 0.01) with herd-specific monophyletic clades and an epidemic multi-herd lineage. In-lactation strain replacement supported contagious transmission. WGS resolved *S. xylosus* isolates into distinct *S. xylosus* and *S. shinii* lineages. Both species, as well as *S. simulans*, displayed phylogenetic divergence between individual pairs and long-branched topologies, consistent with opportunistic pathogen behavior. *S. caprae* possessed an anti-neutrophil arsenal including aureolysin, the *irgAB* operon, T7SS, a leukocidin, and dual *nuc* genes, targeting antimicrobial peptides, immune cells, and neutrophil extracellular traps. The *ica* operon was defective, in line with minimal biofilm formation *in vitro*. The *S. xylosus/shinii* complex featured a defective *ica* operon but carried biofilm protein genes (*cbp/bap*) and formed biofilm *in vitro*. The antimicrobial resistance gene *tet*K was present in all species (20.8%), while only *S. shinii* carried *mph*C. In conclusion, the investigated NAS species, especially *S. caprae*, act as true pathogens able to establish chronic IMI persisting until dry-off, highlighting their key role in caprine udder health and mastitis epidemiology.

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13. Other

P085. DYNAMIC EVOLUTION AND SHIFTING PREVALENCE OF *Canine Parvovirus* TYPE 2 (CPV-2) VARIANTS IN ITALY (2022-2025)

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Canine Parvovirus type 2 (CPV-2) continues to be a major enteric pathogen in dogs worldwide. This study presents molecular surveillance data on CPV-2, examining organ samples from 387 dogs collected over four years (2022-2025). Standardized nucleic acid extraction and molecular assays were employed for the detection and characterization of CPV-2 variants. All dogs tested negative for CPV-2 vaccine strains.

CPV-2 was detected in 51.4% (199) of the dogs. Of the positive cases, the majority 155 (77.9%) presented a single CPV-2 variant infection, while a significant minority 44 (22.1%) were coinfected with two or more variants. Analysis of individual variants revealed CPV-2b as the most frequently detected single variant 67 (33.7%), followed by CPV-2a 48 (24.1%) and CPV-2c 35 (17.6%). A small percentage 5 (2.5%) of detected CPV-2 variants could not be definitively typed. Coinfection patterns indicated that the CPV-2b and CPV-2c combination was most common (11.5%), with other pairwise combinations also identified (CPV-2a and CPV-2b; CPV-2a and CPV-2c). Rare instances of triple infections (CPV-2a, CPV-2b, and CPV-2c) were observed, exclusively in 2022.

Temporal analysis of CPV-2 detection rates across the years indicated a notable peak in 2023, with 65.9% (89) of infected dogs identified in that year. The prevalence in the other years, while lower, remained comparable: 41.7% in 2022, 46.4% in 2024, and 44.4% in 2025. CPV-2b demonstrated a consistent increase in prevalence, establishing itself as the predominant genotype throughout the study duration. Interestingly, the proportion of single infections tended to increase over time, while the proportion of coinfected animals decreased.

These findings underscore the dynamic nature of CPV-2 evolution, highlighting the need for ongoing surveillance to monitor emerging variants. An extension of surveillance to other susceptible domestic animals and wild carnivores would be desirable to better understand the complex ecology of CPV-2 and develop effective disease control strategies.

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This study was funded by the Italian Ministry of Health: IZS ME 08/23 RC, recipient Flora Alfano.

13. Other

P086. IDENTIFICATION OF THE HISTORICAL MALARIA VECTOR *Anopheles labranchiae* (*An. maculipennis* COMPLEX) FROM SOUTH ITALY BY MALDI-TOF MS SPECTROMETRY

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Human malaria is a mosquito-transmitted disease caused by four *Plasmodium* species. Imported cases are annually recorded in countries no longer endemic, including Italy due to international travel and migratory flows (1). In Italy, the historical vector *Anopheles labranchiae* (*An. maculipennis* complex) is still present with different densities in scattered foci, including the coasts of the Apulia and Basilicata regions, such as recently reported (2). Because the *An. maculipennis* complex members are morphologically indistinguishable, their identification currently relies on molecular methods. However, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is increasingly emerging as a rapid and reliable alternative for arthropod identification (3). This study reports preliminary data on the application of this technique to the identification of *An. labranchiae*. A total of 24 female mosquito specimens collected (CDC traps and aspirators) from seven different sites in Apulia and Basilicata regions (Fig.1) and identified as *An. labranchiae* by molecular methods were analyzed. Heads and legs from each specimen were subjected to protein extraction following a previously standardized protocol (4). MALDI-TOF MS analysis was performed by acquiring spectra in linear positive mode over a mass range of 2–20 kDa. The resulting spectra were used to generate a reference library. Mass spectra obtained from both heads and legs (Fig.2) showed high intra-species similarity among specimens; conversely, they differed from spectra of other mosquito species available in the reference in-house database indicating a good discriminatory power. Spectra obtained from mosquito legs showed higher quality and reproducibility compared with those from heads, probably due to the lower protein variability. Our data corroborate the potential of MALDI-TOF MS as a promising tool for the rapid and reliable identification of *An. labranchiae*, providing valuable support for mosquito-borne disease surveillance. *(Funds: ITALIAN MINISTRY OF HEALTH, PROJECT RC IZSPB 06/22)*

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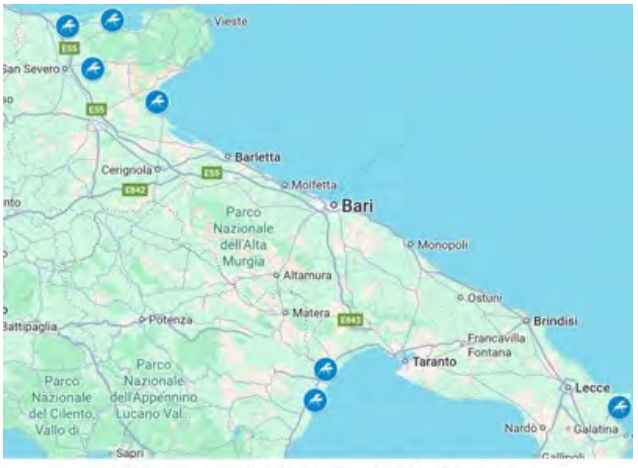


Fig. 1. Mosquito collection sites analyzed in this study.

13. Other

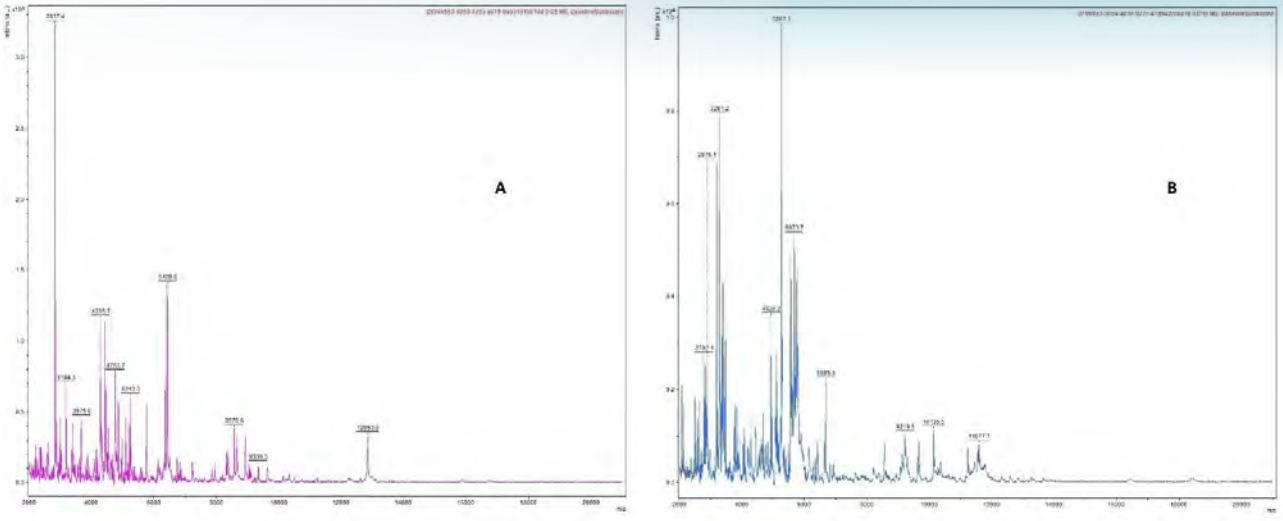


Fig.2 Examples of protein spectra obtained from the head (A) and leg (B) portions of an *An. labranchiae* specimen analyzed in this study.

13. Other

P087. DEVELOPMENT AND VALIDATION OF A NEW RT-PCR TEST FOR MULTIPLEX DETECTION OF PRRSV-1 AND PRRSV-2 IN EUROPE

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant diseases affecting the swine industry worldwide. Effective PRRS management depends on the use of diagnostics, with ELISA and RT-PCR being the most common. This study reports on the development and validation of a new RT-PCR test for detection and differentiation of PRRSV-1 and PRRSV-2 strains circulating in Europe.

The performance of a new RT-PCR test (RealPCR PRRSV-1/ PRRSV-2 Multiplex RNA Test) for detection of PRRSV-1 and PRRSV-2 was evaluated: exclusivity, inclusivity, limit of detection (LOD), diagnostic specificity and sensitivity, calculated as percentage of agreement with reference methods. For this study, 1,326 recent field samples and characterized samples were sourced from multiple European countries. Samples included swine serum, plasma, oral fluids, processing fluids, semen, tongue exudates, lung tissue, lung lavage samples, tracheo-bronchial swabs and abortion samples.

In-silico analysis and experimental testing (both internally and externally) showed high inclusivity for PRRSV-1 and PRRSV-2 strains circulating in Europe and lack of cross-reactivity with other targets. The LOD was estimated at 5 copies per reaction for PRRSV-1 and 10 copies per reaction for PRRSV-2, respectively. Using characterized negative samples, diagnostic specificity of 100% was shown. Diagnostic sensitivity for detection of PRRSV-1 and PRRSV-2 was calculated at 99.7% and 100%, respectively.

This study showed excellent analytical and diagnostic performance of a new RT-PCR for multiplex detection of PRRSV-1 and PRRSV-2 strains circulating in Europe. During development, emphasis was given to detection of contemporary strains of PRRSV, including recently emerged strains such as Rosalia in Spain or Horsens in Denmark. A broad variety of commonly used sample types and new sample types, such as tongue exudates, were successfully validated. Furthermore, it contains an RNA host-based endogenous control to quality control for PRRSV RNA integrity after storage and transport.

13. Other

P088. EVALUATION OF MORPHOMETRIC CHARACTERISTICS IN ERYTHROCYTES CONTAINING INCLUSIONS OF *Babesia canis canis*

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Introduction: Automated morphometric analysis of erythrocytes, based on advanced computer software, provides greater accuracy than conventional microscopy by enabling precise quantification of subtle changes in cell size and shape. Morphometric alterations of erythrocytes in canine piroplasmosis have not yet been clearly defined. The aim of this study was to identify morphometric changes in erythrocytes as a component of the pathogenesis of canine piroplasmosis using automated morphometric analysis, and to determine a possible correlation between the number and/or size of inclusions and the degree of erythrocyte shape deformation.
Materials and methods: The study included blood smears from twelve dogs diagnosed with piroplasmosis prior to treatment. Erythrocytes with and without Babesia canis canis inclusions were imaged using light microscopy at 1000× magnification with oil immersion and a high-resolution digital camera. Morphometric analysis was limited to 381 erythrocytes because the morphology of Babesia-infected erythrocytes was markedly altered, making accurate and consistent morphometric measurements difficult in a larger sample. Measured size indicators included length, width, minimum and maximum radius, perimeter, area, and convex area. Shape indicators included ellipticity, elongation, roundness, form factor, and solidity index. Statistical analysis was performed using SAS 9.4. software using the SGPLOT procedure (SAS Institute, USA).
Results: Significant differences were observed in most morphometric indicators between infected and non-infected erythrocytes. Infected cells were more elongated and elliptical, with higher solidity and reduced roundness. These findings indicate that the presence of inclusions, irrespective of their size or number, induces membrane deformation and disrupts the normal discoid shape of erythrocytes.
Conclusions: The results support the potential application of automated morphometric analysis in the diagnosis of canine piroplasmosis through detection of characteristic deviations in erythrocyte morphology.

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13. Other

P089. BENCHMARKING OF NINE COMMERCIAL LATERAL FLOW ASSAYS FOR THE DETECTION OF FIV ANTIBODIES: A COMPARATIVE PERFORMANCE STUDY

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Feline immunodeficiency virus (FIV) is a globally distributed retrovirus that compromises the feline immune system, rendering infected cats more susceptible to opportunistic infections and chronic diseases. Despite its worldwide presence, epidemiological data remain incomplete in many regions, highlighting the need for reliable diagnostic methods to support disease monitoring and clinical decision-making. Following infection, FIV establishes lifelong persistence within host lymphocytes and enters a latent phase characterized by very low levels of circulating virus. Consequently, direct detection of viral antigens is of limited diagnostic value. Instead, routine diagnosis relies primarily on the identification of virus-specific antibodies, making serological assays the cornerstone of FIV detection in clinical practice. Rapid tests are particularly important for point-of-care screening and timely management decisions. A comprehensive comparative evaluation was performed on nine commercially available lateral flow assays (LFAs) intended for rapid detection of FIV antibodies in whole blood, serum, or plasma. Diagnostic performance was assessed using a newly developed in-house enzyme-linked immunosorbent assay (ELISA) as the reference method, whose reliability was confirmed through full concordance of positive samples with a commercial ELISA reference test. All evaluated LFAs demonstrated 100% diagnostic specificity. Two assays achieved 100% sensitivity and overall accuracy; however, statistical analysis did not reveal significant differences between any of the tested kits, indicating comparable diagnostic performance across all evaluated LFAs. These findings confirm that rapid antibody-based assays are effective and reliable tools for identifying FIV-infected cats. Further studies focusing on the distribution and circulation of FIV subtypes are warranted to address existing knowledge gaps and to support comprehensive epidemiological characterization of the virus.

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13. Other

P090. SUSTAINING THE UK'S BRUCELLOSIS-FREE STATUS: THE IMPORTANCE OF BULK MILK SURVEILLANCE

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Bovine brucellosis (primarily caused by *Brucella abortus*) is a notifiable zoonotic disease commonly characterised by abortions and epididymitis/orchitis. Central to maintaining Official Brucellosis-Free (OBF) status, which Great Britain has held since 1985, is a surveillance system which can quickly identify outbreaks, and enable disease control. This system must be capable of annually detecting one infected holding nationally if the proportion of infected holdings ≥ 0.2% and is primarily achieved through statutory abortion enquiries and bulk milk testing [1]. Covering approximately 1.3 million animals quarterly across England and Wales, bovine bulk milk samples from every dairy holding are submitted to the UK's Animal and Plant Health Agency (APHA) for testing. An indirect enzyme-linked immunosorbent assay (iELISA) is used to detect the presence of IgG anti-*Brucella abortus* antibodies using a *B.abortus* sLPS antigen and anti-ruminant IgG enzyme conjugate. Non-negative samples undergo follow-up serological testing of the individual animals in the contributing herd using a 1/20 serum iELISA for screening, and the Complement Fixation Test (CFT) for confirmation [2]. Across 2024 and 2025, a total of 59,701 bulk milk samples were submitted from >6,500 individual holdings. This resulted in 20 investigations from non-negative samples. Upon further testing of the contributing animals (>4,100 individuals in total), suspicion of brucellosis was negated for each case. This, therefore, provided a diagnostic specificity of 99.97% (95% CI of 99.95%-99.98%) in 2024 and 2025. As this strategy uses routinely obtained, non-invasively collected samples and enables screening of multiple animals through one submission, this component of brucellosis surveillance is not only the most sensitive element but also cost-effective[3]. Historical outbreaks of *Brucella abortus* to GB since obtaining OBF status, including in 1993, 2003, and 2004 underscore the need for continued surveillance. In each instance, early detection preserved OBF status and protected the public health, the environment, and the economy.

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13. Other

P091. DISCRIMINATION OF CEREULIDE-PRODUCING *Bacillus cereus* SENSU STRICTO STRAINS BY MALDI-TOF MASS SPECTROMETRY: PRELIMINARY RESULTS

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Bacillus cereus sensu stricto (*B. cereus* s.s.) is an important foodborne pathogen associated with both diarrhoeal and emetic syndromes. The emetic form is caused by cereulide, a heat-stable toxin encoded by the *ces* gene cluster. Rapid identification of cereulide-producing strains is essential for food safety surveillance and outbreak prevention. This study evaluated the ability of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) to discriminate *ces*-positive from *ces*-negative *B. cereus* s.s. isolates. In this regard, thirty *B. cereus* sensu stricto strains recovered from food sources were included of which ten isolates were ces-positive, whereas twenty were *ces*-negative. The presence of the *ces* gene cluster had been previously determined by real-time PCR and confirmed by whole genome sequencing (WGS). MALDI-TOF MS analyses were performed using the MALDI Biotyper System. Protein extracts were prepared using a standard formic acid/acetonitrile extraction protocol and spectra were acquired in positive linear mode within a mass range of 2–20 kDa. Spectral profiles were compared to identify reproducible differences between the two groups. Distinct and homogeneous spectral patterns were observed among ces-positive isolates, enabling clear discrimination from non-emetic strains. Several protein peaks associated with cereulide-producing isolates were identified (Fig.1). The findings confirm the potential of MALDI-TOF MS as a rapid, reliable and cost-effective screening tool for identifying potentially emetic *B. cereus* strains. Integration of MALDI-TOF MS with molecular and genomic approaches may enhance routine diagnostic workflows, speeding up and strengthening food safety surveillance. Further studies involving larger strain collections are warranted to validate the identified spectral biomarkers and support their implementation in routine laboratory practice.

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13. Other

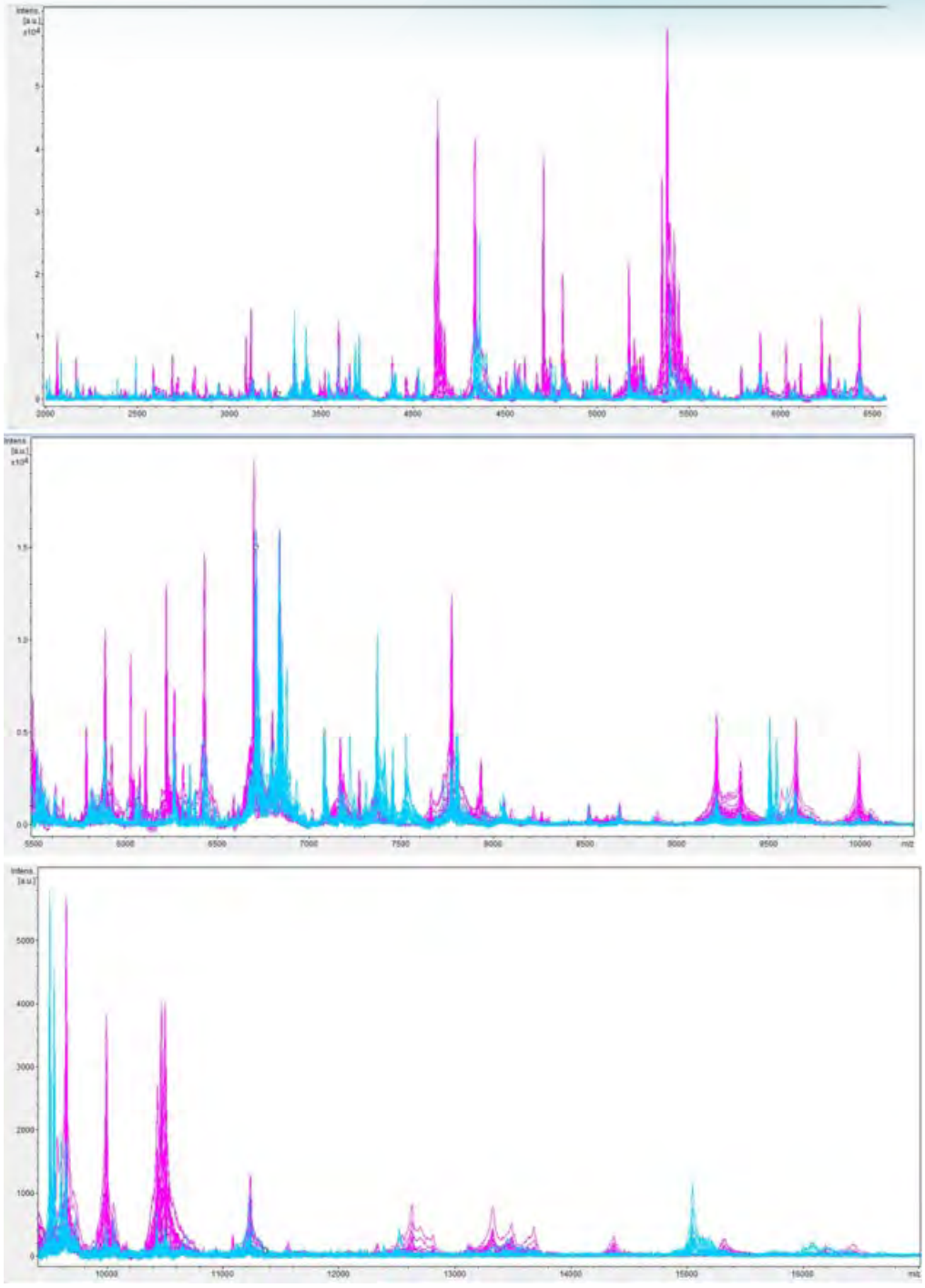


Figure 1. MALDI-TOF MS mass spectra of Bacillus cereus sensu stricto strains in the 2–20 kDa mass range. Spectra obtained from cereulide-producing (magenta) and non-producing (blue) isolates are overlaid to highlight characteristic differences in protein profiles.

13. Other

P092. A NOVEL RAPID IMMUNOCHROMATOGRAPHIC ASSAY FOR FELINE CORONAVIRUS DETECTION IN CAT FECES

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Feline coronavirus (FCoV) is a widespread alphacoronavirus infecting domestic cats, most commonly associated with mild enteric disease. During viral replication in the host, mutations can occur, especially in genes responsible for how the virus enters cells, such as the spike (S) protein gene. These changes may result in the development of a more pathogenic variant that can cause feline infectious peritonitis (FIP). FIP is a largely fatal disease, therefore early detection of FCoV infection is critical for limiting further transmission and implementing appropriate supportive care. Thus, rapid point-of-care diagnostics, such as lateral flow immunoassays (LFAs), constitute a valuable, rapid, and user-friendly approach for routine clinical practice, particularly in high-density environments such as shelters and multi-animal settings, where rapid decision-making is essential. In this study, the diagnostic performance of a newly developed Rapid FCoV Ag Test was evaluated against RT-PCR, the reference standard for FCoV detection, and compared with several commercially available immunochromatographic assays. The novel test demonstrated a sensitivity of 100% and a specificity of 95.2% relative to RT-PCR. These findings indicate that the newly developed Rapid FCoV Ag Test represents a reliable and efficient point-of-care tool for the rapid detection of FCoV infection in cats.

13. Other

P093. SEROLOGICAL AND VIRAL DYNAMICS OF PCV2 IN ENDEMICALLY INFECTED COMMERCIAL PIG HERDS: A CROSS-SECTIONAL FIELD STUDY

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Porcine circovirus type 2 (PCV2) remains one of the most economically significant pathogens in pig production. This study evaluated PCV2 viraemia and serological dynamics in breeding animals from endemically infected farms to clarify the role of breeders in viral persistence and potential vertical transmission. The study was conducted in two farrow-to-finish farms with different levels of PCV2 circulation. Sows and gilts were not vaccinated against PCV2, while piglets were vaccinated at 21 days of age (Mhyosphere PCV ID, HIPRA, Spain). Blood samples from gilts and sows and processing fluids from litters were collected quarterly over two years. Serum samples were tested for PCV2 antibodies using ELISA (BioChek), and pooled sera and processing fluids were analysed by droplet digital PCR (ddPCR). Mortality, clinical performance, and treatment cost data were also recorded. Both farms remained PCV2-positive throughout the study. The high-circulation farm showed higher seroprevalence and antibody titres, with transient viraemia mainly detected in gilts. In the low-circulation farm, seroprevalence and antibody levels were lower, with sporadic viraemia in sows. PCV2 was intermittently detected in processing fluids, indicating possible in utero transmission. Reductions in mortality, clinical signs, and treatment costs were observed in both farms during the study period. Detection of PCV2 in processing fluids supports transplacental viral transmission and highlights the role of non-vaccinated breeding animals in maintaining viral circulation within herds. Detection of PCV2 in litters despite limited maternal viraemia suggests that vertical transmission may occur even in the absence of detectable systemic infection. The observed improvement in health and productivity indicators underlines the importance of strengthened herd immunity and effective PCV2 control strategies.

13. Other

P094. DOES SAMPLE MATRIX MATTER? COMPARATIVE EVALUATION OF FELV ANTIGEN DETECTION ACROSS DIFFERENT SAMPLE TYPES

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Feline leukemia virus (FeLV) remains a significant infectious disease contributing to morbidity and mortality in cats worldwide. Therefore, early detection of the virus is crucial for disease control and transmission prevention. Common approaches for FeLV detection include molecular methods targeting viral RNA or proviral DNA, such as polymerase chain reaction (PCR), as well as immunological assays, including lateral flow assays (LFA) and enzyme-linked immunosorbent assays (ELISA), which detect viral antigens. PCR enables sensitive and specific detection of viral genetic material; however, its application may be constrained by technical complexity and susceptibility to contamination. Lateral flow assays provide a rapid, point-of-care alternative, allowing qualitative detection of FeLV antigen with minimal equipment and fast turnaround time. ELISA is widely used in clinical practice to detect viral p27 antigen due to its high sensitivity and ability to provide quantitative or semi-quantitative results. Although antigen-detecting methods are widely used, there is still limited information available on assay precision, sensitivity, and most importantly the influence of different sample matrices. While serum and plasma are conventionally recommended matrices, whole blood would be preferred due to its convenience. However, differences in sample matrices may influence assay sensitivity, specificity, and overall diagnostic accuracy. Whole blood is generally not recommended for ELISA due to matrix-related factors, including hemolysis, cellular components, and nonspecific interference, which may compromise signal accuracy, especially in low-antigen samples. Understanding matrix effects and analytical limits of detection is essential for accurate result interpretation and may contribute to improved diagnostic reliability.

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13. Other

P095. POINT-OF-CARE DIAGNOSTICS FOR FELINE LEUKEMIA VIRUS (FeLV): COMPARATIVE EVALUATION OF RAPID ANTIGEN DETECTION TESTS

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Feline leukemia virus (FeLV) remains a significant diagnostic challenge in veterinary medicine due to its variable clinical course and impact on feline health. Early and accurate detection is essential for effective clinical management and prevention of viral spread. Although polymerase chain reaction (PCR) is considered the reference standard, its limited availability in routine practice highlights the importance of rapid point-of-care (POC) diagnostics, particularly lateral flow assays (LFAs). This study evaluates the performance of a novel Vet Expert FeLV Antigen rapid test and compares it with several commercially available assays, including the SNAP FIV/FeLV Combo test. Diagnostic accuracy was assessed using PCR-confirmed samples, with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) as key parameters. Both the Vet Expert and SNAP tests achieved 100% sensitivity and specificity, showing complete agreement with PCR results. In contrast, other evaluated LFAs, while maintaining high sensitivity (95–100%), demonstrated variable specificity, leading to false-positive results in some cases. This variability had a notable impact on PPV, particularly in low-prevalence settings, where less specific tests may misclassify healthy animals as infected. In addition to analytical performance, practical aspects of rapid testing were considered. The Vet Expert test offers advantages such as low sample volume requirements, room-temperature storage, and immediate usability, enhancing its suitability for routine clinical use. In conclusion, while rapid antigen tests are essential tools in FeLV diagnostics, their performance differs across products. The Vet Expert test combines high diagnostic accuracy with practical usability, supporting its role as a reliable POC diagnostic tool in veterinary practice and FeLV control strategies.

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13. Other

P096. EVALUATION OF A NOVEL RAPID ANTIGEN TEST FOR FELINE PANLEUKOPENIA VIRUS IN FECAL SAMPLES: A COMPARATIVE STUDY WITH COMMERCIAL ASSAYS AND PCR

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Feline panleukopenia virus (FPV) remains a major infectious threat to domestic cats worldwide, particularly in multi-cat environments where rapid transmission can result in severe outbreaks. Prompt and accurate diagnosis is therefore essential for effective clinical management and infection control. Although polymerase chain reaction (PCR) enables highly sensitive detection of FPV, its routine use in veterinary settings may be limited by the requirement for specialized laboratory infrastructure and longer turnaround times. Consequently, rapid point-of-care assays, particularly lateral flow immunoassays, constitute a highly valuable and clinically effective diagnostic approach, offering a reliable and accessible method in routine practice. In this context, the present study assessed the diagnostic performance of the newly developed Rapid Test FPV Ag, a lateral flow immunoassay intended for the detection of FPV virus in feline fecal samples, using PCR as the reference method. The assay was evaluated alongside several commercially available tests from different manufacturers, enabling a comparative analysis of their diagnostic accuracy. Among the evaluated assays, the newly developed Rapid Test FPV Ag demonstrated the highest overall performance, achieving a sensitivity of 96.2%, specificity of 95.0%, and an overall accuracy of 95.8%. In addition to its strong diagnostic performance, the test offers practical advantages such as rapid result generation and ease of use, supporting its suitability for point-of-care applications. Overall, these findings indicate that the Rapid Test FPV Ag is a reliable tool for the rapid detection of FPV infection and may facilitate timely clinical decision-making in both veterinary clinics and field conditions.

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13. Other

P098. ASFV MONODOSE-LAMP® FOR RAPID ON-FIELD DETECTION OF AFRICAN SWINE FEVER VIRUS IN WHOLE BLOOD

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African swine fever virus (ASFV) is a highly contagious and lethal pathogen affecting domestic pigs and wild boar, requiring rapid and reliable diagnostic tools to support disease control, particularly under field conditions. Loop-mediated isothermal amplification (LAMP) offers a fast and simple alternative to conventional molecular methods, enabling on-site detection without the need for complex laboratory infrastructure.

The aim of this study was to evaluate a fluorimetric LAMP assay for the direct detection of ASFV in whole blood samples from wild boars, analyzed in the Barcelona area and domestic pigs, infected with the Georgia strain. The assay was performed using specific primers targeting ASFV in a ready-to-use MONODOSE-LAMP® format, allowing direct testing without prior nucleic acid extraction. Reactions were carried out using the portable Genie® II device, enabling in situ analysis under field conditions. LAMP reactions were completed within 30 minutes, and ASFV was successfully detected directly from blood samples. The performance of the assay was validated against the WOAH-recommended real-time PCR, showing high concordance between both methods. The ability to detect ASFV without extraction significantly reduced processing time and simplified the workflow.

In conclusion, the fluorimetric LAMP assay, combined with the portable Genie® II platform represent a rapid, robust, and field-deployable approach for ASFV detection. By enabling reliable on-site diagnostics and reducing dependence on centralized laboratories, highly trained personnel, and Biosafety Level 3 (BSL-3) facilities, this method supports early disease detection and the timely implementation of outbreak response measures.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P099. ETIOLOGICAL AND CLINICAL PATTERNS OF BOVINE MASTITIS IN SARDINIAN DAIRY HERDS

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The Arborea district of Sardinia, Italy, is a geographically confined Holstein dairy enclave under veterinary oversight, ClassyFarm monitoring, and IZS-coordinated Milk Quality programmes for contagious pathogens. This survey mapped the clinical and etiological landscape of mastitis[1]. Between February and August 2025, 12 dairy farms with mastitis history were enrolled. A total of 622 quarter milk samples were collected according to NMC guidelines at the time of clinical presentation[2]. For each quarter, a standardised record captured udder signs and milk appearance. Cultures on Hayflick Agar and Blood Agar and MALDI-TOF MS[3] identification enabled direct quarter-level pairing of clinical and microbiological data. Farm-level management variables were collected via questionnaires. Culture was positive in 195 quarters (31%), mixed flora in 67 (11%), and no growth in 360 (58%). Thirty-seven bacterial species were recovered: the three most prevalent pathogens were *Escherichia coli* (46 isolates), *Streptococcus uberis* (24), and *Staphylococcus chromogenes* (17); contagious pathogens were rare, with *Staphylococcus aureus* detected in one quarter from one herd. *Mycoplasma* and *Prototheca* were absent. Clinical-bacteriological associations were observed for eight species (*E. coli*, *Klebsiella pneumoniae*, *Mammaliicoccus sciuri*, *Staphylococcus chromogenes*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *Staphylococcus microti*, *Streptococcus lutetiensis*). Gram-negative infections were predominantly associated with overtly abnormal secretion (flakes, clots, absent milk), while Gram-positive infections presented mainly with serous milk and inflammatory udder signs. Other species, often isolated from high-SCC quarters sampled outside acute events, showed no consistent macroscopic pattern. This field survey revealed a mastitis epidemiology shaped by environmental and opportunistic pathogens (*E. coli*, *S. uberis*, non-*aureus* staphylococci) consistent with epidemiological findings in modern, well-managed dairy farms. The high frequency of culture-negative and mixed-flora quarters reflected the inherent complexity of field-level mastitis diagnosis. The virtual absence of contagious pathogens supports the long-term effectiveness of district-wide control programmes, while confirming the growing relevance of environmental mastitis in modern dairy systems.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P100. LONGITUDINAL ANALYSIS OF INTRAMAMMARY INFECTIONS IN PRIMIPAROUS DAIRY GOATS: SPECIES-SPECIFIC PERSISTENCE PATTERNS AND IMPACT ON SCC

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The epidemiology of intramammary infections (IMI) in dairy goats is complex, largely due to the high prevalence of subclinical mastitis. Sensitive detection and accurate species-level identification is required to understand their specific pathogenic roles. This study investigated the prevalence of intramammary infections in primiparous goats to determine which species establish chronic intramammary colonization versus sporadic infections, and to evaluate their impact on somatic cell count (SCC). A longitudinal study was conducted on 95 primiparous dairy goats from three herds, with half-udder milk samples (n = 2,660) collected twice a month over a 7-month period. Samples were analyzed via bacteriological culture, and isolates were identified using MALDI-TOF MS. Statistical significance of observations was assessed by GLMM. A total of 1,057 IMI cases were recorded. non-aureus staphylococci and mammaliicocci (NASM) were the main identified bacteria. *Staphylococcus caprae* was the most prevalent (33.8%), followed by *S. xylosus* (13.1%), and *S. simulans* (11.1%). Other NASM accounted for a total 15.4%. Notably, *S. caprae* exhibited the highest propensity for chronic infection, occasionally persisting throughout the entire lactation period. *Staphylococcus aureus* and Serratia sp. showed farm-specific behavior. *Corynebacterium* spp. had the lowest tendency for chronic colonization. SCC increased proportionally with infection duration, resulting in elevated counts during chronic infections. Among the predominant NASM, *S. simulans* exhibited the strongest correlation with increased SCC, followed by *S. caprae* and *S. xylosus*. Similarly, *Serratia* sp. and *S. aureus* showed a marked association with elevated SCC. This study highlights the distinct impact of microbial species on goat udder health, particularly concerning staphylococci, emphasizing their varying capacities to establish chronic infections and subsequently affect milk quality.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P101. COMPARISON OF PCR AND SEQUENCING FOR DETECTION OF PRRSV VACCINE STRAINS

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndrome (PRRS), a major viral disease affecting swine. Because modified-live PRRSV vaccines are commonly used, the identification of vaccine strains in diagnostic samples is important for interpretation of laboratory results. The aim of the present study was to assess the detection of PRRSV vaccine strains in PRRSV EU-positive samples collected from PRRS clinical cases in Flanders in 2025, comparing PCR and sequencing.
To characterize the circulating PRRSV strains, seven serum samples were collected from pigs. DNA was extracted using the IndiMag Pathogen Kit (Indical Bioscience, Leipzig, Germany) according to the manufacturer's instructions. On seven sera, ORF1b-ORF7 whole genome sequencing (WGS) was performed within the Belgian PRRS monitoring program(1). In addition, the samples were screened for PRRSV-positive EU or NA strains using the virotype® PRRSV 2.0 RT-PCR kit (Indical Bioscience, Leipzig, Germany) and then tested with virotype® PRRSV EU Vax RT-PCR Kit ((Indical Bioscience, Leipzig, Germany)(Table 1). Five sequenced samples with PRRSV EU vaccine strain result were confirmed by the virotype® PRRSV EU Vax kit. Two sequenced samples with PRRSV NA vaccine strain result were negative with the virotype® PRRSV EU Vax kit, as expected.
The findings of this study shows that results of WGS were confirmed with the virotype® PRRSV EU Vax RT-PCR kit and both methods can be applied in diagnostics. PCR is a faster and cheaper alternative to determine the vaccine strain after obtaining a positive PRRSV EU result in screening. When a more sophisticated analysis is needed, such as phylogenetic tree, outbreak analyse and information on recombination events, WGS is the best option.

Matrix	ID	virotype® PRRSV 2.0 Ct EU	virotype® PRRSV 2.0 Ct NA	Result WGS	Vaccine strain detected in WGS	Result PRRS EU VAX INDICAL	Ct PRRS EU VAX INDICAL
Serum	DGZ-25-006551.0016	30,5		PRRSV1 - VACCINE	Suvaxyn	Suvaxyn	29,57
Serum	DGZ-25-006572.0016		32,6	PRRSV2 - VACCINE	PRRSV MLV NA	/	Undetected
Serum	DGZ-25-011132.0136	26,4		PRRSV1 - VACCINE	Unistrain	Unistrain	26,27
Serum	DGZ-25-013370.0003	26,6		PRRSV1 - VACCINE	Unistrain	Unistrain	21,72
Serum	DGZ-25-013478.0006	34,5		PRRSV1 - VACCINE	Porcilis	Porcilis	34,75
Serum	DGZ-25-015089.0016	31	34,1	PRRSV2 - VACCINE	Field and PRRSV MLV NA	/	Undetected
Serum	DGZ-25-017788.0016	26,9		PRRSV1 - VACCINE	Suvaxyn	Suvaxyn	27,03

Table 1. Results of comparison ORF1b-ORF7 WGS and virotype® PRRSV EU Vax RT-PCR Kit.

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P102. COMPARISON OF ELISA AND PCR FOR DETECTION OF ROTAVIRUS IN PIGS

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Porcine rotavirus is a major etiological agent of enteric disease in pigs. Multiple rotavirus species circulate in swine populations, with rotavirus A being the most frequently detected in diarrheic animals. The aim of the present study was to evaluate rotavirus detection in samples collected from suspected porcine rotavirus clinical cases in Flanders in 2025 by comparing two diagnostic methods: ELISA and PCR.

To compare diagnostic methods for rotavirus, 20 porcine faecal samples were collected. ELISA for rotavirus A was performed using the ProSpecT Rotavirus microplate assay. PCR for rotavirus A, B and C was performed using the VetMAX™ Rotavirus A/B/C reagents and primer/probe design. Amplification was performed on a QuantStudio 5 (Thermo Fisher Scientific).

In total, 20 faecal samples were analysed (Table 1). The optical density (OD) values obtained by ELISA range from 0,002 to 0,859. The cut-off value is calculated by adding 0,200 absorbance units (=OD) to the negative control. Six samples tested negative and 14 samples showed a positive result in ELISA. Of the negative ELISA results, 2/6 were positive for rotavirus A, 1/6 were positive for rotavirus B and 6/6 were found positive in PCR for Rotavirus C. Of the positive ELISA results, all 14 were positive for rotavirus A, four were positive for rotavirus B and six were positive for rotavirus C. In conclusion, all positive rotavirus A ELISA results were confirmed by PCR for rotavirus A, but two negative rotavirus A ELISA results were not confirmed negative by PCR for rotavirus A. Overall, 16 (80%) samples were positive for rotavirus A, five (25%) were positive for rotavirus B and 12 (60%) were positive for rotavirus C.

The findings of this study confirm that rotavirus A remains the predominant genotype circulating in Flanders, according to our sample subset, and shows that PCR Rotavirus A ELISA is less sensitive than the Rotavirus A/B/C PCR.

	ELISA		PCR		
Dossiernummers	Rota A Ag - ELISA Resultaat	Rota A Ag - ELISA OD	Rota A	Rota B	Rota C
DGZ-25-156127.0003	POSITIEF	0,804	15,46	15,72	34,13
DGZ-25-103282.0004	POSITIEF	0,859	12,39	12,95	30,69
DGZ-25-104803.0003	negatief	0,23	15,86	Undetermined	14,54
DGZ-25-222944.0002	POSITIEF	0,456	14,5	Undetermined	Undetermined
DGZ-25-222944.0001	POSITIEF	0,658	11,12	Undetermined	Undetermined
DGZ-26-044024.0001	POSITIEF	0,26	17,73	Undetermined	Undetermined
DGZ-25-203442.0001	POSITIEF	0,546	12,04	14,35	Undetermined
DGZ-26-044024.0002	POSITIEF	0,432	10,52	Undetermined	Undetermined
DGZ-25-224855.0004	POSITIEF	0,611	11,66	Undetermined	Undetermined
DGZ-25-218574.0004	POSITIEF	0,375	14,7	Undetermined	Undetermined
DGZ-26-004432.0001	POSITIEF	0,489	9,01	Undetermined	Undetermined
DGZ-25-204048.0002	POSITIEF	0,332	7,01	11,46	12,07
DGZ-26-045332.0004	POSITIEF	0,331	10,02	18	24,67
DGZ-26-062321.0001	Negatief	0,023	Undetermined	Undetermined	14,55
DGZ-26-066099.0001	POSITIEF	0,584	8,68	Undetermined	12,04
DGZ-26-062311.0001	POSITIEF	0,469	9,92	Undetermined	34,19
DGZ-26-031971.0004	Negatief	0,025	Undetermined	Undetermined	14,9
DGZ-26-005933.0002	Negatief	0,002	Undetermined	Undetermined	13,3
DGZ-26-005933.0001	Negatief	0,006	Undetermined	Undetermined	21,4
DGZ-26-000216.0001	Negatief	0,024	15,7	17	25,4

Table 1. Comparison of ELISA and PCR for Rotavirus detection.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P103. COMPARISON OF PCR AND NANOPORE SEQUENCING FOR PCV2 SUBTYPING

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Porcine circovirus type 2 (PCV2) causes porcine circovirus-associated disease (PCAD). PCV2 comprises nine identified genotypes (PCV2a–i), of which PCV2d has become the predominant genotype globally. The aim was to investigate samples collected from presumed porcine circovirus (PCVD) clinical cases in Flanders in 2025, comparing PCR and sequencing. To characterize the circulating PCV2 field strains, 21 lung tissue, three cardial tissue, five saliva, three tracheobronchial swabs (TBS), three lymph node and four serum samples were collected from pigs. DNA was extracted using the IndiMag Pathogen Kit (Indical Bioscience, Leipzig, Germany). Quantitative PCR (qPCR) targeting the ORF1 gene of PCV2 was performed using the ViroReal kit PCV2 (Ingenetix, Vienna, Austria) and the PCV2 standard (BioChek, Reeuwijk, Nederland). Amplification was performed on ABI7500 (Thermofisher, Massachusetts, USA). The absolute concentration in each PCR reaction was recalculated to copies/g or copies/ml sample. PCV2 genotyping was carried out using the ViroTyping Kit PCV2a-b-d (Ingenetix, Vienna, Austria) and confirmed by whole genome sequencing of PCV2 isolates using Nanopore sequencing technology. In total, 39 samples were genotyped. The detected PCV2 viral loads ranged from 10^6 to 10^{13} genome copies/ml or copies/g. Of the 39 genotyped PCV2 samples, 7 (18%) were identified as PCV2a, one (2,6%) as PCV2b and 31 (79,4%) as PCV2d. Results were confirmed with both PCR and sequencing. The findings of this study confirm that PCV2d remains the predominant genotype circulating in Flanders, according to our sample subset. Genotyping results are concordant between PCR and sequencing, which confirms both methods can be applied in diagnostics. PCR is a cheaper alternative to know the subtype a, b or d. When a more sophisticated analysis, such as a phylogenetic tree, is required for outbreak analysis or determination of subtypes other than a, b, d; sequencing is the best option.

P104. BEYOND TRADITIONAL DIAGNOSTICS: NGS-BASED APPROACH TO DETECT FOOT-AND-MOUTH DISEASE VIRUS IN LOW-YIELD SAMPLES

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Introduction: Foot-and-mouth disease (FMD) is a highly infectious viral disease with a severe socioeconomic impact[1]. Accurate diagnosis is essential; however, samples from endemic regions often contain degraded nucleic acids or are collected on biosafe devices (FTA cards, LFDs) that significantly reduce viral load and viral viability[2]. Consequently, virus isolation frequently fails, while limited template availability hinders reliable serotype characterization via traditional sequencing.
Objectives: Developing and optimizing an NGS-based protocol to detect and characterize FMDV in challenging biological samples without prior knowledge of the viral serotype.
Methods: Viral RNA from FMDV serotypes O and A serial dilutions (10^5–10^0 $TCID_{50}$/mL) was DNase I-treated and rRNA-depleted. To maximize VP1 recovery, amplification-based strategies (SISPA-Illumina and Nanopore PCR-cDNA Barcoding V14) were compared against standard unenriched Illumina RNA-seq. Performance was assessed via sequencing depth and coverage across both the VP1 region and the whole genome. Finally, a custom pipeline (reference-based and de novo) was developed for data processing, consensus generation and full genomic characterization.
Results: Both amplification-based workflows recovered complete VP1 at the highest viral load, with coverage breadth comparable to unenriched Illumina RNA-seq. Across the dilution series, SISPA-Illumina enabled high-fidelity consensus generation down to intermediate-to-low titers (≤ 10^3 $TCID_{50}$/mL). While requiring higher viral inputs for complete VP1 and whole genome coverage, the Nanopore workflow offered advantages in rapid turnaround time and long-read structural resolution. Comprehensive benchmarking of these pre-amplification strategies is ongoing to determine the optimal workflow for routine diagnostic applications, ultimately ensuring the reliable genomic analysis of challenging samples.
Conclusion: In conclusion, the NGS-based approach provides a robust diagnostic framework for FMDV. By overcoming the limitations of traditional Sanger sequencing, it ensures accurate high-resolution viral characterization even from suboptimal samples—critical for outbreak management. Future validations will assess these workflows on complex biological and clinical field samples.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P105. BOVINE VIRAL DIARRHOEA VIRUS (BVDV) IN MILK SAMPLES: A COMPARATIVE VALIDATION STUDY OF TWO COMMERCIAL EXTRACTION KITS

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BVDV is a significant pathogen in cattle that leads to considerable economic losses. The presence of persistently infected (PI) animals within the herd can be demonstrated by analyzing bulk tank milk (BTM) samples with rRT-PCR: nonetheless, this specimen requires a specific validation.
This study aimed to evaluate some pre-analytical and analytical factors potentially affecting the detection of viral RNA in milk, including sample pretreatment, pooling strategies, storage conditions (+4°C, -20°C, and -80°C), and nucleic acid extraction methods.
Ten milk samples collected from PI animals were used to prepare dilutions of untreated and pre-treated samples to simulate herds of up to 800 animals, based on the average farm density of the territory. Automated RNA extraction was performed using ID Gene™ Mag Universal Extraction Kit (Innovative Diagnostics) and MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific). Extracted RNAs were investigated by rRT-PCR using the ID Gene™ BVD/BD Triplex 2.0 Kit (Innovative Diagnostics) and quantified, including a standard curve. One of these samples was used to assess the analytical sensitivity and repeatability of the established protocol.
Both extraction kits demonstrated consistent and reliable performance across all experimental conditions, allowing the detection of viral RNA from a single PI animal in pooled samples simulating herds of up to 800 lactating animals. The analytical limit of detection on milk specimen ranges between 60 and 100 genomic copies per 5 µL for the evaluated extraction kits, maintaining 100% repeatability.
The adoption of automated extraction platforms successfully enabled the direct processing of raw milk samples, eliminating the need for prior pretreatment steps. Moreover, the tested temperatures showed comparable Ct values, suggesting that storing samples at +4°C or -20°C might still maintain RNA integrity if -80°C storage is not available.
This study demonstrates reliability and efficiency of the BTM's use to detect BVDV in lactating herds.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P106. INTERACTIONS BETWEEN ENDOCRINE, INFLAMMATORY AND METABOLIC BIOMARKERS IN IBERIAN PIGS

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The interpretation of biomarkers in swine laboratory diagnostics is complex, as endocrine, inflammatory, and metabolic systems do not operate in isolation, and their interactions under commercial conditions remain incompletely characterized, particularly in less-studied populations such as the Iberian pig, for which breed-specific reference data are limited.
This study examined the relationships between salivary cortisol, two acute-phase proteins—C-reactive protein (CRP) and porcine major acute-phase protein (Pig-MAP)—and non-esterified fatty acids (NEFAs) in 147 Iberian pigs aged 6 to 14 weeks with no clinical signs of disease. Samples were collected under field conditions, and associations among biomarkers were evaluated using Spearman's correlation analysis.
CRP and Pig-MAP showed a strong positive correlation ($\rho = 0.455$, $p < 0.001$), reflecting coordinated activation of the acute phase[1]. Cortisol showed significant negative correlations with both CRP ($\rho = -0.289$, $p < 0.001$) and Pig-MAP ($\rho = -0.211$, $p = 0.009$), suggesting a dissociation between endocrine and inflammatory axes[2]. NEFA showed a weak but significant positive association with Pig-MAP ($\rho = 0.163$, $p = 0.045$), suggesting a potential metabolic contribution to inflammatory activation[3], whereas no significant association was found between NEFA and cortisol.
The inverse relationship between cortisol and acute-phase proteins is consistent with the immunomodulatory effects of glucocorticoids, which vary depending on the intensity and chronicity of exposure[2]. Additionally, interactions between stress and immune function differ between acute and chronic conditions, with implications for the interpretation of cortisol in diagnostic contexts[4].
Taken together, these findings highlight the diagnostic limitations of interpreting biomarkers in isolation in Iberian pigs and advocate for the implementation of integrated multiparametric laboratory panels that jointly address the endocrine, inflammatory, and metabolic dimensions, an approach that would substantially improve the accuracy and clinical relevance of health characterization in this genotype under field conditions.
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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P107. RELATIONSHIPS BETWEEN STRESS, INFLAMMATION AND METABOLISM IN CLINICALLY HEALTHY GROWING PIGS

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The interpretation of stress biomarkers in swine production is influenced by the interaction between the endocrine, metabolic, and inflammatory systems. The profiles obtained in healthy animals may differ significantly from those observed in sick animals; however, the reference physiological characteristics for these markers remain poorly defined. This study aims to elucidate the relationships between salivary cortisol, inflammatory markers [C-reactive protein (CRP) and pig major acute phase protein (Pig-MAP)], and metabolic indicators in clinically healthy, white-coated pigs under commercial conditions.

A cross-sectional study was conducted with 165 (Landrace×Large White)×Pietrain pigs aged between 6 and 14 weeks. The animals were selected based on the absence of clinical signs of disease or inflammatory processes. The biomarkers analyzed included cortisol, CRP, Pig-MAP, non-esterified fatty acids (NEFA), and lactate, and the associations were analyzed using Spearman's correlations.

No significant correlations were found between the inflammatory markers CRP and Pig-MAP ($\rho = 0.071$, $p = 0.367$), suggesting the absence of co-activation of acute-phase markers and confirming a low level of inflammation in the study population[1]. Cortisol was not significantly associated with these markers, reflecting the complex role of glucocorticoids in stress and immune regulation[2]; however, a strong inverse association was observed between NEFA and cortisol ($\rho = -0.431$, $p < 0.001$), which could reflect HPA axis habituation, whereby animals with attenuated stress responses maintain lipid mobilization through alternative mechanisms. Positive correlations were found between lactate and both CRP ($\rho = 0.249$, $p = 0.001$) and Pig-MAP ($\rho = 0.198$, $p = 0.011$), suggesting that metabolic activity, rather than endocrine stress, may be the cause of mild inflammation in healthy pigs under field conditions[3].

These results underscore the importance of health status stratification in studies of porcine biomarkers and support the development of multiparametric diagnostic panels that consider the various physiological factors involved.

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P108. FEATHER CORTICOSTERONE AND HETEROPHIL-TO-LYMPHOCYTE RATIO AS COMPLEMENTARY LABORATORY BIOMARKERS OF STRESS IN LAYING HENS

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Identifying reliable biomarkers in the laboratory to measure stress in poultry production is a challenging task, especially when integrating them throughout the entire production cycle to reflect changes associated with different physiological phases. This study evaluates the diagnostic complementarity of corticosterone in feathers, a biomarker of hypothalamic-pituitary-adrenal axis activity and the heterophil-to-lymphocyte (H:L) ratio, a rapid immunological indicator of acute stress response, both measured in laying hens throughout their production cycle.

A cross-sectional study was conducted with independent cohorts of 258 laying hens sampled at nine different ages (weeks 4 to 60), covering their entire productive cycle. Corticosterone in feathers was quantified using an ELISA assay following extraction, and the H:L ratio was determined by direct counting under a microscope. Both results were analyzed using nonparametric statistical methods; the effect of age was evaluated using Kruskal-Wallis tests with paired post-hoc comparisons. The relationship between biomarkers was assessed using Spearman's correlation, including age-stratified and age-adjusted partial correlations.

Both biomarkers showed significant differences ($p < 0.001$) across the production cycle stages analyzed, confirming marked age-related physiological variation. However, no significant association was found between corticosterone in feathers and the H:L ratio ($p = 0.916$); this lack of correlation persisted after adjustment for age ($p = 0.801$) and in age-stratified analyses ($p > 0.05$).

This lack of correlation is consistent with the time windows reflected by each biomarker. Corticosterone in feathers reflects cumulative HPA axis activity deposited during feather growth, providing a retrospective measure of chronic stress[1]. The H:L ratio responds to acute immunological and physiological changes occurring over the past hours or days[2]. The absence of correlation confirms that both biomarkers capture independent stress dimensions[3,4], supporting the implementation of a combined panel of chronic and acute stress indicators to improve interpretive accuracy in veterinary laboratory diagnostics applied to poultry farming.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P109. COMBINED DIAGNOSTIC APPROACH APPLIED TO A CASE OF *Meningoencephalomyelitis* ASSOCIATED WITH FELINE HERPESVIRUS TYPE 1 INFECTION IN A JUVENILE CAT

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Infectious diseases affecting central nervous system (CNS) in cats are often difficult to diagnose and may remain classified as meningoencephalitis of unknown etiology [1]. This is mainly due to the challenges of sampling in living animals, the use of incomplete diagnostic approaches, and the limited knowledge of pathogens capable of causing CNS disease in this species. This study describes a combined diagnostic approach applied to a case of non-suppurative meningoencephalomyelitis in a 9-month-old cat that died following the acute onset of neurological signs. The investigation integrated pathological techniques (necropsy, histopathology, immunohistochemistry) with molecular analyses (PCR, qPCR, sequencing). No evident or specific gross pathological lesions were detected at necropsy, while histopathology revealed severe multifocal lymphocytic perivascular cuffing in the brain and spinal cord, associated with moderate gliosis and occasional neuronal degeneration. These findings are consistent with non-suppurative meningoencephalomyelitis likely of viral origin. For this reason, molecular analyses were performed to detect RNA and DNA viruses. Brain tissue tested positive for feline herpesvirus 1 (FHV-1) DNA. To confirm positivity and its correlation with clinical signs, sequencing and immunohistochemistry (IHC) were performed. Sequencing confirmed the presence of FHV-1 closely related to strains identified in cats without neurological signs. IHC confirmed positivity for FHV-1 antigen in astrocytes and neurons, demonstrating the ability of the virus to infect CNS cells. This case supports the potential involvement of FHV-1 in severe CNS disease in cats, although the virus is classically regarded as a respiratory and ocular pathogen. To date, only one other case of neurological signs associated with FHV-1 infection has been reported [2]. In conclusion, this report highlights the importance of considering FHV-1 among the differential diagnoses in young cats presenting with acute or progressive neurological signs. It also underscores the value of integrating histopathology with virological and molecular techniques to achieve an accurate diagnosis.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P110. IMPROVING SPECIFICITY IN BOVINE TUBERCULOSIS DIAGNOSIS: A NOVEL IS6110-BASED REAL-TIME PCR ASSAY MINIMIZING CROSS-REACTIVITY WITH NON-TUBERCULOUS MYCOBACTERIA

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Bovine tuberculosis is a chronic infectious disease that primarily affects cattle and represents a public health concern due to its zoonotic nature [1]. It is caused by members of the *Mycobacterium tuberculosis* complex (MTBC), mainly *Mycobacterium bovis* (*M. bovis*) and, less frequently, *Mycobacterium caprae*. Currently, the confirmation of suspected cases is allowed by different methods that detect the presence of the pathogens, such as bacteriological culture or direct PCR in animal tissues. Molecular methods have gained importance due to their rapid result and high diagnostic performance [2;3]. Real-time PCR targeting the IS*6110* insertion sequence is a widely used method for MTBC detection, although cross-reactivity with certain non-tuberculous mycobacteria (NTM) can compromise its specificity (Sp) [3]. To address this limitation, the aim of this study was to design and validate a novel real-time PCR assay targeting an alternative region of the IS*6110* to improve its diagnostic Sp. The *in vitro* parameters evaluated included efficiency, linearity (R^2), limit of detection with 95% confidence (LOD95), diagnostic sensitivity (Se) and specificity (Sp), and the Cohen's kappa index (κ). The qPCR yielded an efficiency of 96.3% and an R^2 of 0.999 between 1 ng/µl and 11 fg/µl of a standard curve generated from genomic DNA extracted from a pure culture of *M. bovis* BCG Danish. The LOD95 was established at 55 fg/reaction (11 genomic equivalents/reaction), whereas analytical Sp was 100% in a panel of 29 DNA extracts from NTM and other microorganisms. Diagnostic Se and Sp were 95% (CI:86.1-98.9%) and 100% (CI:95-100%), respectively, with a κ index of 0.96 indicating excellent agreement with respect to the reference. Overall, this optimized assay shows improved specificity compared to the routinely used IS*6110*-based PCR method with slightly lower but comparable sensitivity. Further studies are needed to evaluate the assay performance while maintaining the objective of minimizing cross-reactivity with NTM.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P111. MOLECULAR CHARACTERIZATION OF *Mycobacterium bovis* FROM TUBERCULIN NON-REACTOR CATTLE IN ARGENTINA

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Mycobacterium bovis (*M. bovis*), the causative agent of bovine tuberculosis (bTB), affects livestock, wildlife, and humans. In Argentina, as in many countries, the tuberculin skin test (TST) is routinely used to identify infected cattle. However, a subset of *M. bovis*-infected animals fails to respond to this test, complicating disease control and surveillance efforts (1).

This study aimed to molecularly characterize *M. bovis* in tuberculin non-reactor cattle. Three *Holstein* cows—two from Buenos Aires Province and one from Córdoba Province—negative to the TST but positive by bTB ELISA were included. Lesions were recorded, and lymph nodes (cranial, respiratory, and digestive) and mammary gland tissues were collected. Infection confirmation was performed by direct tissue PCR targeting the *Rv2807* sequence (PuriPrep-T, InbioHighway) and culture on Stonebrink medium followed by Ziehl-Neelsen staining. Molecular typing of *M. bovis* was conducted by spoligotyping from tissue samples (2). All animals (100%) presented granulomatous lesions. Two of them (2/3. 66.7%) showed multi-organ involvement, one in respiratory and cranial lymph node, and the other one in cranial, respiratory and digestive lymph nodes and mammary gland tissue. The remaining animal (1/3. 33.3%) showed lesions only in one organ system (respiratory). No bacterial growth was obtained from cultures. Infection was confirmed by *Rv2807* PCR in all animals: three were positive in respiratory lymph nodes, and one in cranial and respiratory lymph nodes. Spoligotyping identified genotypes SB0140 in two animals and SB0131 in one, from tissue samples positive by *Rv2807* PCR. These findings highlight the value of spoligotyping directly from tissue samples as a complementary approach for molecular characterization of *M. bovis* in cases where culture results are negative. Moreover, they underscore the epidemiological importance of identifying false-negative TST animals, which may contribute to the persistence of bTB in endemic regions (1).

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P112. *Dermatophilus congolensis* – A MISSED PATHOGEN IN EQUINE DERMATOLOGY?

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Dermatophilosis, caused by *Dermatophilus congolensis*, is a bacterial, exudative multifactorial dermatitis characterized by pustule and crust formation followed by hair loss, occurring primarily in horses, cattle, and sheep. Zoospores are released and activated in a warm, humid environment; consequently, clinical cases occur more frequently during periods of prolonged rain. It represents an important differential diagnosis to dermatophytosis in horses. The aim of the study was to investigate how frequently *Dermatophilus congolensis* can be detected in samples with clinical suspicion for dermatophytes. 307 hair samples of horses from different European countries, which were submitted to a diagnostic laboratory for dermatophyte PCR between January 2022 and January 2023, were retrospectively tested for the presence of *Dermatophilus congolensis* DNA via qPCR. Samples originated from Germany (n=206), Sweden (n=44), Norway (n=23), Austria (n=17), Czechia (n=6), Switzerland (n=5), Netherlands (n=3) and Spain (n=3). 19.9% (61/307) of the samples were positive for dermatophytes, while *Dermatophilus congolensis* was detected in 3.3% (10/307) of the samples from Germany (n=5), Norway (n=2), Austria (n=2) and Netherlands (n=1). Two mixed infections were observed. The majority of the *Dermatophilus congolensis*-positive samples were collected between October and March, whereas dermatophyte-positive results occurred throughout the year. Given that hair is not the ideal material for the detection of *Dermatophilus congolensis* – for which scabs or skin biopsies would be more suitable – the true proportion of positive samples is likely even higher. Since the pathogen is transmitted both through direct animal-to-animal contact and via inanimate vectors such as brushes and blankets, accurate diagnosis is of essential importance to prevent its spread and to treat it appropriately. In addition, *Dermatophilus congolensis* has zoonotic potential, even if human cases are rare. In summary, samples should also be examined for *Dermatophilus congolensis* to avoid missing this important disease.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P113. DIAGNOSTIC CHALLENGES OF ATYPICAL CANINE TRANSMISSIBLE VENEREAL TUMOR IN GEORGIA: THE CRITICAL ROLE OF ONCOCYTOLOGY AND RISKS IN STRAY POPULATIONS

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Background: Canine Transmissible Venereal Tumor (CTVT) represents a major contagious neoplastic challenge in veterinary medicine, particularly in regions with high populations of stray and free-roaming dogs. In Georgia, unmonitored reproductive contact accelerates CTVT transmission. While genital lesions are classic, atypical extragenital forms often present diagnostic dilemmas, leading to misdiagnosis, therapeutic delay, and silent transmission.
Objective: To evaluate the diagnostic value of fine-needle aspiration (FNA) cytology in detecting atypical CTVT presentations and highlight the epidemiological risk associated with free-roaming dogs.
Methods/Case Series: A clinical and cytological evaluation was conducted on 20 confirmed CTVT cases at Veterinary Clinic "Aibo" and the Tamaz Elizbarashvili Shelter. Atypical presentations were systematically investigated:
1. A shelter Husky presented with severe ocular CTVT and systemic depression following chronic diagnostic delays;
2. A 7-year-old Hound ("Max") managed for months as a traumatic nasal wound with flushing and drains;
3. A stray mixed-breed managed unsuccessfully for chronic dermatological ulcerations;
4. A mixed-breed ("Listik") presenting solely with periodic epistaxis, diagnosed via rhinoscopy and tissue sampling after ruling out infectious etiologies.

All cases underwent FNA cytology and were treated with weekly Vincristine sulfate (0.5 mg/m^2 IV).
Results: Extragenital atypical forms accounted for 30% of cases, with 70% of total patients being stray dogs. Initial clinical misdiagnoses were common in atypical cases (e.g., chronic pyoderma, open trauma, rhinitis). Oncocytology provided rapid, definitive identification based on characteristic round cells with eccentric nuclei and cytoplasmic vacuolation. Vincristine monotherapy achieved 100% complete clinical and cytological remission.
Conclusion: The high stray dog population in Georgia creates a significant reservoir for CTVT transmission. Atypical extragenital forms frequently mimic traumatic or dermatological conditions, resulting in misdiagnosis and increased risk of transmission to healthy animals. Timely oncocytology (FNA) is an indispensable, minimally invasive tool for early diagnosis, ensuring effective chemotherapy and disease containment.
1. Stray Dog Prevalence, Transmission Risk: Explicitly stresses the large free-roaming population (70%) as a key driving factor for rapid disease transmission.
2. Atypical Presentations, Misdiagnosis: Details all 4 specific cases (ocular Husky, mismanaged nasal Hound, dermatological mixed-breed, internal nasal "Listik").
3. Diagnostic Role of Cytology: Emphasizes FNA cytology as the gold-standard, rapid diagnostic tool for atypical forms to prevent diagnostic delays.

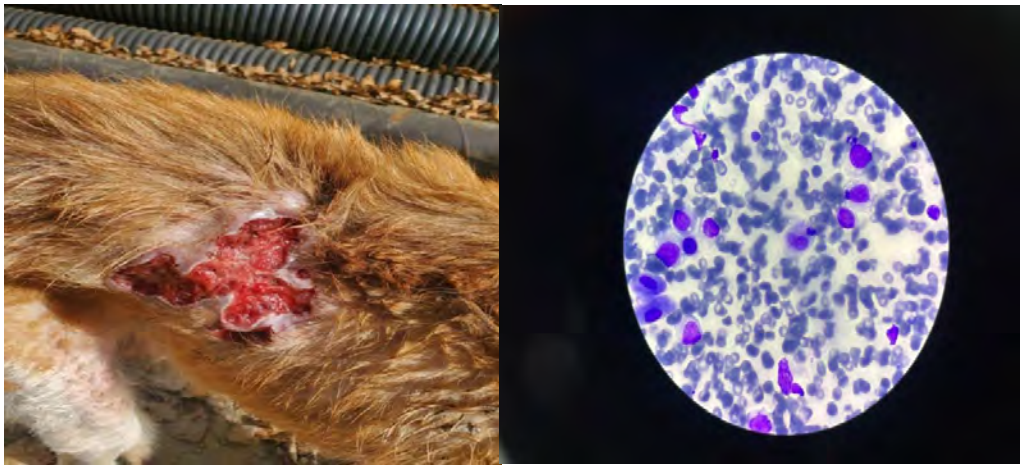


Case 1: A 7-year-old Hound ("Max") managed for months as a traumatic nasal wound with flushing and drains.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics



Case 2: A shelter Husky presented with severe ocular CTVT and systemic depression following chronic diagnostic delays



Case 3: A stray mixed-breed managed unsuccessfully for chronic dermatological ulcerations



Case 4: A mixed-breed ("Listik") presenting solely with periodic epistaxis, diagnosed via rhinoscopy and tissue sampling after ruling out infectious etiologies.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P114. INTESTINAL MICROBIOME OF BROILER CHICKENS AS A REFLECTION OF WELFARE QUALITY

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The chicken intestinal microbiota contributes to digestion, nutrient absorption, immune function, and protection against pathogens. Dysbiosis may arise from improper feeding, breeding conditions, or health disturbances. Slow-growing chickens are increasingly used as an alternative to conventional broilers to enhance welfare, differing in fattening duration, stocking density, and access to outdoor areas. This study compares the intestinal microbiome of chickens from different breeding systems to evaluate their impact on gut homeostasis and animal welfare.
Cecal samples were collected from conventional broilers aged 35 (BC1, n=7) and 43 days (BC, n=10), and slow-growing broilers reared in: limited free-range (BZ, 58 days, n=10), unlimited free-range (BW, 76 days, n=10), and organic systems (BB, 80 days, n=10). Microbial profiling was performed by 16S rRNA gene sequencing on Illumina MiSeq platform. Bioinformatic analysis using QIIME 2 included DADA2 denoising and ASV inference, taxonomic classification against the SILVA_138 database, and alpha- and beta-diversity analyses.
Alpha-diversity analyses showed BW and BZ had the highest microbial richness, while BC/BC1 exhibited the lowest. BW displayed the most even bacterial distribution. Beta-diversity revealed distinct clustering among groups. *Prevotella and Veillonellaceae* were detected exclusively in BB, where *Prevotella* was dominant. *Megamonas, Elusimicrobium, and Odoribacter* were absent in conventional broilers but present in slow-growing birds. *Rikenellaceae_RC9_gut_group*, *Alloprevotella*, *Methanobrevibacter*, and *Olsenella* were detected exclusively in BB and BW.
Each breeding system harbored a distinct microbial community; among them, BW was the most diverse, and BC/BC1 the least. BB exhibited a microbiome associated with fiber-rich diets typical of organic systems. Although beneficial bacteria were present in all systems, free-range access and longer fattening periods were associated with increased gut microbiota diversity and maturation, potentially enhancing chicken welfare through polysaccharide fermentation, SCFA production, nutrient utilization, gut integrity, immune homeostasis, and pathogen exclusion. After further research, microbiome analysis may become a useful indicator of chicken welfare.

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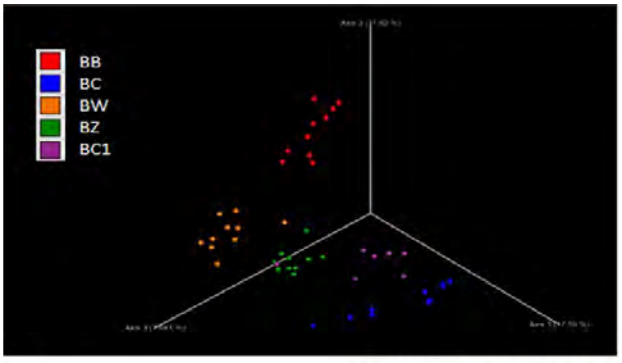


Fig. 1. β-diversity analysis

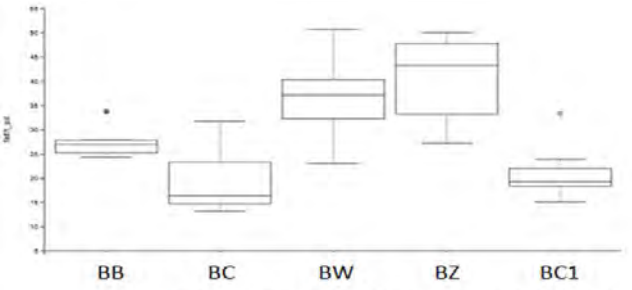


Fig. 2. α-diveristy analysis: microbial richness

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P115. COMPILATION OF MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING METHODS IN A RAPID WORKFLOW FOR DETECTION AND CHARACTERIZATION OF CHICKEN RESPIRATORY PATHOGENS

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Bacterial and viral respiratory pathogens represent significant microbial threats to the poultry industry, causing severe disease outbreaks and substantial economic losses. We implemented a set of detection and characterisation methods and established an integrated diagnostic workflow targeting ten different viral and bacterial agents representing the most common chicken respiratory pathogens.

The diagnostic panel was developed by adapting previously published detection assays and integrating them into four multiplex qPCR reactions, each of which can be applied individually or in combination depending on the diagnostic needs:

- RNA viruses: infectious bronchitis virus (IBV), avian influenza virus (AIV) and Newcastle disease virus (NDV)
- duplex reaction for the simultaneous detection and typing of avian metapneumovirus (AMPV) A and B
- bacterial respiratory pathogens: *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Ornithobacterium rhinotracheale*
- other DNA-genome pathogens: infectious laryngotracheitis virus (ILTV), *Avibacterium paragallinarum* and *Gallibacterium anatis.*

Samples positive for either of IBV, AIV, ILTV or *Avibacterium*, are submitted to further characterisation by amplicon sequencing using the Oxford Nanopore MinION platform:

- IBV: spike gene sequencing and genotyping;
- AIV: whole genome sequencing and subtyping;
- ILTV: field/vaccine typing based on sequences of distinctive genes;
- *Avibacterium:* genotyping based on hemagglutinin gene sequences.

The presented diagnostic workflow has been successfully applied to pooled oronasal swab samples as well as to specimens collected on FTA cards. The broad-spectrum diagnostic workflow is suitable not only for detecting the respiratory pathogens but for their accurate genetic characterization.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P116. GENOMIC CHARACTERISTICS OF *Salmonella enterica* serovar *Gallinarum biovar Gallinarum* ISOLATES FROM SOUTH KOREA BETWEEN 2002 AND 2020

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Salmonella enterica serovar Gallinarum biovar Gallinarum (SG) is a host-adapted pathogen that causes fowl typhoid, resulting in significant economic losses to the poultry industry in many regions of the world, particularly in Africa, Asia, and Latin America. Although fowl typhoid has become rare in Korea in recent years due to improved biosecurity and control measures, genomic epidemiological information on SG isolates from South Korea remains limited. This study aimed to investigate the phylogenomic relationships and major genomic features of SG isolates from South Korea between 2002 and 2020. Twenty-two representative isolates recovered from fowl typhoid outbreaks in South Korea were subjected to whole-genome sequencing on an Illumina MiSeq platform. Whole-genome SNP analysis was performed together with 34 publicly available genomes retrieved from public databases. The Korean isolates were additionally screened for antimicrobial resistance (AMR) genes and resistance-associated point mutations, virulence genes, and *Salmonella* pathogenicity islands (SPIs). Whole-genome SNP phylogeny resolved the dataset into three broad phylogenetic groupings. All Korean isolates formed a distinct and relatively homogeneous clade, whereas most Brazilian genomes were concentrated in a second, Brazil-enriched clade, and the remaining international genomes were distributed within a more heterogeneous assemblage that included a closely related Colombian subclade. Korean isolates harbored approximately 110 of 130 virulence targets in the selected screening panel and showed a largely conserved predicted pathogenicity-island profile, including C63PI, CS54, and multiple SPI loci. The chromosomal aminoglycoside resistance-associated gene *aac(6')-Iaa* and quinolone resistance-associated *gyrA* substitutions at codons 83 and 87 (Ser83Tyr/Phe and Asp87Gly/Tyr/Asn) were identified by AMR analysis. These findings suggest that Korean SG isolates are genetically distinct from most publicly available international genomes and possess a conserved virulence repertoire with recurrent quinolone resistance-associated mutations, providing a genomic baseline for molecular epidemiology and fowl typhoid control.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P117. DIAGNOSTIC APPLICATION OF OCT IN CHICKEN EGGSHELL ABNORMALITIES RELATED TO BACTERIAL INFECTIONS

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Optical Coherence Tomography (OCT) is an imaging technique based on low-coherence light, enabling visualization of complex internal structures. Different light sources, including superluminescent diodes and supercontinuum lasers, may be used, influencing both imaging depth and resolution. OCT generates cross-sectional and volumetric images through the interference of reference and backscattered light. The intensity of light scattering correlates with the attenuation coefficient, an additional optical parameter of the examined tissue that can be visualized parametrically. The aim of this study was to evaluate whether bacterial pathogens associated with reproductive tract disease and eggshell abnormalities, including *Gallibacterium anatis* (GA) and *Ornithobacterium rhinotracheale* (ORT), are associated with changes in eggshell structure and optical properties assessed by OCT (Fig. 1). OCT imaging was performed on eggshells collected from a healthy flock without visible abnormalities and from flocks in which GA and ORT infections were confirmed by bacterial culture and PCR. All investigated flocks had previously been vaccinated with a live *Mycoplasma synoviae* vaccine strain, which was also detected in the hens' oviducts. In infected flocks, eggshell structural abnormalities and deformations were observed. Necropsy examinations additionally revealed abnormalities of the egg yolk and oviduct in some birds.
The results demonstrated that eggshell optical properties were associated with the severity of eggshell abnormalities observed in flocks in which GA and ORT were detected. In healthy eggs, the OCT attenuation coefficient showed a uniform distribution with values around 30. In contrast, eggshells from infected flocks exhibited variable attenuation patterns depending on the extent of visible lesions. Shells without apparent external changes displayed localized regions of increased attenuation, whereas visibly deformed shells showed elevated attenuation coefficients across the entire surface (Fig. 1,2). Structurally altered eggshells also showed increased optical heterogeneity, which may reflect changes in shell microstructure potentially associated with reduced mechanical integrity.

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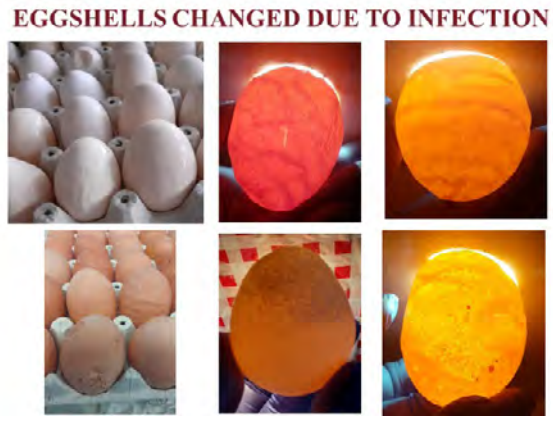


Fig 1

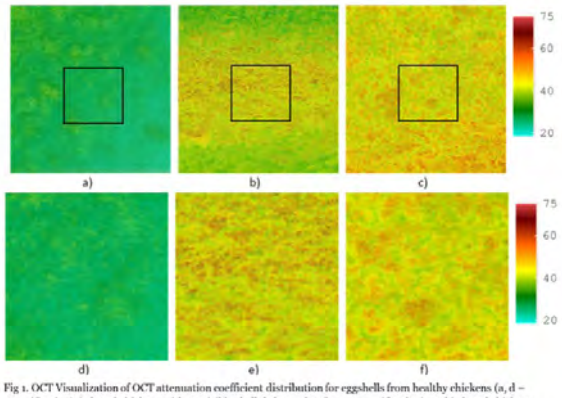


Fig 2. OCT Visualization of OCT attenuation coefficient distribution for eggshells from healthy chickens (a, d – magnification), infected chickens with no visible shell deformation (b, e – magnification), and infected chickens with visible shell deformation (c, f – magnification).

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P118. COMPLETE GENOME SEQUENCING OF CANINE DISTEMPER VIRUS IN DOMESTIC DOGS FROM CUBA IN 2025

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Canine distemper virus (CDV) is a highly contagious viral pathogen affecting a wide range of carnivores globally. Despite its broad distribution, genomic information on CDV circulating in the Caribbean is lacking. This study aimed to investigate the presence and genetic characteristics of CDV in domestic dogs in Cuba, providing the first genomic data from the country. Oral, nasal, and rectal swab samples were collected from n= 17 domestic dogs in Cuba from 2025, all presenting clinical signs consistent with CDV infection. CDV RNA was confirmed to be using Real-Time RT-PCR. Complete genomes were sequenced on-site with a pan-genotype CDV-specific amplicon-based sequencing method developed by our laboratory and optimized for the Oxford Nanopore Technologies platform (1). Phylogenetic analysis was performed using the complete genome sequences to determine lineage and genetic relationships of the Cuban strains in the context of global CDV diversity. Phylogenetic analysis based on the complete genomes showed that all Cuban sequences clustered within the America-3 lineage, indicating circulation of this lineage among domestic dogs in Cuba. These findings represent the first genomic evidence of CDV in the country and confirm a significant geographic expansion of this lineage into the Caribbean. The presence of America-3 in Cuba suggests an epidemiological bridge connecting canine populations across the Americas, highlighting how viral lineages can transcend borders through animal movement or trade (2). The application of Oxford Nanopore sequencing on-site demonstrates its utility as a rapid and reliable tool for monitoring CDV, providing essential data to guide vaccination strategies, disease control measures, and risk assessment for domestic dog populations in previously uncharacterized regions. Furthermore, Cuba's island characteristics, combined with its role as an epidemiological bridge, create a unique ecological scenario that could facilitate viral adaptation and potential coevolutionary processes within local host populations.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P119. OPTIMIZED NUCLEIC ACID EXTRACTION PROTOCOLS FOR DETECTING AVIAN INFLUENZA A VIRUS IN EXTENDED BOVINE SEMEN

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The emergence of highly pathogenic avian influenza (HPAI) A(H5N1) in U.S. dairy cattle in March 2024 marked its first documented occurrence in bovine, raising animal health, public health, and trade concerns. Epidemiologic and experimental studies illustrated a strong tropism of H5N1 for the mammary gland, with high viral loads detected in mammary tissue and milk. Despite evidence suggesting H5N1 is unlikely in bovine semen, the risk of pathogen transmission through artificial insemination and trade requirements for PCR-certified disease-free semen necessitate validated semen-specific diagnostic protocols. The objective of this study was to validate a universal nucleic acid extraction protocol for extended bovine semen that effectively removes PCR inhibitors and supports sensitive molecular detection of infectious agents, including HPAI. Sixty-four negative semen samples extended in milk- or egg-based media were evaluated using exogenous extraction controls added at the lysis step. Extraction performance was assessed using manufacturer-recommended and laboratory-modified protocols for the MagMAX CORE and IndiMag Pathogen kits, followed by testing with the NAHLN-approved Influenza A matrix PCR assay. Diagnostic sensitivity was further evaluated using 31 naturally infected semen samples representing a range of pathogens. Significant PCR inhibition was observed with a 200µL semen input, with variability associated with extender type. The IndiMag Pathogen standard protocol using a 75-100µL semen input, or 100µL with heating in ATL buffer, demonstrated acceptable performance with 93.8-100% exogenous control recovery while the MagMAX CORE protocol performed optimally using a 12.5µL input with proteinase K pretreatment. Diagnostic sensitivity for bovine viral diarrhea virus, bovine gerpers-1 and Mycoplasma bovis detection was 100% for IndiMag Pathgen (100µL), and 90.3% for MagMAX CORE (12.5µL) input. While HPAI has not been demonstrated in bull semen, evolving outbreak dynamics supports proactive diagnostic readiness. This study validates robust semen extraction protocols adaptable for reliable HPAI surveillance and broader pathogen detection in extended bovine semen.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P121. MOLECULAR AND SEROLOGICAL INVESTIGATION OF BOVINE CORONAVIRUS (BCoV) CIRCULATION IN DAIRY HERDS OF SOUTHERN ITALY

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Bovine Coronavirus (BCoV) is a widespread Betacoronavirus associated with enteric and respiratory disease in cattle, including neonatal calf diarrhea and winter dysentery. Despite the high seroprevalence reported worldwide, the duration and protective role of the immune response following natural infection or vaccination remain poorly understood. In addition, the genetic variability of circulating strains may contribute to viral persistence and reinfection within herds.

The aim of this study was to investigate the circulation of BCoV in cattle farms located in Southern Italy, evaluate the serological response following infection, and characterize circulating viral strains through molecular analyses.

A total of 113 serum samples from 22 farms in the Apulia Region and 164 serum samples from 38 farms in the Basilicata Region were analyzed using a commercial indirect ELISA assay for the detection of anti-BCoV antibodies. Additional field investigations were conducted in dairy farms showing enteric and/or respiratory clinical signs compatible with BCoV infection. Nasal and rectal swabs, and sera were collected from symptomatic animals. Viral RNA was extracted and analyzed by real-time RT-PCR targeting the BCoV nucleocapsid gene. Positive samples were subjected to virus isolation on HRT-18 cell cultures. Follow-up serological analyses were performed six months after the initial sampling. Serological positivity was detected in 89% of cattle from Apulia Region and 80% from Basilicata Region, confirming widespread viral circulation. Molecular analyses identified BCoV infection in samples collected from two affected farms. Virus isolation was successful in one positive sample. Longitudinal monitoring demonstrated persistent seropositivity in one herd, whereas 20% of animals in another herd seroreverted during follow-up. These preliminary findings support the endemic circulation of BCoV in the investigated areas and indicate variability in the persistence of the humoral immune response following natural infection. Ongoing genomic and immunological investigations will contribute to a better understanding of BCoV epidemiology and immune dynamics in cattle populations.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P122. DIAGNOSTIC EVIDENCE OF PRRSV-1 MLV VACCINE TRANSPLACENTAL TRANSMISSION: VIROLOGICAL, SEROLOGICAL, AND HISTOPATHOLOGICAL FINDINGS IN NEWBORN PIGLETS

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is among the most economically significant swine pathogens globally, with modified live virus (MLV) vaccines forming the cornerstone of its control. However, the widespread use of mass ("blanket") vaccination protocols carries the risk of inadvertently inoculating gilts during late gestation, potentially resulting in congenital fetal infection. This study evaluated the virological, serological, histopathological, and scanning electron microscopic (SEM) consequences of PRRSV-1 MLV heterologous vaccination on the 100th day of gestation. The research was conducted across four commercial farrow-to-finish pig farms in Greece. In total, 125 RT-PCR-negative gilts, previously immunized with four PRRSV-1 MLV vaccines (strains DV, VP-046 BIS, 94881, and 96V198), were allocated to five experimental groups per farm (one control and four vaccine groups). Blood samples were collected from 250 newborn piglets (≤9 hours post-partum, poor clinical performance) and tested by ELISA and RT-PCR; RT-PCR-positive samples underwent ORF5 phylogenetic sequencing. Lung tissue from euthanized piglets on one farm was examined histopathologically, and by SEM. RT-PCR confirmed PRRSV viraemia in 10% of piglets, while 90.5% were seropositive. Antibody levels increased significantly with time post-partum (linear regression; all $p<0.001$), consistent with colostral passive transfer and de novo fetal synthesis. Phylogenetic analysis demonstrated 98.7–99.2% ORF5 nucleotide similarity between field isolates and vaccine strains VP-046 BIS and 94881, confirming vaccine-strain transplacental transmission. Piglets from VP-046 BIS-vaccinated gilts showed the greatest clinical compromise and, on histopathology, moderate to severe interstitial pneumonia with diffuse alveolar septal thickening, lymphocytic-plasmacytic infiltration, and type II pneumocyte hyperplasia; SEM corroborated these findings through alveolar exudation and leukocyte accumulation. These multi-modal diagnostic findings confirm that PRRSV-1 MLV vaccine strains can act as pathogenic agents when administered in late gestation, causing congenital infection and clinically significant pulmonary lesions. Vaccination of pregnant gilts within the last two weeks before parturition should be strictly avoided.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P123. A STANDARDIZED MICROARRAY PLATFORM FOR PRACTICAL HEALTH SURVEILLANCE IN COMMON CARP

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Reliable laboratory tools for early health assessment in aquaculture are needed to support veterinary decision-making before overt clinical disease develops. The objective of this study was to develop and analytically verify a standardized transcriptomic platform for common carp that could be translated into practical laboratory surveillance of infection-, stress-, and environment-associated host responses. A species-specific Agilent 8 × 60 K oligonucleotide microarray was designed using a gene-level probe strategy to reduce redundancy from paralogous sequences and alternative transcripts. Head kidney RNA from clinically healthy carp was used to establish a diagnostic baseline, and platform performance was evaluated through dye-balance assessment, technical reproducibility testing, probe-level quality control, functional enrichment analysis, and comparison with RT-qPCR for selected immune-associated markers. The final array comprised 50,770 unique probes representing 15,096 gene-level targets. Analytical evaluation showed stable hybridization, absence of relevant dye bias, and good repeatability across independent RNA preparations. The baseline expression profile consistently captured pathways associated with hemoglobin metabolism, erythrocyte oxygen transport, oxidative-stress defense, innate and humoral immune activity, and iron homeostasis, while RT-qPCR comparison confirmed good agreement for selected targets. From a diagnostic perspective, these results indicate that the platform can provide a reproducible host-response reference framework for differentiating normal physiological expression from transcriptional deviations linked to infectious challenge, environmental stress, or deteriorating water quality. In conclusion, this microarray platform represents a standardized laboratory tool with practical potential for aquatic animal health surveillance, biomarker-informed assay development, and future integration into veterinary diagnostic workflows for common carp. The study was funded from subsidy provided by the Ministry of Science and Higher Education under the grant no. S/545 performed in 2023 at the National Veterinary Research Institute, Puławy, Poland.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P124. DETECTION OF DIFFERENT SPECIES OF *Flavobacterium* IN FARMED CYPRINID AND SALMONID FISH

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The most flavobacteriosis are caused by major pathogenic species: *F. psychrophilum*, *F. columnare* and *F. branchiophilum*. However, many other bacteria belonging to *Flavobacteriaceae* are overlooked due to difficulties in diagnosis and uncertain pathogenicity. The aim of this study was to identify the strains with morphology characteristic of the *Flavobacteriaceae* isolated from fish. In 2022-2024, 63 bacterial strains were collected from different fish species of *Salmonidae* and *Cyprinidae*. Identification was performed by proteomic biotyping using MALDI-TOF mass spectrometry and sequencing the 16S rRNA gene. Serious fish pathogens *F. columnare* and *F. psychrophilum* were detected in cyprinid and salmonid, respectively. Atypical species *F. oncorhynchi*, *F. branchiarum*, *F. succinicans*, *F. tructae* and *F. bizetiae* were detected in both fish family. However, *F. branchiicola*, *F. collinsii*, *F. psychrolimnae*, *F. saccharophilum*, *F. frigoritolerans*, *F. frigidimaris* were isolated from salmonids, unlike *F. acetivorans*, *F. chryseum*, which were detected only in cyprinid. MALDI-TOF MS are useful to identify *Flavobacterium* species, especially *F. psychrophilum*, *F. columnare* and *F. oncorhynchi*. Molecular studies based on 16S rRNA gene sequencing is a valuable tool for the identification and phylogenetic analysis of *Flavobacterium* species. The study was funded from subsidy provided by the Ministry of Science and Higher Education under the grant no. S/546 performed in 2023-2025 at the National Veterinary Research Institute, Puławy, Poland.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P125. VALIDATION OF A PL3-TARGETED ddPCR ASSAY FOR *Bacillus anthracis* SPORES DETECTION IN FOOD AND BIOLOGICAL MATRICES

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Anthrax is a zoonotic disease caused by *Bacillus anthracis*, a Gram-positive, spore-forming bacterium that is widely distributed worldwide and capable of causing life-threatening infections in both animals and humans. *B. anthracis* is classified as a Category A priority pathogen by the Centers for Disease Control and Prevention (CDC).
In this study, we propose a molecular method for the detection of *B. anthracis* spores using droplet digital PCR (ddPCR), targeting the highly conserved chromosomal marker lambda prophage type 3 (PL3), which is present as a single-copy gene. The assay was evaluated using different food and biological matrices.
Method validation was performed in accordance with the guidelines of the WOAH Terrestrial Manual (12th edition). The validation protocol included the assessment of analytical accuracy and determination of the limit of detection (LOD). Spleen homogenates, coffee and tea powder were artificially contaminated with known concentrations of *B. anthracis* spores. Digital PCR assays were carried out using the QX200™ Droplet Digital™ PCR System with customized dPCR probe assays.
The results indicate that PL3 is a reliable marker for both the identification and quantification of *B. anthracis* spores. A satisfactory limit of detection was achieved, corresponding to 4,700 spores/mL for biological matrices and 470 spores/mL for food matrices.
This approach represents a robust and powerful novel molecular technique for the detection of B. anthracis, offering high specificity and rapid absolute quantification of genomic copies.
In conclusion, these findings support the standardization of an alternative molecular assay for the detection of *B. anthracis*. The ddPCR approach showed high sensitivity, specificity, and reliable absolute quantification across different matrices, confirming PL3 as a robust target for spore detection. This method may represent a valuable tool for rapid diagnostics, food safety, environmental surveillance, and biodefense applications.

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P126. SUPPORTING MOLECULAR DIAGNOSTICS IN VETERINARY PRACTICE: STABILITY OF BACTERIAL DNA DURING SAMPLE STORAGE AND TRANSPORT

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In veterinary clinical practice, biological samples are frequently collected in field settings, farms, or remote clinics where immediate processing is not feasible. This is particularly relevant for infectious disease diagnostics and microbiome investigations, where preservation of nucleic acids during transport and storage is critical to ensure reliable molecular results. This study evaluated the performance of Copan eNAT®, a nucleic acid preservation medium, in maintaining bacterial DNA stability under different conditions. A standardized suspension of Neisseria gonorrhoeae (NG) was prepared, diluted, and spiked into filtered urine as a surrogate matrix. Samples were transferred into eNAT® medium and either analyzed immediately (T0) or stored at 4°C and 30°C. Stability was assessed at multiple time points up to 1.5 years (1 week, 3 weeks, 5 weeks, 10 weeks, 6 months, 1 year, and 1.5 years). Detection of bacterial DNA was performed using two independent molecular workflows: the Cepheid Xpert® CT/NG assay and the Qiagen NG assay following nucleic acid extraction. Ct values of NG targets remained stable across all storage conditions and time points. For the Cepheid assay, average Ct values were consistently around 29–30 for both temperatures compared to baseline values of approximately 32–33. Similarly, the Qiagen-based workflow demonstrated minimal variation, with Ct values remaining around 32–33 throughout the study. No significant differences were observed between refrigerated and elevated temperature conditions, indicating that prolonged storage and transport at up to 30°C did not compromise nucleic acid detection. eNAT® effectively preserves bacterial DNA in conditions representative of veterinary clinical workflows, including extended storage and non-controlled temperature transport. Its ability to maintain nucleic acid stability, combined with rapid microbial inactivation, supports its use for safe and reliable molecular diagnostics in veterinary settings. Further studies will evaluate its performance with additional pathogens and matrices relevant to animal health.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P127. RELATIONSHIP BETWEEN ESTRADIOL PEAK AT OVULATION AND PLATELET DYNAMICS IN MARES

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Estradiol-17β (E2) reaches its highest systemic concentrations during the peri-ovulatory period in mares, coinciding with maximal follicular development [1]. In several species, E2 modulates platelet activation, vascular reactivity, and megakaryocyte function, influencing platelet size, turnover, and aggregation capacity [2]. Although the reproductive role of E2 in mares is well established, its influence on platelet morphology and function remains insufficiently characterized. This study evaluated the association between circulating E2 concentrations and platelet indices in clinically healthy Spanish Purebred mares during the breeding season. Fourteen mares were sampled during the peri-ovulatory period, and serum E2 concentrations were measured using chemiluminescent immunoassays. Platelet indices were determined using an ADVIA 2120i hematology analyzer. Mean E2 concentration was 51.4 pg/mL (range 40–60 pg/mL). Platelet count remained relatively stable, whereas indices related to platelet mass and heterogeneity exhibited substantial variability. Correlation analyses revealed moderate positive associations between E2 and MPV ($r = 0.42$, $p < 0.05$) and MPM ($r = 0.47$, $p < 0.05$), indicating that mares with higher peri-ovulatory E2 concentrations tend to present larger and more metabolically active platelets. Correlations with PDW, PCT, P-LCR, and PMDW were weak, and no association was observed with platelet count. Overall, these findings suggest that the peri-ovulatory E2 surge is associated with changes in platelet size and functional mass, supporting the value of integrating hormonal and platelet biomarkers in equine reproductive assessment.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P128. OXIDATIVE SHIFT IN MARE MILK DURING EARLY LACTATION: TOS AND AOPP DYNAMICS

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Although the pro-oxidant markers during early-lactation have been extensively described in ruminant species, they remain poorly characterized in mares. The aim of this study was to evaluate TOS and AOPP dynamics during the transition from colostrum to milk in mares during the first 30 days postpartum. Twenty-nine clinically healthy mares were sampled at 0, 3, 7, 15, and 30 days after foaling. TOS and AOPP were determined in whey using validated spectrophotometric methods. Both markers exhibited significant temporal variation ($P < 0.001$), with the highest concentrations observed in colostrum, followed by a marked decline during early-lactation. TOS decreased progressively from day 0 to day 15 and remained relatively stable thereafter, with significantly higher values at days 0 and 3 compared to subsequent time points. AOPP showed a more pronounced reduction, characterized by a sharp decline between day 0 and day 7, followed by consistently lower levels from day 15 onwards. Both parameters were negatively correlated with lactation time (TOS: $\rho = -0.314$; AOPP: $\rho = -0.373$; $P < 0.01$). Overall, these findings indicate that mare mammary secretions undergo a rapid shift from an oxidatively active colostrum to a more balanced and stable redox state within the first month of lactation. This pattern likely reflects the physiological transition from the highly protective and bioactive composition of colostrum—rich in reactive species and immune-related components—to the more regulated composition of mature milk, optimized for sustained neonatal nutrition and development. This shift may also be associated with the resolution of postpartum inflammatory processes and the progressive closure of mammary tight junctions, supporting the existence of a conserved physiological adaptation during early-lactation.

14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P129. CIRCULATING SEROTONIN CONCENTRATIONS STRONGLY REFLECT PLATELET MASS AND AGGREGABILITY IN MARES DURING THE BREEDING SEASON

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Serotonin (5-HT) is a key neuromodulator in equine reproductive physiology, with increased serotonergic pathways during the breeding season, as ovarian activity and neuroendocrine responsiveness rise [1]. In several mammalian species, fluctuations in ovarian steroids across the estrous cycle influence peripheral serotonergic activity, affecting circulating 5-HT availability. Platelets (PLT) constitute the major peripheral reservoir of 5-HT, regulating its uptake, storage, and release, and establishing a close functional link between 5-HT and PLT physiology. This study evaluated the association between circulating 5-HT concentrations and platelet indices in healthy Spanish Purebred mares during the breeding season. Twenty-five mares were sampled, and serum 5-HT was quantified using a validated ELISA. Platelet indices included platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR), mean platelet component concentration (MPC), platelet component distribution width (PCDW), mean platelet mass (MPM), platelet mass distribution width (PMDW), and platelet aggregation (AGREG), all measured using an ADVIA 2120i hematology analyzer. Mean serum 5-HT concentration was 392.1 ng/mL, consistent with neuroendocrine activation during the reproductive season. PLT count remained regulated (mean 131.9 × 10^3/µL), whereas parameters related to PLT mass, size, and heterogeneity exhibited broad dispersion. Significant positive correlations were observed between 5-HT and MPM ($r = 0.60$, $p < 0.01$) and AGREG ($r = 0.61$, $p < 0.01$), indicating that 5-HT is closely linked to functional PLT mass and aggregability. A moderate positive correlation with MPV ($r = 0.45$, $p < 0.01$) further suggests that larger or more metabolically active PLTs tend to store more 5-HT. Results demonstrate robust 5-HT–PLT interactions during the breeding season, supporting the diagnostic value of integrating neuroendocrine and hematological biomarkers in equine reproductive assessment.

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P130. EARLY LACTATION SHIFTS IN PROTEIN BALANCE: PHYSIOLOGICAL INSIGHTS FROM THE ALB/GLOB RATIO IN MARE MILK

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Mare colostrum and early milk undergo rapid biochemical remodeling as the mammary gland shifts from passive immune transfer to neonatal nutrition. Although the major protein changes of this transition are well described, the contribution of serum derived fractions—particularly the albumin/globulin (ALB/GLOB) ratio—remains less explored. This study evaluated the temporal patterns of total whey proteins (TWP), immunoglobulin G (IgG), and the ALB/GLOB ratio in 29 healthy Spanish Purebred mares, sampled at 0, 3, 7, 15 and 30 days of lactation, with protein fractions quantified using spectrophotometric methods. All measured parameters decreased significantly over time ($p < 0.05$), with maximum values recorded at day 0 and minimum values at day 30. Specifically, TWP decreased from 3.05 to 1.18 g/dL, ALB from 1.05 to 0.12 g/dL, GLOB from 2.05 to 1.07 g/dL, IgG from 383.35 to 40.09 mg/dL, and the ALB/GLOB ratio from 0.51 to 0.11. Early lactation showed a marked restructuring of protein balance, with the greatest shift between day 0 and day 3. TWP declined sharply during this period, reflecting the rapid depletion of colostral immunoglobulins. GLOBs decreased more steeply than ALB, consistent with the high GLOB and IgG content of equine colostrum and their swift reduction once passive immune transfer is achieved. This differential decline was mirrored by the ALB/GLOB ratio, which fell markedly between day 0 and day 3 and stabilized thereafter. Overall, the ALB/GLOB ratio provides an integrated indicator of the shifting contribution of serum derived proteins as colostrum transitions to early milk, capturing the progression from an Ig-rich secretion optimized for passive immunity to a more stable early milk profile adapted to sustained neonatal nutrition.

Day	TWP (g/dL)	ALB (g/dL)	GLOB (g/dL)	IgG (mg/dL)	ALB/GLOB
0	3.05 (4.73)[a]	1.05 (1.68)[a]	2.05 (2.79)[a]	383.35 (235.85)[a]	0.51 (0.30)
3	1.68 (0.24)[b]	0.26 (0.10)[b]	1.44 (0.25)[b]	101.65 (40.19)[b]	0.17 (0.04)
7	1.52 (0.32)[c]	0.19 (0.72)[c]	1.32 (0.26)[c]	77.13 (14.72)[c]	0.14 (0.03)
15	1.29 (0.16)[d]	0.13 (0.04)[d]	1.15 (0.13)[d]	57.07 (17.26)[d]	0.12 (0.02)
30	1.18 (0.22)[e]	0.12 (0.04)[e]	1.07 (0.17)[e]	40.09 (14.32)[e]	0.11 (0.02)

Superscript letters within a column indicate significant differences among sampling times ($P < 0.05$).

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P131. COMPARATIVE SURVEILLANCE OF ATYPICAL PORCINE PESTIVIRUS IN SCOTLAND: 2018 AND 2022

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Atypical porcine pestivirus (APPV), a member of the genus *Pestivirus* within the family *Flaviviridae*, was first identified in 2015 and is primarily associated with congenital tremor (CT) in neonatal piglets. Although CT is not generally considered a major cause of mortality, affected piglets may show reduced survival, impaired growth and increased susceptibility to secondary infections. Since its initial description, APPV has been detected across North America, South America, Europe and Asia, indicating widespread circulation. However, information on APPV prevalence, molecular epidemiology and temporal dynamics within the UK remains limited. This study investigated APPV circulation in Scotland and compared epidemiological patterns and strain distributions using archived serum samples collected from approximately five-month-old pigs at slaughter in the two most recent QMS biobank collection years, 2018 and 2022. Samples from the biobank were tested using in-house qPCR and ELISA. The preliminary analysis included 1,077 samples from 109 farms in 2018 and 370 samples from 38 farms in 2022. Molecular detection rates were 4.46% (48/1,077) in 2018 and 2.97% (11/370) in 2022, while serological prevalence was 22.56% (243/1,077) and 23.24% (86/370), respectively. The consistently higher seroprevalence relative to qPCR positivity in both sampling periods suggests that APPV exposure is more widespread than active viraemia detectable at slaughter age and supports ongoing circulation in clinically normal production systems. APPV-positive samples were selected for sequencing to evaluate the genetic diversity and distribution of circulating strains between years. Overall, these preliminary findings strengthen current understanding of APPV circulation in Scotland, highlight the value of retrospective biobank analysis for surveillance, and support continued monitoring of APPV in Scottish pig populations.

P132. SEROTYPE DISTRIBUTION OF *Actinobacillus pleuropneumoniae* ISOLATED FROM SWINE LUNGS IN SOUTH KOREA

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Actinobacillus pleuropneumoniae(APP) causes porcine pleuropneumonia, a highly contagious disease characterized by acute onset, short clinical course, and high morbidity and mortality in pigs. It is essential that surveillance and serotyping of APPs should be done to set up the preventative measures and control program due to serovar distribution across regions and over time. This study aimed to isolate APPs and determine the distribution of their serovars in South Korea. The pneumonic lungs (n = 558) were from the slaughterhouse and pig farms showing pneumonia from 2021 to 2026. All isolates were grown on blood with nurse streaking of *S. aureus* and chocolate agar and identified by PCR targeting the a*px* IV gene. Serotyping (1-19) was determined by the method previously described. A total of 80 isolates (14.3%) were recovered from the lungs. Among them, serovar 2 (33.8%) was the most prevalent, followed by 1 (26.3%), 7 (16.3%), 5 (8.8%) and 8 (7.5%). Compared to finisher pigs (12.5%, 4/32), serovar 1 was highly detected in post-weaing and grower pigs (36.7%, 18/49) but serovar 2 showed a similar incidence within growth stages. Interestingly, the outbreak of serovar 7 has increased on pig farms since 2024. These findings highlight the need for continued surveillance and monitoring of APP serovas in Korea. It seems that serovar 1 is more pathogenic in grower pigs rather than other serovars, resulted in economic losses in pig farms. In addition, APP vaccine contains serovar 1, 2, and 5 in South Korea. Accordingly, there is a need to develop vaccines against APP serovar 7 to alleviate the severity of pleuropneumonia.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P133. MULTIPLEX REAL-TIME PCR FOR SEROTYPING OF *Actinobacillus pleuropneumoniae* 1, 2, 5, 7 AND 8 USING PEPTIDE NUCLEIC ACID (PNA) PROBES

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Actinobacillus pleuropneumoniae (APP) causes porcine pleuropneumonia, resulting in economic losses including sudden death, growth retardation in pigs. Nineteen serovars have been identified and reported in variation of virulence and distribution according to area and time. Serotyping is generally based on molecular techniques with a single testing for each serotype due to high genetic similarity of capsular polysaccharide loci (*cps* gene) of APP. Therefore, we developed a mutiplex real-time PCR system based on PNA based probes for differentiation of APP serovars (1, 2, 5, 7, and 8) circulating in Korea. Primers were designed by the sequence of capsular polysaccharide (*cps*) genes in serotype 1, 2, 5, 7 and 8. The *apx*IV gene was used as common primer target to detect APP. PNA probes were also developed to differentiate the serovars. The reaction conditions were optimized based on annealing temperatures of primers and concentrations of the probes. The detection limit and specificity were tested by serial dilutions of APP in broth and relevant pulmonary bacteria (*P. multocida, G. parasuis, S. suis* and *T. pyogenes*), respectively. The *apx*IV gene was amplified at all APPs with FAM and melting temperature (MT) of probes was 68°C. Serovar 1 and 2 were detected by HEX probe and MT of 1 and 2 was 69°C and 57°C, respectively. Cy5 probe differenntiated serovar 7 and 8 with a MT of 74°C and 58°C, respectively. Serovar 5 was amplified in quasar probe showing melting temperature of 67°C. The detection limits (CFU/mL) were serovar 1(10^3), 2 (10^3), 5(10^2), 7(10^3) and 8(10^2). No amplification was detected in *P. multocida, G. parasuis, S. suis and T. pyogenes*.
PNA-based real-time PCR assay is a reliable and powerful tool for the rapid detection and serotyping of APP, with applications in diagnostics and surveillance.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P134. INTEGRATED DIAGNOSIS OF *Mycobacterium thermoresistibile* FROM A CHRONIC CUTANEOUS LESION IN A DOMESTIC CAT

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Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms increasingly recognized as opportunistic pathogens in human and veterinary medicine. This study reports the isolation and characterization of *Mycobacterium thermoresistibile* from a domestic cat presenting a chronic, non-healing dorsal skin lesion characterized by thickened, fragile epidermis with ulcerated or easily bleeding areas and small pyogranulomas releasing dense, opalescent fluid upon digital pressure. Histology was suggestive of granulomatous dermatitis and panniculitis containing intralesional Ziehl–Neelsen (ZN)-positive bacteria. The microorganism was isolated from the lesion, cultured on selective Middlebrook 7H10 medium and incubated aerobically at 36±1 °C for up to 14 days. Preliminary identification was achieved by ZN staining and MALDI-TOF mass spectrometry (MycoEX protocol, Bruker Daltonics-Biotyper database), followed by molecular confirmation through PCR amplification and 16S rRNA gene sequencing. Sequences were analyzed by BLASTn against GenBank reference sequences. After 14 days of incubation, large, flat, irregular, ivory-colored colonies compatible with mycobacteria were isolated and acid-fast bacilli were observed by ZN staining. MALDI-TOF mass spectrometry identified the isolate as *Mycobacterium thermoresistibile* (log-score 1.774). Molecular analysis confirmed the identification: genus-specific PCR (Myc-gen F/R) and universal 16S rRNA PCR (27F/338R) yielded amplicons of the expected sizes (~1030 bp and ~300 bp, respectively). Sequencing generated an 838-bp consensus sequence showing 100% identity with two different *M. thermoresistibile* reference strains, unequivocally confirming species-level identification. Antimicrobial susceptibility testing showed no growth on Middlebrook 7H11 medium (Liofilchem, Italy) supplemented with amikacin-4µg/mL, rifampicin-1µg/mL or ofloxacin-2µg/mL, whereas growth occurred in the presence of streptomycin-2µg/mL. Although *M. thermoresistibile* is considered a rare pathogen, its isolation from a companion animal highlights its potential clinical relevance. The integration of advanced microbiological and molecular techniques proved essential for accurate identification and supports their inclusion in the diagnostic workflow of atypical mycobacterial infections in veterinary practice.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P135. COMPARATIVE PERFORMANCE OF TWO TRANSPORT SYSTEMS FOR PRESERVATION OF FUNGAL VIABILITY IN DIAGNOSTIC SETTINGS

F. Bonometti, F. Stretti, C. Sabelli
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Fungal infections are an important and often underdiagnosed cause of disease in animals, ranging from superficial to systemic conditions. Their diagnosis is challenging and strongly depends on proper sample collection and preservation to ensure pathogen viability[1-2]. This study aims to evaluate the ability of two transport and preservation systems, Copan eSwab™ and semisolid-based Transystem™, to maintain the viability of clinically relevant fungal strains and support accurate diagnostic outcomes. ATCC strain dilutions (*Candida albicans, C. tropicalis, S Saccharomyces cerevisiae, C. auris*) were prepared to obtain 25-250 CFU at time zero (T0) and used to inoculate FLOQSwabs (100 µL) and rayon swab (100 µL). After inoculation, all the swabs were transferred to their transport medium tube (FLOQSwabs into Copan eSwab™ and rayon swab into Transystem™). For each device, 21 swabs were prepared:

- 3 for T0 inoculum determination
- 6 (3 stored at 4°C; 3 stored at room temperature (RT)) for determination at 24 hours
- 6 (3 stored at 4°C; 3 stored at RT) for determination at 48 hours.

Following plate incubation (35°C in appropriate culture medium), colonies were counted. Results were expressed as mean CFU from triplicate samples. Acceptable criteria (ref. CLSI M40-A2) were defined as 25-250 CFU/plate at T0 and ≤ 1 Log at T24-48h versus T0.

Both systems maintained fungal viability up to 48h at RT and 4°C, meeting acceptable criteria. At T0, eSwab™ showed higher CFU recovery than Transystem™, indicating superior absorption and release capacity of the flocked swab in liquid Amies compared to the semisolid-based system. At RT, eSwab™ continued to yield higher recovery in line with accetable criteria, whereas Transystem™ showed lower recovery rate. Storage at 4°C improved stability for both transport devices. Both systems preserved viability up to 48 hours at different storage temperatures, with eSwab™ providing higher or comparable recovery across all time points.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P136. A DISCUSSION ON VALIDATING VETERINARY DIAGNOSTIC TESTS WITH A LIMITED SAMPLE PANEL

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APHA's testing capability plays a critical role in safeguarding animal health, supporting national surveillance programmes, and enabling international trade. Test performance is fundamental to early disease detection, informing control strategies and government policy, and maintaining confidence in the UK's animal health status. Validation assesses whether an assay has been optimised and developed for its intended purpose, as stated in the WOAH Manual[1]. A pragmatic approach can be used where ideal validation conditions cannot be met, for instance a limited sample panel size. The availability of samples with sufficient volume and provenance is a significant challenge for validation. In such circumstances, provisional recognition may be appropriate. The WOAH Manual recognises that no single approach is universally applicable and that methods should be adapted according to the available validation evidence. One approach prioritises analytical sensitivity, using standardised methods to characterise limits of detection, repeatability, and reproducibility. Alternatively, matrix spiking may be employed, whereby a positive sample of known provenance is diluted in negative serum of known provenance to increase sample volume. This enables further analytical assessment and supports evaluation of assay cut-off values. Where no gold standard test exists, Bayesian methods may be applied to integrate multiple sources of validation evidence. Sensitivity and specificity estimates derived from limited sample panels should be interpreted with caution. Although the calculated values may be mathematically correct, their confidence and representativeness may be reduced due to limited biological diversity. As outlined in the WOAH Manual, these estimates should be viewed as evolving rather than definitive. While these methods can provide valuable validation evidence of test performance, they do not capture real-world biological and field variability. Evidence weighting can therefore contextualise validation data and aid interpretation and decision-making. Test validation is a pragmatic process, with evidence continually added and refined over time as new data becomes available.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P137. AGREEMENT OF THREE PCR PLATFORMS (QPCR/DDPCR/DPCR) FOR QUANTIFICATION OF *Bovine* LEUKEMIA VIRUS PROVIRAL LOAD

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Bovine leukemia virus (BLV) is the etiologic agent of enzootic bovine leukosis (EBL), the most prevalent neoplastic disease in cattle worldwide. BLV proviral load (PVL) in blood is associated with disease progression and transmission risk, making this marker useful for control programs in highly prevalent regions where the classical strategy based on testing and culling of seropositive animals is economically impossible. Comparison of several quantitative real-time PCR (qPCR) assays revealed a wide discordance in the ability to detect and quantify BLV infection [1]. Digital PCR is the last-generation PCR methodology that allows highly accurate and absolute quantification of nucleic acids, offering a promising tool for evaluating BLV PVL. However, a recent inter-laboratory comparison showed a moderate agreement among qPCR and digital PCR assays [2], underscoring the need of further harmonization between PCR tests to improve quantitation accuracy. In this study, we compared three PCR methods targeting the BLV *pol* gene and bovine RPP30 housekeeping gene using the same primers and probes, including a qPCR assay, a droplet dPCR (QX200 system, Bio-Rad) and a microwell dPCR (QIAcuity One system, Qiagen). Using whole blood samples from 100 naturally infected cattle with variation in their PVL, a stronger correlation of measurement was found between digital PCR tests (Spearman's coefficient r = 0.943 *vs* 0.867-0.886 between qPCR and digital PCR tests). The qPCR and dPCR were further compared by evaluation of the limit of detection (LOD) and quantification accuracy. The two methods showed a similar LOD of 5 copies BLV/µL of blood. In contrast, accuracy profiles revealed that dPCR was the only valid method for accurate quantification of PVL at the threshold level associated with risk of BLV transmission. Altogether, these results indicated that digital PCR is a more suitable method for the detection and quantification of BLV.

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14. Other subjects pertaining to Veterinary Laboratory Diagnostics

P138. EVALUATION OF THE PERFORMANCE OF BULK TANK MILK TESTING UNDER LABORATORY AND FIELD CONDITIONS FOR THE SURVEILLANCE OF IBR IN CATTLE HERDS

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Testing of bulk tank milk (BTM) is a valuable method for the serological surveillance of infectious bovine rhinotracheitis (IBR) in dairy cattle herds. Previous comparative evaluation of commercially available ELISA tests (indirect, gB and gE) have been performed using a very limited number of BTM samples [1], preventing robust estimation of their relative diagnostic accuracy. In this study, a panel of 500 BTM samples collected from three countries with different IBR prevalence was analyzed in parallel by four reference laboratories using routine diagnostic ELISA tests in their respective country, including three whole-virus indirect tests (IDEXX Pool, IDEXX Tank Milk, and ID Screen Milk Indirect) and two blocking tests (Priocheck gB and IDEXX gE XM). Different Bayesian latent class models (BLCM) were fitted to determine the performance of each test. As expected, all BLCM analyses revealed a higher sensitivity of the indirect ELISA tests. By excluding BTM samples from the vaccinated population, the sensitivity was 41% and 79% for the gE and gB tests, respectively, and ranged from 92% to 97% for the indirect tests. All tests showed a high specificity (>99%). The tests with highest sensitivity were further evaluated using experimental BTM samples mimicking different within-herd prevalence. For this purpose, individual milk samples from 495 seropositive cows (19 infected herds) were titrated by ELISA. Pools of 100 milk samples were then created by combining representative positive milk samples with negative milk samples. Thirty unique pools were created for each within-pool prevalence. The IDEXX Tank Milk, ID Screen Milk Indirect and IDEXX Pool indirect tests detected all pools with a within-herd prevalence ≥ 2%, 4% and 6%, respectively. This study highlights the potential for applying BLCM in an inter-laboratory setting for evaluation of diagnostic methods, and the ability of the different ELISA tests to support the surveillance of IBR in BTM.

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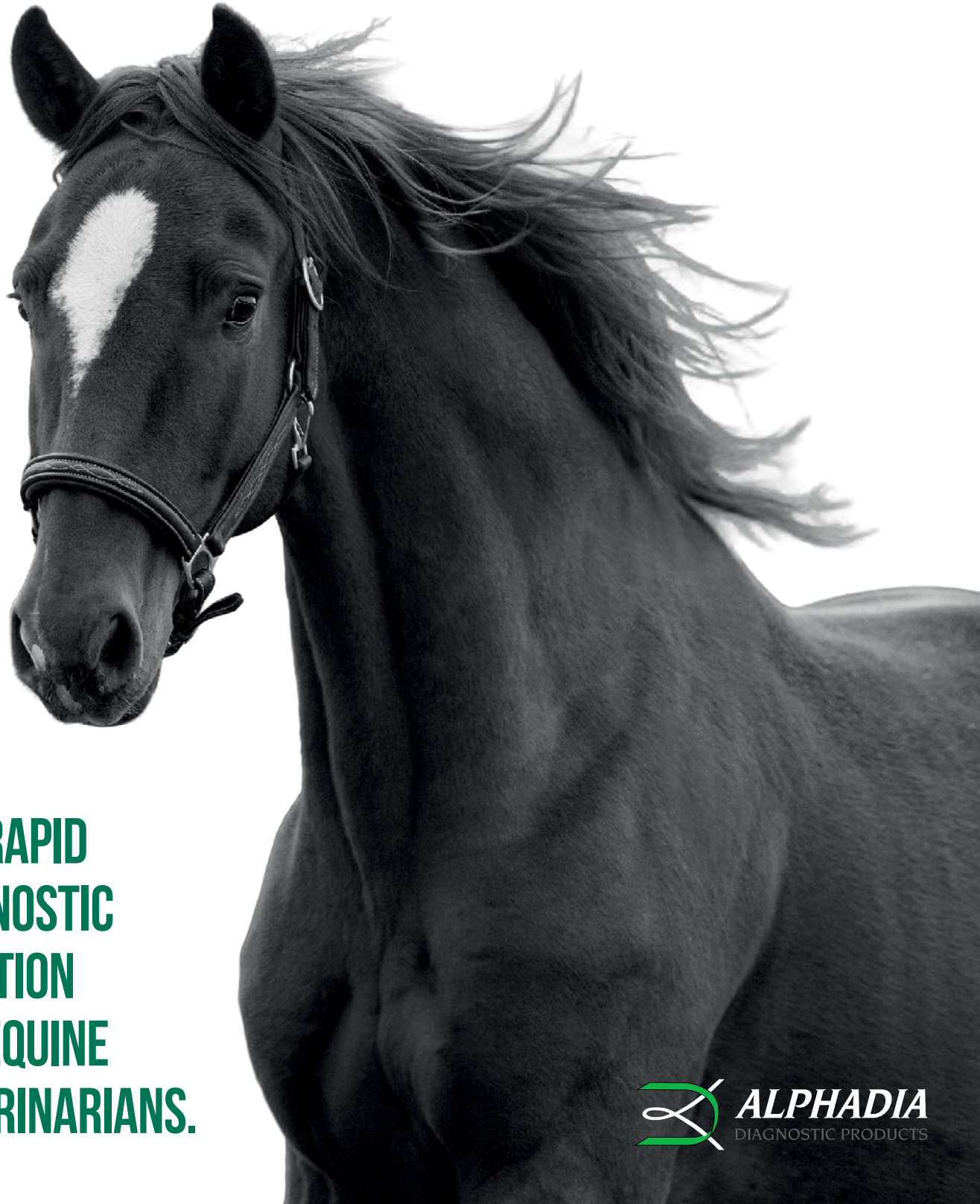
P139. ALTERATIONS IN WHITE BLOOD CELLS DERIVED INFLAMMATORY INDICES IN NEONATAL DIARRHEIC LAMBS WITH OR WITHOUT SIGNS OF BACTEREMIA

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Neonatal diarrhea is a major cause of morbidity and mortality in lambs and may be complicated by secondary bacterial overgrowth and bacteremia. The aim of this study was to compare hematological inflammatory indices among healthy lambs, lambs with diarrhea, and lambs with diarrhea and signs of bacteremia, and to evaluate their discriminatory ability. Fifty-nine neonatal lambs were classified as healthy controls (HC; n=18), lambs with diarrhea alone (D; n=23), or lambs with diarrhea and signs of bacteremia (DB; n=18). Neutrophil-to-lymphocyte ratio (NLR), derived NLR (dNLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), and aggregate index of systemic inflammation (AISI) were assessed at diarrhea onset (D1) and on the third day of diarrhea (D3). Because variables were non-normally distributed, comparisons were performed using non-parametric methods. At D1, NLR and dNLR were significantly higher in D than HC lambs ($P < 0.05$), while differences between HC and DB showed a tendency ($P=0.057$ and $P=0.063$, respectively). At D3, NLR and SII were significantly higher in DB than D lambs ($P < 0.05$). MLR and AISI were significantly higher in DB than HC lambs ($P < 0.05$), while dNLR, SIRI, and AISI showed tendencies toward higher values in DB than D ($P=0.06$, $P=0.082$ and $P=0.076$, respectively). These findings suggest that NLR and dNLR differentiate healthy from diarrheic lambs at disease onset, whereas AISI may be more closely associated with bacteremic status at D3. ROC analysis showed that AISI had the highest AUC among the indices (AUC=0.648, 95% CI: 0.548–0.740; $P<0.05$); however, its sensitivity (61.1%) and specificity (77.7%) at a cut-off >369.73 indicated only modest discriminatory performance, limiting its utility as a standalone marker for identifying bacteremic lambs. Composite hematological inflammatory indices may provide useful adjunctive information on systemic inflammation in neonatal lambs with diarrhea and signs of bacteremia.

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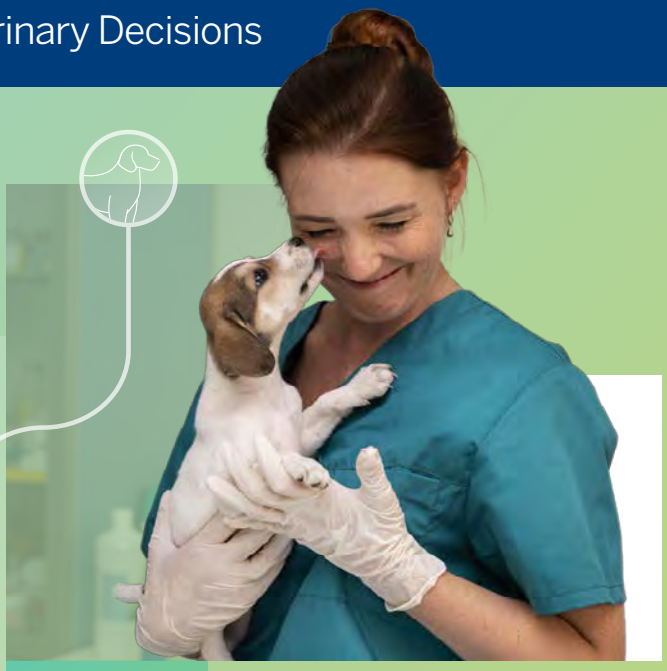


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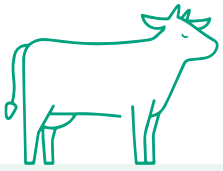
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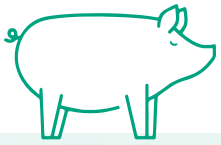
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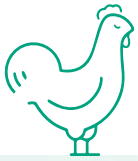
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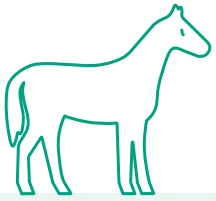
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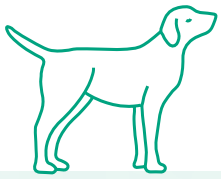
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