ISWAVLD 2025

CALGARY | JUNE 12 - 14

DIGITAL ABSTRACT BOOKLET

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ABSTRACTS

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JUNE 12, 2025

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08:30 - 10:30	**Plenary I - Panel Discussion:** Interplay between parasites/ vector-borne diseases with humans, animals and the environment - Drs. Chelsea Himsworth, Catherine Bouchard, John Gilleard			
10:30 - 11:30	Coffee / Tea Break 1 - Day 1 *(Exhibition & Posters open)*			

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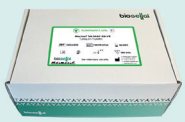

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SESSION LISTINGS

JUNE 12, 2025

Time	Exhibition Hall D	Exhibition Hall E	Glen 202	CAVP Glen 206
11:00 - 12:30	One Health		AMR Surveillance	Welcome to CAVP Invited Presenters
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SESSION LISTINGS

JUNE 12, 2025

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16:00 - 17:30	Lab Diagnostics in Resource-Limited Countries		AMR Surveillance and Approaches to Combating AMR	Pathology Case Reports - 2 Provincial Diagnostic Lab Reports & CAVP Business Meeting
17:30 - 18:30		Poster Session: Student Poster Competition Judging		
18:30 - 20:30		Welcome Reception		

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SESSION LISTINGS

JUNE 13, 2025

Time	Exhibition Hall D	Exhibition Hall E	Glen 202	WOAH - Bioprvail **Glen 206**
08:00 - 09:00	**Keynote speaker Presentation** Dr. Craig Stephen			
09:00 - 10:30	**Plenary III - Joint WOAH-ISWAVLD Panel Discussion:** Disease detection in a changing world – Dr. Primal Silva (Chair): Dr. Isabella Monne, Dr. Theophilus Odoom, Dr. Aruna Ambagala, Dr. David Williams			
10:30 - 11:00	Coffee / Tea Break 1 - Day 2 *(Exhibition & Posters open)*			
11:00 - 12:30	Innovative Technologies for Disease Detection		One Health and Multi-Host Spillover	Welcome & Introduction to BioPrevail
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13:30 - 15:30	Improved Lab Diagnostics		Artifiical Intellegence/ Machine Learning, Digital Diagnostics and Laboratory Networks	Interactive Brainstorming Sessions

SESSION LISTINGS

JUNE 13, 2025

Time	Exhibition Hall D	Exhibition Hall E	Glen 202	WOAH - Bioprvail Glen 206
15:30 - 16:00	Coffee / Tea Break 2 - Day 2 *(Exhibits & Poster open)*			
16:00 - 18:30	**WILDER INSTITUTE / CALGARY ZOO** *Ticket event - Name badges must be worn*			
18:30 - 21:30	**SYMPOSIUM DINNER AT CALGARY ZOO**			

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SESSION LISTINGS

JUNE 14, 2025

Time	Exhibition Hall D	Exhibition Hall E	Glen 202
08:30 - 10:30	**Plenary IV - Panel Discussion:** Highly Pathogenic Avian Influenza (HPAI) - Drs. Dayna Goldsmith, Clarice Lulai-Angi, Isabella Monne		
10:30 - 11:00	Coffee / Tea Break 1 - Day 3 *(Exhibits & Posters open)*		
11:00 - 12:30	Disease Detection and Improved Lab Diagnostics		Surveillance
12:30 - 13:30	Lunch - Day 3 *(Exhibitors & Posters open)*		
13:30 - 15:30	Outbreak Preparedness and Response		a) Deadly Secrets: bioweapons, bioterrorisms, and deliberate biological threats
			b) Clinical Conundrums
15:30 - 14:00	Closing Remarks and Awards		

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Keynote Presentation

Exhibition Hall D, June 13, 2025, 8:00 AM - 9:00 AM

The need for disruptive thinking to combat the global polycrisis

Dr. Craig Stephen
McEachran Institute

The world is being increasingly impacted by multiple, interconnected crises occurring together and amplifying each other's effects, making them more difficult to manage or resolve. This is the world of the polycrisis. This talk explores why we need to challenge conventional ways of thinking to create new solutions and innovations in a polycrisis era. Suggested ways to become less surprised by events like emerging diseases, become more resilient to problems like climate change and become more effective in mobilizing knowledge into action will be discussed. The goal of the presentation is to suggest pathways to future ready animal health in a complex, interconnected and rapidly changing world.

Plenary Presentations

Plenary I

Panel Discussion: Interplay between parasites/vector-borne diseases with humans, animals and the environment - Drs. Chelsea Himsworth, Catherine Bouchard, John Gilleard,

Exhibition Hall D, June 12, 2025, 8:30 AM - 10:30 AM

The Great Prince of the Forest Effect: Unexpectedly High Prevalence of Zoonotic Anaplasma phagocytophilum (Ap-ha) in White-Tailed Deer

Dr. Catherine Bouchard
PHAC

Human granulocytic anaplasmosis (HGA) is an emerging tick-borne disease in southeastern Canada, caused by Anaplasma phagocytophilum (Ap), specifically the zoonotic "human-active" strain (Ap-ha). While white-tailed deer (Odocoileus virginianus) are established reproductive hosts for Ixodes scapularis, they are not traditionally considered competent reservoirs for tick-borne pathogens. However, given their role in tick population maintenance and the recent outbreak of HGA cases in Estrie, Québec, this study aimed to evaluate deer as sentinel species and potential reservoirs of Ap-ha.

In November 2023, a targeted surveillance effort was conducted at three butcher stations in the Estrie region. A total of 246 hunter-harvested deer were examined for ectoparasites, and samples of blood and ear tissue were collected. Pathogen detection was performed by multiplex PCR. We used a negative binomial model to assess the effects of age and sex on tick infestation, and logistic regression to examine predictors of Ap-ha infection. Spatial cluster analysis was used to identify patterns in tick burden and pathogen distribution.

Of the 246 deer, 94 (38%) were infested with adult I. scapularis, yielding 642 ticks. Ap-ha-infected ticks were found on 30 deer (32%), predominantly males. The prevalence of Ap-ha in deer-associated ticks (31.3%) was significantly higher than that in questing ticks from the same region (3%, $p < 0.0001$). Male ticks exhibited a higher infection prevalence (55.2%) than females (19.4%). Ap-ha was also detected in 8 deer blood samples and 10 ear biopsies—the first such detection reported in North America.

Tick burden was greater in male deer and declined with age. Ap-ha infection likelihood increased with infestation intensity. Spatial clusters of infected ticks were observed in southwestern Estrie. These findings challenge current assumptions about deer as non-competent hosts and support the use of tick xenosurveillance of harvested deer as an efficient tool for monitoring emerging zoonotic pathogens like Ap-ha.

Beyond Microscopy: How DNA Metabarcoding is Transforming Our Understanding of Parasite Genetic Diversity, Distribution and Drug Resistance.

Dr. John Gilleard
University of Calgary

Parasitic nematodes are important human and animal pathogens with extremely high levels of genetic diversity. Until recently, diagnostic parasitology relied on traditional microscopy, sometimes supported by PCR and Sanger sequencing of taxonomic markers. The recent explosion of next-generation sequencing now allows us to define complex parasite communities at a scale and depth that was previously impossible. I will discuss how we are applying next-gen sequencing to precision parasite diagnostics using two recent examples. The first example, is for the hookworm Ancylostoma caninum, an important canine pathogen worldwide which is also zoonotic. We first identified benzimidazole drug resistance mutations in the A. caninum isotype-1 β-tubulin gene and then used deep amplicon sequencing on >700 hookworm positive fecal samples from pet dogs and racing greyhounds across the USA. Two resistance mutations were widespread with prevalences of 49.7% (overall mean frequency 54.0%) and 31.1% (overall mean frequency 16.4%) for F167Y(TTC>TAC) and Q134H(CAA>CAT), respectively. Molecular epidemiological analysis supports the hypothesis that benzimidazole resistant A. caninum originated in racing greyhound kennels and spread into the pet dog populations as retired greyhounds were rehomed. A second example was for human Trichuriasis, an important soil transmitted helminth in low/middle income countries for which albendazole/ivermectin combination therapy is being trialed for Mass Drug Administration Programs (MDA). We used fecal DNA metabarcoding to genetically characterize Trichuris populations in patient samples from Côte d'Ivoire showing lower albendazole/ivermectin sensitivity than those from Laos and Tanzania. Internal transcribed spacer ITS1 and ITS2 metabarcoding revealed the Côte d'Ivoire Trichuris was phylogenetically distinct from T. trichiura found in Laos and Tanzania. Mitochondrial genome sequencing confirmed its status as a newly recognized species Trichuris incognita which is closer to Trichuris suis than the human pathogen T. trichiura. Sequences corresponding to this new species from three captive non-human primates suggest this may be a zoonotic parasite.

From Data to Knowledge to Wisdom: How Combining Laboratory Diagnostics with Other Disciplines Can Change Our Understanding of Rat-Associated Zoonoses.

Dr. Chelsea Himsworth[1]
[1]BC Ministry Of Agriculture, Abbotsford, Canada

Over the past 15 years, the Vancouver Rat Project has been studying the health risks associated with urban rats. This interdisciplinary research has shown is that it is unhelpful to assume that the harm associated with rats is a function of the number of rats present. Rather, the estimation of harm needs to focus on the interface between rats, people, pathogens, and the environment.
Similarly, though each scientific discipline can provide data to help characterize rat-associated harms, it is the interface between these disciplines from which the intelligence to solve current rat problems emerges, and from which we can create the wisdom needed to predict and prevent future issues.
In this presentation, I will describe how we combined veterinary laboratory diagnostics with ecology, epidemiology, experimental medicine, population genetics, policy analysis, social sciences and more, to change the way we assess, monitor, and mitigate diverse risks associated with urban rats.

Plenary II

Panel Discussion: Antimicrobial resistance in agriculture and aquaculture - Drs. Tim McAllister, Diego Nobrega, Claire Burbick

Exhibition Hall D, June 12, 2025, 1:30 PM - 3:30 PM

Antimicrobial Susceptibility Testing in Aquatic Animals: A CLSI Overview and Update

Claire Burbick
Washington State University

Antimicrobial susceptibility testing (AST) is an essential tool for clinical decision making, surveillance for acquired resistance and assessment of interventions to mitigate resistance development. While phenotypic AST methodology has been standardized for decades for common mammalian pathogenic bacteria, standard methods for aquatic pathogens have lagged behind. Unfortunately, these bacteria of aquatic origin are typically more nutritionally fastidious and require a range of temperatures for optimal growth rendering current mammalian standard methods unable to be used in most situations. One of the only standard development organizations pursuing both methodologic standardization as well as interpretive criteria, in the form of clinical breakpoints and epidemiologic cutoff values (ECV) for global harmonization in aquatic AST is the Clinical and Laboratory Standards Institute (CLSI). Under the Veterinary Antimicrobial Susceptibility Testing (VAST) Subcommittee the Aquatic Animal Working Group has made significant strides in both methodology, quality control, and ECVs. We will review the CLSI organization and current work being performed, including a new document edition updates.

Leveraging Peer-Reviewed Data for Filling Gaps in Antimicrobial Resistance Surveillance in Animals

Dr. Diego Nobrega
University of Calgary

Antimicrobial resistance (AMR) is a growing global threat, calling for innovative surveillance strategies that move beyond conventional clinical and laboratory approaches. In agricultural settings, where routine AMR monitoring can be limited or resource-intensive, peer-reviewed literature and other non-traditional data sources offer a valuable yet underutilized opportunity within Canada. Building on our experience with human clinical data, this talk will explore the challenges and opportunities of incorporating alternative data sources into AMR surveillance frameworks to enhance their scope and effectiveness.

HARNESSING GENOMICS TO EVALUATE ONE HEALTH AMR RISKS

Dr. Tim McAllister[1], Mrs Krysty Thomas[1], Dr Sara AndresLasheras[1], Dr. Rahat Zaheer[1]
[1]Agriculture And Agri-food Canada, Lethbridge, Canada

Antimicrobial resistance (AMR) has been identified as one of the grand health challenges to humanity and is predicted to be responsible for 10 million deaths and 1 trillion $ loss yearly to global GDP by 2050. Antimicrobials play an essential role in promoting health in humans companion animals and livestock, but inappropriate use can select for resistance and reduce their efficacy at controlling infectious disease. Accurate risk assessments are essential to identifying antimicrobial use practices that reduce the emergence of AMR pathogens and optimize health outcomes. Genomics is increasingly being used to assess AMR risk in a variety of ways.

Description

Metagenomics can characterize the resistome in food production, hospital, urban and livestock waste streams as well as in pristine environments. Phylogenetic analysis and molecular bacterial identification can identify the level and types of pathogenic bacteria that may be present in an One Health environment. Antimicrobial resistant genes (ARGs) that confer resistance to antimicrobials of greatest concern to human health can be identified. Furthermore, their association with mobile genetic elements (MGE) can be ascertained and used to assess the risk of horizontal gene transfer. Metatranscriptomics can gage the extent to which ARGs are expressed. Comparative genomics of bacteria isolated from an array of One Health environments can identify AMR hot spots. Metagenomic and genomic screening for virulence genes can inform risk and the level of pathogenicity.

Discussion

Further exploration of genes associated with metabolic pathways can shed light on the potential environmental persistence of AMR pathogens and identify mitigation practices that impede survival. Compilation of AMR data along with other relevant metadata in global databases support the development of machine learning approaches that will further enhance the prediction of AMR risk throughout the One Health Continuum.

Plenary IV

Panel Discussion: Highly Pathogenic Avian Influenza (HPAI) - Drs. Dayna Goldsmith, Clarice Lulai-Angi, Isabella Monne

Exhibition Hall D, June 14, 2025, 8:30 AM - 10:30 AM

Avian influenza in wildlife: A changing global landscape

Dr. Dayna Goldsmith
Diagnostic Services Unit

Historically, despite its potential pathogenicity to domestic poultry, avian influenza was considered a disease of minimal clinical significance to wild birds. Wild birds, especially dabbling ducks, are the natural host and reservoirs for this virus, and infection was historically not associated with clinical disease. This perspective has dramatically changed in light of the current global outbreak of an H5N1 strain of highly pathogenic avian influenza. H5N1 has become a major source of mortality for several avian species including waterfowl, raptors and corvids. More recently this virus has also spilled over into several wild mammalian species including skunks, canids, felids, bears and a variety of marine mammals. This presentation will review the evolution of the current outbreak of H5N1 in wildlife including the gross and histologic pathology associated with this infection and the importance of collaborative surveillance efforts.

HPAI in Europe: An evolving threat in a complex ecosystem

Dr. Isabella Monne
Istituto Zooprofilattico Sperimentale Delle Venezie

Since late 2005, Europe has been repeatedly targeted by incursions of highly pathogenic avian influenza (HPAI) H5 viruses descending from the goose/Guangdong (Gs/GD) lineage first detected in China in 1996. These incursions typically occurred as sporadic events and were driven by the seasonal migratory movements of wild birds from Asia during autumn and winter, with no evidence of year-round persistence.
However, a significant shift has occurred since October 2020 with the re-emergence and sustained circulation of clade 2.3.4.4b, a descendant of the Gs/GD lineage HPAI A(H5) viruses. This transition has been marked by multiple ecological, epidemiological, and evolutionary changes that underscore the growing complexity of this threat. These include: (i) the geographic expansion of the virus into previously unaffected northern and western European regions; (ii) a broader range of affected wild bird species, indicating a diversification of ecological niches; (iii) a rising number of mammalian infections, including sporadic events suggestive of potential mammal-to-mammal transmission; (iv) an unprecedented degree of viral reassortment, with over 90 distinct genotypes identified in Europe alone;(v) and the virus's persistence through summer months, challenging previous assumptions about the timing and duration of risk periods for epidemic outbreaks in birds across the European region.
This presentation will explore the evolving nature of HPAI H5 in Europe, reflecting on how a complex and interconnected region such as Europe is striving to maintain a coordinated One Health response in the face of a pathogen that knows no borders.

High (and Low) Pathogenicity Avian Influenza in Canada, 2022- 2025

Dr. Clarice Lulai Angi
Canadian Food Inspection Agency

Since 2020, Highly Pathogenic Avian Influenza (HPAI) clade 2.3.4.4b has caused widespread outbreaks globally, presenting a complex One Health challenge that spans wild and domestic animals, as well as humans. In Canada, this clade has resulted in the loss of over 14 million domestic birds and more than 2,800 wild birds, with multiple mammalian species affected, including one confirmed human infection. Unlike earlier HPAI clades, 2.3.4.4b not only circulates in wild birds but also causes significant mortality in wild birds and mammals. Globally there have been over 280 million domestic birds affected by HPAI, while it is estimated that over 1 million wild birds may have also died of HPAI worldwide.

The virus was first detected in the Americas in Newfoundland in December 2021 and has since spread to Central and South America including to Antarctica, affecting previously unaffected regions of the world. Notably, in March 2024, infection in lactating dairy cattle was confirmed in the United States—an unprecedented spillover event—with outbreaks now reported in over 1,000 herds across 17 states. Despite extensive surveillance in Canada, including targeted testing of symptomatic cattle and raw milk screening, HPAI has not been detected in Canadian dairy herds.
To better understand transmission dynamics and inform biosecurity, the Canadian Food Inspection Agency (CFIA) applies Transmission Network Analysis. This approach integrates genomic sequencing data from viral isolates with epidemiological information from affected premises to elucidate likely introduction routes and transmission chains. The resulting insights guide actionable biosecurity measures at the farm level to mitigate future outbreaks.

For decades, countries have avoided the use of vaccination against avian influenza, because of concerns about the safety and efficacy of HPAI vaccines. In the face of the current outbreak, the World Organization for Animal Health (WOAH) began highlighting the importance of vaccination as a tool for preventing HPAI outbreaks. For influenza vaccines to be effective they must be well matched to circulating strains and be used in carefully controlled ways to allow for surveillance to detect any possible viral mutations or transmission from vaccinated birds. As many countries begin to explore using vaccination to prevent HPAI outbreaks as they face the increased probability that waves of HPAI will continue to occur in future years, government and industry in Canada are discussing the possible use of vaccines. Three vaccines have been approved for emergency use in Canada, but vaccination is currently prohibited.

This presentation will provide an overview of the current epidemiological situation in Canada and North America, discuss the role of Transmission Network Analysis in outbreak response, and review progress on vaccination strategies—all framed within the broader One Health context emphasizing the interconnected risks to animal, human, and ecosystem health.

A One Health Approach to Surveillance for Japanese encephalitis in Papua New Guinea

Dr. David Williams[1], Ms Joelyn Goi[2], Ms Bridgit Kavana[3], Dr Melanie Koinari[4], Ms Shaylee Latimore[1], Mr Antonio Di Rubbo[1], Mr Stephen Gideon[2], Ms Donna Gillies[1], Mr Darcy Beveridge[1], Dr Jianning Wang[1], Dr Petrina Johnson[2,4], Ms Julie Cooke[1], Mr Orlando Mercado[3], Mr Martin Paina[3], Mr Pius Clement[3], Dr Tim Bowden[1], Mr Muker Sakur[2], Ms Rebecca Vinit[2], Dr Lisa Rigby[5], Mr David Tenakanai[3], Dr Ilagi Puana[3], Professor Willie Pomat[2], Dr Stephan Karl[2,4]
[1]CSIRO Australian Centre for Disease Preparedness, Geelong, Australia, [2]Vector-borne Diseases Unit, Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]National Agriculture and Quarantine Inspection Authority, Port Moresby, Papua New Guinea, [4]Australian Institute of Tropical Health and Medicine, James Cook University, Smithfield, Australia, [5]Australian Defence Force Malaria and Infectious Disease Institute, Enoggera, Australia

Introduction

Japanese encephalitis (JE) is an emerging zoonotic mosquito-borne disease affecting humans and livestock. Little is known about its epidemiology in Papua New Guinea (PNG), the most populous Pacific Island nation, located to the north of Australia. Using a One Health approach, pilot JE surveillance activities were undertaken in PNG using a combination of sentinel animal (swine) and mosquito trapping methods between 2019-2021 and 2023-2024 to investigate the prevalence and distribution of JE in PNG.

Methods

Serological surveillance of animals utilised sites located in the provinces of National Capital District, Central and Morobe, including commercial and village farming systems. ELISAs were employed to detect antibodies to JE virus and antigenically related flaviviruses. Mosquitoes were collected using CDC light traps (UV and incandescent) and BG traps, targeting Culex mosquitoes, the major vectors of JE. Trapped mosquitoes were identified, pooled and tested by real-time RT-PCR.

Results

Serological evidence of JE virus infection was found in sentinel swine at all study sites. For mosquito surveillance, CDC traps with ultraviolet light were found to be optimal for trapping Culex species. During 2019-21, from a total of 18,951 Culex spp. mosquitoes tested, JEV was detected in 2 pools of Cx. gelidus mosquitoes collected in Morobe. Between 2023-24, a further 33,851 Culex spp. were trapped, and JEV was detected in 2 more pools of Cx. gelidus from Morobe and Central provinces. Sequence analysis revealed that these viruses are closely related and belong to JEV genotype 4.

Discussion

Underpinned by laboratory and field capacity building, this study demonstrated continued circulation of JE in PNG and the earliest detection genotype 4 in the Australasian region. Our study highlights the value of a One Health approach for leveraging resources and capabilities in resource-limited settings as well as regional collaboration to facilitate surveillance and control of priority zoonotic pathogens.

AMR Surveillance, Glen 202, June 12, 2025, 11:00 AM - 12:30 PM

Antimicrobial resistance genes and associated mobile genetic elements in Escherichia coli: genomic insights from hybrid assemblies of surveillance isolates across One Health sources

AUTHORS: Alyssa Butters[1,2], Juan Jovel[1], Cheryl Waldner[3], Sheryl Gow[3,4], Karen Liljebjelke[1,2], Sylvia Checkley[1,2]
AFFILIATIONS: [1]University of Calgary, Faculty of Veterinary Medicine, [2]AMR One Health Consortium, [3]University of Saskatchewan, Western College of Veterinary Medicine, [4]Public Health Agency of Canada (CIPARS/FoodNet),

Introduction

Understanding antimicrobial resistance (AMR) transmission requires consideration of not only antimicrobial resistance genes (ARGs) but also the hierarchical layers of mobile genetic elements (MGEs) that mobilize and disseminate them. Phenotypic AMR surveillance cannot delineate the genomic location or MGEs associated with resistance elements, limiting insights into transmission dynamics. The aims of this study are to identify ARGs and associated MGEs in hybrid assemblies of generic *Escherichia coli* surveillance isolates and identify patterns of gene presence, location, and co-location within and between sources in a One Health continuum.

Methods

Escherichia coli isolates (n=148) collected in Alberta, Canada, in 2018 and 2019 for routine surveillance of retail meat (beef and chicken), production animal feces (feedlot cattle and broiler chickens), wastewater, and well water were subjected to short-read (Illumina) and long-read (Nanopore GridION) sequencing. Hybrid assemblies were produced using Trycycler or Unicycler, then scrutinized for ARGs, plasmids, integrons, transposons and insertion sequences, integrative conjugative elements, and phages with AMRFinderPlus, PlasmidFinder, IntegronFinder, MobileElementFinder, ICEFinder, and PHASTEST, respectively. Annotated Genbank files were created using a custom Python script, then visualized using Geneious Prime. Plasmid similarity was assessed using pling, and proximity network analysis identified resistance and mobile elements co-located within 10,000 base pairs on bacterial chromosomes or plasmids.

Results

Isolates with similar phenotypic resistance often bear different ARGs associated with variable MGEs, frequently differing by source. Co-located ARGs and MGEs also demonstrated differences in genomic location (plasmid or chromosome).

Discussion

As the genomic location (plasmid versus chromosome) and characteristics of the associated MGEs could potentially influence transmissibility of a resistance element, the specific MGEs and ARGs mediating resistance are important considerations when assessing AMR surveillance results or integrating surveillance information within AMR transmission models.

Antimicrobial Resistance, the role of wild avifauna

Miss Loredana Capozzi[1], Rosa Fraccalvieri[1], Marta Caruso[1], Laura Maria Difato[1], Adelia Donatiello[1], Domenico Galante[1], Paola Pino D'Astore[2], Gianluca Nocco[3], Antonio Camarda[4], Elisabetta Catalano[1], Michela Galgano[1], Giulia Schino[1], Antonio Parisi[1]
[1]Istituto Zooprofilattico Sperimentale Puglia E Basilicata, Foggia, Italy, [2]Centro Fauna Selvatica Provincia di Brindisi - Santa Teresa, Brindisi, Italy, [3]Centro di Recupero Fauna Selvatica di Calimera, Calimera, Italy, [4]Università degli Studi di Bari Aldo Moro, Bari, Italy

Introduction
Antimicrobial resistance is a global health emergency, and the overuse of antibiotics has encouraged the spread of multi-resistant bacterial strains. Wild birds, increasingly in contact with humans and exposed to drug residues with pollution, can act as reservoirs and vectors of resistant pathogens, contributing to their dissemination. Resident species pose a risk to urbanized areas, while migratory species spread resistance along routes. The study aims to understand the role of avifauna in the transmission of resistance genes, providing useful data for surveillance and management strategies for this global issue.

Methods
Cloacal swabs from wild birds were collected in Apulia and subjected to enrichment and culture on selective media for resistance to carbapenems, beta-lactams, vancomycin and colistin. Zoonotic and resistant bacterial strains were selected, evaluated by MIC, sequenced with Illumina MiSeq and analyzed by bioinformatics protocols.

Results
Between July 2024 and February 2025, 99 wild birds were sampled to assess the spread of resistant bacteria. 74.74% of the samples (74/99) showed the presence of potentially zoonotic antibiotic-resistant bacteria, while 25.25% were negative. From the 74 positive samples, 145 bacterial strains with relevance to human health were isolated and cryopreserved: 59 E. coli, 27 Enterococcus spp., 20 Klebsiella spp., 8 Acinetobacter spp., 8 Enterobacter spp. along with other species. On 36 selected bacterial strains, MIC was evaluated and genomic sequencing conducted. All bacteria, with the exception of one Salmonella strain isolated on ESBL agar, showed in vitro or in silico resistance.

Discussion
These data highlight the role of avifauna in the spread of multi-resistant bacteria, underscoring the importance of microbiological surveillance. Continuous monitoring and genomic analysis are key tools for understanding the transmission of antimicrobial resistance and developing containment strategies, reducing public health risks.

FECRT for Determining Anthelmintic Resistance to Equine Strongyles – A Retrospective Study (2019-2025)

Maria Finnerty[1], Holly White[1], Rebecca Young[1], Danielle Maguire[1], Dr. Manigandan Lejeune Virapin[1]
[1]Animal Health Diagnostic Center & New York State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, USA

Introduction

The Fecal Egg Count Reduction Test (FECRT) is an important parasitological diagnostic tool for assessing the efficacy of various classes of anthelmintics for treatment and control of equine strongyles. FECRT determines the percent reduction in strongyle eggs post-treatment and compares it with the suggested cutoff values for interpreting results. This test was recommended by the American Association of Equine Practitioners (AAEP) in its Parasite Control Guidelines published in 2016. The Cornell Animal Health Diagnostic Center (AHDC) offers FECRT for equine clients to encourage evidence-based management of anthelmintic resistance.

Methods

Practitioners who submitted equine fecal samples to the AHDC parasitology laboratory for strongyle egg counts were alerted to avail FECRT when an animal with >500 eggs per gram (EPG) of feces was detected using the Wisconsin double centrifugation floatation assay. The post-treatment sample obtained from days 10-14 was subjected to the same floatation technique, EPG was determined, and percent egg count reduction was calculated and reported. In this retrospective study, we analyzed FECRT data from 2019 to 2025. The primary anthelmintics classes evaluated were benzimidazoles (fenbendazole & oxibendazole), tetrahydropyrimidines (pyrantel pamoate), and macrocyclic lactones (ivermectin & moxidectin).

Results

In total, 215 FECRT results were studied. Resistance to Fenbendazole was noted in 19 of the 25 animals (76%) treated with this drug. All three horses (100%) treated with oxibendazole were resistant. Resistance to pyrantel pamoate was noted in all four animals (100%). Four percent of 130 animals treated with Ivermectin showed resistance. One of the 40 horses (2.5%) showed resistance to moxidectin. A combination treatment of fenbendazole with macrocyclic lactones was effective (91% of 11 animals); whereas fenbendazole with pyrantel pamoate was 100% ineffective (n=2).

Discussion

Macrocyclic lactone performed better than other anthelmintic classes; however, the small percentage of resistance observed for this drug class was not confirmed by retesting.

Quebec Veterinary Antimicrobial Surveillance Program – New interactive report

Julie-Hélène Fairbrother

Geneviève Côté, Lauriane Duplaix, Julie-Hélène Fairbrother
Direction générale Expertise en santé et bien-être des animaux
Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec

In Quebec, surveillance of bacterial resistance to antibiotics is carried out by the Programme Québécois d'Antibiosurveillance Vétérinaire (PQAV). Analyses for this program are carried out by the Laboratoire de Santé Animale (LSA) of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, in collaboration with the Centre de Diagnostic Vétérinaire de l'Université de Montréal (CDVUM) and the Public Health Agency of Canada (PHAC).

The objectives of the program are to monitor temporal variations in the susceptibility and multidrug resistance of certain pathogenic bacteria to antimicrobials of importance in veterinary medicine in Quebec, and to circulate the results in a timely manner. The results of this surveillance are mainly used to guide treatment strategies, to decide whether measures to reduce antimicrobial resistance should be implemented, and to monitor the effectiveness of these measures, when appropriate. Since 2023, we are very proud to present the results of this surveillance with a new interactive report available online and accessible to all for consultation.

Screening of indoor airborne microbial communities and antimicrobial resistant genes from layer barns across Alberta, Canada

Mr. Awais Ghaffar[1], Karen Liljebjelke[1], Sylvia Checkley[1], Dr. Frank Meer[1], Muhammad Farooq[1], Dr. M. Faizal Abdul-Careem[1]
[1]Faculty Of Veterinary Medicine, University Of Calgary, Calgary, Canada

Introduction

Bacteria carrying antimicrobial resistant genes (ARGs) can become airborne within poultry barns and can be transmitted to animals and poultry workers, presenting a serious One Health concern. The current study aimed to characterize the microbiome and resistome of bioaerosols sampled from layer chicken barns across Alberta.

Method

A total of 15 layer poultry barns were sampled in this study with a microbial air sampler to characterize the microbiome and resistome using a shotgun metagenomic sequencing approach.

Results

The bacterial species, Klebsiella pneumonia and Corynebacterium ulcerans were significantly more relatively abundant in bioaerosols of cage housed barns when compared to that of floor housed barns. The potential pathogenic bacterial species including Staphylococcus aureus and Salmonella enterica were relatively present more in bioaerosols of floor housed when compared to cage housed barns whereas the bacterial species, Campylobacter jejuni was present relatively more in the bioaerosols of cage housed when compared to floor housed ones. The respiratory pathogens including Gallibacterium anatis, Ornithobacterium rhinotracheale and Pasteurella multocida were relatively more frequently observed in air of cage housed when compared to floor housed layer barns, while Escherichia coli and Avibacterium paragallinarum were relatively observed more in bioaerosols of floor housed when compared to cage housed layer barns. Based on the presence of ARGS, resistance to tetracycline, lincosamide's and macrolides were more frequently observed in the bioaerosols of cage housed layer barns when compared to floor housed layer barns while aminoglycoside resistance was observed more frequently in floor housed layer barns. Overall, both the microbiome and resistome were found more relatively in the bioaerosols of cage housed when compared to floor housed layer barns. Discussion: The current study improved understanding of complex microbial communities in the layer barn bioaerosols and also highlighted the potentially opportunistic pathogens important for birds and human health.

One Health, Exhibition Hall D, June 12, 2025, 11:00 AM - 12:30 PM

COVISelect: A Novel Duplex RT-LAMP Assay for the Surveillance of SARS-CoV-2 in Wild Animals

Dr. Yogesh chander[1], Alyssa Hassinger[1], Muhammad Luqman[2], Parfait Kada Florent[2], Sunil Mor[2], Binu Velayudhan[3], Hemant Naikare[4]
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Introduction

Coronavirus is a major public health challenge. Besides humans, many animal species are susceptible to SARS-CoV-2 infection and could act as reservoirs. Global dissemination of SARS-CoV-2 among humans provides opportunities for spillover transmission into non-human hosts and the emergence of novel variants with the potential for spillback to humans.
Rapid tests for detecting existing SARS-CoV-2 infections and assessing virus spread are critical in both humans and animals. Here we report on development of a novel duplex RT-LAMP assay for rapid detection of SARS-CoV-2 at point -of-care.

Methods

This duplex RT-LAMP assay consists of two primer sets, one specific for SARS-CoV-2 and another for 18S rRNA gene (internal control). Reactions were carried out at 64°C for 30 minutes on a benchtop isothermal instrument. Reaction conditions were optimized for the detection of SARS-CoV-2 directly from the clinical samples, including nasopharyngeal (NP) swabs in VTM tissue samples with low virus titer (CT=35) without using any RNA extraction.

Results

Sensitivity of the COVISelect test kit was evaluated using spiked samples, and results showed the limit of detection (LoD) 200 copies/mL. In addition, 30 NP swab samples collected from wild deer were also tested by COVISelect kit. Of these 30 NP swab samples, 6 were known positives (confirmed by RT-qPCR). Results obtained with the COVISelect kit showed >95% concurrence with RT-qPCR results. Only those samples that had Ct >36 tested negative by COVISelect kit. The specificity of the COVISelect kit was evaluated by testing a panel of respiratory pathogens (humans and animals), and results showed 100% specificity.

Conclusion

The High specificity, sensitivity, faster time to result, ease of use, and low cost of COVISelect test kit make it a promising candidate for rapid screening for SARS-CoV-2 in low-resource settings.

Findings from an Acute Erysipelas Outbreak in a High Arctic Muskox (Ovibos moschatus) Population

Dr. McCaide Wooten[1], Dr. Taya Forde[2], Amélie Roberto-Charron[3], Matthew Fredlund[3], Dr. Sreejith Radhakrishnan[2], Dr. Dayna Goldsmith[1,4], Dr. Beverly Morrison[1], Angela Schneider[1], Dr. Frank Meer[1], Ronan Donovan[5,6], Dr. Susan Kutz[1]
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Introduction

The muskox is an emblematic Arctic species that has been vital to the health of northern ecosystems and Indigenous Canadians for millennia. However, this ungulate now faces an infectious disease threat – Erysipelothrix rhusiopathiae – that has arisen in concert with regional population declines. Mortality events attributed to this pathogen have been observed in muskoxen across the Canadian Arctic Archipelago since 2010. Our research aims to identify the underlying factors that are promoting the success of this bacterium.

Methods

From 2021-2024, we investigated a mortality event and its aftermath on Ellesmere and Axel Heiberg Islands, Nunavut, Canada. We collected samples for culture and PCR from 138 unique muskox carcass sites, including 46 sites re-visited over multiple years. Tissues from 17 muskoxen were analyzed histologically. Whole genome sequencing and phylogenetic analyses were performed on >200 bacterial isolates.

Results

Histological findings supported E. rhusiopathiae septicemia as a definitive cause of death for 2 muskoxen and presumptive cause for 29. Erysipelothrix rhusiopathiae was found in association with >80% of sampled carcasses. Most recovered isolates were of the same clonal lineage that has repeatedly been identified in mortality events since first reported in 2009 (>1200km away). The bacterium remained viable at carcass sites for up to 3 years and was found in the feces of muskox predators and scavengers.

Discussion

Investigation of the roles of other candidate hosts, outbreak modelling, and detailed virulence profiling are priority areas for further research. Our results have contributed greatly to our understanding of pathogen-associated factors facilitating these impactful disease outbreaks.

Marine Mammals as Sentinels of One Health

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Introduction

Marine mammals, including whales, dolphins, seals, manatees, sea otters, and polar bears, inhabit diverse marine ecosystems and play important roles in the trophic web, making them indicators of environmental health. They face numerous stressors such as habitat degradation and emerging diseases, which can reflect human impacts on ecosystems. Pinnipeds, being long-lived and occupying high trophic levels, accumulate contaminants that provide insights into environmental pollution. Chile is a significant producer of salmon and copper, adding stress to the environment. We investigate the interactions of environment, health, contaminants, and selected pathogens in two pinniped species: the South American sea lion (SASL, Otaria byronia) and the South American fur seal (SAFS, Arctocephalus australis).

Methods

We sampled pups from three sites with varying human impacts during South American summers between 2020 and 2024, plus females from SAFS in one site. Selected pathogens such as influenza virus, herpesvirus, and adenovirus, were investigated using molecular techniques to assess their relationship with animal health. We also examined antibiotic-resistant bacteria through bacterial culture and MALDI-TOF analysis and Mercury levels.

Results

During the 2022-2023 season, an influenza virus outbreak resulted in significant mortality among South American sea lions, Humboldt penguins, and various marine birds, marking the first instance of this virus affecting sea lions in the area. We were allowed to carried out necropsies during the summer of 2024 on four pups, two of them revealed pathological evidence of viral infection, most likely influenza virus. In addition, herpesvirus was identified in females of SAFS, Furthermore, we detected antibiotic-resistant bacteria and confirmed early mercury exposure in pups, likely through vertical transfer.

Discussion

Our work highlights the role of marine mammals in monitoring the intricate relationships between animal and environmental health under the One Health framework. Ongoing investigations will assess influenza virus circulation among marine birds and pinnipeds in Chile.

West Nile and Usutu Viruses, Introduction and Dynamics of Two Emerging Orthoflaviviruses in France

Mathilde Gondard[1], Camille Victoire Migné[1], Marine Dumarest[1], Mrs. Teheipuaura Helle[1], Yannick Blanchard[2], Sylvie Lecollinet[3], Cécile Beck[4], Stéphanie Desvaux[5], Anouk Decors[5], Christel Marcillaud-Pitel[6], Coralie Lupo[6], Thierry Petit[7], Hanae Pouillevet[7], Thomas Charpentier[7], Clément Bigeard[1,8], Alexandre Duvignaud[8], Denis Malvy[8], Raphaelle Klitting[9], Laura Pezzi[9], Albin Fontaine[9], Xavier de Lamballerie[9], Stephan Zientara[1], Sandra Martin-Latil[1], Gaëlle Gonzalez[1]
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Introduction

West Nile virus (WNV) and Usutu virus (USUV) are neurotropic and zoonotic arboviruses of the Orthoflavivirus genus (Flaviviridae family), both maintained in an enzootic cycle involving ornithophilic Culex mosquitoes and avian reservoir hosts. Originating from Africa, where they were first described at the beginning of the twenty century, WNV spreads globally (nine lineages) while USUV emerged only in Europe (eight lineages). WNV strains belonging to lineages 1 and 2 are considered the most important causative agent of viral encephalitis in human and horses, and WNV infection is a notifiable disease at the EU level in humans, equids and avifauna. Almost all USUV lineages have been described in Europe and are responsible for major epizooties in wild and/or captive birds. In France, WNV and USUV are considered as emerging pathogens due to the extension of their geographical distribution and an increase frequency of the outbreaks.

Methods

The European and French National Reference laboratory is involved in a multidisciplinary One Health surveillance system for WNV and USUV with partners from the medical, veterinary and entomological sectors. In addition to the diagnostic activity of clinically symptomatic horses or captive birds (serological and molecular tools), whole genome sequencing of the circulating strain integrated the surveillance workflow to better understand viral emergence and dynamics.

Results

Here, we will present the emergence and contrasted dynamics of WNV strains belonging to lineage 2 in southern France since 2018. Additionally, we will discuss the molecular epidemiology of avian USUV infections in France since its introduction in 2015.

Discussion

The intensification of WNV and USUV circulation in France underline the need to extend and reinforce the surveillance system over the whole territory. Alerting the authorities at an early stage would enable public decision-makers to better prevent and control virus spread and limit public health consequences.

Pathology Case Reports - 1, Glen 206, June 12, 2025, 1:30 PM - 3:30 PM

Hyperplastic Gastritis with Presumed Trichostrongylus axei Infection in a Miniature Donkey

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Introduction

Trichostrongylus axei is a gastrointestinal nematode that commonly parasitizes cattle and sheep. Equids are infrequently infected, more often occurring when sharing pastures with ruminants. This case presents characteristic hyperplastic gastritis with intralesional nematodes, consistent with T. axei, in an aged miniature donkey.

Description

A 27-year-old male neutered miniature donkey was brought to an animal rescue in November 2024, then euthanized shortly afterward due to severe laminitis. The animal was examined post-mortem at the Animal Health Centre, BC Ministry of Agriculture and Food. The miniature donkey was emaciated and had markedly overgrown front hooves with chronic, severe laminitis. The distal phalanx of both front feet were severely sunken, with marked osteolysis. The pyloric gastric mucosa had 15-20 exophytic, verrucous white to light pink, soft nodules ranging from 2-5 mm in diameter. In the glandular fundus, there were several well-demarcated, irregular shaped, multifocal to coalescing white plaques with 2-3 mm diameter wide, raised margins. Histologically, there was marked gastric hyperplasia of surface mucous cells with intraepithelial adult nematodes, presumed to be Trichostrongylus axei, with minimal inflammation. The gastrointestinal tract contained some sand with watery feed. Rare larval nematodes were present within the lung with associated pleural and pulmonary fibrosis. Fecal flotation yielded 4+ Strongylidae, with a fecal egg count of 800 eggs per gram.

Discussion

This miniature donkey was diagnosed with chronic, severe laminitis and multifocal to coalescing hyperplastic gastritis with intralesional nematodes, most likely Trichostrongylus axei based on histologic features and appearance in the stomach. Confirmation of exact nematode species often requires further molecular or morphologic examination, highlighting a limitation of histopathology in parasite identification. The source of T. axei infection is unknown in this miniature donkey based on limited clinical history, but likely was due to co-grazing with cattle or sheep.

Leptospirosis in a Puppy in BC

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Introduction

In late 2024/early 2025 there was concern about a perceived increase in leptospirosis in dogs noted by companion animal clinicians in British Columbia, Canada. In January 2025 the Animal Health Centre (AHC) diagnosed leptospirosis, complicated with an E. coli cystitis and pyelonephritis, as the cause of death in an 11-week-old female German Shepherd cross puppy. This is the only canine necropsy case diagnosed with leptospirosis out of the more than 900 canine necropsies conducted in the past 10 years at the AHC.

Description

On gross examination there was diffuse icterus and moderate petechiation of the kidneys on both the capsular and cortical cut surface. Histologically there was moderate mononuclear cortical interstitial nephritis, often associated with mild tubular epithelial necrosis, with numerous spirochetes visible in affected tubules with Steiner-Chapman modified silver stain. In addition to this there was a marked suppurative ascending pyelonephritis with frequent tubulorrhexis associated with myriad small coccobacilli. In the urinary bladder there was a chronic-active cystitis with marked lymphoid nodule hyperplasia, and scattered neutrophils and colonies of coccobacilli along the mucosa. Leptospira spp. PCR was positive on the kidney and was further identified as Leptospira interrogans by DNA sequencing. Bacterial culture of the kidney yielded 4+ Escherichia coli.

Discussion

This puppy had two concurrent bacterial infections targeting the kidney. There was a chronic interstitial nephritis with intralesional spirochetes consistent with leptospirosis, as well as a suppurative pyelonephritis with intralesional coccobacilli consistent with Escherichia coli. Leptospirosis is an uncommon diagnosis in BC. This was the first canine case diagnosed on necropsy at AHC in at least 10 years. Between 2015 and 2024, 639 canine whole blood and urine samples were submitted for Leptospira PCR by clinicians. Of these samples 12 were positive and 3 suspect with the highest percentage positive in 2024 at 3.6%.

Perirenal Hemorrhage Associated with Feline Infectious Peritonitis (FIP): A Novel Presentation of a Classic Disease

Madeleine Gauthier[1], Dr. Carolyn Legge[1], Dr. Dayna Goldsmith[1], Dr. Maria Bravo-Araya[1], Dr. Jennifer Davies[1]
[1]University Of Calgary Faculty Of Veterinary Medicine, Calgary, Canada

Introduction

Feline infectious peritonitis (FIP), caused by a mutated biotype of feline coronavirus (FCoV), is a significant disease affecting felids. Since 2014, anatomic pathologists at the Diagnostic Services Unit (DSU) in Calgary, Alberta have observed perirenal hemorrhage in FIP cases, a previously undescribed lesion. This retrospective study aims to describe this novel lesion and determine its prevalence among FIP cases diagnosed at the DSU since 2010.

Description

The DSU's Laboratory Information Management System (LIMS) was queried for FIP cases from June 30, 2010, to June 30, 2024, and necropsy reports reviewed. Only cases with histologic lesions (perivasculitis, vasculitis, and pyogranulomatous inflammation) compatible with FIP and verified by FCoV IHC were included in the study. Among 51 FIP cases, 5 (9.8%) exhibited perirenal hemorrhage in the right retroperitoneum. Four had concurrent subcapsular renal hemorrhage, and 1 displayed sublumbar muscular hemorrhage and hemoabdomen. One case presented with additional hemorrhages in the brain and cervical spinal cord. Concurrent gross lesions typical of FIP included pyogranulomatous inflammation in various organs and protein-rich cavitary effusions. Histologic lesions typical of FIP (vasculitis and pyogranulomatous inflammation) were present in the kidneys and retroperitoneal fat of 4 cases, with FCoV antigen demonstrated in the hemorrhagic regions of 3 cases.

Discussion

The mechanism of this hemorrhage is unknown but may result from FIP-associated vasculitis, supported by renal vasculitis in 4/5 cases, the close association of the perirenal hemorrhage with FCoV antigen in 3/5 cases, and the presence of meningitis and vasculitis in the cat with brain and cervical spinal cord hemorrhage. Consumptive coagulopathy represents an alternate pathogenesis. A key limitation is the study's retrospective nature, leading to incomplete historical data. Although uncommon, perirenal hemorrhage represents a novel manifestation of FIP that clinicians and pathologists should consider in cases of fluid accumulation or space-occupying lesions in the retroperitoneum of cats.

Systemic Amyloidosis in a Siamese Cat

Dr. Ann Britton[1]
[1]Animal Health Centre, Abbotsford, Canada

Introduction

In April 2024, an intact male Siamese cat was submitted for necropsy with a multi-day history of lethargy, loss of appetite and no response to doxycycline.

Description

The 3.6 kg cat was in good body condition with pallor of the mucous membranes and marked dehydration. The thyroid glands were light tan in colour and enlarged. 105 ml of free blood was harvested from the abdomen. The liver was enlarged with clotted blood scattered over the capsular surface and multiple capsular fractures.
On microscopic examination, marked multifocal hepatic hemorrhage, marked periacinar hepatocellular atrophy, and deposition of amyloid in sinusoids, terminal hepatic venules, portal tracts, venules and hepatic capsule was noted. Moderate lymphoplasmacytic cholangiohepatitis, splenic sinus plasmacytosis, chronic lymphoplasmacytic bronchitis, chronic plasmacytic rhinitis, mild tubulointerstitial disease and marked myeloid bone marrow hyperplasia was indicative of multisystemic inflammation. Thyroid follicles were widely separated by amyloid. Amyloid was also detected in spleen, kidney, pancreas, heart, adrenal gland and stomach.

Discussion

Systemic amyloidosis (SA) is a condition whereby protein fibrils of light chain immunoglobulin or serum amyloid A are deposited in tissues. The condition has been described mainly in purebred, often Oriental type breeds but more recently reports of the disease in shelter cats of any breed have been published. In Siamese cats, amyloid deposition is reported to occur predominantly in the liver as well as the thyroid gland, kidney and spleen. Affected cats are at risk of liver capsular rupture, hemoabdomen and acute death.
SA is thought to involve a chronic inflammatory disease process superimposed on a familial predisposition in Siamese cats. It is generally thought that the majority of SA cases in cats are associated with deposition of serum amyloid A.

The importance of whole genome sequencing for a reliable and robust diagnostic service: An example of an ETEC:ExPEC hybrid E. coli isolate

Dr. Dominique Fournier[1]
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Introduction

Bacterial identification plays a crucial role in the treatment and prevention of diseases in animal production. In Escherichia coli, the classical diagnostic approach is based on the PCR detection of virulence genes specific to pathotypes of importance depending on the animal species. However, this technique is based on the search for a limited range of genes, limiting the detection of atypical isolates.

Description

A strain of E. coli isolated from the filter organs of several lambs from the same farm was initially classified as enterotoxigenic (ETEC). These lambs were submitted for necropsy from April to August 2024, for neonatal mortality and presented histological lesions compatible with bacterial septicemia due to extraintestinal E. coli (ExPEC). Using PCR, genes encoding two characteristic ETEC toxins (STa/STb) were detected, however, genes associated with the routinely tested ExPEC pathotype were not identified. Genome sequencing analysis revealed a high diversity of virulence genes. Among these genes, those encoding two hemolysins (hlyE and hlyA), as well as two genetic markers involved in invasion and survival (iss and ompT), were detected, confirming membership in the ExPEC pathotype. The presence of genetic characteristics of both pathotypes suggests the acquisition of mobile genetic elements. Further genomic analysis is underway.

Results

These results highlight the ability of genome sequencing to characterize the emergence of hybrid pathotypes, thus providing essential support for diagnosis and the development of clinical intervention plans.

Transplacental Transmission of Canine Distemper Virus and Abortion in a Linnaeus's 2-Toed Sloth (Choloepus didactylus)

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Introduction

Canine Distemper Virus (CDV) causes a highly contagious and often fatal disease in a broad range of mammals. The virus is regarded to have pronounced tropism for epithelial, lymphoid, and nervous tissues. In dogs, canine distemper can manifest as a multisystemic disease, typically involving respiratory, gastrointestinal, cutaneous, or neurological signs.

Description

After aborting a fetus mid-gestation, a 4-year-old female Linnaeus's 2-toed sloth (Choloepus diadactylus) succumbed to respiratory and gastrointestinal disease. Autopsies were performed on both the adult sloth and the fetus. On histology, endothelial cells in maternal and fetal tissues contained amphophilic intranuclear and intracytoplasmic viral inclusion bodies. Endothelial syncytia were also noted. Microscopic evidence suggests that the abortion was the result of CDV infection inducing acute, necrosuppurative placentitis with vasculitis. Immunohistochemistry and real time PCR support a transplacental CDV infection. Sequencing identified the causative CDV strain as one that was first isolated from a raccoon in Iowa, United States.

Discussion

Reproductive failure caused by CDV has not been reported in sloths before now. Rare instances of CDV-induced abortion have been reported in domestic dogs, though these cases are often attributed to the stress of clinical disease. While maternal stress may have been a contributing factor in this case, histologic lesions demonstrate a critical disruption of the maternal-fetal interface. Along with a novel viral tropism and reproductive disease presentation, this case represents the first reported transplacental transmission of CDV in this host species.

Pathology Case Reports - 2, Glen 206, June 12, 2025, 4:00 PM - 5:30 PM

Respiratory Disease with a Precipitous Drop in Egg Production in Egg Layers.

Dr. Ashish Gupta[1], Dr. Teryn Girard[2], Dr. Hayley Bowling[2], Dr. Beverly Morrison[1]
[1]University Of Calgary, Calgary, Canada, [2]Prairie Livestock Veterinarians , Red Deer, Canada

Introduction

A case of leghorn chickens which was experiencing acute respiratory signs such as swollen faces, eyelids, combs and wattles, lacrimation, and nasal discharge with an acute drop in egg production of up to 14% following an introduction of pullets from a farm with unknown disease history.

Description

Two 27-week-old white leghorn chickens were submitted for post-mortem examination to the Diagnostic Services Unit, Faculty of Veterinary Medicine, University of Calgary. The face, eyelids, and wattles of the chickens were markedly swollen and red. Mucoid discharge from the nostrils, marked subcutaneous edema, marked necrosis, and hemorrhage in the wattles were also noted. Histopathological examination revealed severe acute fibrino heterophilic inflammation in the nasal cavity, skull bones, and internal ears. Necrohemorrhgic lesions in the wattles were remarkable. Avibacterium Paragallinarum (AP) was isolated in pure culture from wattles and sinus swabs.

Discussion

AP causes an acute respiratory infection in chickens with a significant drop in egg production in egg layers and broiler breeders and airsaculitis and condemnation in broilers. Several pathogens such as Pasteurella multocida, Ornithobacterium rhinotracheale, Gallibacterium anatis, Mycoplasma gallisepticum, gamma coronavirus (Infectious bronchitis), Gallid herpesvirus-1 (Infectious laryngotracheitis), Avian metapneumovirus can cause clinical symptoms and lesions we observed in this case. In addition to AP, weak positive signals were observed for infectious bronchitis virus in PCR. Similar disease was reported in some other flocks in Alberta, Manitoba, and Saskatchewan. All the disease outbreaks were linked to a common source.

Emerging Turkey Reovirus with neuro- and hepatotropism in commercial Turkeys in Alberta.

Dr. Ashish Gupta[1], Dr. Hayley Bowling[2], Dr. Teryn Girard[2]
[1]University Of Calgary, Calgary, Canada, [2]Prairie Livestock Veterinarians , Red Deer, Canada

Introduction

In the fall of 2023 and in 2024, seven cases of Turkeys were submitted for diagnostic work to the Diagnostic Services Unit, Faculty of Veterinary Medicine at the University of Calgary with a history of lameness, and a few cases had unique nervous presentation or high early mortality.

Description

Six of eight cases were field necropsies with the submission of assorted tissues, and two cases were full-body necropsies. Seven cases had tenosynovitis, 2/8 cases had encephalitis, 2/8 cases had lymphocytic myocarditis, and 1/8 had necrotic hepatitis and splenitis. Variant ARVs (TRVs) were genotyped with nucleotide identity (>90%) to viruses identified in Pennsylvania (US), and one virus had >90% identity to variant ARVs identified in Ontario (Canada).

Discussion

Avian reoviruses (ARVs) have been ubiquitous and known for decades for their role in causing enteritis (helicopter disease) and tenosynovitis (viral arthritis) in commercial broilers, broiler breeders, and commercial turkeys. ARVs are emerging globally, and newer variants are reported every year. There have been reports of ARVs affecting turkeys (TRVs) to cause encephalitis and hepatitis in commercial birds in the US and Eastern Canada (Quebec). We have also observed a wave of TRV outbreaks in Alberta in 2023 and 2024, which caused encephalitis, hepatitis, and tenosynovitis. The strains that affected the liver had more severe outcomes in young poults.

AMR Surveillance and Approaches to Combating AMR, Glen 202, June 12, 2025, 4:00 PM - 5:30 PM

Surveillance of Antimicrobial Resistance in American Foulbrood: Local and Global Perspectives

Dr. Oleksii Obshta[1], Belarmino Eugenio Lopes Neto[1], Rosephine Enadeghe[1], Julia Tregobov[1], Marina Carla Bezerra da Silva[1], Emilio Tellarini[1], Thanuri Edirithilake[1], Dr. Midhun Sebastian Jose[1], Alvaro De la Mora Pena[1], Marcelo Polizel Camilli[1], Dr. Muhammad Fahim Raza[1], Sarah Wood[1], Elemir Simko[1]
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Introduction

Endospore-forming Paenibacillus larvae is the causative agent of American foulbrood (AFB), a fatal infectious disease of honeybee larvae. Once established in honeybee larvae, P. larvae inevitably leads to colony collapse. To prevent outbreaks of AFB, in North America, antimicrobials are widely prescribed by veterinarians for colony metaphylaxis, resulting in selective pressure for the emergence of antimicrobial resistance in P. larvae. In contrast, there is a ban on use of antibiotics in beekeeping in the EU and New Zealand. Therefore, objectives of this study were to investigate the suitability of honey for surveillance of antibiotic-resistant P. larvae and to assess the P. larvae spore contamination in honey samples collected from Saskatchewan (SK) and globally.

Methods

For SK surveillance, 36 commercial beekeepers submitted a total of 1459 samples in 2023-2024. For global surveillance, 268 honey samples from 39 countries were purchased online through Amazon. Honey samples were cultured on selective media to screen for antimicrobial resistance to the three antimicrobials approved for AFB. Antimicrobial-resistant isolates were further characterized by the broth microdilution technique to determine the MIC of antibiotic, as well as by PCR to evaluate for the presence of tetracycline resistance genes.

Results

P. larvae was identified in 46% of SK honey samples and 44% of global honey samples at spore concentrations ranging from 0.12-12876 spores/g of honey. Oxytetracycline-resistant P. larvae was identified in 6% of SK samples, representing 7 beekeepers, in 1 sample from each of Manitoba and British Columbia and 4 samples from the USA, with MICs ranging from 64-128 µg/mL and with the presences of tetracycline resistance genes confirmed by PCR.

Discussion

Overall, results of this pilot study highlight the suitability of honey as a matrix for surveillance and monitoring of antimicrobial resistance in P. larvae and support evidence-based prescribing and antimicrobial stewardship in the beekeeping industry.

Colonization of Humans and Companion Animals by Antimicrobial-Resistant Organisms: Prevalence, Risk Factors and Implications for Antimicrobial Resistance Surveillance

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[1]Faculty of Veterinary Medicine University of Calgary, Calgary, Canada

Introduction

Antimicrobial resistance (AMR) is a growing public health concern, with companion animals identified as potential reservoirs for resistant bacteria. Close contact between dogs and humans facilitates bacterial transmission, yet AMR prevalence and risk factors for colonization with resistant bacteria in dogs and their owners remain underexplored. This study assesses AMR prevalence in zoonotic and indicator bacteria, including Staphylococcus aureus, Staphylococcus pseudintermedius, and Escherichia coli, in dogs and their owners in Calgary, along with associated risk factors.

Methods

Between July and November 2024, fecal and oral samples from dogs and nasal samples from their owners were collected at off-leash parks in Calgary. Samples were cultured on selective media for the identification of methicillin-resistant S. aureus (MRSA), S. pseudintermedius (MRSP), and extended-spectrum beta-lactamase (ESBL)-producing E. coli. Surveys gathered data on antimicrobial use, diet, and other potential risk factors. Proportions and 95% confidence intervals (CIs) were calculated to describe the prevalence of resistant bacteria in each sample type, as well as the survey data.

Results

A total of 245 samples were collected from eight off-leash dog parks. Presumptive MRSA was detected in 11.4% (95% CI: 5.9%–21.0%) of human nasal swabs and 30.0% (95% CI: 22.8%–38.4%) of dog oral swabs. ESBL-producing bacteria were identified in 19.2% (95% CI: 12.8%–27.8%) of dog fecal samples. Survey findings from 77 dogs and their owners showed that 25.4% (95% CI: 16.7%–36.6%) of dog owners and 24.7% (95% CI: 16.4%–35.4%) of dogs had received antibiotics in the past year. Regarding diet, 87.2% (95% CI: 78.0%–92.9%) of dogs consumed commercial dry food, and 20.5% (95% CI: 13.0%–30.8%) received raw meat.

Discussion

These findings provide early insights into AMR prevalence and colonization risk factors in dogs and their owners in Calgary. They offer valuable guidance for AMR surveillance and mitigation efforts, reinforcing the importance of a One Health approach.

Emergence of Multidrug-Resistant Staphylococcus aureus in Cattle Mastitis: Molecular Insights and Resistance Discrepancies

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Introduction
Multidrug-resistant Staphylococcus aureus is rapidly emerging at the interface of livestock animals, posing a hidden but escalating threat to both animal and human health. The current study aimed to investigate the molecular prevalence and characterization of methicillin-resistant (MRSA), beta-lactam resistant (BRSA), tetracycline-resistant (TRSA), aminoglycoside-resistant (ARSA), and multidrug resistant S. aureus (MDRSA). Moreover, the discrepancies in phenotypic and molecular methods for detection of resistant strains and antibiotic susceptibility of MDRSA isolates were also investigated.

Methods
A total of 384 cattle milk samples were screened for subclinical mastitis (SCM) followed by isolation and molecular confirmation of S. aureus. MDRSA was confirmed phenotypically by Kirby-bauer disc diffusion test and genotypically by detection and sequencing of mecA, blaZ, tetK, and aacA-aphD genes responsible for methicillin, beta-lactam, tetracycline, and aminoglycoside resistance respectively. The antibiogram profiling of MDRSA was assessed by disc diffusion test.
The results showed a 40.35% prevalence of S. aureus in SCM. Among these isolates, 50.72%, 36.23%, 40.58%, and 33.33% were identified as MRSA, BRSA, TRSA, and ARSA via disc diffusion. Gene confirmation indicated 37.68% as MRSA, 18.84% as BRSA, 27.54% as TRSA, and 36.23% as ARSA.

Results
Overall, 15.84% of isolates were confirmed as MDRSA. This study also revealed a high incidence of discrepancies between genotypic and phenotypic methods for resistance evaluation. Phylogenetic analysis revealed close genetic relationship among the study isolates and with previously reported Pakistani isolates. Antimicrobial susceptibility trials showed that MDR isolates exhibited high resistance to penicillin, ceftriaxone, cefotaxime, and amoxiclav. Conversely, the highest susceptibility was observed for gentamicin, followed by streptomycin.

Discussion
This study highlights the high prevalence of antimicrobial resistant strains of S. aureus especially MDR S. aureus in subclinical mastitis. The genetic similarity among isolates suggests potential transmission within and between animals. Effective control strategies, including targeted antibiotic use and alternative therapeutic approaches, are crucial to mitigate its spread.

Exploring the Relationship Between Antimicrobial Resistance Genes and Virulence Factor Content in Streptococcus Isolated from Canadian Dairy Herds

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Introduction

The majority of the antimicrobials used to treat bovine mastitis are also classified by the World Health Organization as critically and highly important antimicrobials in human medicine. Exploring the current status of antimicrobial resistance and virulence determinants in bovine mastitis Streptococcus and Enterococcus is necessary to comprehensively understand the burden of AMR and clinical mastitis in the Canadian dairy industry.

Objective

The objective of this study is to estimate the prevalence of Streptococcus and Enterococcus antimicrobial resistance genes (ARGs) in Canadian dairy cattle and investigate the association of virulence factors with ARGs in these isolates.

Methods

Nearly 1000 Streptococcus and Enterococcus isolates obtained from 89 dairy herds were whole-genome sequenced. ARGs were detected against several databases. Virulence factor genes were determined using VFDB and VirulenceFinder. Biomass of isolate biofilms was quantified using an in vitro crystal violet assay on 96-well microtiter plates, and characterized as negative, weak, moderate, or strong biofilm-forming isolates based on volume of biofilms.

Results

Out of a total of 897 isolates sequenced, the majority were S. dysgalactiae (29%), S. uberis (26%), other Streptococcus spp. (16%), and Enterococcus spp. (14%). Approximately 25% of isolates produced moderate-to-strong biofilms in vitro, and were primarily formed by S. uberis isolates that contained ≥ 2 biofilm-associated genes. The majority of isolates (> 50%) contained ARGs mediating resistance to aminoglycosides—ANT(6) and APH(3')—and tetracyclines—tetM and tetS. No significant associations were discovered between the presence of ARGs and virulence factors.

Conclusion

Comprehensively studying ARG prevalence within the context of clinical status and isolate virulence in this work leads to a better understanding of Streptococcus infection dynamics. These results can be immediately useful for dairy producers and veterinarians to guide better treatment decisions based on regional ARG patterns.

Isolation and Characterization of Pseudomonas aeruginosa Bacteriophage

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Introduction

Bacterial skin infections are highly presented in small animal practice; among which, Pseudomonas aeruginosa is a significant pathogen associated with otitis in canine species. Treatment challenges are significant for the biofilm producer with evolving fluoroquinolones resistant clones. Bacteriophages are recognized as an alternative option to treat drug resistant infections. Thus, the present study is aimed at the isolation and characterization of bacteriophages against P. aeruginosa with a primary goal of developing a topical bacteriophage therapy to tackle antibiotic resistance.

Methods

In this study, lytic Pseudomonas bacteriophages were isolated from the sewage wastewater using canine clinical isolates of P. aeruginosa as the propagating hosts. Briefly, the 0.45 µM membrane filtered water was enriched with P. aeruginosa and spotted on the lawn culture of P. aeruginosa. Individual lytic zones (plaques) were harvested in salt-magnesium phage buffer and subjected to amplifications in spot assays to obtain a high titer of 108 PFUs (plaque forming units)/ml. The growth kinetics of PA_phage-1 versus P. aeruginosa was examined in trypticase soy broth. The lytic activity of the phage was examined on fifty clinical isolates of P. aeruginosa. The phage DNA was sequenced.

Results

The antimicrobial action of the isolated phages generated 1 mm clear lytic zones. In vitro growth kinetics of P. aeruginosa versus PA_phage-1 exhibited pronounced clearing at 4 h. The lytic efficiency of phage-1 was observed on 50% of the clinical isolates of P. aeruginosa tested so far. The sequenced genome of 6.9 Kbp carries 58% GC content, and belongs to the class of Caudoviricetes.

Discussion

The PA_phage-1 genome lacks toxin and antibiotic genes, and is a potential therapeutic candidate. The kinetics of PA_phage-1 indicated complete clearing of the pathogen at 4 h corresponding to a high phage titer in liquid phase. More detailed characterization of the phage is underway.

Foot-and-Mouth Disease Virus Circulation in the Sultanate of Oman (2018-2023) and Associated Risks

Dr. Guillaume Girault
Anses
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Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, posing significant economic losses for the livestock industry in affected countries. The disease is caused by an RNA virus with seven distinct serotypes, each further divided into topotypes and lineages based on geographical and phylogenetic analyses. In the Sultanate of Oman, FMDV is endemic, with serotypes O, A, and Asia1 having been reported in the past. Rapid identification and characterization of circulating FMDV strains is essential for implementation of adequate control measures. Here we report serological and molecular investigations of FMDV outbreaks occurred during 2018-2023.

This study was conducted on 340 animals (cattle, goats, sheep) distributed across ten governorates of Oman. A total of 514 samples (epithelium, swab, whole blood, serum, milk, saliva) were collected between 2018 and 2023. Real-time RT-PCR duplex assays for 3D/B-actin and IRES/B-actin were performed on all samples except serum. Positive samples underwent viral isolation on two cell lines. The obtained isolates were analyzed using an Ag capture ELISA to determine the viral serotype. Based on these results, the VP1 coding region of each isolate was sequenced. All serums and whole blood samples were analyzed using NSP ELISA.

The seroprevalence of NSP/3ABC antibodies was 43,5%. For serotype O, four lineages have been identified: ME-SA/IND-2001e, ME-SA/PanAsia-2ANT-10, ME-SA/SA-2018 and EA-3. The identification of A/AFRICA/G-I strain suggests a connection with Est Africa. Finally, the sixth strain detected in Oman between 2018 and 2023 is the SAT2/XIV strain, also found in other countries of the region (Iraq, Bahrain) and in Est Africa.

These results indicate multiple independent incursions of FMDV in the Sultanate of Oman, likely due to livestock imports. The rapid virus spread emphasizes the risks of the propagation of the FMDV in the region and highlights the importance of surveillance, health controls, and vaccine evaluation.

Sensitivity and Specificity of a Non-Invasive Rapid Diagnostic Device for African Swine Fever Detection

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Introduction
Timely detection of African swine fever antigen from swine saliva on-farm is not a common method in Nigeria for detection of ASFV-infected pigs in spite of being non-invasive and potentially useful in the monitoring and surveillance of the endemic high consequence disease in Africa. In this study, we assessed the performance of a novel portable diagnostic devices for detecting ASFV VP72 antigen, using biosensors and disposable screen-printed electrodes. The test was compared to a reference PCR (qPCR) test for viral genome detection.

Methods
Oral swab samples were collected from 64 pigs on university farm and two selected pilot farms. Samples were transported on Virus Transport Medium and DNA shield. They were subjected to test upon collection, and a subset each transported to a virology laboratory and the national veterinary research institute's reference laboratory, respectively for analysis using standard qPCR assay.

Results
The biosensor-based test detected two positive cases out of 64 samples (3.13%) while the standard qPCR test at both laboratories detected four positive samples (6.25%). The sensitivity of biosensor-based test was 50% and specificity 100%, compared to the reference qPCR assay.

Discussion
The biosensor-based test showed high specificity but reduced sensitivity for African swine fever detection. In spite of noted issues with dilution factor, there is prospects for using the novel biosensor-based rapid diagnostic test as pen-side device for swine disease surveillance Decision Support System in Nigeria.

Seroprevalence and Associated Risk Factors of Brucellosis in Livestock Population: A Cross-sectional Study from South Punjab, Pakistan

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Introduction
Brucellosis is an important zoonotic disease with considerable economic impact worldwide. It primarily affects livestock and wildlife, posing risks to public health, especially in developing countries. This study aims to assess the seroprevalence of brucellosis in bovine population and the associated risk factors in southern Punjab, Pakistan.

Methods
A total of 550 serum samples of cattle, buffalo, sheep, and goats were collected from 102 livestock farms in two districts (Layyah=40 farms and Dera Gazi Khan=62 farms) of South Punjab, Pakistan. Samples were initially tested for anti-Brucella antibodies using two different Rose Bengal Test (RBT) reagents (one from Veterinary Research Institute (VRI), Lahore and the other from IDEXX, France) and the positive samples were later confirmed using I-ELISA. Data on animal- and farm-level factors were also collected to identify the potential risk factors associated with the occurrence of brucellosis on livestock farms.

Results
The herd-level seroprevalence of brucellosis in Dera Ghazi Khan district was 22.6% (14/62), while no herd was positive in Layyah district. The animal-level seroprevalence was 10% (25/250) in Dera Ghazi Khan district. The seroprevalence was highest in buffaloes (13/100, 13%) followed by cattle (11/100, 11%) and small ruminants (1/50, 2%). Herd size >10 animals ($p = 0.003$), and abortion history at the farm ($p<0.001$) were found as risk factors for the occurrence of brucellosis on livestock farms. There was a substantial agreement between the RBT reagent from VRI and IDEXX (Cohen's kappa coefficient = 0.7, 95% CI = 0.56–0.84, Percent agreement = 97%), however, when compared with I-ELISA results, VRI reagent performed better than IDEXX reagent.

Discussion
Brucellosis prevalence in Dera Gazi Khan district underscores the need for effective control measures. These findings suggest that although RBT and I-ELISA can be used together for improved diagnosis, however, VRI reagent can be used for surveillance in endemic areas.

Innovative Technologies for Disease Detection, Exhibition Hall D, June 13, 2025, 11:00 AM - 12:30 PM

Development of Influenza A subtyping multiplex assay for domestic animals

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Introduction

Influenza is highly contagious and causes acute infections of the respiratory tract worldwide in many vertebrate hosts. Influenza virus type A (IAV) is the most important type that induces disease in humans worldwide, as well as in wildlife and domestic animals such as cats, dogs, horses, and livestock animals such as poultry and pigs. Recently, it also has been shown to affect cattle. The IAV type is further classified as subtypes based on the genotype of the two viral surface proteins, Haemagglutinin (H) and Neuraminidase (N). Among all the possible IAV subtypes, the vast majority have been found in waterfowl (especially ducks, geese, and swans), their natural host. Generally, IAVs isolated from a given species can replicate effectively in a specific host. Thus, the terms “human strains”, “avian strains”, “equine strains”, etc., are commonly used. However, their high genetic variability facilitates adaptation to other host species, causing outbreaks with “novel” influenza genotypes. For this reason, it is important to quickly identify the influenza virus genotype. Real-time qPCR (RT-qPCR) is the molecular method of choice to enable a rapid screening and identification of IAV’s strains.

Methods

The novel RT-qPCR -based panel has been developed to detect any influenza A virus (pan-Influenza A) and the main subtype genotypes circulating in cats (H1N1, H5N1 or H7N2), in dogs (H3N2), or in horses (H3N8). All assays have been validated analytically in multiplex with synthetic positive controls. For clinical validation, 26 H5NX samples (24 waterfowl, 2 dogs), 10 H7NX (waterfowl), 42 H3N2 (canine), 6H1N1(5 swine, 1 cat) and 4 H3N8 (equine) have been included.
Results and discussion: All samples were positive for the pan-IAV assay. All samples, except one H5, were detected for the corresponding subtype after Sanger sequencing confirmation. No cross-reaction with other subtypes was observed.

A Scalable Software Framework for PRRSV Genomic Surveillance: The SDRS PRRSV Sequencing Database

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Introduction
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) remains a global challenge to swine health. Detecting emerging variants rapidly is essential for disease management, but managing large-scale PRRSV sequencing data requires a robust computational system. To address this, the Swine Disease Reporting System (SDRS) PRRSV Sequencing Database was developed, integrating data from six major U.S. Veterinary Diagnostic Laboratories (VDLs). This scalable platform enables real-time PRRSV surveillance, efficient data storage and retrieval, and comprehensive epidemiological analysis to support informed decision-making in disease management.

Methods
The database was built using a relational structure optimized for high-throughput sequence storage and retrieval. A processing pipeline standardized data by calculating ambiguities and sequence length, classifying sequences by lineages, variants, assigning restriction fragment length polymorphism (RFLP) patterns, aggregating metadata, and detecting novel sequences. An integrated BLAST system was used to identify the closest sequence matches and facilitate external similarity searches. Parallelized data processing was used for handling large datasets to enhance scalability and efficiency.

Results
As of December 2024, the database, updated daily, stored >112,000 PRRSV sequences. Lineage 1A (n = 22,232) and 5A (n = 19,645) were the most detected lineages. RFLP analysis identified ten dominant patterns, and novel sequence detection, identified 167 novel sequences based on similarity thresholds, sequence length, and ambiguity limits. Temporal and geographical analyses provided insights into variant trends and regional PRRSV spread. Analysis of sequence metadata, including sample origin, age group, and collection date, further strengthened disease monitoring efforts.

Discussion
By providing a centralized, scalable, and accessible platform for PRRSV sequence analysis, the SDRS PRRSV Sequencing Database enhances real-time disease detection and epidemiological assessments. Standardizing sequence data across VDLs ensures consistency in monitoring trends, while integrated similarity searches streamline the identification of emerging sequences. The system streamlines PRRSV sequence monitoring and facilitates real-time disease detection.

Bovreproseq – Bovine reproductive pathogen sequencing panel

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Introduction

Bovine reproductive failure refers to abortion or pregnancy failure in cows. It causes significant economic losses to beef and dairy producers. The current diagnostic approach for infectious causes focuses on the most likely pathogen, followed by additional testing if the initial test fails to confirm the suspected cause. This stepwise approach is expensive and time-consuming. The objective of this study is to develop a syndromic sequencing panel (one-for-all test) for the detection of pathogens associated with bovine reproductive failure using targeted amplicon sequencing and to compare the performance of the developed syndromic panel (Bovreproseq) with methods currently used in the diagnostic laboratory.

Method

A total of 37 PCR assays were designed to target 17 pathogens associated with bovine reproductive failure. A partial sequence encoding green fluorescent protein was added as an internal amplification control to identify false negatives due to PCR inhibition. An ultra-multiplex PCR with pooled primers (38 primer pairs) was designed to amplify pathogen specific targets and the resulting amplicons from each sample were sequenced using the Oxford Nanopore sequencing platform. Sequence data was analyzed using a custom bioinformatics pipeline. The results from the Bovreproseq assay were compared to diagnostic assays that are currently used in the laboratory.

Results

A total of 116 samples (61 positive and 55 negative) were tested using the Bovreproseq method and lab methods. The total agreement between Bovreproseq and lab method was 98.8 %. Most of the disagreements were on samples that had a very low pathogen load. Additionally, the Bovreproseq assay was able to detect pathogens for which specific testing was not originally requested, demonstrating the advantage of this approach.

Conclusion

This study demonstrates that the Bovreproseq method can reliably and simultaneously detect multiple pathogens associated with bovine reproductive failure, while reducing the cost and turn-around time relative to conventional diagnostics.

INNOVATIONS IN THE DIAGNOSIS OF PARATUBERCULOSIS: DEVELOPMENT OF NEW TOOLS FOR ACCURATE DETECTION AND QUANTIFICATION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP)

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Introduction

The paratuberculosis, caused by Mycobacterium avium subsp. paratuberculosis (MAP), is a chronic enteric disease in ruminants leading to significant economic losses. A threshold of 104 MAP/gram of feces is considered critical for distinguishing active infections from passive shedding. This data is crucial for controlling and managing this disease through regular herd surveillance and accurate diagnostic methods like quantitative PCR (qPCR). Most of the existing qPCR assays, targeting the IS900 gene, can result in inaccurate quantifications due to a high variability in gene copy numbers across bacteria .

Methods

In collaboration with Labocéa (Quimper, France), BioSellal has developed and validated new digital PCR (dPCR) and qPCR workflows using a Reference Material (RM) calibrated with the monocopy gene F57. These methods target fecal and environmental samples and comply with French regulations (NF U47-600) and MIQE guidelines. The dPCR solution provides accurate MAP quantifications, while the qPCR method offers relative quantification based on the RM.

Results

We have developed the RM and two quantification methods: a direct dPCR method quantifying IS900 and F57, and a qPCR RM-based relative quantification method with the same targets. Results show significant advantages in combining IS900 for sensitivity and F57 for specificity and accurate quantification. The RM, calibrated at 104 MAP bacteria/gram, proved to be reliable for harmonizing and comparing results through different extraction and PCR methods . The dPCR method is proving particularly valuable in livestock environments, thanks to its enhanced inhibitors resistance and its accuracy, both of which help to better establish herd status.

Discussion

These innovative qPCR and dPCR tools offer enhanced accuracy in paratuberculosis diagnostics. Their integration into routine herd surveillance could improve disease management and reduce the economic impact on livestock industries.

IR-Biotyper: A New Diagnostic Tool in Microbiology

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Introduction

Since 2017, human microbiology has seen the rise of the IR-Biotyper (IRBT), using Fourier transform infrared spectrometry. It compares the absorption spectra of molecules that vary between strains. IRBT is proposed to group bacterial strains by 1) sequence-type, 2) serotype and clone, facilitating 3) nosocomial and 4) epidemiological surveillance. Already effective for typing Staphylococcus aureus and Klebsiella pneumoniae [1,2], this fast, simple and economical tool could offer an alternative to whole-genome sequencing (WGS) for applications in animal health.

Methods

A linear discriminant analysis (LDA), a user-supervised method, were used to analyse the data obtained. The spectra of 125 sequenced (cgMLST) and serotyped Pseudomonas aeruginosa strains were analysed to evaluate the capability of IRBT to group them. Nosocomial surveillance was evaluated with eight Enterobacter hormaechei strains isolated during a microbiological control of a veterinary clinic. Twenty-six Rhodococcus equi strains from eight farms collected over a decade were used to confirm epidemiological surveillance ability.

Results

P. aeruginosa strains clustered effectively by sequence type and serotype. In nosocomial surveillance, six beta-lactamase-producing E. hormaechei strains had distinct spectral profiles, except for those from the neonatal unit and operating room, which belong to the same clone. Epidemiological surveillance showed potential clonal strains of Rhodococcus equi in three of the eight farms sampled from different horses sampled in the same year.

Discussion

Ours promising results confirm the potential of IRBT to characterize strains, consistent with previous literature comparisons of IRBT and WGS [2-4], and for nosocomial and epidemiological surveillance. However, despite reduced variability with LDA, residual differences hinder the visualization of many spectra on a single plot. Standardizing inoculum concentration will improve result clarity and reliability. This innovative tool could bring real-time strain typing within reach!

[1]Hong, 2022. Antibiotics ; [2]Hu, 2020. Microb. Biotechnol. ; [3]Martak, 2019. Front. Microbiol. ; [4]Hu, 2023. Front. Microbiol.

One Health and Multi-Host Spillover, Glen 202, June 13, 2025, 11:00 AM - 12:30 PM

Highly Pathogenic Avian Influenza Virus Isolated from Urine from Neurologic Cat After Raw Milk Ingestion

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Introduction

In March 2024, highly pathogenic Avian Influenza (HPAI) H5N1 virus was detected in milk from dairy cattle. HPAI infects felids, causing encephalitis and death. High viral loads have been detected in the brain and lung tissue of deceased cats, but little information is available regarding best sample types from live cats. Here we describe the clinical signs, detection of HPAI in a urine sample, and seroconversion from a household cat that was fed raw milk and survived.

Methods

An owner of four indoor only cats purchased raw milk, which he and three of his cats consumed. The milk was included in a recall by the California Department of Public Health after products tested positive for HPAI. Two of the cats developed fevers, anorexia, lethargy, and died. A third cat with similar signs was hospitalized and progressed to hind end paresis and blindness. The cat received supportive care, including Oseltamivir phosphate (Tamiflu®) and recovered. Urine was tested for HPAI by PCR and virus isolation. HPAI virus neutralization was performed on an acute plasma sample from the third cat, and a convalescent serum sample from the third and fourth cat that did not consume raw milk.

Results

The urine was positive for HPAI H5N1 by RT-PCR. The remaining urine was used for virus isolation in ten-day old embryonated chicken eggs. Sequencing of the isolate confirmed H5N1 clade 2.3.4.4b genotype B3.13. The plasma H5N1 virus neutralization titer was 1:512, indicating robust early seroconversion after exposure. Seven weeks later, the recovered cat had a titer of 1:1,024 and the cat that did not consume raw milk had no titer.

Discussion

Urine may be an ideal sample type to test cats with a history of consumption of raw milk showing clinical signs consistent with HPAI.

Applying One Health Principles at a Local Public Health Laboratory

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A One Health approach is needed more than ever due to our changing interactions with animals, plants, and our environment (e.g., population growth, climate change and land use, and increased cross-border movement of people, animals, and animal products). The County of San Diego (COSD) identified this need for a local One Health approach, saw an opportunity to improve the health of humans, animals, and the environment, and in May of 2023, launched the COSD One Health Epidemiology Program (OHEP).

OHEP consists of a cross-sectoral group of subject matter experts that includes epidemiologists, physicians, veterinarians, nurses, laboratorians, and other support personnel. A network of connectivity with federal, state, academic, commercial, and local partners has been created with relationship-building and communication key features of the program.

Four primary goals of OHEP are to: 1) enhance disease outbreak management (e.g., robust animal specimen testing capability), 2) develop a centralized data hub consisting of local human, animal, and environmental disease information, 3) conduct surveillance for spillover risk management, and 4) provide education and outreach to our local partners and beyond.

An example of OHEP's efforts is our influenza A virus (IAV) testing program. Through collaboration with a commercial veterinary diagnostic laboratory, specimens from local animals are tested for IAV via PCR with a turn-around-time of <7 days. The results are communicated to the COSD Epidemiology Unit, and California Department of Public Health (CDPH) and CDC guidelines are followed for monitoring exposed humans. Non-negative specimens get forwarded to USDA's National Veterinary Services Laboratory for confirmation, subtyping, sequencing, and uploading into the national database. Results are shared with partners such as California Department of Food and Agriculture, California Department of Fish and Wildlife, and CDPH.

Scrutinizing the human-animal interface and rapidly identifying potential spillover events will create an opportunity for enhanced surveillance of animals and humans.

Impact of a chronic wasting disease vaccination on prion shedding

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Chronic Wasting disease (CWD) is a fatal neurogenerative prion disease of cervids, it is caused by the conversion of the normal cellular prion protein (PrPc) to the infectious misfolded scrapie prion protein (PrPsc). CWD is the most contagious prion disease, and there is no treatment or vaccine available. Our previous studies showed that vaccination targeting the PrPc overcomes the self-tolerance and induced self-antibodies and increase the survival time in mice models. Our current study aims to test the efficacy of our vaccine candidates on CWD shedding in feces. We used gene targeted mice Knock In (KI) 138 to mimic the CWD pathogenesis in real host. In this study, there are three groups of KI mice: two vaccinated groups and one control group, dimeric recombinant protein (Ddi) and Monomeric mouse recombinant protein (Mmo) and CPG control group. The ELISA results of the post-immune sera showed that both vaccine candidates are effective in producing high antibody titers. By using IPR technique which is a combination of iron oxide magnetic extraction (IOME), Protein misfolding cyclic amplification (sPMCA) and real-time quaking-induced conversion (RT-QuIC) showed that the Ddi candidate has less CWD shedding in feces and urine at different time points. Our findings are promising as our vaccine can be used as a preventive measure against CWD by decreasing the shedding and save the environment as well through breaking the disease transmission cycle. This is the first study tests the efficacy of active vaccination on CWD shedding and using IPR technique for detection of CWD in cervidized mouse model.

Molecular Investigation of the Emerging Zoonotic Parasite Echinococcus multilocularis in Wild Muskrats and Domestic Dogs

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Introduction
The zoonotic tapeworm, Echinococcus multilocularis is of emerging importance for human and dog health in western Canada. The parasite normally cycles between canids and rodents. Incidental ingestion of eggs can result in severe alveolar hydatid disease in humans and domestic dogs. Coyotes and domestic dogs are main definitive hosts. Historically, there were few cases of human alveolar echinococcosis (AE) in North America. Recently, AE cases in humans and domestic dogs has increased in Alberta (2009-2021:18 dog cases; 2013-2021:17 human cases). A recent introduction of the higher virulence European strain may have caused the emergence. The relatedness among human, dog and rodent cases remains unexplored. Using a One Health approach, the objective of this study was to use genomic analyses to determine the relatedness of E. multilocularis from AE cases in muskrats, domestic dogs and humans (intermediate host stage).

Methods
We analyzed samples of histopathology confirmed lesions collected from 7 muskrats and 4 domestic dogs using an RT-PCR assay to detect the NADH dehydrogenase subunit 2 (nad 2) gene of E. multilocularis. Genotyping was performed by amplification and sequencing of relevant SNPs in the cob, cox1 and nad1 genes to classify the strains.

Results
The sequenced samples resembled the E4 strain found in Austria and contained the unique SNP from A to G at position 235 of the cob gene defining the ECA strain.

Discussion
These results correlate to the predominant genotype in human cases in Alberta. Recent increases in human and dog case numbers in Alberta appear out of proportion with the situation elsewhere in Canadian. The presence of the European-like ECA strain in most samples confirms new local endemicity. The reason for a disproportionate increase in case numbers and their clustering is not fully understood, but nevertheless poses a serious medical, veterinary and public health threat.

The Wildlife Health Intelligence Network: Connecting a Global Community to Strengthen Wildlife Health Surveillance

Mathieu Pruvot[1, 2] , Sarah H. Olson[2], and the WHIN steering committee.

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The Wildlife Health Intelligence Network (WHIN) is designed to transform global wildlife health surveillance from a fragmented, reactive system into a cooperative, proactive network.
Effective wildlife health surveillance is essential for early detection of emerging threats, preventing zoonotic spillovers, protecting biodiversity and ecosystems, and reducing the economic impacts of health emergencies. Despite international guidance from the World Organisation for Animal Health (WOAH), together with the Quadripartite UN Organizations and the One Health Joint Plan of Action, most countries lack consistent, long-term wildlife health surveillance systems. Existing efforts remain fragmented and underfunded, undermining global One Health strategies and pandemic preparedness.
WHIN addresses these gaps by fostering an open, global community of practice that connects experts, organizations, governments, field practitioners, and communities. It emphasizes locally led, context-specific surveillance systems built through ethical engagement and co-development, aligned with global standards, and supported by robust IT infrastructure.
By amplifying local solutions across a global community, WHIN seeks to transform wildlife health surveillance into an effective tool for One Health implementation.

Artifiical Intellegence/Machine Learning, Digital Diagnostics and Laboratory Networks, Glen 202,
June 13, 2025, 1:30 PM - 3:30 PM

CFIA-Approved Laboratory Networks: Strengthening Animal Health Testing Across Canada

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Introduction

The Canadian Food Inspection Agency (CFIA) approves external animal health laboratories across Canada to expand testing capacity for federally regulated diseases, ensuring early detection and rapid response to minimize risks to animal health, public health, and the economy. These laboratories, including federal, provincial, university, and private labs, are organized into networks for better administration and oversight by the CFIA, offering collaboration opportunities and increased exposure.

Methods

CFIA approval requires ISO/IEC 17025 accreditation, participation in a CFIA proficiency testing program, and adherence to specific operating policies and procedures. Each network has a CFIA reference laboratory responsible for establishing diagnostic test parameters, proficiency testing programs, and providing confirmatory testing. Some networks also feature automated data uploading to a central repository accessible by national users.

Results

Network laboratories have significantly contributed to disease response and surveillance. For example, during the ongoing highly pathogenic avian influenza (HPAI) outbreak, the Canadian Animal Health Surveillance Network (CAHSN) Foreign Animal Disease (FAD) testing laboratories conducted initial screening testing linked to 525 infected premises, affecting nearly 15 million birds as of February 2025. These laboratories also participate in ongoing peacetime surveillance for African swine fever (ASF), as part of Canada’s multijurisdictional CanSpotASF program, and have helped Canada achieve negligible risk status for bovine spongiform encephalopathy (BSE). Additionally, they support export testing for various diseases to maintain market access with trading partners.

Discussion

The success of these networks is demonstrated by the high quality of laboratory results and the collaborative nature of the laboratories. Ongoing efforts aim to increase testing capacity, enhance data transfer, and develop strategic plans moving forward for Canada's animal health testing network.

From Research to Reality: Discovery and Validation of a Novel Artificial Intelligence-Enabled Molecular Diagnostic for Johne's Disease.

Dr. Ryan Farr[1], Carlos Rodrigues[1], Annaleise Wilson[1], Christina Rootes[1], Jenny Su[1], Rachel Hodgeman[2], Christopher Cowled[1], Nagendra Singanallur Balasubramanian[1], Marina Alexander[1], Cameron Stewart[1]
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Introduction
Johne's Disease (JD) is a chronic wasting disease of ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP) that results in >$AUD4B/year in losses to the global dairy industry. For decades, JD management has been hampered by the lack of a sensitive diagnostic test. MicroRNAs (miRNAs) are small, non-coding RNAs that have promise as biomarkers of infectious diseases. We developed an artificial intelligence (AI) driven pipeline that identifies miRNA biomarkers and deploys a classification model that converts lab results into a diagnostic output. This optimized model is then integrated with a molecular assay to form the final diagnostic test.

Methods
Serum miRNAs were profiled using next-generation sequencing from uninfected (n=87) and MAP-infected (n=50) cattle across Australia and New Zealand. Infected cattle were selected based on results from ante-mortem faecal culture assays or a combination of ELISA (S/P > 200%) and faecal PCR (>103 genomes/mL). Uninfected cattle were from a herd that has tested negative using reference tests for >20 years. A qRT-PCR assay for the biomarker miRNAs underwent further validation following the guidelines of the World Organisation for Animal Health manual of diagnostic tests and vaccines for Terrestrial Animals.

Results
The AI-pipeline identified three miRNAs that, when combined, predict MAP infection (ROC AUC=0.97, P<0.001). A qRT-PCR assay with AI user software was developed. Using Bayesian Latent Class Analysis it displayed superior DSe (median 92.8% vs 75.5%) and comparable DSp (median 94.8% vs 96%) to the current reference tests (combined performance of faecal culture, ELISA, and fPCR). The positive/negative predictive values (PPV/NPV) of this assay were 1.0/47.1 (combined reference tests 1.0/18.8).

Discussion
We have discovered and validated a novel microRNA-based diagnostic assay to detect JD. Our study highlights the utility of biomarkers for other difficult to diagnose diseases and details methods to validate them for use as routine diagnostic tests.

Rapid identification and discrimination of bacteria using advanced MALDI-TOF MS and FT-IR methods in Veterinarian Medicine

Americas Event Manager Elaine Evans

Fourier-transform infrared (FT-IR) spectroscopy, particularly the IR Biotyper® from Bruker, has emerged as a powerful tool for the discrimination of Escherichia coli (E. coli) and Salmonella strains from various animal sources. This study evaluates the efficacy of the IR Biotyper® in distinguishing avian pathogenic E. coli (APEC) strains, with a focus on serogroups O1, O2, O18, and O78, which are commonly associated with avian colibacillosis. The ability of the IR Biotyper® to differentiate these serogroups is crucial for epidemiological studies and infection control in poultry industries. Additionally, the IR Biotyper® Salmonella classifier was evaluated for its ability to discriminate various Salmonella O-serogroups. With the MALDI Biotyper® System, confirmation of Salmonella spp. has been shown and validated. MALDI-TOF MS is the first step in this workflow solution followed by FT-IR. Salmonella enterica, a significant pathogen in veterinary medicine, comprises numerous serogroups that require precise identification for effective outbreak management. The IR Biotyper®, combined with machine learning algorithms, demonstrated robust performance in differentiating multiple Salmonella O-serogroups, providing a rapid and reliable alternative to traditional serotyping methods. The IR Biotyper® demonstrated high discriminatory power. The integration of the IR Biotyper® into routine veterinarian diagnostic workflows offers several advantages, including reduced time and cost compared to whole-genome sequencing as one good example.

Real-Time Pathologist-Assisted Field Postmortem Examinations of Beef Cattle

Dr. Jennifer Davies[1], Dr. Lindsay Rogers[1], Dr. Dayna Goldsmith[1], Dr. Grace Kwong[1,2], Dr. Carolyn Legge[1], Dr. Erin Zachar[1]
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Introduction

Postmortem examination (PM) of deadstock with appropriate ancillary testing is fundamental to determining causes of morbidity and mortality. Reaching a definitive diagnosis is crucial to evidence-based herd management and treatment decisions. Many factors influence submission to a veterinary diagnostic laboratory meaning not all carcasses are examined. PMs of livestock are often performed on-farm by the attending veterinarian with tissue samples submitted to a veterinary diagnostic laboratory; these can be associated with lower diagnostic rates. The purpose of this study was to implement real-time video assistance by veterinary pathologists to beef cattle veterinarians (rVets) performing field PMs to see if it could improve the likelihood of attaining a definitive diagnosis and to determine if this was a potential service option to offer veterinary practitioners.

Methods

rVets in Alberta were enrolled and selected cases of deceased beef cattle over 48h-old for submission. Initially, rVets performed unassisted field PMs and submitted samples to the lab. In the second part, rVets performed real-time, pathologist-assisted field PMs (rtPAPs) via video conferencing. Histopathology and ancillary testing were done with an outcome of reaching a final diagnosis or partial/no diagnosis. rVets and pathologists were surveyed following the study to assess the viability of rtPAPs as a service.

Results

We demonstrated that rtPAPs improve the odds of reaching a definitive diagnosis by 13 times compared to unassisted field PMs. Both the rVets and pathologists saw benefits to rtPAPs.

Discussion

Our proof-of-concept study shows the positive role of rtPAPs in diagnosing beef cattle disease, speaking to the need for telepathology services supporting livestock veterinarians and producers. The higher rate of diagnosis associated with rtPAPs translates into better use of producer and veterinary resources. Additionally, this service would better support animal disease surveillance and could extrapolate to support all livestock species and underserved animals and veterinarians in remote regions.

Successes and Challenges of the CAHSS Surveillance Dashboards

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Introduction

The Canadian Animal Health Surveillance System (CAHSS), a division of Animal Health Canada, has collaborated with provincially inspected abattoirs and provincial veterinary diagnostic laboratories in western Canada since 2019 to bring together data-driven information for national animal health surveillance. To achieve this, CAHSS developed dashboards to display these complex and technical data in a real-time and interactive format that aids in the visualization and analysis of large datasets.

Methods

The two dashboards are 1) the abattoir dashboard, with data contributions from provincially inspected abattoirs in Manitoba, Saskatchewan and Alberta, and 2) the veterinary diagnostic laboratory dashboard, with participating laboratories in Manitoba, Saskatchewan, Alberta, and British Columbia. Data were collected into the highly secure and permissioned CAHSS data platform storage centre using Amazon Web Services. The data management, integration, and visualization capacities were advanced with a period of rapid development in 2024. Stakeholders were involved in the development process through engagement meetings and granting access to the dashboards to collect additional feedback and use cases. The dashboards are expected to be used to monitor endemic and re-emerging disease trends and to generate quarterly reports for managers and government representatives.

Results

The most recent stakeholder feedback has been positive, indicating that the product satisfied laboratory and abattoir data visualization needs. Furthermore, the capabilities of the CAHSS data platform have stimulated interest from other stakeholders. Discussions are underway to explore the feasibility and challenges of incorporating additional provincially inspected abattoirs and veterinary diagnostic laboratories into the CAHSS data platform.

Discussion

The CAHSS dashboards are a unique example of interprovincial collaboration toward the creation and use of a national tool to aid in animal disease surveillance.

Use of Digital Post-Mortem Images to Capture Feedlot Necropsy Information

Dr. David McPhee[1]
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Introduction

The collection of necropsy data is an integral component of veterinary feedlot consulting. A standardized necropsy protocol has been developed in which trained personnel collect digital images and forward them to a veterinarian for diagnosis.

Methods

71 postmortem examinations on a commercial feedlot in Nebraska were prosected by a trained technician following a written protocol, capturing digital images. An on-site veterinarian then established a necropsy diagnosis based on grossly visible postmortem signs. This diagnosis was considered to be the gold standard for the study. The images were then transferred by the technician to two veterinarians that were blinded to the on-site veterinarian's diagnosis. Agreement between each veterinarian and the gold standard was compared.

Results

Diagnoses obtained from the digital necropsy images agreed with the gold standard diagnosis on average 95% of the time (92% agreement for one veterinarian and 98% agreement for the other).

Discussion

Feedlot postmortem signs are often grossly visible and a diagnosis can typically be made using between 4 and 8 photos. Agreement between off-site and on-site veterinarians is high and disagreements usually relate to image quality rather than interpretation of the gross findings.

The study was first published in the year 2000. Limitations that were present at the time such as digital image quality, data storage capacity, and internet connectivity have been substantially improved. Trained technicians (and feedlot personnel on remote sites) can now upload and store necropsy images using TELUS Digital Post Mortem, a secure web-based tool. A veterinarian can then return a diagnosis to the client, typically within 24 hours. The timeliness of this digital necropsy protocol, as well as the large number of necropsies that can be done, enables rational decision-making and refinement of animal health protocols.

Improved Lab Diagnostics, Exhibition Hall D, June 13, 2025, 1:30 PM - 3:30 PM

Development of a Modified Indirect ELISA for Caseous Lymphadenitis Surveillance

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Introduction

Caseous lymphadenitis (CL), caused by Corynebacterium pseudotuberculosis, is a chronic bacterial infection of sheep and goats with internal and external abscess development a hallmark of the disease. While culture of abscess material is definitive, flock screening utilizes the synergistic hemolysis inhibition (SHI) test, which is labor-intensive protocols and has a long turnaround time. This study developed a modified indirect (MI)-ELISA utilizing recombinant phospholipase D (rPLD) antigen as an alternative to the SHI test to improve assay uniformity, reduce complexity and time, and enable higher-throughput screening.

Methods

The MI-ELISA utilizes plate-bound and horseradish peroxidase (HRP)-conjugated rPLD antigen for enhanced antibody detection, specificity and assay robustness. Caprine serum samples (n = 161) were collected and classified as SHI-positive (n = 20) or SHI-negative (n = 141) were tested by the ELISA and sample-to-positive control OD (S/P) ratios calculated. Serial dilutions of a strong positive sample were used to assess the relative sensitivity of the ELISA and SHI tests.

Results

With a S/P cutoff of 0.2 based on ROC analysis, the sensitivity and specificity of the ELISA was 95% and 99.3% (kappa = 0.942). Additionally, three 2-fold dilutions of a strong positive were detected by the ELISA compared to two for the SHI test. There was no reactivity with MAP antibody positive samples or samples from recently immunized goats.

Discussion

Despite a small positive cohort and reliance on SHI for classification, results indicate strong agreement with the SHI test with many usability enhancements including no sample dilution, water wash and an assay time of 75 minutes. Future work will expand validation using culture-confirm cases or Bayesian latent class analysis to refine diagnostic accuracy estimates.

Enteric Viral Multiplex Assay for Swine Health Surveillance

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Introduction

Animal health surveillance is crucial for sustaining food security, animal welfare and international trade in animals and animal products; however, surveillance testing can be cost-prohibitive. A common technology used for targeted surveillance testing is qPCR (singleplex to triplex). A multiplex assay to detect six swine enteric viruses (SEVs), in a single test was developed. This assay is based on a fluidic bead-based system, where each bead is color-coded with a distinct fluorescent signature, and each bead coated with a specific DNA capture probe. Multiplex technology may prove efficient and cost-effective for targeted animal health surveillance testing.

Methods

Modified DNA primers were designed to detect North American strains of six SEVs and an internal control, including degenerate primers to enhance inclusivity for divergent isolates. Assay parameters were optimized, and specificity for target amplification was confirmed, by capillary electrophoresis and Sanger sequencing. Multiplex and qPCR assays were compared for sensitivity and specificity using laboratory samples consisting of quantified, targeted reverse transcribed RNA in SEV negative sow feces collected from a SPF PEI farm.

Results

Limit of detection, for each North American reference SEV tested, is reported as RNA copy-number(s)/µl, for the multiplex assay and qPCR assays respectively: Porcine Deltacoronavirus (1, 1); Transmissible Gastroenteritis Virus (1, 1); Porcine Epidemic Diarrhea Virus (16, 80); Porcine Rotavirus A, PRVA (200, 40); PRVB (500, 4), and PRVC (tbd).

Discussion

Our SEV multiplex assay demonstrated test sensitivity, analytical specificity, and inclusivity for divergent isolates, comparable to qPCR. Multiplexing technology may make animal health surveillance more economical. This SEV assay will be expanded to include additional viral targets and will be further evaluated in a national proficiency program for SEVs, currently being established at the International Veterinary Proficiency Testing Centre at the AVC. This assay will be implemented in a swine health surveillance program in Atlantic Canada.

Evaluating Salivary Anti-CarLA IgA as a Method to Manage Gastrointestinal Parasitism in Canadian Pastured Sheep

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Introduction

Identifying sheep with superior immunity to gastrointestinal nematodes (GIN) is of great interest for Canadian sheep producers. Measuring the concentration of salivary IgA against carbohydrate larval antigen (CarLA) found on all third-stage GIN larvae has shown promise in New Zealand sheep, where salivary anti-CarLA IgA exceeding 1.0 U/ml has been associated with 20–30 % lower fecal egg counts (FEC). However, it remains unclear whether these findings translate to other sheep-producing regions, especially northern climates where GIN epidemiology and flock management differ from New Zealand. Accordingly, this study investigated salivary anti-CarLA IgA testing in sheep under Canadian conditions.

Methods

In 2022, an average of 25 ewe lambs per farm were randomly selected on 18 farms in Ontario, Canada, after grazing pasture for a minimum of 60 consecutive days. Body condition, fecal consistency, FAMACHA© score, liveweight, hematocrit, FEC, and salivary anti-CarLA IgA concentration were recorded for each study animal after the grazing season. Study animals returned to pasture in 2023 and were re-sampled 4 weeks after turnout.

Results

Multivariable linear regression demonstrated that the salivary anti-CarLA IgA response in 2022 predicted the salivary anti-CarLA IgA response in 2023 ($\beta = 0.213$; $p < 0.001$). In addition, salivary anti-CarLA IgA in 2022 was negatively associated with FEC in 2023 ($\beta = -0.167$; $p = 0.025$).

Discussion

Measurement of salivary anti-CarLA IgA appears a useful diagnostic test for identifying Canadian sheep with superior immune responses to GIN. Considering the problem that GIN pose to sheep producers, selection of sheep for superior immunity to GINs using the CarLA Saliva Test could be a promising tool for GIN management on Canadian farms. In order for the CarLA Saliva Test to be widely adopted by Canadian sheep producers, it is essential that the salivary anti-CarLA IgA ELISA is offered by regional veterinary diagnostic laboratories.

Fowl Aviadenovirus (FAdV) Genomic Diversity in Poultry Diagnostic Liver Samples Collected From 2020 to 2024 in Quebec, Canada

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Introduction

Fowl aviadenoviruses (FAdV) are icosahedral viruses with double-strand DNA genomes of approximately 44k nucleotide base pairs in length. FAdV species type D and E viruses are the etiological agents of inclusion body hepatitis (IBH). No treatment is available to cure infected fowls, but autogenous vaccines are vastly used in Quebec, Canada, to prevent IBH. The autogenous vaccine's efficacy, however, depends on its genomic/antigenic similarity to the virus strain in the field that is involved. Poultry veterinarians have reported that the type usually found in Quebec is type E, but cases of type D/2 were identified in 2024.

Methods

Liver samples were either directly sent to the Molecular diagnostic laboratory of CDVUM for FAdV qPCR analysis by poultry veterinarians, or by the Quebec Ministry of Agriculture, Fishery and Food following the necropsy of dead birds. Positive samples were selected and whole genome sequencing of FAdV was carried out using a MiSeq (Illumina) sequencing platform. Bioinformatic analyses were done using CLC Genomics Workbench and Geneious Prime.

Results

Out of 112 qPCR FAdV positive cases, 105 complete and 2 partial FAdV sequences were obtained. Among those sequences, 76, 20, 10, and 1 sequences were classified as genotypes E/8b, E/7, D/2, and E/8a, respectively. Only type E strains were found between 2020 and 2023, while type D/2 strains were newly identified in 2024. The sequence nucleotide identities of the strains within each cluster varies depending on the type identified (85.34% and 99.93% for E/8b, 93.02% and 99.83% for E/7, and 90.09% and 99.77% for D/2).

Discussion

This study gives us a better insight into the genomic diversity of FAdV strains present in Quebec poultry flocks. Rapidly identifying new strains could help practitioners adapt their autogenous vaccine strategy.

Increased Molecular Workflow Efficiency for Avian Influenza Virus Testing in Milk

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Introduction

The United States has been enduring a poultry H5N1 highly pathogenic avian influenza (HPAI) outbreak since January 2022, which spilled over to the cattle population in March 2024. The virus spread within the cattle and spilled back to the poultry population in multiple states, bringing new challenges to controlling HPAI. The outbreak of HPAI in poultry and cattle increased the demand for high-throughput testing of samples in the National Animal Health Laboratory Network (NAHLN) laboratories.

Methods

Increased capacity for high throughput testing explored the Integra and Biomek Span-8platforms to add diluent and transfer samples from collection tubes into 96 well plates, prefilled RNA extraction with the IndiMag Pathogen KF96 Cartridge on the KingFisher Flex, and the Biomek i5 multi-channel equipment to transfer the master mix and samples into the 96 and 384 well PCR plates. NAHLN PCR assay were optimized in 384 well formats using Quant 7 Pro PCR system, and the fast PCR chemistry IndiMix JOE was optimized for the ABI7500.

Results

Videos illustrated during the presentation highlight the Integra and Biomek Span-8 platforms, prefilled IndiMag Pathogen Kit, and Biomek i5, which yield a saving about 60 minutes or $35 in technician time per 96 samples. The IndiMix JOE fast chemistry saved 40 minutes per run while the 384-format saved 4.5 hours compared to 96 sample format. A further 20% cost saving of PCR reagents with the 20 ul reaction volume with both formats. The increased molecular workflows yielded a 100% diagnostic sensitivity and specificity, precision, and limit of detection compared to the current NAHLN-approved methods.

Discussion

Some or all of these workflow efficiencies were easily adaptive and transfer to the demand of HPAI or future major disease outbreaks testing while significantly reducing the risk of errors due to manual transfering/pipetting of samples, staff time, and laboratory expenses.

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) as a Diagnostic Tool for the Rapid Identification of Arthropod Species

Farah Kandil[1], Dr. Sawsan Ammar[1,2], Dr. Beverly Morrison[2], Mai Farghaly[2], No Kathryn Duncan[3], No Daniel Barrantes Murillo[3], Muhammad Bilal[1], No John Soghigian[1], No Brian Herrin[4], Dr. Jennifer Davies[2], Dr. John S. Gillerad[1]
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Introduction
Morphological and genetic approaches are frequently used to identify arthropod vectors and parasites. Both approaches are time-consuming and require specialized knowledge, and genetic methods require expensive equipment and consumables. Matrix-assisted laser desorption/ionization time of flight (MALDI TOF) mass spectrometry compares protein profiles of various organisms to a library to identify the species. MALDI-TOF is commonly used for bacterial identification in diagnostic bacteriology labs, yet its use in parasitology is limited, and its ability to identify arthropods has not been fully explored. Our ongoing project aims to validate MALDI-TOF as an alternative to traditional identification methods.

Methods
Arthropods were collected from a wide range of domestic and wild animal host species, undergoing a detailed morphological examination prior to dissection. DNA was prepared from half of each specimen for 18S rDNA and Cox-1 DNA molecular barcoding whilst the other half was prepared for MALDI-TOF. Arthropods tested so far are lice, fleas, mosquitoes and ticks. Tick samples are the focus of this abstract.

Results
Two species of ticks (Dermacentor variabilis (DV) and Ixodes scapularis (IS)) were successfully tested using MALDI TOF, and specific protein profiles were generated. Using our standardized protocol on 58 ticks, we produced 633 successful spectra (454 from IS, 179 from DV) out of a total replicate count of 652. Among these spectra, were the library entries. After testing the library, 100% of DV matched the library to the species level (score>2). 92% of IS matched to the species level, while the remaining matched to the genus level (2>score>1.7).

Discussion
Preliminary results suggest that MALDI-TOF has the potential to serve as a reliable identification tool for these tick species. As the project progresses, incorporating more arthropod species will further assess MALDI-TOF's broader applicability, strengthening its role as an alternative to traditional identification methods.

Surveillance, Glen 202, June 14, 2025, 11:00 AM - 12:30 PM

Diagnosis of Q Fever in bulk tank milk in dairy cattle: Herd-level prevalence based on reported clinical signs

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Introduction

Coxiella burnetii is a gram-negative bacterium causing Q fever in animals and humans. The disease is present worldwide, except in New Zealand. Ruminants are the most affected and are the primary source of human cases. In small ruminants, Q fever mainly causes abortions, while in cattle, it can also lead to metritis/endometritis, retained placenta, and fertility issues.
The study aimed to compare the prevalence of Q fever infection between farms reporting APSW complex (abortions, premature delivery, stillbirth, weak born offspring) with those with only other reproductive disorders.

Materials and Methods

PCR testing of bulk tank milk (BTM) using QTest, developed by Ceva and Qualyse, was used to detect Coxiella burnetii. QTest uses a FTA® card for sample collection and real-time PCR analysis. The study analyzed results from October 2020 to September 2024, categorizing herds into two groups: those with the APSW complex and those with other reproductive disorders.

Results

A total of 2014 samples were collected from various countries in Europe, North Africa and Canada. APSW complex was reported in 62.6% of farms, with 44.0% testing positive for Coxiella burnetii. Other reproductive disorders were reported in 37.4% of farms, with 40.4% testing positive. The difference in prevalence between the two groups was not statistically significant (Fisher exact test, p=0.11).

Discussion

These results suggest that the presence of Coxiella burnetii in dairy cattle can be associated without difference with major reproductive disorders (abortions, premature delivery, stillbirth, weak born calves) or other reproductive disorders (metritis/endometritis, retained placenta, infertility). Testing for Coxiella burnetii should therefore be considered in herds with reproductive problems, even in the absence of abortion.

Swine Disease Reporting System: Using Veterinary Diagnostic Laboratories Data for Real-time Monitoring of Swine Pathogens

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Introduction

Veterinary diagnostic laboratories (VDLs) in the U.S. receive thousands of samples daily for diagnostic testing, with testing results stored in each VDL's database. The Swine Disease Reporting System (SDRS, www.field.org/SDRS) was created to collate diagnostic data and report generated information. The SDRS database includes PCR results for nine swine pathogens and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) ORF5 sequences. This study demonstrates the methodology of creating the SDRS database and its contributions to the U.S. swine industry.

Methods

VDL clientele, producer, and veterinarian anonymized PCR submission data, including tests and results, were collected from six VDLs, cleaned, and collated. A web-based application written in C# 10 using the .NET 6 framework collated and organized data at the submission level, using the VDL-assigned ID as a unique identifier. A "case" refers to all samples associated with a single ID. PCR results reported by the VDLs established the final case result in the database. Positive cases required at least one PCR-positive sample, while negative cases had all samples with negative PCR results. PRRSV ORF5 sequences were collated at a sample ID level.

Results

The final dataset includes 1,603,499 PCR cases and 116,484 PRRSV ORF5 sequences. Highlights include: a) TGEV had its last positive case in March 2021, marking 3 years since the last detection; b) Decreased positive cases of M.hyopneumoniae from sow farms, reaching 15 years lowest positivity (6.86%) in April 2024; c) PRRSV detection dynamics vary among U.S. states, with regional differences for predominant strains; d) PEDV and PDCoV positivity remains low in summer but increases in colder months, mainly in finishing sites.

Discussion

The SDRS demonstrates the importance of aggregating, analyzing, and sharing data on endemic and emerging diseases in the U.S. swine industry, aiding veterinarians and producers in making informed disease prevention and management decisions.

Efficacy and feasibility of diagnostic tests for early detection and risk assessment of American foulbrood in beekeeping operations

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Introduction

American foulbrood (AFB), caused by Paenibacillus larvae, is a devastating contagious disease that is often controlled by antimicrobials in North America. Destruction of affected colonies is mandatory in many jurisdictions. Therefore, early detection of AFB is crucial. However, the efficacy of diagnostic tests and risk assessment strategies is influenced by sample types, timing of sample collection and size of operation.

Methods

We conducted multiple clinical investigations of AFB outbreaks and assessed levels of P. larvae spores (hereafter, AFB spores) in various samples using quantitative bacterial culture. The number of AFB spores in bees, bottom board debris, brood-chamber honey, and extracted honey was comparatively evaluated for their suitability for AFB risk assessment.

Results

The validity and feasibility of various sample types for risk assessment will be discussed based on the results of our ongoing clinical investigations of AFB outbreaks. In general, for both small and large beekeeping operations, the most feasible AFB risk assessment method is based on the number of AFB spores in extracted honey at the end of the honey harvesting season (late summer). Additionally, the number of AFB spores in nurse bees during the brood-rearing season, and potentially in bottom board debris during early spring, may also be useful for AFB risk assessment in small beekeeping operations. The number of AFB spores in brood chamber honey could be used for surveillance of colony exposure to AFB over time, similar to the use of serology tests in "herd health" management.

Discussion

The selection of an appropriate AFB risk assessment strategy depends on the size of the beekeeping operation, the season, and the availability and accuracy of diagnostic/risk thresholds. Future research should focus on refining these diagnostic/risk thresholds to enhance evidence-based AFB control, ultimately reducing reliance on antimicrobials and mitigating the development of antimicrobial resistance.

Identification of circulating beef calf scours causing pathogens and evaluation of efficacy of the current scours' vaccination strategies in Western Canada

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Introduction

Calf scours remains a significant economic concern for producers, despite the availability of scours vaccines. Common causative agents include rotavirus, coronavirus, Escherichia coli(E.coli), Clostridium perfringens, Salmonella species, Cryptosporidium, and coccidia. Scours vaccines typically target enterotoxigenic E. coli K99(F5), Clostridium perfringens type C, rotavirus strains G6 P[1], G8, and/or G10 P[11], and coronavirus. This study aims to determine whether the currently circulating scours pathogenic strains match those in licensed vaccines.

Methods

During calving seasons, producers with scouring calves were requested to submit faecal samples to Prairie Diagnostics Services, Saskatoon for scours panel initial screening. All samples were then shipped to the University of Calgary, AB for characterization using PCR and Sanger sequencing. The PCR analyses targeted Clostridium perfringens alpha and beta toxins, bovine rotavirus NSP3, VP6, and VP7 genes. Optimization of epsilon, iota, and rotavirus VP4 genes is ongoing.

Results

Preliminary results from 136 scouring calves' samples revealed mixed infections as follows;14% rotavirus, 10% coronavirus, 14% Clostridium perfringens,5% Cryptosporidium species, 9% Giardia species,1% Eimeria species, less than 1% Moniezia and Trichostrongylus. In 47% of the samples, E.coli was detected and further screening of its virulent genes using PCR revealed 23 samples had aea,13 with stx1, 9 contained stx2, no Sta were detected and 1 isolate was positive for K99(F5). Sequencing results of 15 rotavirus positive samples (7 NSP3, 6 VP7, and 2 VP6) revealed a mixture of G-types1,2,6,8,10 and 29 with G6 being the most predominant. Additionally, beta toxin gene was detected in 8 Clostridium perfringens isolates and 27 isolates contained the alpha toxin gene.

Conclusion

The presence of E.coli aea, stx2 and stx1 virulent genes, and the diversity of rotavirus G-types illustrates the need for surveillance. Additionally, the detection of K99(F5) in only one sample suggests a low level of circulation of enterotoxigenic E. coli with this colonizing factor.

Disease Detection and Improved Lab Diagnostics, Exhibition Hall D, June 14, 2025, 11:00 AM - 12:30 PM

DEVELOPMENT OF REAL-TIME RT-PCR ASSAYS FOR SPECIFIC DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) LINEAGES OF SEROTYPES O AND SAT2

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease caused by FMDV. Seven antigenically distinct FMDV serotypes and multiple lineages exist. Laboratory diagnosis relies on virological, molecular, and serological tests. During an outbreak, it is crucial to monitor circulating strains in real-time. The rtRT-PCR is a tool of choice for this purpose. Some assays already exist, targeting serotypes or higher levels of discrimination. In this study, we propose three new rtRT-PCR assays, targeting serotypes O and SAT2.

Methods

The rtRT-PCR assays designed in this study were either a response to an emergency (SAT2/V) or targeted lineages considered at high risk for Europe (O/EA-3 and O/ME-SA/SA-2018). VP1 sequences were retrieved from public databases to design primers and probes (validated in silico). Then, the assays were tested in vitro against a panel of FMDV strains from the EU-Reference Laboratory collection.

Results

The SAT2/V assay was tested with 65 strains and 100 % of SAT2/V samples were detected (36/36). The O/EA-3 assay was tested with 34 strains, and 94% of O/EA-3 samples were detected (17/18). Finally, the O/ME-SA/SA-2018 assay was challenged with 32 strains, and 92% of samples were detected (11/12).

Discussion

The three rtRT-PCR assays developed in this study present a good potential of detection (100%, 94% and 92% for SAT2/V, O/EA-3 and O/ME-SA/SA-2018 respectively). All three assays did not detect other strains tested (some exceptions for old isolates in two cases), selected according to specific rules (strains circulating in the same geographic area and/or involved in recent epizooties). Two of these assays have already been shared with laboratories due to outbreaks linked to the targets (SAT2/V in Algeria, and recently O/ME-SA/SA-2018 in Germany). Even if more samples should be tested to precisely define the accuracy of the assays, these PCR assays will be an additional tool to combat FMD.

Metabarcoding to Identify Species Mislabeling in Pet Food

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Introduction

The increasing popularity of pets has highlighted the need for transparency and accuracy in the pet food industry, particularly due to rising concerns about pet health, food fraud, and regulatory compliance. In this study we developed a metabarcoding methodology, targeting cytB and 16S rRNA mitochondrial markers, to identify animal species and evaluate authenticity of commercial pet food.

Methods

The study concerned 100 pet food samples of single-protein and regular kibbles from each brand available in a specialized pet store, a supermarket, and a discount store. DNA was extracted with Qiagen mericon Kit, quantified with Promega QuantiFluor® ONE System, and sequenced adapting the Illumina 16S Metagenomic Library Preparation protocol. Amplicon sequence variants (ASVs) were generated using DADA2 R package and were taxonomically assigned through BLAST against GenBank. Due to the high number of sequences with incorrect species metadata, for cytB a fully curated database with 332468 records was created, to be used with the QIIME 2™ platform.

Results

Data analyses revealed that the cytB and 16S targets did not differ in species attribution, with cytB being more consistent in estimating the proportion of the different ingredients. The results revealed mislabelling across 82.36% of single-protein samples, and 75.76% of mixed-species samples, exposing prevalent substitution with undeclared species, and absence of listed species. Generally, higher-value products were substituted with lower-cost ingredients, like chicken and pork. In fact, substitution events were more common in the more expensive premium diets.

Discussion

Our findings suggest economic motivations behind adulteration, raising concerns for regulatory gaps and consumer trust. The developed methodology proved effective in uncovering mislabelling, which other than a merely financial issue, can be a food safety problem jeopardizing the health of pets with dietary restrictions. However, the absence of a public truly curated dataset, still limits the diffusion and broad use of metabarcoding.

Predictive Modelling for the Rapid Diagnosis of Myxomatous Mitral Valve Disease in Dogs based on Blood microRNA Expression Profiles

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Introduction

Myxomatous Mitral Valve Disease (MMVD) is a common, acquired, and progressive canine heart disease [1]. Current cardiac biomarkers (CBs) are useful in MMVD cases [2,3], but are not sufficiently discriminatory for staging individual patients [4]. Consequently, refined tools to diagnose and stage canine MMVD are needed. MicroRNA profiling is an interesting alternative. MicroRNAs regulate gene expression, are tissue specific (e.g. heart) [5] and found in biofluids (e.g. blood) [6]. They have received increasing recognition in veterinary cardiology, although currently their use as CBs is limited due to the lack of standardized units of measurement and incapacity to use single markers in isolation because of synergies and interactions between microRNAs [7,8].

Methods and Materials

Multicenter, cross-sectional, prospective investigation based on data from healthy controls (n=50) and MMVD cases (n=47). MicroRNA expression profiles were run through a dedicated data preprocessing pipeline and used to train and fine-tune a curated collection of machine learning algorithms. Their performance in distinguishing both healthy controls from MMVD patients and pre-clinical from clinical MMVD patients was thoroughly evaluated.

Results

Analysis of miRNA expression patterns by predictive classification algorithms could differentiate healthy controls from dogs with MMVD (sensitivity=0.85; specificity=0.82) (Figure 1). Discrimination of pre-clinical from clinical MMVD cases resulted in promising results (sensitivity=0.61; specificity=0.79) (Figure 2). The methodology also compared advantageously to current CBs in a limited population.

Discussion

The analysis of miRNA expression profiles by predictive classification algorithms within an artificial intelligence framework provides a useful diagnostic tool to distinguish healthy from MMVD dogs and shows promises in staging MMVD. Further analysis on larger and more varied canine cohorts is expected to improve the predictive system's resolution and allow extensive comparison to existing CBs. The current study suggests that microRNA diagnostic technology has great upcoming potential in veterinary cardiology and in the broader veterinary sphere.

Validation of a Real-Time PCR Assay Targeting Two Conserved Regions of Canine Adenovirus (-1 & -2)

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Introduction

Canine adenovirus-1 (CAV1) causes a systemic infection that may present as acute hepatitis, respiratory disease, or ocular disease in dogs and wild canids. In contrast, canine adenovirus-2 (CAV2) infection is typically limited to respiratory disease. CAV testing is a part of the canine respiratory PCR panel offered at the AHDC, and our objective was to develop and validate a real-time PCR assay that detects CAV (-1 & -2) multiplexed with MS2 exogenous control.

Methods

Seventeen CAV1 and CAV2 genome sequences were aligned to identify regions with 100% identity. To mitigate the effects of mutations that might emerge, we chose two conserved CAV regions for simultaneous detection: ORF13 which encodes the core protein V, and ORF18 encoding a viral DNA-binding protein. MS2 was used as an extraction control. Assays were run with Path-ID™ Multiplex One-Step RT-PCR Kit on the QuantStudio 5 platform.

Results

Real-time PCR amplification efficiency, linearity, intraassay, and interassay variation were evaluated with serially diluted viral isolates, and all metrics were acceptable. The limit of detection was determined to be 10 copies in singleplex and triplex formats based on diluted synthetic fragments. Analytical specificity was characterized in silico and in vitro. No off-target hits were identified in the NCBI nucleotide database. Of 20 viral and bacterial isolates, only CAV1 and CAV2 were detected. Multiplex performance was evaluated with competition experiments, and no changes in sensitivity or efficiency were observed. To characterize diagnostic performance, 60 archived specimens from dogs and 6 wildlife species were tested. The CAV triplex assay performed as expected for the 30 positive and 30 negative samples and showed better sensitivity in two cases.

Discussion

Overall, the ORF13, ORF18, and MS2 targets met validation criteria individually and in a triplex format for detecting CAV. The triplex format will provide better quality control, efficiency, and cost savings.

Outbreak Preparedness and Response, Exhibition Hall D, June 14, 2025, 1:30 PM - 3:30 PM

Building Efficiencies for Testing Multiple Sample Streams for Highly Pathogenic Avian Influenza (HPAI) in a Laboratory of the United States National Animal Health Laboratory Network (NAHLN)

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Introduction

The on-going HPAI outbreak has posed unprecedented challenges and opportunities for veterinary diagnostic laboratories in the United States Department of Agriculture (USDA) National Animal Health Laboratory Network. After initial focus on testing domestic and wild birds, the list of susceptible and infected species has continuously expanded into mammalian, in particular feline species, and since early 2024 into livestock, most notably dairy cattle with close to 1,000 dairy herds across 17 US States reported infected as of February 28, 2025.

Methods

The Animal Health Diagnostic Center (AHDC) as a Level 1 NAHLN laboratory has deployed and enhanced PCR capability and capacity for high volume rapid throughput testing of samples in its molecular diagnostics laboratory. Operational bottlenecks include notification for and processing of specimens from various sample streams, resulting and messaging data to regulatory State and federal agencies, and forwarding non-negative samples for confirmation to the USDA National Veterinary Services Laboratories.

Results

The AHDC in anticipation of high testing demand introduced automation and IT applications at multiple steps in its testing processes. The greatest reduction of operational bottlenecks was achieved through a dedicated web portal-based submission process, that reduces data entry errors and facilitates result messaging and forwarding of specimens for confirmation. The request for uniform 15 ml screw cap tubes and additional streamlining of operations by sample stream added further efficiencies to the testing program. Preservation of appropriate specimens for research investigations, in particular sequencing, was also achieved.

Discussion

The AHDC, as of February 28, 2025, has tested 49,060 samples for diagnostic and surveillance purposes since early 2022, of which 17,433 were milk samples from dairy cattle outbreak and non-outbreak states tested since April 2024. Continuous expansion of the outbreak and necessary testing activities with appropriate safety considerations led to workflow improvements across all aspects of the testing process.

Early Detection of High-pathogenicity Avian Influenza Viruses (H5 clade 2.3.4.4b) in Sediment from Wetland Habitats Preceding an Epizootic in Wildlife and Poultry

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Introduction
High-pathogenicity avian influenza viruses (HPAIVs) are a major threat to poultry and wildlife health and a perennial pandemic threat to humans. Environmental surveillance is a promising new strategy for monitoring these threats, especially panzootic lineages like H5N1 that are spread globally by migratory birds. Diverse avian influenza viruses have been previously detected in sediment from wetland habitats, so we deployed and evaluated a sediment-based surveillance program during fall 2024 in British Columbia (BC), Canada.

Methods
Surveillance was conducted during the fall migration season at seven wetlands in the Greater Vancouver region. Each site was visited bi-weekly between 5 Sept 2024 and 28 Nov 2024. Twelve sediment specimens were collected per site visit (504 total for the season). Sediment specimens were screened for influenza viruses by RT-qPCR, and positives were further characterized by a custom targeted genomic sequencing method. Detection rates in sediment were compared to local HPAIV trends in wildlife and poultry. Phylogenetic analysis was conducted on HPAIV genomic sequences from sediment, wildlife, poultry, and humans in the Pacific Northwest region.

Results
HPAIVs from H5 clade 2.3.4.4b were first detected in sediment on 16 Sept. This preceded detections in wildlife, poultry, and a local human case by 17 days, 35 days, and 53 days respectively. Sediment detections increased over the migratory season, peaking the week of Oct 21 with 50% specimen positivity at some sites. Sediment detection trends foretold and reflected subsequent infection trends in wildlife and poultry. Phylogenetic analysis confirmed that HPAIVs in sediment were closely related to those infecting wildlife, poultry, and humans in BC, Alaska, and Washington state. Additional influenza diversity was also detected in sediment (12 different HA and 9 different NA subtypes).

Discussion
Sediment surveillance is a powerful tool that provides early warning of HPAIV incursions during migration seasons and situational awareness during epizootics.

From Detection to Control: Managing the UK's Bluetongue Virus Outbreak

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Introduction

In November 2023, the UK reported its first bluetongue virus (BTV) case in over 15 years. The following summer, BTV reemerged during peak vector activity. The response required prompt decision-making from policymakers and a flexible, scalable approach from diagnostic labs. This talk covers the timeline of events, control measures implemented, laboratory adaptations, and preparations for future outbreaks. It will also explore ongoing projects aimed at understanding BTV in endemic countries from where future outbreaks may emerge.

Methods

Blood and tissue samples for BTV testing were submitted to the Pirbright Institute, UK, where a RT-qPCR assay targeting BTV segment 10 was used to detect BTV RNA. Positive samples were then tested using a competition ELISA to detect antibodies against BTV VP7. Additionally, virus was cultured using insect KC cells, genotyped with BTV segment 2-targeted RT-qPCR, and sequenced using NGS on the Illumina MiSeq.

Results

Between late 2023 and early 2025, 87,761 UK samples were tested by RT-qPCR, with 890 (1%) testing positive for BTV RNA. Of these, 75 were seronegative (8.4%). The majority were identified as BTV-3 (99.9%), though one animal tested positive for BTV-12.

Discussion

During the BTV outbreak, the Pirbright Institute scaled up testing from around 250 samples per week to over 6,000 samples per week, requiring significant adaptations. Results were shared with UK authorities, prompting nationwide control measures to limit the disease's spread.

Improving Australian laboratory network preparedness for emergency animal disease responses through a laboratory simulation exercise

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Introduction

In 2023, Australia undertook a nationwide laboratory simulation exercise, named “Exercise Waterhole”, to test and evaluate national laboratory network preparedness and surge response capacity for major animal disease incursions, intending to improve national animal health laboratory systems. Key lessons learned from the exercise will be discussed, which may be relevant to other laboratory networks worldwide.

Methods

All state and territory animal health laboratories, Australian Centre for Disease Preparedness and the Department of Agriculture, Fisheries and Forestry participated in the exercise. Key laboratory, industry and public health stakeholders were also involved as observers. The planning was guided by a scoping study, in line with various international simulation exercise guidelines, including those from the World Organisation for Animal Health. The Australian Animal Disease Spread Model was used to simulate a possible spread of a lumpy skin disease incursion in cattle in northern Australia, followed by concurrent outbreaks of high pathogenicity avian influenza in poultry in the south. Two desk-top discussion exercises and one small-scale functional exercise in Tasmania were conducted before a 3-day nationwide functional exercise. Many diagnostic and laboratory activities were discussed with some identified as focuses for functional testing. Self-assessment and independent evaluation also occurred throughout the exercise.

Results

A range of findings specific to laboratory-related regulations, surge capacity demands, communication and information management arrangements, and resource and logistical needs were identified. Their respective recommendations to enhance Australia’s network laboratory preparedness for disease responses were also developed and prioritized.

Discussion

Exercise Waterhole, contributing to Australia’s National Animal Health Diagnostics Business Plan 2021-26, represents a significant milestone for improving Australia’s animal health laboratory systems in recent years. The findings and recommendations have proven useful in guiding a roadmap to enhance future laboratory emergency animal disease responses, with some laboratories implementing changes immediately and relevant new improvement projects developed post exercise.

Production Impacts and Estimated Seroprevalence of Highly Pathogenic Avian Influenza H5N1 virus in a Dairy Herd

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Introduction
A dairy herd in Ohio, USA experienced an outbreak of Highly Pathogenic Avian Influenza (HPAI) H5N1 virus after the importation of cattle from Texas in March 2024. Analysis of the farm records was performed to determine risk factors associated with clinical influenza in the Ohio dairy, its effects on milk production, mortality and herd removal in affected cows. Serological testing post-outbreak was performed on a subset of the herd.

Methods
We studied the association between milk production prior to the outbreak, somatic cell count, parity, days in milk (DIM), breed and the risk of clinical influenza using Cox proportional hazards regression. The relationship between clinical influenza, mortality, and herd removal over a two-month period was investigated. Three months after the outbreak, 637 serum samples were collected to assess seroconversion rate, and compare seropositivity to clinical influenza in the herd.

Results
776/3,433 (23%) lactating cattle were diagnosed with clinical influenza, with 24 cases confirmed by polymerase chain reaction. Among clinically affected cows, 53 died, with affected cows having an increased risk of death (relative risk [RR]: 6.0 [95% CI: 3.9, 8.9]), and 245 animals were culled during the study (RR: 3.9 [95% CI: 3.3, 4.5]). DIM and parity were associated with the risk of new clinical influenza (Type III P-value <0.01). Breed, baseline milk production, and SCC were not significantly associated with the risk of clinical influenza (P > 0.05). Milk losses of 901.2 kilograms were recorded for clinically affected cattle two months post-outbreak. Convalescent serum samples revealed 89.5% (570/637) seroconversion in the cattle tested, and 76.1% (485/637) were seropositive but lacked clinical signs.

Discussion
Clinical influenza significantly impacts milk production, herd removal, and mortality in affected cows. Herd level seroconversion may be high, while clinical disease may affect a smaller subset, and should be considered in disease management.

b) Clinical Conundrums, Glen 202, June 14, 2025, 1:30 PM - 3:30 PM

An Outbreak of Severe Necrotic Tongue Lesions in Heavy Cattle in a Canadian Feedlot

Meghan Brookhart, Greg Dimmers, Dr. Dayna Goldsmith, Dr. Eugene Janzen, Dr. Vanessa Cowan, Assitant Veterinarian Felipe Reggeti, Francisco Uzal, Timothy W.J. Olchowy, Dr. Beverly Morrison, Dr. Erin Zachar, John Remnant, Dr. Lindsay Rogers, Katie Waine[1]
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Introduction

In July 2023 three heavy cattle in a feedlot in Canada were found with severe necrotic lesions of the rostral tongue during a morning pen ride. By January 2024 over 1,000 animals had been affected. This presentation will describe the extensive investigation that took place including the clinical presentation, treatment and management of animals involved, and the diagnostic testing that was conducted.

Methods

Initial investigations began on the feedlot where pens, feeders, and water troughs were examined for sharp objects. Handling systems were also inspected, and cattle behaviour was observed. Samples of feed and water were collected for toxicology, chemical and microbial analysis. Laboratory investigation included gross examination of heads and tongues, with tissues collected for histopathology, bacteriology, and virology. A clinical case discussion was also held with national and international colleagues to discuss possible aetiologies.

Results

There was no evidence found at the feedlot to support an external traumatic cause. Gross and histopathological evaluation found necrotizing glossitis with abundant mixed bacteria. Bacteriology results suggested these to be commensals or secondary invaders. There was no evidence of viral involvement, and no evidence of primary bacterial infection, including clostridial myositis. Initial mycotoxin analysis of feed was unremarkable, but subsequent analysis of hard clusters found in the dried distiller's grains with soluble feed additives found toxic levels of ergot alkaloids.

Discussion

This case describes an outbreak of severe tongue lesions in Canadian feedlot cattle, where localized ergot alkaloid toxicosis is considered the likely cause.

Poster Session: Student Poster Competition Judging, Exhibition Hall E, June 12, 2025, 5:30 PM - 6:30PM

Antimicrobial Resistance of Salmonella spp. Isolated From Horses in Poland

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Introduction

Salmonella infections and antimicrobial resistance pose significant threat to global public health. Horse infections are usually asymptomatic but may result in clinical illness of different severity. Bacteria shedding leads to widespread environmental contamination. Outbreaks with high fatality rates and financial losses were reported. As infection significance used to be neglected and the bacterium was not included in routine bacterial examinations data regarding horses in Poland are scarce.

The situation changed in 2023 when Salmonella was detected in horses suffering from severe diarrhea and septicaemia increasing the number of investigated samples.

Methods

The antibiotic susceptibility data (January 2023 – February 2025) of Salmonella spp. horse isolates acquired from two diagnostic laboratories were analysed.

Results

Thirty-eight isolates were obtained from tissues (2), milk (1), rectum swabs, and faecal samples (35) collected from septicaemia, diarrhoea, colic cases, and asymptomatic animals as part of implemented monitoring protocols. The disc diffusion method was used, and antibiotic susceptibility profile differed between the laboratories.

All isolates were found sensitive to florfenicol, colistin, lincomycin, and amoxicillin-clavulanate. Marbofloxacin was effective against 96.9% of isolates, trimethoprim-sulfamethoxazole, and enrofloxacin 60.5%.

All isolates were resistant to tylosin and most to ceftiofur (91.7%), trimethoprim-sulfadiazine and ampicillin (88.2%), penicillin (66.6%), and amoxicillin (65.7%)

100% of isolates showed intermediate resistance to doxycycline, 31.6% to enrofloxacin, and 28.6% to penicillin.

Discussion

This study provides the first report of equine Salmonella infections and antibiotic susceptibility profiles from Poland. While available data did not allow for detailed epidemiological analysis, those confirm the threat of antibiotic resistance. Taking into account that most of horses in Poland are kept for meat production and that horse meat could be a source of food-borne infections continuous monitoring and surveillance are crucial for equine medicine and mitigating public health risks.

A diagnostic multiplex qPCR panel for the detection and identification of gastrointestinal parasites in cattle.

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Introduction

Gastrointestinal parasitism is a health issue and major cause of production loss among grazing livestock. Amongst the most important gastrointestinal parasites affecting cattle are gastrointestinal nematodes (GIN) (Haemonchus spp., Ostertagia ostertagi, Cooperia spp., Trichostrongylus spp. and Nematodirus spp.) and coccidia (including Eimeria bovis and E. zuernii). The emergence of drug resistant parasites and environmental concerns of parasiticide use are driving the need to develop better diagnostic tools to support more evidence-based control. However, the diagnosis of parasitic infections in cattle has barely changed in decades and is generally limited to enumerating eggs and oocysts in feces using floatation and microscopy methods such as Wisconsin and mini-Flotac. However, eggs and oocysts of the majority of species are difficult to differentiate, which is a major limitation due to differences in pathogenicity, epidemiology and anthelmintic sensitivity.

Methods

Biovet, a division of Antech, has recently developed a set of real-time multiplex PCR (qPCR) tests to identify and quantify the main GIN and coccidia of cattle, namely Ostertagia spp., Trichostrongylus spp., Haemonchus spp., Cooperia spp. and Nematodirus spp. for GIN and Eimeria bovis, Eimeria zuernii, Eimeria auburnensis, and Eimeria alabamensis for coccidia. Next Generation Sequencing (NGS) methods such as “nemabiome metabarcoding”, have been recently developed to characterize the intestinal parasite communities of different animal species, including cattle. Multiplex qPCR are less comprehensive than NGS but offer a less expensive solution with faster turnaround time (TAT) and higher throughput.

Results and discussion

We used NGS metabarcoding to help refine primer design and to validate qPCR tests using pilot sets of cattle fecal samples. Although the numbers examined to date is relatively small, there is good overall agreement between the two methods for relative abundance of GIN and coccidia species in cattle fecal samples.

A freeze-dried RT-qPCR with ambient temperature shipping allowing for an efficient detection of Foot and Mouth Disease Virus in 40 min

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Introduction

Foot and mouth disease (FMD) is one of the most contagious viral diseases in ruminants, causing epizootic diseases in a few weeks and with devastating economic consequences. Rapid and specific identification of the agent is of utmost importance. Innovative Diagnostics has developed a freeze-dried molecular diagnostic tool to detect all known serotypes of FMDV. Using the ultra-rapid amplification program, the ID Gene LYO™ FMDV Triplex provides results in less than 40 minutes and can be used on multiple type of samples: blood, serum, tissues, organs, swabs, milk and nucleic acid storage cards.

Methods

Inclusivity was evaluated on a panel of 10 inactivated strains from all 7 serotypes. Diagnostic specificity was assessed on 310 samples: 160 bovine and 50 swine whole blood samples and 100 goat milk from non-infected area (France). Diagnostic sensitivity was assessed on 3 positive bovine serum samples part of an inter-laboratory trial (2018, TPI, UK). These samples and nine additional spiked samples at two different loading rates were tested to compare the performance of the new kit with other commercially available RT-qPCRs (kit A&B).

Results

Measured specificity was 100% (95%CI [99-100], n=310). 3/3 samples were found positive giving a measured sensitivity of 100% (95%CI [51-100], n=3). Strains from all serotypes were detected and the ID Gene LYO™ kit gave earlier Cq values than kit A (ΔCq = 4).

Discussion

The ID Gene LYO™ FMDV Triplex exclusively detects all FMDV known serotypes in less than 40 minutes thanks to an ultra-rapid amplification program. The ID Gene LYO™ kit's superior performance enables confident and early identification of FMD virus and is available in a freeze-dried format for a cost-effective and eco-friendly shipment around the world.

A new double antigen ELISA for a highly specific assessment of herd status toward Sheep Scab infestation

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Introduction

Sheep scab is a highly contagious and pruritic disease caused by Psoroptes ovis, causing serious economic losses for the livestock industry. Mites are mainly transmitted by direct contact with infested sheep. As animals may be asymptomatic, caution should be taken when introducing new animals in the herd. There is no vaccine against Sheep scab yet and treatments are heavy, not always effective, and associated to negative environmental impact. Therefore, Innovative diagnostics has developed The ID Screen® Sheep Scab Double Antigen ELISA, a double antigen ELISA to detect antibodies against Psoroptes ovis to assess presence of the parasite at herd-level as well as antiparasitic treatments efficacy.

Methods

Diagnostic specificity was assessed with 457 negative sheep serum samples from 3 different French herds (absence of clinical signs for at least 5 years prior to sampling). Diagnostic sensitivity was assessed : on an individual level with 55 samples from animals with clinical signs and at herd level on 7 herds containing clinically affected animals. 49 infested animals were tested before and after treatment (two dip-baths, Dimpylate (Dimpygal®), two weeks apart).

Results

Measured specificity was 99.6% (95%CI [98.4-99.9], n=457). 49/55 samples were found positive : measured sensitivity was 89.1% (95%CI [78.2-94], n=55), with no correlation between S/P% values and severity of skin lesions. All the 7 herds presumed positive were found positive using the ID Screen® ELISA. After 2 dip-baths, 17 days post-treatment, the mean S/P% value decreased by 55% and 18,4% of animals became seronegative.

Discussion

The new ID Screen® kit shows high performance enabling a reliable detection of Sheep Scab at herd level, even before clinical signs appear, and allows an efficient assessment of antiparasitic treatment effectiveness. The ID Screen® kit is not only “animal-friendly” by being less traumatic than skin scraping but also user-friendly thanks to a ready-to-use format.

A new freeze-fried RT-qPCR with ambient temperature shipping to detect and differentiate Bluetongue and Epizootic Hemorrhagic Disease in a single well

Anna Greatrex[1], Lea Despois[1], Emilie Bianchini[1], Loïc Comtet[1], Philippe Pourquier[1]
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Introduction

BlueTongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are Orbiviruses which were reported in 2023/2024 across Europe. Both have huge economic impacts and similar clinical symptoms, making laboratory testing essential for diagnosis. Innovative Diagnostics offers a freeze-dried triplex RT-qPCR, ID Gene LYO™ BTV & EHDV Advantage Triplex, enabling the detection and differentiation, in a single well, of both BTV & EHDV. Results can be obtained in 50 min with a rapid amplification program, compatible with all ID GeneTM kits, making possible to test on the same run with different Orbiviruses RT-qPCRs therefore offering maximum flexibility & lab ressources optimization.

Methods

Diagnostic specificities for BTV and EHDV were assessed with 327 negative samples. Sensitivities were tested on 70 BTV-positive samples and 143 EHDV-positive samples. Inclusivity was assessed on 3 reference panels: 13 EHDV RNAs, 36 BTV RNAs (French national reference laboratory for BTV & EHDV, Anses), 7 EHDV RNAs (The Pirbright Institute, UK) and 10 BTV RNAs (FLI, Germany).

Results

The ID Gene LYOTM kit measured specificities with respect to both BTV and EHDV were 100%, CI95% [99.1-100], n=327. Measured diagnostic sensitivity with respect to BTV and EHDV targets was respectively 100%, IC95% [99.1-100], n=70 and 98.9%, IC95% [98.2-100], n=143. All BTV and EHDV strains tested, including the BTV and EHDV strains detected in Europe in 2023/2024, were efficiently detected by the new ID Gene LYOTM kit, indicating a perfect inclusivity.

Discussion

The new kit enables to efficiently detect and differentiate BT and/from EHD in only one reaction. Its freeze-dried format allows for an eco-friendly shipment at room temperature. In regions where both viruses can co-circulate, this RT-qPCR is, in complement to the existing ID GeneTM qPCRs, the ideal tool for differential diagnosis testing, disease surveillance and testing before animal movements.

A Retrospective Analysis of Pathogens Associated with Bovine Diarrhea from Clinical Specimens Submitted to a Veterinary Diagnostic Laboratory

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Introduction

Bovine diarrhea causes significant economic losses to the cattle industry worldwide. The common infectious causes of bovine diarrhea include bovine viral diarrhea virus (BVDV), bovine rotavirus (BRV), bovine coronavirus (BCoV), bovine enterovirus (BEV), Salmonella enterica, Escherichia coli, Clostridium perfringens, Mycobacterium avium subsp. paratuberculosis (Johne's) and Cryptosporidium parvum.

Methods

Data from clinical specimens submitted to the Athens Veterinary Diagnostic Laboratory (AVDL) at the University of Georgia over a three-year period (2021 to 2023) was collected and evaluated. The data analysis focused on the detection of agents by two PCR/RT-PCR panels, an adult and a calf diarrhea panel. The adult panel included BEV, BCoV, Johne's, Salmonella spp., and BVD types 1and 2. The calf panel included S. enterica, C. parvum, E. coli, BCoV, BRV, and BVD types 1and 2.

Results

A total of 68 cases were analyzed using the adult diarrhea panel, of which only 11 cases (16.2%) had positive results, and two cases had a coinfection with two agents. The agents identified most frequently were S. enterica (7.4%, n=5), MAP (7.4%, n=5), BEV (2.9%, n=2), and BCoV (1.5%, n=1). A total of 70 calf diarrhea panels were analyzed in this study, of which 30 cases (42.9%) had at least one positive result, and 6 cases (8.6%) had a coinfection with two agents. The agents most frequently isolated were C. parvum (27.1%, n=19), S. enterica (12.9%, n=9), and BRV (8.6%, n=6). Most of the calves were less than four weeks of age (60%, n=42), and beef breeds were the most common (62.3%, n=38).

Discussion

This study provided an update on the prevalence of the common bovine diarrheal agents in Southeast USA. Clear information on infectious agents and their prevalence can help veterinarians and producers develop effective prevention and intervention strategies. This would help the industry to better prepare for future outbreaks.

A tool for the post eradication era of Rinderpest virus: The development of a serological assay for the detection of antibodies against Rinderpest virus.

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Introduction

Following the declaration of the global eradication of Rinderpest (RP) in 2011, it is no longer a requirement for countries to re-confirm absence of RP virus (RPV) infection on an annual basis. However, countries are expected to remain vigilant and notify WOAH/FAO of any potential cases of RP for full investigation to determine if they meet the definition of a suspected case. Should a suspected case arise, samples would need to be submitted to a WOAH/FAO Reference Laboratory for diagnosis and confirmation. At present, there is no available serological test for the diagnosis of RPV that does not involve handling RPV. Diagnosis would require confirmation using both molecular and serological techniques, currently only available in RPV WOAH/FAO reference laboratories, who are also recognized Rinderpest holding facilities.

Methods

A recombinant RPV H protein was expressed and purified. The Pirbright institute have a hybridoma expressing a mAb that specifically detects the RPV H protein. The hybridoma was sequenced and cloned into a mammalian expression vector. The antibody was expressed and purified. The recombinant H protein and antibody have been used in combination to develop and ELISA.

Results

Preliminary results for the optimization and initial validation of the ELISA will be presented.

Discussion

The provision of an ELISA for RP would allow countries, in the event of an outbreak, to perform serological surveillance, helping to mitigate the impact of a potential global animal health emergency. Additionally, a non-infectious serological diagnostic test would remove the requirement for countries to hold live RPV stocks and encourage them to destroy or sequestrate any remaining RPV. This, in turn, would greatly reduce the potential risk of accidental or deliberate release of RPV into the environment, which would have devastating global effects.

ADVANTAGES OF DRIED BLOOD FILTER-PAPER SAMPLES FOR PRRSV DETECTION WITH THE BIOLISA® KIT PRRSV AB

Mrs Céline CASTERAN[1], Mr Eric SELLAL[1], Mr Johan Guilloux[1], Mrs Victoria PEYRON[1]
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Introduction

PRRSV (Porcine Reproductive and Respiratory Syndrome Virus) infection is one of the leading causes of economic losses in the swine industry. Early detection of anti-PRRSV antibodies is crucial for disease management and prevention. Traditionally, serum samples have been used for diagnosis, but dried blood filter-papers offer an appealing alternative, particularly in field settings or when difficult blood collection is encountered.

Methods

The BioLisa® kit PRRSV Ab, an indirect colorimetric ELISA test, allows the detection of anti-PRRSV antibodies in serum, plasma, and more recently, dried blood filter-paper samples. This method provides a practical, cost-effective, and reliable solution for monitoring antibody presence in swine populations. Using dried blood filter-papers, especially in pools of five samples, reduces testing costs while maintaining high result accuracy.

Results

The benefits of dried blood filter-papers include ease of transport and storage, minimizing logistical constraints. Additionally, this method enables sampling in diverse conditions, offering greater flexibility compared to traditional serum samples. Studies have shown that the sensitivity of the test with dried blood filter-papers is comparable to that of individual serum samples, ensuring reliable results for PRRSV detection.

Discussion

In conclusion, the use of dried blood filter-papers with the BioLisa® kit PRRSV Ab provides an innovative and convenient solution for PRRSV surveillance, enabling rapid and accurate diagnosis, particularly suited to field environments.

AmpliSeq Approach Using Third-Generation Sequencing Technologies For Equine Infectious Anemia Virus Characterization

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Introduction

Equine infectious anemia (EIA), a notifiable viral disease, is a lifelong persistent infection affecting equids, transmitted mainly by hematophagous insects. It is caused by a macrophage-tropic lentivirus that can integrate into the genome of the infected host throughout its life. The characterization of each newly isolated strain of EIAV is challenging due to the high genetic variability of EIAV, which complicates the development of molecular biological detection methods. In 2010, 2019 and 2023, three EIA outbreaks with 15 EIAV-infected horses occurred in the same department of France. This study aims to analyze the genetic proximity of the viral strains from these outbreaks in order to establish phylogenetic links.

Methods

In 2010 and 2019, tissue samples (mainly spleen) were collected from 5 and 9 euthanized horses, respectively. For the 2023 outbreak, blood samples were collected to isolate peripheral blood mononuclear cells (PBMC), from the EIA-infected horse, which was isolated and kept alive until further notice due to ongoing legal proceedings. DNA was then extracted from tissue and PBMC samples. Nested PCR was used to amplify a portion of the gag gene, followed by sequencing.

Results

After DNA extraction from PBMC samples and some challenging tissue samples, the concentration of pure and high-quality DNA was too low to be sequenced by the Sanger method. However, by optimizing a specific protocol using Oxford Nanopore Technology (ONT), we successfully overcame this limitation and were able to recover and characterize gag sequences from these three EIA outbreaks.

Discussion

In conclusion, this new technical approach allowed us to characterize the gag sequence from blood samples collected from a live animal for the first time. This could be a new step in detecting EIAV-infected animals and studying the genetic evolution of EIAV even before the animal is euthanized.

AN ANCYLOSTOMA CANINUM ISOLATE FROM A BRAZILIAN GOLDEN RETRIEVER BREEDING KENNELS WITH THREE INDEPENDENT BENZIMIDAZOLE RESISTANCE MUTATIONS

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Introduction

Multiple anthelmintic resistance is an emerging problem in the canine hookworm, Ancylostoma caninum. Molecular studies have revealed Q134H and F167Y mutations in the isotype-1 β-tubulin gene are widespread in pet dogs across the USA, Canada, and Australian greyhounds. This study reports the molecular characterization of an A. caninum population from a Golden Retriever breeding kennel in São Paulo, Brazil. This is the first time an A. caninum population has been identified containing three independent benzimidazole resistance mutations (Q134H, F167Y, and F200Y).

Method

The KeyScreen® GI Parasite PCR Test, Antech Diagnostics (Mars Petcare Science & Diagnostics product), used to test fecal samples from Golden Retriever breeding kennels in São Paulo, Brazil, detected the presence of benzimidazole-resistant A. caninum, and then subjected to further molecular characterization.

Result

qPCR assays applied to the isolate revealed the presence of three benzimidazole resistance mutations in the A. caninum isotype-1 β-tubulin gene: Q134H, F167Y, and F200Y. ITS-2 metabarcoding confirmed the presence of A. caninum with no other hookworm species. Targeted Illumina amplicon sequencing confirmed all three mutations with mutation frequencies of Q134H (6.7%), F167Y (18.2%), and F200Y (12.3%). Nanopore sequencing of full-length gene amplicons independently confirmed these mutations Q134H (24.1%), F167Y (38.9%), and F200Y (8.0%) and showed these to be on separate haplotypes. Finally, the whole isotype-1 β-tubulin open reading frame was screened by nanopore amplicon sequencing and revealed additional non-synonymous mutations at codons 27 (S27N) and 368 (V368A).

Discussion

The presence of three independent canonical isotype-1 β-tubulin benzimidazole resistance mutations in an A. caninum population from a breeding kennel is likely the result of intense drug selection pressure, which is a consequence of the anthelmintic treatment regimens applied in many such environments. This work illustrates the application of Illumina and Nanopore sequencing to confirm the qPCR assay results and characterize anthelmintic-resistant A. caninum populations.

An exclusive and highly performant double antigen ELISA for Corynebacterium pseudotuberculosis antibody detection

Marina Gaimard[1], Kevin Martin[1], Celia Derendinger[1], Loïc Comtet[1], Philippe Pourquier[1]
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Introduction

Corynebacterium pseudotuberculosis (Cp) is a bacterium that causes Caseous Lymphadenitis (CL) disease in small ruminants. Horses, cattle, camels and humans are often affected as well. Characterized by external abscesses, the infection has severe economic consequences. Control of CL relies on strict biosecurity measures, vaccination and serological surveillance. As vaccines doesn't show 100% efficacy, reliable serological tools are essential to prevent the spread of the bacteria. Consequently, IDvet developed ID Screen® CLA Double Antigen, a double antigen ELISA enabling the exclusive detection in multiple species of antibodies directed toward the phospholipase D (PLD) exotoxin, which is considered as the most relevant virulence factor of the bacterium.

Methods

Diagnostic specificity was assessed with 603 sheep/goat serum samples, from European CL-free herds. Diagnostic sensitivity was assessed using 42 samples: 37 goat/sheep samples (Netherlands) with clinical signs and positive on another serological technique and 5 sheep samples (European countries), qualified positive on another ELISA. The analytical sensitivity of the new ID Screen® kit was compared to 3 serological techniques.

Results

Measured specificity was 100% (95%CI [99.5-100], n=603). 41/42 samples were found positive and 1/42 was doubtful. Considering the doubtful sample as positive, measured sensitivity was 100% (95%CI [96.8-100], n=42). The latest positive dilution measured on the new ID Screen® kit, a previously available ELISA prototype: ID Screen® CLA Indirect ELISA, a commercial ELISA (kit A) and another serological technique (kit B) were respectively 1:4, 1:4, 1:2 and 1:4.

Discussion

The ID Screen® CLA Double Antigen shows high diagnostic specificity and sensitivity, and superior detection and discrimination capacity compared to other techniques. The new ID Screen® kit is a perfectly suited tool for high throughput testing for Caseous Lymphadenitis surveillance and monitoring, validated in small ruminants. This species-independent ELISA could also be used in all mammalian species, including cattle, horse and camelids.

Antimicrobial Prescribing Patterns Among Livestock Veterinarians in Punjab, Pakistan: A Multicenter Analysis of HPCIA and CIA Use

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Introduction

Antimicrobials, once revolutionary in treating infectious diseases are being widely used in veterinary practices across the world for treatment, prevention, and growth promoting purposes, particularly in developing countries. However, imprudent use of antimicrobials in animals has accelerated the antimicrobial resistance (AMR), posing significant threats to both animal and human health.

Methods

We collected all prescriptions for livestock species (buffalo, cattle, goat and sheep) recorded between January and December 2021 across five veterinary hospitals to analyze antimicrobial prescribing patterns among veterinarians. The data included treatment date, hospital location, animal species, presenting complaints, and prescribed antimicrobials. We mainly focused on the use of Highest Priority Critically Important Antimicrobials (HPCIA) and Critically Important Antimicrobials (CIA) in livestock species.

Results

During the entirety of the study period a total of 32,728 prescriptions were noted, of these 5,062 (15.47%) prescriptions contained anti-microbial agents. The highest number of prescriptions belonged to buffaloes (15,591), followed by cattle (9,162), goat (4,710) and sheep (3,265), while the percentage of antimicrobials was highest in goat (27.05%) followed by sheep (22.6%), cattle (14.73%) and buffalo (10.9%). Among the prescribed antimicrobials, 1,582 (31.25%) were classified as the HPCIA, while 994 (19.63%) as the CIA. Across species, sheep had the highest rate of HPCIA prescriptions (40.51%) followed by goat (34.62%), cattle (27.93%) and buffalo (27.35%). Oxytetracycline (27.70%) was the most prescribed antimicrobial followed by enrofloxacin (27.60%). About half (52.5%) of the antimicrobials were prescribed for respiratory problems alone.

Discussion

Our study highlights the use of antimicrobials across animal species, emphasizing the need to evaluate prescribing practices to combat antimicrobial resistance. It calls for disseminating the guidelines to veterinary practitioners and developing data collection systems to monitor antimicrobial usage trends, facilitating stewardship programs and promoting prudent antimicrobial use by veterinarians.

Antiretroviral Strategies Against EIAV, an “In-Vitro” Study: A Step Toward Non-Lethal Disease Management.

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Introduction

Equine infectious anemia virus (EIAV) is a lentivirus related to human immunodeficiency virus type 1 (HIV-1). Unlike HIV-1, EIAV causes a lifelong infection in equids without progressive immune dysfunction. No approved treatment exists for EIAV, whereas HIV-1 is managed with antiretroviral therapy. This study evaluates the in vitro antiviral effects of 18 FDA-approved antiretroviral compounds from different drug classes against EIAV using an equine cell infection model.

Methods

Equine dermal cells (ED cells) and equine peripheral blood mononuclear cells (ePBMCs) were treated with non-cytotoxic concentrations of antiretroviral drugs and infected with the EIAV Wyoming strain. Viral replication was assessed by quantifying viral RNA in culture supernatants via RT-qPCR and proviral DNA integration in cells using qPCR.

Results

Out of 18 tested drugs, 13 demonstrated a significant antiviral effect against EIAV. Nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), and integrase strand transfer inhibitors (INSTIs) effectively reduced viral replication. Tenofovir (TDF) showed the strongest inhibitory effect among NRTIs, while darunavir (DRV) and bictegravir (BIC) significantly impaired viral replication within their respective drug classes. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors exhibited no efficacy against EIAV .

Discussion

This study provides the first evidence that FDA-approved antiretroviral drugs effectively inhibit EIAV in vitro, highlighting similarities between EIAV and HIV-1 replication mechanisms. These findings pave the way for future investigations into antiviral strategies for equids, potentially reducing the need for euthanasia in infected horses. Further in vivo studies are necessary to explore the therapeutic potential of these compounds for controlling EIAV dissemination.

Botulism Outbreak and Diagnosis in cattle in South Korea

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Introduction

Botulism is an intoxication caused by botulinus toxin (BOTN) produced by genus of Clostridium, mostly C. botulinum in ruminants. It is mostly associated with ingestion of BOTNs (Type B, C, D, C/D and D/C) detected in around the world and varies from the region and countries. Recently, botulism has been diagnosed by clinical and laboratory testing and characterize the incidence in cattle in South Korea.

Materials and Methods

A total of 90 farms from 2021 to 2024 were suspected based on the clinical examinations including sudden death, recumbency and progressive paralysis of affected cattle and goats despite intensive care. Mouse neutralization and ruminal culture were performed to detect toxin and genes of Botulism B, C and D. The association between botulism and weather (temperature. and precipitation) was compared to identify factors influencing the outbreaks.

Results

A total of 90 cases suspected to bovine botulism. 50 (55.6%) cases were confirmed by clinical and laboratory testing. The botulism outbreak was significantly associated with precipitation rather than temperature. Also, leftover has been regarded as one of the most common causes of bovine botulism in order to decrease the expenses of feedstuffs.
Discussion: Bovine botulism generally occurs in the cattle ingesting feeds and forages contaminated by BOTN. Although the feed was contaminated the bacteria, the production of toxin is associated with anaerobic and appropriate circumstances (temperature and humidity). In this study, the botulism outbreak was significantly associated with precipitation rather than temperature.

Cataloging Gastrointestinal Helminths of the Virginia Opossum (Didelphis virginiana)

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Introduction

The Virginia opossum (Didelphis virginiana) is a common synanthropic marsupial mammal found across North America, often in proximity to human settlements. They play an important ecological role by controlling pests, but their presence in suburban and urban areas brings them into contact with humans and domestic animals creating a potential role in parasite dynamics. Opossums are reservoirs for a variety of parasites, including both host-specific and zoonotic species, however a catalogue of opossum gastrointestinal helminths is lacking. Our research aims to elucidate the species present and the prevalence of these parasites and provide an accessible identification key to aid future parasitological research and diagnostics.

Methods

We necropsied 11 opossums found as roadkill along a 50-mile route from suburban Knoxville, Tennessee, USA, to rural Harrogate, Tennessee, USA. We preserved helminths in 70-80% ethanol until morphological identification. We also conducted fecal flotations to identify ova and parasites. Our goal is to compile a comprehensive key of helminths commonly found in opossums.

Results

One hundred percent (11/11) of the opossums had parasites collected distal to the duodenum and 63.6% (7/11) also had worms in the stomach. Parasites were viewed microscopically for identification. We found at least 5 species of helminths including the zoonotic species Ancylostoma caninum, the canid whipworm Trichuris vulpis, and the opossum nematodes Turgida turgida and Cruzia americana. Our findings illustrate that parasites from opossums may have health implications for domestic animals and humans, but that most of the parasites found are host-specific to opossums.

Discussion

The absence of resources for opossum parasites is a hindrance in the research and identification of their parasites. Understanding the ecological roles and health impacts of these parasites will improve our ability to diagnose and manage parasitic infections from a One Health perspective and assess the broader ecological and health impacts of these parasites.

Causes of Equine Sarcoids in New Zealand and Australian Horses

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Introduction

Equine sarcoids are common skin neoplasms of horses. They can be infiltrative and difficult to treat. Sarcoids are thought to be caused by cross-species infection by bovine papillomavirus (BPV) types 1, 2, and 13. Previous studies, especially in Europe, have revealed BPV1 causes around 90% of sarcoids. In contrast, 80% of sarcoids in Canadian horses were caused by BPV2. No previous studies have been done in Australasian horses. The causative BPV type is increasingly important due to the development of vaccines to prevent sarcoid development.

Methods

Equine sarcoids were identified through searching diagnostic submissions. DNA was extracted from formalin-fixed paraffin-embedded tissue scrolls and the presence of amplifiable DNA confirmed by amplifying a housekeeping gene. PCR primers that specifically amplified BPV1, BPV2 or BPV13 were the used.

Results

A total of 119 sarcoids were included in the study. This included 104 sarcoids from New Zealand and 15 sarcoids from Australian horses. All samples contained amplifiable beta actin DNA. Papillomaviral DNA was amplified from 109 of 119 sarcoids. Of the 94 sarcoids from New Zealand horses that contained PV DNA, 83 contained only BPV2 DNA, 9 contained both BPV1 and BPV2 DNA, and 2 contained only BPV1 DNA. BPV2 DNA was amplified from 13 of the 15 Australian sarcoids with BPV1 amplified from 2 sarcoids.

Discussion

These results suggest that BPV2 is the predominant cause of equine sarcoids in New Zealand and Australia. BPV2 was also more commonly detected in sarcoids from Canadian, but not European, horses. Infections by multiple BPV types were detected in almost 10% of sarcoids. Mixed infections have only rarely been previously reported. The absence of identifiable PV DNA in 10 sarcoids may be due to an incorrect histological diagnosis or due to formalin-induced fragmenting of small quantities of BPV DNA in the sample.

Characterization and Composition of the Gastrointestinal Microbiota of the North American Raccoon (Procyon lotor) and the Virginia Opossum (Didelphis virginiana) in East Tennessee, USA

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Introduction

Gastrointestinal microbiota with high species richness and diversity are associated with the overall health of humans and animals while conversely, microbiota dysbiosis can lead to disturbances of bodily functions and may indicate poor fitness. In wildlife, microbiome composition can be associated with anthropogenic changes in the environment, which may provide an early indicator of population health and can potentially serve as a proxy to assess ecosystem health.

Methods

We necropsied 4 North American raccoons (Procyon lotor) and 1 Virginia opossum (Didelphis virginiana) collected as roadkill along a 100-mile route from suburban Knoxville, Tennessee, USA to rural Lee County, Virginia, USA. We extracted DNA from ingesta samples taken from 3 anatomic locations along the gastrointestinal tract of each animal and submitted the extracted DNA for 16S microbiome sequencing.

Results

We found the GI tract of the opossum had a higher species richness (Chao richness 4,007 vs. 1,047) and was more diverse (Shannon diversity 3.57 vs. 2.16) compared to the raccoons. Twenty percent of the community composition as a whole was explained by host species ($F=8.73$, $p=0.001$), indicating fundamental differences in gut bacteria and possible health implications. We have processed an additional 6 raccoons and 9 opossums doubling our sample size and may reinforce the host species and GI locations differences we have uncovered.

Discussion

We have shown statistically significant differences in the microbiota of raccoons and opossums despite our initial limited sample size. By doubling our sample size and incorporating the geographic location of animals found, we can add a gradient of human disturbance as a variable to understand how environmental changes, human impacts, and habitat or diet changes may be affecting wildlife. These potential impacts on animal microbiomes due to human disturbance may help target conservation interventions to protect wildlife health and ultimately One Health dynamics.

Characterization of Avian Infectious Bronchitis Virus using Pan-coronavirus Probe

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Introduction

Avian Infectious Bronchitis Virus (IBV) is a member of genus Coronavirus of the family Coronaviridae. The frequent recombination events and mutations of the S1 gene of IBV makes it difficult to amplify the virus consistently. Whole genome sequencing (WGS) or targeted sequencing are options for amplification of IBV. Pan-coronavirus probes (Illumina, CA) were designed to cover different coronaviruses and have been widely used for SARS-CoV2 but rarely for avian IBV. Pan-coronavirus probe was used to sequence IBV that were difficult to amplify by conventional RT-PCR or with inconsistent results between real time RT-PCR and sequencing.

Methods

Five samples were used in this study to test the application of Pan-coronavirus probes, one trachea swab, four virus isolations (two from cecal tonsils, two from tracheal swabs). Viral isolation was conducted in SPF chicken embryonated eggs. RNA was extracted from allantoic fluid or directly from tracheal swabs. RNA clean and concentrator kit (Zymo Research, CA) was used to prepare the RNA for whole genome sequencing, TURBO DNase (Thermo Fisher Scientific, MA) was used to remove the DNA from the extraction. Illumina RNA Prep with Enrichment (L) Tagmentation and Pan-coronavirus (Illumina, CA) were used for library prep and amplification of target IBV sequences. Sequencing data was analyzed using Dragon Targeted Microbial from BaseSpace (Illumina, CA).

Results

The full genome (27,562 bp) was assembled from a tracheal swab sample with a Ct value of 24. More than one genome was assembled with the rest of the samples, which may indicate the presence of more than one IBV strain.

Discussion

Based on these observations, Pan-coronavirus targeted sequencing may be an alternative to viral isolation with samples with high viral concentration or difficult to amplify by RT-PCR. More analysis on different samples and determining the threshold of Ct values required to successfully sequence IBV is warranted.

Characterization of Infectious Bursal Disease Virus Associated with a Disease Outbreak in White Leghorn Layers in Ghana

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Introduction

Infectious bursal disease (IBD) remains a major threat to poultry production, causing significant economic losses to the industry. There are two serotypes of IBDV, 1 and 2, of which, serotype 1 is pathogenic in chickens. In this study, we discuss the pathology and molecular characterization of an IBDV associated with a clinical outbreak in Ghana.

Methods

Pathological and molecular characterizations of an IBDV strain from an outbreak in a four-week-old White Leghorn layer flock in Accra, Ghana were performed. Following necropsy on eight carcasses, gross and histopathological lesions were evaluated. RNA extraction and RT-PCR were performed on formalin-fixed paraffin-embedded tissues. A phylogenetic analysis using Maximum-Likelihood method was performed on the VP-2 capsid gene of IBDV along with 62 other sequences retrieved from GenBank. Evolutionary analysis with 1000 bootstrapping was performed using MEGA11 program.

Results

Histopathology of the bursa of Fabricius revealed scattered to effacing hemorrhages on the plicae, extensive necrosis with expansion of the stroma between the bursal follicles, and depletion of lymphocytes within the interfollicular epithelium. RT-PCR and subsequent sequencing of the VP2 gene showed the presence of IBDV in formalin-fixed paraffin-embedded tissues. Comparative phylogenetic analysis with 62 IBDV sequences from different parts of the world placed the Ghanaian IBDV in genogroup 3 (vvIBDV), closely related to IBDV from Nigeria. There were amino acid substitutions at positions 252, 254, and 300 when compared with reference IBDV strain.

Discussion

The findings of this study align with the studies conducted in Namibia that reported genetic similarity of a Namibian IBDV to IBDV circulating in Zambia. There could be biosecurity breaches between neighbouring African countries that contribute to disease outbreaks. To the best of our knowledge, this is the first report of an IBDV from a disease outbreak in Ghana that has been sequenced and used in a phylogenetic analysis.

Characterization Of Isolated Equine Herpesvirus-1 Strains Using Long Fragment Sequencing Technology

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Introduction

Recurrent epidemics of equine rhinopneumonitis show that vaccination is sometimes ineffective in preventing disease transmission, resulting in significant animal and financial losses to the equine industry. Equine rhinopneumonitis is caused by infection with equine herpesvirus type 1 (EHV-1), a Herpesviridae virus belonging to the subfamily Alphaherpesvirinae. As the number of EHV-1 outbreaks in Europe appears to be increasing since 2021, molecular characterization of circulating strains each year could help establish epidemiological links or improve vaccine strategy decisions. This study used Oxford Nanopore Technology (ONT) to sequence long fragment DNA, and to classify them into their respective phylogenetic groups.

Methods

Since 2012, when we suspect an EHV-1 infection based on macroscopic anatomopathological observations during the autopsy of aborted fetuses, tissues are collected for testing by EHV-1 real-time PCR. EHV-1 isolation on RK13 cells is then performed from all EHV-1 positive tissue homogenates. DNA is extracted from the positive supernatants and sequenced using ONT.
Results: Twenty different isolated EHV-1 strains were amplified on RK13 cells. A supernatant virus concentration protocol was set up before DNA extraction, allowing us to improve DNA concentrations by 10 to 100 times. Our sequencing results using ONT were satisfactory. Our phylogenetic analysis allowed us to characterize the genetic proximity between the EHV-1 strains isolated in France and those already described in the literature.

Discussion

In conclusion, the use of ONT is a step forward that can help determine faster whether a newly isolated strain of EHV-1 is new or a recirculating strain in the equine population and will also help identify their virulence markers. In addition, this new molecular technology will enable us to produce more detailed reports for the relevant authorities, who will be able to define future EHV-1 surveillance and prevention plans and alert the equine industry more quickly to implement effective preventive measures.

Circulation of Pathogenic Bacteria in Ukraine: Analysis of Laboratory Diagnostic Data (2022-2024)

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Circulation of Pathogenic Bacteria in Ukraine: Analysis of Laboratory Diagnostic Data (2022-2024)
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Introduction

Monitoring pathogenic bacteria circulation provides both valuable epidemiological insights and treatment strategies. This study presents a three-year analysis (2022-2024) prevalence of key bacterial pathogens in poultry, companion animals, swine and cattle in Ukraine.

Methods

A total 625, 1478, 4108 of samples were received in 2022, 2023 and 2024, respectively. Samples from poultry, companion animals, swine and cattle were collected and tested using routine culture. Pathogen identification was performed using MALDI-TOF MS. Data was then combined and analyzed to assess trends and changes in pathogen distribution.

Results

The total number of analyzed samples from poultry increased from 242 (2022) to 599 (2024). Escherichia coli infections remained the predominant poultry pathogen within the surveillance period accounting for 81.4%, 75.8% and 73.3% of cases in 2022, 2023 and 2024, respectively. Hovewer, Salmonella spp. declined from 6.2% to 2.0%. In companion animals significant increase in urinary tract infections was noted, with Escherichia coli (13.3%) and Staphylococcus spp. (6.1%) as major pathogens. Same trend was observed in swine submissions, were Escherichia coli rate remained 50% within 3 year period, whereas Actinobacillus pleuropneumoniae increased from 1.1% (2022) to 12.7% (2024), highlighting a rise in respiratory infections. For bovine pathogens, Mannheimia haemolytica emerged in 22.6% of cases (2023), while Pasteurella multocida prevalence fluctuated. Lastly, Staphylococcus aureus (51.4%) and Enterobacteriaceae (33.3%) overtake as a major mastitis-causing pathogens.

Discussion

The study reveals a high prevalence of Escherichia coli across species, a rising incidence of Actinobacillus pleuropneumoniae in swine and shift in mastitis etiology towards Staphylococcus aureus. These findings underscore the need for ongoing pathogen surveillance and antimicrobial susceptibility to enhance veterinary disease control strategies.

Comparative Analysis of Conventional PCR and In-Clinic qPCR for the Rapid Detection of Babesia gibsoni in Dogs

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Introduction

Veterinarians often face challenges regarding the time required for diagnostics and accessing trained professionals. The in-clinic qPCR device addresses these issues with its rapid operation and user-friendly design, delivering real-time results at the point of care. Babesia gibsoni is a protozoan parasite causing canine babesiosis. Its diagnosis can be challenging due to variable clinical presentations, making molecular testing crucial for accurate detection. This study evaluated the effectiveness of the Qmini point-of-care qPCR system for detecting B. gibsoni.
Methods: A comprehensive analysis was conducted on a total of 173 clinical samples collected from 21 different clinics using both conventional PCR and an in-clinic qPCR assay (Qmini Real-time PCR Analyzer, BVQ-1100, Bioguard) to detect the presence of Babesia gibsoni DNA. To thoroughly evaluate the diagnostic capabilities of the point-of-care qPCR system, blood samples were tested simultaneously, allowing for a detailed assessment of its sensitivity, specificity, and overall accuracy in identifying B. gibsoni infections.

Results

The in-clinic qPCR method demonstrated a sensitivity of 97.44% (95% CI: 84.93% - 99.87%), a specificity of 98.51% (95% CI: 94.17% - 99.14%), and an accuracy of 98.27% (95% CI: 94.70% - 99.56%). Despite slightly lower sensitivity compared to conventional PCR, qPCR's reduced preparation and processing time makes it highly suitable for point-of-care diagnostics.

Discussion

The Qmini point-of-care qPCR system demonstrates clinical performance comparable to the PCR test conducted in a reference laboratory. This study highlights the advantages of in-clinic qPCR for rapidly diagnosing B. gibsoni infection in under 90 minutes. By providing quick and reliable results, this approach allows veterinarians to make early diagnoses and initiate timely treatment, which is essential for preventing potential complications associated with the disease.

Concurrent Infections Associated with Squamous Cell Carcinoma in an African Spurred Tortoise (Centrochelys sulcata)

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Introduction

Reports of squamous cell carcinoma in tortoises are relatively rare; however, it remains a significant cause of morbidity and mortality in affected individuals. Few reports of this neoplasm in tortoises originated from the plastron and carapace, while in this tortoise, the mass involved the face and oral cavity. As the animal continued to clinically deteriorate, euthanasia was performed.

Methods

A 16-year-old male African spurred tortoise (Centrochelys sulcata) had a left facial mass invading the oral cavity. The tortoise underwent CT scan and multiple incisional biopsies. A necropsy was performed, and tissue samples were collected for histopathology and fungal culture.

Results

Grossly, the left facial area, left mandible, and hard palate were severely occupied by ulcers and bone lysis. The lung had mild multifocal irregular-shaped yellowish-white masses with plugs of purulent exudate in the bronchioles. The ventral side of the brain and cribriform plate was attached to caseous and had extensive hemorrhage. Histologically, the facial mass revealed an infiltrative neoplasm, consistent with squamous cell carcinoma. The meninges of the ventral part of the brain exhibited extensive hemorrhage, necrosis, and infiltrates of granulocytes, accompanied by thick sheets of fungal hyphae, which were highlighted by PAS and GMS stains, and Fusarium spp. was cultured. In the lung, multiple sections of nematodes, consistent with Strongyloides spp., associated with fungal infection and infiltrations of granulocytes were identified.

Discussion

Squamous cell carcinoma in tortoises and animals has been associated with various predisposing factors, including herpesvirus, as well as environmental and husbandry-related stressors. Facial skin and oral cavity are rare origin of this neoplasm in tortoise. In this case, the co-infection with fungal infection and nematode infestation further exacerbated its condition, significantly worsening its quality of life. To date, there are no documented reports of neoplasia occurring alongside both fungal infection and nematode infestation in tortoises.

Creation of Interactive, Near Real-Time Dashboards for Surveillance of Vector-Bourne Diseases in Ontario Equids.

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Introduction

West Nile and eastern equine encephalitis viruses (WNV and EEEV, respectively) are mosquito-borne pathogens endemic to Ontario that can cause severe neurological signs in equids and humans. Although uncommon, the provincial incidence of these infections has increased in recent years, likely due to changes in vector distribution and population density driven by climate change and other factors. The severity of equine cases and potential for zoonotic disease underscores the need for timely surveillance and reporting of cases to veterinarians, government and industry stakeholders.

The Interactive Animal Pathogen Dashboard (IAPD) project is an initiative by the University of Guelph that aims to create interactive, near real-time surveillance dashboards displaying temporal and spatial trends using data from the Animal Health Laboratory (AHL). The objectives for this project include creating WNV and EEEV dashboards to aid in One Health surveillance and inform veterinarians involved in clinical decision-making.

Methods

Demographic, temporal, geographical and test data are queried daily from the AHL Laboratory Information Management System (LIMS) and aggregated by time period (week, month, quarter, and year), county, and test type using R software. Number of submissions, positive count, and percent positive are then presented graphically in interactive dashboards using Tableau®. Spatial maps are created using submitting veterinary clinic postal codes as a proxy for animal location. Postal codes were aggregated at the provincial county level.

Results

The WNV and EEEV dashboards were added to the IAPD website (https://iapd.lsd.uoguelph.ca) in March 2025, and are accessible via a login to Ontario veterinarians, researchers, and government agencies. The dashboards are refreshed daily and display the previous 10 years of test results.

Discussion

Veterinarians and surveillance groups can use these dashboards to help inform response and prevention programs, identify areas at increased risk, and educate clients. Future initiatives include disease trend forecasting and signal detection programming.

Detection of Retroviral Integration in the de novo Genome Assembly Of Endangered Steppe Eagle (Aquila nipalensis)

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Introduction

The steppe eagle (Aquila nipalensis, SE) is an endangered predatory bird in the family Accipitridae with limited genetic information currently available. Retroviral integration, observed in various human and animal genomes, can drive evolutionary and potentially impact host immunity. This study aimed to investigate retroviral integrations within the de novo SE genome.

Methods

Genomic DNA was extracted from whole blood of an adult male SE. Hybrid de novo genome assembly was performed, using Nanopore long-read and MGISEQ-2000 short-read sequencing platforms. Raw reads were assembled, polished and annotated against the genomes of Gallus gallus, Meleagris gallopavo, Taeniopygia guttata, Columba livia and Aquila chrysaetos. Endogenous retrovirus sequences were identified, quantified, and assessed for their proportions in the SE genome. Additionally, species-specific gene families associated with retroviral genes in the genomes of Accipiter gentilis, Harpia harpyja, Haliaeetus leucocephalus and Sarcogyps calvus were analyzed using OrthoVenn3.

Results

The assembled SE genome was 1.2 Gb in size with a 154× coverage and a scaffold N50 of 23.49 Mb. A total of 10 endogenous retrovirus-related genes (43 copies) were identified, including pol (86.05%), env (6.98%) and gag (6.98%) genes. Compared with other avian species, SE had unique gene families associated with envelope glycoproteins for virion attachment to host cells (GO:0019062), Gag-Pol polyprotein for viral genome integration (GO:0044826) and virion assembly (GO:0019068).

Discussion

The SE genome size aligned with other Accipitridae members (1.18-1.27 Gb). Endogenous retroviral integrations have been documented in the genomes of various bird species, including the turkey vulture and the Asian-king vulture. These integrations provide insights into the evolutionary relationships and interactions between birds and viruses over millions of years, offering valuable knowledge for studying viral evolution and host-virus dynamics.

Developing Nanopore Nemabiome Metabarcoding as a Routine Diagnostic Tool for Monitoring Nematode Burdens in Small Ruminants and Cattle

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Introduction

Gastrointestinal nematodes are a major challenge in cattle and small ruminant production, reducing growth rates and the overall health of animals. Traditional monitoring methods, such as fecal egg counts, are limited in identifying specific worm species and detecting anthelmintic drug resistance. Our current Nemabiome metabarcoding based on Illumina sequencing is a powerful research tool; however, has some limitations, including short read lengths, challenges with primer design, inflexibility, high cost per unit (when low sample numbers), and long sequencing run time. Consequently, we are transitioning this technology to Oxford Nanopore (ON) sequencing, which offers a faster and more flexible alternative, potentially suitable for smaller research and diagnostic labs.

Method

Nanopore metabarcoding of the ITS-1/5.8S/ITS-2 rRNA locus was done in three independent replicates using the NC5/NC2 primer pair for genomic DNA samples from 43 UK and 34 Western Canadian ovine GIN populations that were originally used for ITS-2 Illumina metabarcoding. Nanopore metabarcoding was directly applied to fecal DNA extracted from sheep and ewe feces. After bioinformatic and statistical analysis ITS-1/5.8S/ITS-2 rRNA Nanopore metabarcoding data was compared to existing data from Illumina ITS-2 metabarcoding.

Results

Among the replicates, there was a high level of repeatability of the ITS-1/5.8S/ITS-2 nanopore metabarcoding data and a high level of agreement regarding the relative species abundance data between the Illumina and Oxford nanopore. ON MinION reported a lower turnaround of 15 hours compared to the three-day turnaround of Illumina nemabiome metabarcoding.

Discussion

We have provided proof of concept for nemabiome metabarcoding using Oxford nanopore sequencing and are currently working to improve accuracy and practical workflows. Specifically, assessing target copy number to remove biases, developing absolute quantitation (as opposed to relative quantitation to remove the need for fecal egg counts), and transitioning to work directly on fecal DNA.

Keywords

Illumina, ITS-1/5.8S/ITS-2, Nemabiome metabarcoding, Nematodes, Oxford nanopore

Development and validation of a specific recombinant-based ELISA for detection of anti-borrelia antibodies in equine serum

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Introduction

Equine borreliosis, is a bacterial infection caused by Borrelia burgdorferi and transmitted by ticks of the Ixodidae family. This zoonotic disease can infect many animal species and humans. Equine borreliosis presents a challenge for diagnosis due to its vague clinical signs; main symptoms being lameness, fever and performance loss. Due to low concentration of the bacteria in bloodstream, direct detection by PCR or culture is reliable only in tissue samples. Therefore, serology is the method of choice for effective diagnosis. Western-blot is a gold-standard but it is not adapted to rapid routine screening test. That is why we have developed the ID Screen® Borreliosis Double Antigen Multi-species ELISA, based on highly conserved proteins from Borrelia burgdorferi sensu lato, for a first intention screening in horses.

Methods

This study describes the validation of the new ELISA. Specificity was evaluated on 249 horse sera from Iceland, where circulation of ticks and Borrelia is very low. Sensitivity was measured using 27 Western-Blot positive sera (Anti-borrelia Euroline Horse IgG, EUROIMMUN).

Results

Measured specificity was 100% (CI95% : 98.5-100%, n=248). Out of 27 Western-Blot positive sera, 26 were detected by the ID Screen® ELISA, giving a sensitivity of 96% (CI95% : 81.3-99.3%, n=27).

Discussion

The ID Screen® ELISA shows excellent specificity and high analytical sensitivity. It is a reliable tool for anti-Borrelia antibody detection. Positive results should be taken into account together with clinical symptoms and may be confirmed by Western Blot.

Development of a Lateral Flow Immunochromatography Test for the Rapid Detection of Mycoplasma agalactiae in Small Ruminants

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Introduction

Mastitis is the one of the most common health problems affecting dairy sheep. Sardinia, an island located in the middle of the Mediterranean Sea, has approximately 3.2 million milking Sarda sheep, corresponding to half of the total Italian stock. The control of intra-mammary infections (IMI) is of greatest importance for dairy farmers. IMI outbreaks are usually caused by microorganisms belonging to Staphylococcus, Streptococcus and Mycoplasma genus [1]. Mycoplasmopsis (M.) agalactiae is the main aetiological agent of contagious agalactia (CA) [2].

Material and methods

In this study, we developed a lateral flow immunochromatography (LFIA) system for the rapid diagnosis of CA using experimental sera [3] labelled with 40 nm colloidal gold particles. To understand whether the test could be used in the whole region of Sardinia, we genotyped 126 M. agalactiae strains by MLST. All isolates, collected during the period 2012-2022, were also analyzed by immunoblotting to check whether the antibodies present in the experimental sera were able to recognize and bind mycoplasma antigens. LFIA strips were assembled using the double-antibody sandwich strategy, while the box containing the strip was made from polylactic acid filament, a biodegradable material, using a 3D printer.

Results and discussion

Almost all (96%) of the isolates belonged to ST5. The remaining percentage was ST2 and ST3. To assess the sensitivity of LFIA, increasing amounts (10 to 10_{10} CCU/ml) of M. agalactiae were resuspended in the running buffer. A sharp coloured band formed in the test line from 10_4 CCU/ml of mycoplasma. Direct use of negative milk, mycoplasma-positive milk, and milk from sheep with contagious agalactia did not give good results due to the immediate saturation of the sample pad with interruption of the lateral flow.
Further work is needed to automate antibody dispensing, standardise strip sizes and use milk directly.

Development of a Rapid, Cost-Effective Amplicon Sequencing Method for ILTV Genomic Analysis

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Introduction/background

Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory disease in chickens caused by a Gallid herpesvirus 1, also referred to as ILT virus (ILTV). ILTV causes frequent outbreaks in both commercial and backyard poultry flocks reported across Canada, greatly affecting high poultry-producing regions. Currently, ILTV outbreaks are controlled through vaccination and biosecurity strategies, and monitored by diagnostic qPCR and histopathology. It is believed that transmission occurs through contaminated fomites and aerosols, however, vaccine reversion events may also play a role. To better understand transmission, we developed a multiplex amplicon sequencing approach for genotyping of ILTV specimens collected across British Columbia (BC) outbreaks.

Methods

DNA was extracted from trachea and lung tissues collected from chickens associated with BC ILTV outbreaks (n = 85; mean Ct = 21.32, range = 15.7 - 36). Three ILTV vaccine products, and an ATCC reference strain were included in the sequencing experiments (AviProLT, LT-IVAX, LT-Blen, and VR-783, respectively). Multiplex PCR amplification of eight different genes of the ILTV genome (gG, gM, ORF a/b, UL 0/1, TK, gB, gC, gDIE) was performed for each specimen. Amplicons were sequenced on the Oxford Nanopore platform and assembled using a custom bioinformatics pipeline.

Results

ILTV outbreak specimens were sequenced at ~$17.4 per specimen and a turnaround time of 3 days. An average of 26,000 reads were generated for each specimen over a 17-hour sequencing run time. Bioinformatic analysis confirmed 83% of specimens assayed generated >95% complete genomes. Phylogenetic analysis demonstrates specific clustering for the majority of ILTV BC outbreak specimens, with several grouping among vaccine strains.

Discussion

The multiplex amplicon sequencing method presents a cost and time efficient method while providing valuable sequence information for ILTV specimens. The high-quality data and scalability support the application of the amplicon sequencing method for ILTV surveillance and outbreak analysis.

Development of an Bartonella henselae specific ELISA

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Introduction

Bartonella henselae causes cat scratch disease (CSD), an often self-limiting lymphadenitis. While cats are the natural reservoir for B. henselae, the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable test for B. henselae infections is highly desirable. Immunofluorescence assays (IFA) are widely used for serodiagnostics of B. henselae-infections, but are laborious, time consuming and interpretation is subjective. An easy and reliable method for the serological diagnosis of B. henselae infections is needed to overcome the shortcomings of the current IFA. The aim of this study is to design an ELISA for detection of B. henselae in humans and animals to improve the shortcomings of the immunofluorescent test (IFT).

Methods

Test development is based on different B. henselae strains and quality assured patient (humans, cats, and dogs) sera [(a)sera positively tested for anti B. henselae antibodies via IFT, (b) patients/animals with typical symptoms, (c) sera of patients/animals with PCR-based infection diagnosis]. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were further tested and optimized for ELISA.

Results

Patients (humans, cats, and dogs) with B. henselae infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. However, our tests show that there are certain protein fractions from B. henselae which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity.

Discussion

By the use of well-characterized sera from the strictly quality-controlled serum library of the German National Consiliary Laboratory for Bartonella infections, sensitivity of this ELISA was 100% for molecular proven infections and 76% for clinically suspected infections at a specificity of 93%, respectively.

Development of Novel Molecular Diagnostic Tools for the Detection of Louse DNA on the Bovine Skin Surface Using Simple Skin Swabs

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Introduction

Parasitic lice are animal welfare and production problems for the beef and dairy cattle industries, particularly in colder climates, causing economic losses of ~$120 million annually in North America. Control depends on blanket macrocyclic lactones and pyrethroids treatment, raising concerns about drug resistance and environmental impacts. Current detection relies on visual inspection, which is time-consuming and insensitive, making more targeted use of parasiticides difficult.

Methods

Adult lice specimens and DNA skin swabs were collected from beef and dairy herds in Western Canada and Northern USA. A longitudinal study was also conducted from January to April 2025, collecting skin swabs multiple times. We undertook PCR amplification and metabarcoding of the 18S rDNA gene from genomic DNA extracted from lice and skin swabs. Amplicon Sequence Variants (ASVs) were mapped to reference databases to identify and assess the relative abundance of lice species. We also developed real-time fluorescence LAMP (Rt-LAMP) assays.

Results

We successfully identified Linognathus vituli and Bovicola bovis using 18s PCR metabarcoding on DNA skin swabs. The PCR results from the longitudinal study reflected the expected seasonal infestation patterns. We also developed several Rt-LAMP assays that could detect louse DNA from the skin swabs: PE-1 which was specific for L. vituli, PE-2 which was specific for B. bovis, and PE-3 which targeted multiple lice species. Sensitivity assessments revealed limits of detection of about 1,000 DNA copies/µL and about 10 DNA copies/µL for PE-1 and PE-2, respectively.

Discussion

We have shown proof-of-concept that louse DNA can be detected on the surface of cattle skin by either PCR-based metabarcoding or Rt-LAMP. We have developed both species-specific and pan-louse Rt-LAMP assays that we plan to transition to colorimetric platforms to explore their potential as rapid pen-side tests.

Development of Standardized RT-QuIC Reagents for the Detection of CWD in Diagnostic Laboratories

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Introduction

Chronic wasting disease (CWD) is an emerging prion disease affecting cervid species predominantly in the United States and Canada Real-time quaking induced conversion (RT-QuIC) is a next generation test for CWD that is currently in the process of USDA approval. RT-QuIC is unique in that it amplifies prions to a detectable level compared to currently approved techniques that rely on degradation of host PrPC and the stability of prions for detection.

Methods

The ability to amplify CWD prions is achieved by production of a soluble PrPC protein that will amplify in the presence of CWD prions but remain stable when no prion is present. In order to bring RT-QuIC technology to the diagnostic laboratory multiple advancements in stability, ease of use and longevity needed to be made.

Results

Here, we report 4 C stability of the RT-QuIC substrate and on advancements in composition of other required reagents for RT-QuIC that increase ease of use. We compared the lag phase at a 10-4 dilution of a homogenized retropharyngeal lymph node with RT-QuIC substrate that was freshly prepared or held at 4 C for 3, 6, 9, and 12 months. We observed an average lag phase of 11.6 hours +/- 1.55 hours for all time points. There was no significant change in the false positive rate for any time point tested. We also tested a premade 5x buffer including all necessary reagents for RT-QuIC testing compared to a buffer where each reagent was added and filtered as previously published. We observed lower variability in lag phase (3.03 hours) in the ready-to-use buffer compared to the typically prepared buffer (8.89 hours).

Discussion

Overall, we have made improvements in 4-degree stability and workflow of RT-QuIC testing which should translate to easier adoption by diagnostic laboratories and aid in USDA approval process.

Distribution of Avian Influenza Viruses in Ukraine, 2020-2024

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Introduction

Avian influenza viruses (AIVs) are highly contagious viruses of the Orthomyxoviridae family that pose a major threat to poultry industry, leading to substantial economic losses. AIV surveillance is important for the prevention of virus spreading and spillover to mammals. The aim of the work was to summarize and analyze results of AI surveillance in Ukraine.

Methods

The tests were conducted in the framework of state control of infectious animal diseases during 2020-2024. 53,120 samples of biological material (14,311 and 38,809 samples from wild and domestic birds, respectively) were tested by RT-qPCR. For testing following commercial kits and reagents were used: “IndiSpin Pathogen Kit” (INDICAL BIOSCIENCE); “Virus DNA/RNA Purification Kit” (Biocomma); “Bio-T kit Avian&Swine Influenza Virus”, “Bio-T Kit AIV genotypes H5 & H7 V2” (Biosellal); “AIV REAL TIME”, “AIV H5-H7 REAL TIME” (ADIAVET), “AgPath-ID™ One-Step RT-PCR Reagents” (Applied Biosystems) with N1 and N8 primers and probes (Hoffmann et al., 2016).

Results

During 2020-2024, 42 AI outbreaks were registered (9 outbreaks in 2020, 12 in 2021, 2 in 2023, and 19 in 2024) among poultry in backyards (27 outbreaks) and poultry farms (3), also wild (11) and zoo birds (1). AI was registered in 13 regions of Ukraine, namely: Mykolaiv – 16 outbreaks, Kyiv – 5, Kherson – 4, Sumy – 3, in Donetsk, Chernivtsi, Odesa, and Kharkiv – 2 outbreaks each, Vinnytsia, Volyn, Ternopil, and Rivne – 1 each. All outbreaks were caused by AIV of the H5 subtype, among which the H5N8 subtype were mostly detected in 2020-2021 and H5N1 – in 2023-2024.

Discussion

In 2020-2024, AIV of the H5 subtype mainly H5N8 and H5N1 were widely distributed in Ukraine. The majority of outbreaks were recorded among poultry from backyards in regions located at the intersection of wild bird migration routes, with prevalence in Mykolaiv, Kherson, and Kyiv regions.

Diversity of Equine Strongyles in Northern Iran Assessed Through Morphological Identification and Nemabiome Metabarcoding

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Introduction

Equine strongyles pose significant health risks to horses globally. Strongyle infections, which comprise over 60 species, can cause severe morbidity and mortality, with species such as Strongylus vulgaris posing greater risks due to their migratory behavior. Routine diagnostic methods, including fecal egg counts, lack of species-level resolution, and traditional morphological identification require advanced taxonomic expertise. DNA metabarcoding offers a powerful alternative, enabling species-level identification via high-throughput sequencing. This study explored the diversity of equine strongyles in northern Iran using both morphological and nemabiome metabarcoding approaches.

Methods

Morphological identification was performed on 1,558 adult strongyles recovered from 23 horses following anthelmintic treatment. In parallel, ITS-2 nemabiome metabarcoding was conducted on larval cultures collected from fecal samples of 25 untreated horses. Species were identified using both morphological keys and nemabiome sequencing. Comparative analyses of strongyle community composition between farms were performed using PERMANOVA.

Results

Morphological identification was conducted on 1,558 worms recovered from 23 horses after anthelmintic treatment, while ITS2 nemabiome metabarcoding was performed on larval cultures from 25 untreated horses. Overall, 33 species belonging to 13 genera were detected across both molecular and morphological methods, with nemabiome metabarcoding identifying more species than morphological methods. Notably, S. vulgaris was prevalent across all provinces. Furthermore, the ITS2 DNA sequence of Triodontophorus tenuicollis was added to public databases. Comparative analyses using PERMANOVA revealed significant differences in strongyle community composition among farms, underscoring the influence of management practices on parasite diversity.

Discussion

This study utilized the classical and molecular methods to study equine strongyle diversity in Iran. The detection of Strongylus vulgaris reflects ongoing risk from pathogenic species, likely linked to suboptimal anthelmintic practices. This study highlighted the strength of nemabiome metabarcoding as a valuable diagnostic tool for studying the clinically important and rare species of equine strongyles.

Effects of nanoselenium on the performance, blood indices, and milk metabolites of dairy cows during the peak lactation period

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Introduction

The impact of nanoselenium and sodium selenite on performance, blood indices, and milk selenium contents of dairy cows during peak lactation period was studied.

Methods

Two groups of dairy cows under the same conditions were selected as the control group (CON) and treatment group (NSe) for a 38-day (10 days for adaptation and 28 days for sampling) experiment. The control group was provided a basal diet +3.3 g/d of sodium selenite (purity1%), whereas the Nano selenium group was offered the same diet +10 mL/d of Nano selenium (selenium concentration 1,500 mg/L).

Results

NSe significantly increased the milk yield, milk selenium content, and feed efficiency($p < 0.05$), but had no significant effect on other milk components($p > 0.05$). NSe significantly increased blood urea nitrogen and alkaline phosphatase($p < 0.05$), but had no significant effects on malondialdehyde, superoxide dismutase, glutathione peroxidase, blood total antioxidant capacity, or blood selenium ($p > 0.05$). In addition, the nontargeted metabolomics of the milk was determined by LC–MS technology, and the differentially abundant metabolites and their enrichment pathways were screened. According to these findings, NSe considerably increased the contents of cetylmannoside, undecylenoic acid, 3-hydroxypentadecanoic acid, 16-hydroxypentadecanoic acid, threonic acid, etc., but decreased the contents of galactaric acid, mesa conic acid, CDP-glucose etc. The enriched metabolic pathways that were screened with an impact value greater than 0.1 included metabolism of niacin and niacinamide, pyruvate, citrate cycle, riboflavin, glycerophospholipid, butanoate and tyrosine. Pearson correlation analysis revealed relationships between different milk metabolites and blood selenium, as well as between milk selenium and blood biochemical indices.

Discussion

Compared with sodium selenite, nanoselenium improves the milk yield, feed efficiency, and milk selenium content of dairy cows and regulates milk metabolites and related metabolic pathways in Holstein dairy cows during the peak lactation period, which has certain application prospects in dairy production.

ENHANCING CANINE AND FELINE DIAGNOSTICS: MULTIPLEX RT-PCR TECHNOLOGY FOR ACCURATE AND RAPID PATHOGENS DETECTION

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Introduction

In veterinary diagnostics for both feline and canine species, rapid, sensitive, and specific pathogen detection is essential. Infectious agents responsible for respiratory, digestive, neurological, and reproductive disorders pose significant challenges in clinical practice. Accurate pathogen identification enables veterinarians to make informed treatment decisions and prevent disease transmission. The BioSellal PETS line offers multiplex real-time PCR kits, providing fast and precise detection of major pathogens across various syndromic assessments.

Methods

The PETS line kits are multiplex assays based on TaqMan technology, enabling the simultaneous detection of multiple pathogens in a single test. Each kit includes internal positive controls (IPC), either exogenous or endogenous, ensuring quality control across all sample types. Developed and validated according to the French AFNOR NF 47-600-002 standard, the kits are optimized for the MIC qPCR cycler, a next-generation thermal cycler utilizing magnetic induction for superior performance and faster results.

Results

BioSellal has developed and validated a wide range of real time PCR kits for multiple sample types, capable of detecting various pathogens within the same syndromic assessment. The integration of internal process controls minimizes the risk of false negatives due to sample inhibition. All kits use a common PCR run, providing results in just 40 to 50 minutes on MIC thermal cycler. These kits are compatible with all thermal cyclers supporting the detection of major fluorophores such as FAM, VIC (HEX), Cy5, and Texas Red (Rox). The multiplex approach reduces diagnostic time, allowing for better veterinary decision-making.

Discussion

The PETS line kits represent the first validated commercial real-time PCR solution for feline and canine diagnostics. Offering superior technical performance compared to current diagnostic methods, including rapid tests, non-commercial techniques, and LAMP technology. This makes the PETS line an ideal solution for laboratories seeking to enhance diagnostic capabilities, streamline workflows, and improve disease management.

Equine Herpesvirus 1 and 4 Multiplex Real-Time PCR Assay

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Introduction

Equine herpesvirus (EHV) 1 and EHV-4 are ubiquitous viral pathogens of horses that can cause upper respiratory disease, neurological disease, abortion, or potentially death. Control of EHV-1 and EHV-4 is difficult as both viruses establish latency and existing vaccines induce short-term immunity with partial protection. Some EHV-1 assays amplify EHV-8, resulting in misdiagnosis of at least two abortion cases. Rapid, accurate diagnosis of EHV-1 and EHV-4 is imperative considering their highly infectious nature and potential for outbreaks. Our aim was to validate a sensitive and specific multiplex real-time PCR diagnostic test (EHV1-4MP) that simultaneously detects EHV-1, EHV-4, an equid genome target, and an exogenous extraction control.

Methods

De novo assays were designed for EHV-1, EHV-4, and the equine MC1R gene. A second EHV-1 target was added to improve specificity. An existing MS2 exogenous control assay was also incorporated. Real-time PCR was performed on viral isolates, synthetic fragments, and archived specimens with the SensiFAST Probe No-ROX Kit on ABI 7500 Fast instruments.

Results

Analytical validation criteria were met for each assay. The limit of detection was 15 copies of EHV-1, EHV-4, and equid MC1R per reaction. Analytical specificity was characterized in silico and on a panel of 28 equine respiratory pathogens and commensal organisms. The EHV-1 genomic target exclusively detected EHV-1, although the EHV1gB assay also detected EHV-8 and -9 despite mismatched sequences. Diagnostic performance was evaluated using 60 clinical specimens and the EHV1-4MP assay results were in 100% agreement with singleplex assays. Additionally, 16 donkey samples containing herpesvirus were tested, resulting in identification of EHV-8 in 3 samples.

Discussion

Two EHV-1 assays were integrated into the EHV1-4MP to enhance sensitivity and enable EHV-8 and -9 detection. The EHV1-4MP real-time assay offers simultaneous, sensitive and specific detection of EHV-1 and EHV-4 while confirming sample integrity and assay performance.

ESTABLISHING A DIAGNOSTIC PANEL TO DETERMINE BACTERIA AND FUNGI THAT IMPACT URINARY TRACT INFECTIONS IN DOGS

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Introduction

Urinary tract infections (UTIs) are common in dogs, particularly older dogs and those with conditions like diabetes. Traditional diagnostic methods often fail to detect all pathogens, leading to ineffective treatments and recurrent infections. Next-generation sequencing (NGS) provides a more comprehensive approach by identifying bacterial and fungal species in a single test. This retrospective study analyzed the urinary microbiome of 300 dogs, including healthy dogs (n=81), those with UTIs (n=150), and those with other conditions like diabetes and Cushing's disease (n=68).

Methods

A Linear Discriminant Analysis of Effect Size (LefSE) was used to identify bacterial and fungal species significantly more prevalent between groups. Bacterial and fungal species that were greater than 5% prevalent in each group were included.

Results

Among healthy dogs, Ralstonia detusculanense-pickettii and Phytobacter ursinigii bacteria (possible contaminants), and three fungal species in the Cladosporium genus were significantly abundant. In the UTI group, two common bacterial pathogens related to UTIs, Escherichia coli and Staphylococcus epidermidis, and one fungal species in the Alternaria genus were significantly dominant. In dogs with other clinical conditions, Paraburkholderia fungorum and two fungal species within the Malassezia genus were significantly prevalent. In addition, various AMRs related to common UTI treatments were prevalent, including Methicillin genes.

Discussion

Bacteria in dog urine may indicate a UTI or result from environmental contamination, particularly via drinking water. While E. coli and Staphylococcus are known UTI pathogens, bacteria like Ralstonia and Sphingobium, commonly found in soil and water, may be incidental. Proper urine collection and culture are crucial to distinguishing true infections from contaminants, ensuring accurate diagnosis and treatment.

Evaluating the Impact of Pre-Partum Vaccination on Passive and Mucosal Immunity in Newborn Calves

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Introduction

Pre-partum vaccination against calf scours, a multifactorial disease associated with pathogens like rotavirus, coronavirus, E. coli, and Clostridium perfringens, is widely practiced in the cattle industry to mitigate its impact on calf health, including diarrhea and reduced growth. However, studies conducted across various farms in Canada have found no association between vaccine use and protection against the disease occurrence. To monitor the vaccine induced immune response, we are developing ELISAs to quantify “anti-Bovine Ig” isotypes A, M, G1 and G2 in serum, colostrum/milk, and calves' gastrointestinal mucosa (feces).

Methodology

Healthy pregnant heifers from WA ranches were vaccinated with a killed virus and bacterin-toxoid combination vaccine against scours. Serum samples were collected pre-initial dose and pre-booster (4 weeks later). Additional blood samples will be taken one week pre-calving and post-parturition (after first milking). Colostrum samples will be collected 5 times each 12 hrs apart. Blood samples from colostrum-fed calves will be taken at 24- and 48-hours post-feeding. Fecal samples from these calves will assess mucosal immunity dynamics. A quantitative Indirect ELISA assay is currently being validated to be able to quantify the total immunoglobulin isotypes, A, M, G1 and G2 in serum, milk and feces samples using capture and secondary antibody. This assay based on the “anti-bovine Ig” will be optimized using a checkerboard dilution method.

Expected Findings

We hypothesize, feeding colostrum produced by vaccinated dam will ultimately enhance immune response in the offspring’s gastrointestinal mucosa.

Discussion

Assessing the mucosal immune response in calves is crucial, as protection against scours pathogens requires high levels of pathogen-specific antibodies at the surface of the gastrointestinal tract. This study aims to assess the feasibility of isolating different immunoglobulin subtypes from three matrices (blood, milk/colostrum and feces), providing a foundation for the next step of our study in understanding pathogen-specific immunoglobulin responses.

EVALUATION OF CRISPR-CAS-BASED RNA DEPLETION FOR METAGENOMIC SEQUENCING OF VIRAL PATHOGENS

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Introduction

The detection and genetic characterization of emerging viral pathogens require metagenomic sequencing approaches that enhance sensitivity by reducing host and bacterial ribosomal RNA (rRNA) content. CRISPR-Cas technology has been proposed as an alternative strategy for rRNA depletion to support virus discovery. In this study, we compared CRISPR-Cas-based and enzymatic rRNA depletion methods using clinically positive samples for avian influenza virus and rabies lyssavirus that had previously been characterised using targeted methods.

Methods

Total RNA was extracted from brains of mice (N=3) and turkeys (N=2) with known viral loads of rabies lyssavirus and avian influenza virus, respectively. Both depletion strategies were applied using commercial kits prior to sequencing on an Illumina MiSeq. Viral read enrichment was assessed by comparing the proportion of viral reads recovered by the two depletion strategies in respect to shotgun sequencing. The two depletion strategies were also tested in their ability to reconstruct consensus sequence and in calling minority variants.

Results

The shotgun approach detected the lower levels of viral RNA. Both depletion methods improved viral RNA recovery, with one of the tested depletion strategies yielding on average the highest enrichment (16X), while the CRISPR-Cas-based depletion showed an average enrichment of ~8.9X. While both methods provided uniform genome coverage, the ribosomal RNA depletion via enzymatic digestion approach recovered about twice the number of viral reads as CRISPR-Cas, making it more efficient at equivalent sequencing depth and cost.

Discussion

This study highlights both the potential and current limitations of CRISPR-Cas in metagenomic pathogen discovery. The results indicate that while CRISPR-Cas-based depletion is a promising alternative for host rRNA removal, it currently demonstrates lower efficiency compared to established depletion strategies for viral metagenomics. Further optimizations are needed to simplify reaction conditions and enhance cost efficiency.

First case study on Antimicrobial resistance and pathogenicity of prevalent bacterial pathogens isolated from bovine mastitic milk in Sikkim-A Himalayan state in India

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Introduction

Mastitis is a widespread issue that adversely impacts animal health, quality of milk and the economies of milk production with implications across countries engaged in the dairy industry. Antimicrobial therapy is commonly implemented for mastitis prevention and control. Unfortunately, despite the best possible antimicrobial treatments, failures of bacteriological cure are common, especially for mastitis and antimicrobial resistance (AMR) which is considered to be one of the reasons for low cure rate.

Methods

In the present study, 114 mastitis suspected milk samples were analysed for isolation and identification of bacterial pathogens followed by its antibiogram. The samples were screened by California Mastitis Test to confirm the presence of Mastitis. Samples were further processed for isolation of prevalent bacterial pathogens viz., Staphylococcus spp, Streptococcus spp. and E. coli. A total of 82 bacterial isolates including coagulase positive Staphylococcus (34), coagulase negative Staphylococcus (12), Escherichia coli (28) and Streptococcus spp. (08) were isolated from the milk samples. All the isolates were subjected to antibiotic susceptibility test against 15 antimicrobials . Ciprofloxacin, Enrofloxacin and Gentamicin were found to be highly effective (85 to 100 percent). There were a total of 27 isolates (26.7%) showing resistance against ≥4 antimicrobial agents. Multidrug resistance was shown by Staphylococcus aureus (11), coagulase negative staphylococci (4) and Escherichia coli (12). The multidrug resistance could not be detected in Streptococcus spp.

Discussion

Detection of antibiotic resistance genes in isolates from mastitis showed the prevalence multidrug resistance in cow milk collected from Sikkim India. The possible transmission of such organisms to human via the food chain cannot be dismissed. Therefore, strict regulations and usage of antibiotics in veterinary practices is needed to prevent the emergence and dissemination of these strains among animals and humans.

Keywords: AMR, Mastitis, CMT

Gross Anatomy Diagrams of Reptiles and Amphibians: The Importance of Updating Resources with Digital Images

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Introduction

Reptile and amphibian necropsies can be challenging due to the relatively low case numbers and scarcity of anatomic descriptions. Between 2019-2023, the Purdue Animal Disease Diagnostic Laboratory received 68 reptiles and amphibians for necropsy. The species seen included 18 snakes, 3 turtles, 13 lizards, 7 frogs, and 27 salamanders with 88.9% of the salamander cases being hellbenders (Cryptobranchus alleganiensis).

Methods

High resolution, colored, gross anatomy images were taken during the necropsy of a ball python (Python regius), an ornate box turtle (Terrapene ornata ornata), and a hellbender (Cryptobranchus alleganiensis) to create detailed and anatomically accurate diagrams. Images were taken at various stages of the necropsy to assist with clear identification of the organs and confirmed via histology. Anatomical drawings or digital diagrams were utilized to assist with determination of various organs. A survey was given to necropsy technicians, pathology residents, and senior pathologists to determine effectiveness of new diagrams.

Results

A survey was taken by technicians, pathology residents, and senior pathologists to determine how useful these diagrams are. The responses to the survey agreed 100% that the gross anatomy diagrams were useful during necropsy for organ identification.

Discussion

While the anatomical drawings or digital diagrams were beneficial, they are not a clear representation of how the organs appear in-situ. It was determined that gross anatomy diagrams and resources of various amphibian and reptile species are limited. Gross anatomy images will provide a more detailed and accurate diagram for use during necropsies for students, residents, and pathologists unfamiliar with these species. Creating and utilizing comprehensive gross anatomy images and diagrams will assist in more accurate diagnosis and treatment of exotic animals.

HEPATITIS E VIRUS IN FOOD-PRODUCING ANIMALS AND IN HUMAN FROM SLOVAKIA

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Introduction

Hepatitis E virus (HEV) is the causative agent of viral hepatitis E reported from all over the world. HEV belongs to the family Hepeviridae. Three genotypes HEV-3, -4, -7 out of eight have been zoonotic so far. The objective of study was to detect RNA HEV in food-producing animals and meat products, to characterize genetically detected isolates and to search relations with HEV isolates of HEV positive patients.

Methods

Liver and muscle tissues from wild boars (n=284) and different wild ruminant species (n=107) were collected from 35 hunting areas. Pork liver and meat (n=111), farm rabbit liver (n=24), sausages and liver pâté (n=83) were collected from supermarkets and butcher shops. Serum/stools of human patients (n=78) were obtained from hospitalized HEV suspected patients. Total RNA was extracted by QIAamp Viral RNA Mini QIAcube kit (Qiagen).

HEV RNA was detected by real-time RT-PCR and nested RT-PCR. PCR products of partial ORF1 were sequenced commercially by Sanger sequencing (Microsynth). MEGA X was used for phylogenetic analysis of HEV sequences.

Results

HEV RNA was detected in wild boar liver tissues (14.2%), muscle tissues (10.5%), in domestic pig livers (3.6%) but no sample was positive in wild ruminants. HEV RNA was detected in 41/78 (53%) patients. Phylogenetic analysis of food-producing animals HEV sequences revealed that belonged to genotype HEV-3, mostly group 2, subtypes HEV-3a, 3i, 3e. Human HEV sequences clustered mostly to group 1, subtypes HEV-3e, 3f, 3g.

Discussion

The study showed that wild boars are the largest reservoir of HEV. However, in the phylogenetic tree HEV sequences of wild boars and pigs clustered predominantly in different phylogenetic groups than human HEV sequences. We did not find a direct relationship between the HEV incidence in patients and analysed meat products and foods.

The study was supported by the VEGA 1/0220/24

High performance freeze-dried triplex qPCR for diagnosis of Equine Herpesvirus 1 and 4 infections

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Introduction

Herpesvirus 1 and 4 (EHV-1 / EHV-4) are worldwide common in horses, causing respiratory symptoms. EHV-1 can lead to severe symptoms, neurological disorders and abortions. It affects sport horse performance and breeding causing significant economic loss. Hence, a sensitive and specific diagnostic tool is crucial to determine during outbreaks which herpesvirus is involved. IDvet has developed and evaluated a freeze-dried triplex qPCR kit for EHV-1 and EHV-4, ID Gene LyoTM Equine Herpes virus 1 and 4 Triplex, containing an exogenous internal control.

Methods

Limit of detection of the PCR (LDPCR) was determined with EHV-1 and EHV-4 synthetic nucleic acids. The Method Detection Limit (MDL) was determined by using equine whole blood, respiratory swabs and fetal organ samples spiked with EHV-1 (ATCC® VR-700TM) or EHV-4 (ATCC® VR-2230 TM) strains. Specificity was evaluated using equine respiratory viruses and bacteria.

Results

The LDPCR (95%) was established around 3.12 copies/PCR for EHV-1 and EHV-4. The MDL obtained for EHV-1 was 379 copies/mL for respiratory swab, 5833 copies/mL for whole blood and 8750 copies/g for fetal organs. The MDL obtained for EHV-4 was 123 copies/mL for respiratory swab, 1891 copies/mL for whole blood, and 71 061 copies/g for fetal organ. The new qPCR kit shows no cross-reactions with other pathogens except EHV-8, which is known to be closely related to EHV-1.

Discussion

The new ID Gene LyoTM EHV-1/EHV-4 qPCR kit allows for rapid detection of both viruses simultaneously, with high reliability thanks to the internal control included. It is freeze-dried, allowing for easy shipment at ambient temperature, reducing shipping costs and the environmental footprint.

Histopathology as a Tool to Detect Zoonotic Helminth Parasites in Wild Rats from Hong Kong

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Introduction

Free-ranging rats can carry various parasites capable of causing human disease (i.e., zoonotic). Angiostrongylus cantonensis, commonly known as rat lungworm, is a nematode significant to public health, as incidental infection of people can result in eosinophilic meningitis and death. Wild rats are the definitive host, and the larval stage can be detected when histologically examining rat lungs. Wild rats can also be infected with parasites in the liver that rarely cause disease in people, including the nematode Capillaria hepatica, and the metacestode stage of Taenia taeniaeformis, known as Cysticercus fasciolaris. Our objective was to histologically detect helminth parasites in wild rats trapped in Hong Kong.

Methods

From 2020-2021, 221 wild rats (Rattus norvegicus, n=144; Rattus tanezumi, n=67; Rattus andamanensis, n=8; and Niviventer huang, n=2) were trapped across 16 geographic regions in Hong Kong. Sites included urban residential areas in Kowloon area and Hong Kong Island, while semi-rural areas in the New Territories included chicken farms, swine farms, and horse-riding schools. Lung, liver, kidney, spleen, skeletal muscle, and tissues with macroscopic abnormalities were examined histologically.

Results

Larval nematodes, presumably A. cantonensis, were identified in the lungs of 5.9% (13/221) of rats, often associated with eosinophilic, granulomatous pneumonia and hemorrhage; only Rattus norvegicus were infected. Adult nematodes and eggs with bipolar plugs consistent with C. hepatica, were identified in the livers of 33.8% (73/216) of rats. 5.1% of rats (11/216) had hepatic cysts, presumably C. fasciolaris.

Discussion

Zoonotic parasites in Hong Kong wild rats were identified by histopathologic examination. An important limitation is that helminth histopathologic identification relies on morphologic parasite features and knowing which parasites are most likely given the geographic region. Further molecular characterization is needed to confirm exact species. Nonetheless, histopathology is a valuable tool to survey for zoonotic helminthic parasites in wild rodents.

Hypermucoviscous Klebsiella pneumoniae Bacteremia in a Raccoon (Procyon lotor) in Thailand

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Introduction

Hypermucoviscous Klebsiella pneumoniae is a hypervirulent, gram-negative, facultative anaerobic, bacteria that is highly contagious and zoonotic with a high potential to express multi-drug resistance genes. Infection can occur in many organs leading to bacteremia and death. Raccoons in Thailand are considered as exotic species and are kept as household pets. To date, there have been no reports of Hypermucoviscous Klebsiella pneumoniae in raccoons in Thailand.

Materials and methods

A 1-year-old, female, raccoon (Procyon lotor) was sent to the Pathology unit for necropsy at Chulalongkorn University Small Animal Teaching Hospital after developing signs of acute anorexia and died without previous sickness. After necropsy, tissue samples were collected for histopathology and bacterial culture.

Results

Grossly, the most notable lesions included severe purulent pharyngitis, myositis of adjacent cervical muscles, and severe pneumonia. The regional lymph nodes were expanded due to purulent exudate. Histological findings indicated bacteremia, characterized by significant neutrophil infiltration, fibrin, and colonies of rod-shaped bacteria in the brain, lungs, and kidneys. The spleen and liver contained rod bacteria within the parenchyma with absent of an inflammatory response. Gram staining revealed gram-negative bacteria in multiple sections. Klebsiella pneumoniae was cultured and tested positive for the string test, indicating a hypermucoviscous type.

Discussion

Based on the gross and histopathological findings, along with bacterial culture results, the cause of death in this raccoon is determined to be bacteremia due to hypermucoviscous Klebsiella pneumoniae. The source of infection remains ambiguous. Given the hypervirulence potential of hypermucoviscous Klebsiella pneumoniae, even healthy animals or humans can be susceptible. In this case, inhalation is a possible route of infection, supported by the severe lesions observed in the pharynx and respiratory tract. This report represents the first documented case of hypermucoviscous Klebsiella pneumoniae causing bacteremia in a raccoon in Thailand.

Identification of Potential Serum Protein Biomarkers for Canine Oral Cancers Using GeLC Tandem Mass Spectrometry

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Introduction

Diagnosing canine oral cancers typically involves invasive biopsies, limiting their feasibility for routine screening. Serum biomarkers offer a promising, less invasive alternative, yet their potential in canine oral cancer diagnosis remains underexplored. This study aimed to investigate serum protein biomarkers capable of distinguishing canine oral cancers from non-cancerous conditions, using GeLC-MS/MS analysis.

Methods

Serum samples were collected from 7 healthy dogs, 5 dogs with periodontitis, 12 dogs with benign oral tumors and 43 dogs with oral cancers (5 early-stage and 28 late-stage oral melanomas, 10 oral squamous cell carcinoma). Proteins were identified via GeLC-MS/MS and analyzed against the NCBI mammal database. Differential protein expression between cancerous and non-cancerous groups was examined using MetaboAnalyst 6.0. A volcano plot highlighted candidate proteins at fold change thresholds >1.5 or <0.67 with FDR-adjusted p-values <0.05. A recursive support vector machine (R-SVM) algorithm pinpointed critical proteins with a 0% error rate by filtering out noise. And protein-chemotherapeutic interactions were explored using Stitch.

Results

Out of 3,874 proteins identified, 181 showed significant alteration in cancerous groups. R-SVM analysis revealed 37 potential biomarkers, with 19 up-regulated proteins confirmed via heatmaps. Among these, eight up-regulated proteins, including E3 ubiquitin-protein ligase UBR5 (UBR5), phosphatidylinositol 3-kinase catalytic subunit beta (PI3KCB), cyclin-dependent kinase 14 (CDK14), adenylate kinase (AK), Four-point-one, Ezrin, Radixin, Moesin (FERM) domain, centrosome-associated protein CEP250 (CEP250), A-kinase anchor protein 8 (AKAP8), and TNF receptor-associated factor 3 interacting protein 1 (TRAF3IP1) exhibited interactions with chemotherapeutics: doxorubicin, cisplatin and cyclophosphamide.

Discussion

This study identified eight key proteins as potential biomarkers and therapeutic targets in canine oral cancer, contributing to insights on oral cancer pathology and treatment. Further validation of these findings may unlock novel therapeutic strategies for these malignancies.

Immune Checkpoints As Biomarkers For Differentiating Intestinal Lymphoma From Lymphoplasmacytic Enteropathy In Cats

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Introduction

Intestinal lymphoma (FIL) is the most prevalent type of lymphoma in cats, with low-grade intestinal T cell lymphoma (LGITL) being the most common variant, typically leading to chronic enteropathy (CE). Another frequent cause of CE in cats is lymphoplasmacytic enteritis (LPE), also known as inflammatory bowel disease. Differentiating LPE from FIL, particularly LGITL, poses a diagnostic challenge due to overlapping clinical signs and histologic findings.

Currently, no biomarker exists to distinguish LPE from FIL. Diagnosing feline CE necessitates the accumulation of various laboratory data, including imaging, standard serum chemistry panels, complete blood counts, and specific serum markers that indirectly evaluate intestinal health. This is often combined with the more invasive yet necessary histopathologic evaluation of intestinal biopsies, frequently paired with immunohistochemistry and/or lymphocyte clonality determination. Despite various laboratory analyses, histopathology remains the primary method for accurately differentiating between LPE and FIL.

The PD-1/PD-L1 and CD80 or CD86/CTLA4 pathways are crucial immune checkpoints that control T cell-mediated immunity by deactivating cytotoxic T cells. Tumor cells may exploit these mechanisms to evade immune-mediated destruction. Overexpression of these molecules has been reported in various human cancers and is often correlated with poor prognosis. Currently, there is no data on the expression of immune checkpoints in feline enteropathies.

Methods

This study evaluated the mRNA expression of PD-L1, PD-1, CTLA4, and CD86 in 40 cats with gastrointestinal disease diagnosed as FIL (22 cases) or LPE (18 cases) using quantitative qPCR.

Results

Results showed significantly ($P<0.05$) higher expression of all immune checkpoints in the FIL compared to the LPE group.

Discussion

Higher expression of immune checkpoints in FIL suggests their potential as diagnostic biomarkers to distinguish these diseases in cats. However, further evaluation with a larger sample size is necessary to establish cut-off values with acceptable confidence levels.

Innovative Automated System for Gram-Positive AST Testing in Veterinary Medicine

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Innovative Automated System for Gram-Positive AST Testing in Veterinary Medicine

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Introduction

Antimicrobial resistance (AMR) is a significant concern in veterinary medicine due to its impact on animal health, public health, and the environment. In this context, the VITEK 2 System is essential to provide fast, automated susceptibility testing for a wide variety of veterinary isolates including Gram-positive organisms such as Staphylococci and Group B Streptococci. In order to accommodate veterinary-specific breakpoints as listed in CLSI VET01S-Ed7, calling ranges were expanded for amoxicillin/clavulanic acid (AMC), doxycycline (DO), enrofloxacin (ENR), and minocycline (MNO) MIC tests* on the VITEK® 2 platform Amoxicillin/clavulanic acid data include Staphylococcus species and Streptococcus agalactiae. Doxycycline, enrofloxacin, and minocycline data include only Staphylococcus species.

Methods

Throughout this study, a total of 1315 isolates, including strains obtained from veterinary labs, were tested in VITEK 2 investigational use only (IUO) cards containing varying concentrations of the different antimicrobials. All strains were tested with both IUO cards and the CLSI/ISO broth microdilution reference method. Growth data were collected from the VITEK 2 cards and compared to the reference results.

Results

Overall Essential agreement (EA) for the development isolates are shown in Table 1.

Table 1.

%EA	AMC	DO	ENR	MNO
Overall	93.4	98.2	99.2	97.7

Discussion

The overall essential agreement for all MIC tests exceeded 90%, demonstrating that the VITEK® 2 system can accurately determine MICs for the specified antimicrobials in various Gram-positive bacteria. These results are crucial for guiding appropriate antibiotic therapy in veterinary institutions and contribute to the One Health approach as they provide essential data to combat antimicrobial resistance (AMR) across human, animal, and environmental health sectors.

* These new tests are not yet available for commercial use.

Interstitial Pneumonia in Fetal and Neonatal Beef Calves with Bronchiolar Associated Lymphoid Tissue (BALT) Hyperplasia and Interstitial Emphysema

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Introduction

Lung pathology is a common finding at necropsy of fetal and neonatal calves. The reaction pattern of extensive interstitial emphysema with interstitial pneumonia and bronchiolar associated lymphoid tissue hyperplasia (BALT) is however unusual. This case series describes a syndrome affecting beef calves in Western Canada.

Methods

Fifteen aborted, stillborn or non-viable neonatal beef calves were received for necropsy examination over a ten-year period. A range of fresh tissues were collected for bacteriology, molecular and mineral analysis. Tissue samples were also fixed in neutral-buffered 10% formalin and processed for histopathology, special histochemical stains, and immunohistochemistry.

Results

On gross pathological examination the lungs were diffusely swollen with extensive interlobular and subpleural emphysema which often extended across the pericardium. Few cases had large bullae in the caudal lung lobes or expanding the mediastinum. On histopathology alveolar septa were often expanded by mononuclear inflammatory cells and there was bronchiolar associated lymphoid tissue (BALT) hyperplasia. Interlobular septa and subpleural spaces were markedly expanded by clear space. Mixed bacteria were found on bacteriology. Molecular analysis for pathogens including Bovine herpes virus-1, Respiratory syncytial virus, Bovine viral diarrhea virus, Parainfluenza virus 3, Bovine coronavirus, Ureaplasma diversum and Mycoplasma bovis were negative.

Discussion

This case series describes interstitial pneumonia of unknown cause in fetal and neonatal beef calves, with unusual features of bronchiolar associated lymphoid tissue (BALT) hyperplasia and interstitial emphysema.

Investigating Pestivirus Infections in Barren-Ground Caribou: Strain Identification and Diagnostic Test Refinement

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Introduction

Caribou populations in the Arctic have been declining. One unexplored aspect of caribou health is viral ecology. Virus research is limited by sampling challenges and the lack of caribou-specific diagnostics. Pestiviruses are concerning as they are highly infectious, pathogenic and abortogenic in livestock. Antibodies against pestiviruses were detected in Canadian caribou, but the species in circulation have not been identified. This project aims to identify circulating pestivirus strains through RT-PCR and develop caribou-specific antibody tests (ELISA).

Methods

Pestivirus presence in caribou samples was assessed in 100 samples of harvested spleens and 23 placentas using a 'pan-pesti' RT-PCR targeting conserved 5’ UTR region. Concurrently, an ELISA was developed for BDV strains 1-5 and 7-8 to detect caribou antibodies from filter-paper eluates and serum against these viruses (n=200). BDV type 6, E2 genomic information is unknown. The E2 segment of these BDV strains were expressed in HEK-S cells using pFRT-E2-vector and confirmed via Western blot. The ELISA utilizes anti-deer HRP secondary antibodies which have been validated in caribou ELISA studies.

Results

The Indirect ELISA has been optimized for antibody types and concentrations for both eluates using HIS and STREP tags which are added to the E2 envelope protein expressed. BDV 1,2,3,8 strain expression in eukaryotic HEK-S cells succeeded. Initial testing using ELISA has identified antibodies against BDV strains in eluates. RT-PCR analysis is in progress, positive result has been sent for Sanger-sequencing. Data analysis of the samples is ongoing, results will be presented at the conference.

Discussion

Preliminary ELISA results show the presence of antibodies to pestiviruses in caribou eluate samples. It remains to be determined whether this reflects cross reactivity, or the presence of multiple strains within caribou populations. With pestivirus genetic sequencing and a caribou-specific ELISA this project aims to better monitor caribou health in the Arctic.

INVESTIGATING SARS-COV-2 PRESENCE IN CATS LIVING WITH COVID-19-POSITIVE OWNERS IN IRAN

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Introduction

The emergence of SARS-CoV-2 infections in companion animals has raised concerns regarding zoonotic transmission, particularly in households with infected individuals. Cats, known for their susceptibility to SARS-CoV-2, serve as critical subjects to study the virus's transmission dynamics between humans and pets. This study aimed to detect SARS-CoV-2 in household cats living with COVID-19-positive owners, providing insights into potential zoonotic risks.

Methods

Deep oropharyngeal and rectal swabs were collected from 96 cats residing in 81 households in Kerman and Tehran, Iran, between April 2021 and 2023. These regions were selected due to their high human COVID-19 caseloads and diverse urban demographics. The inclusion criteria required households to have at least one confirmed COVID-19-positive individual. Confirmation of COVID-19 in owners was based on a positive real-time reverse transcription polymerase chain reaction (RT-PCR) test or a documented clinical diagnosis by healthcare providers. All cat samples were analyzed using RT-PCR targeting specific SARS-CoV-2 genes, following WHO-approved protocols to ensure accuracy and reliability.

Results

Of the 96 cats tested, 17 (17.7%) were positive for SARS-CoV-2. All positive cats exhibited symptoms such as severe anorexia and lethargy and had prolonged close contact with their infected owners. Interestingly, no other pets within these households were reported or tested, narrowing the findings to feline hosts. These results suggest potential owner-to-pet transmission, aligning with similar international studies.

Discussion

This study highlights the role of household cats as potential secondary hosts of SARS-CoV-2, emphasizing the need for infected individuals to minimize close contact with their cats during illness. Limitations include the focus on a single species and the inability to confirm direct transmission routes. Future research should explore transmission dynamics across broader species and geographic regions. Findings contribute to global efforts in zoonotic disease management and underline the importance of monitoring companion animals during pandemics.

Johne's Disease in Bison: Correlations Between Fecal qPCR, Bacterial Culture, and Histopathology During Mycobacterium avium subspecies paratuberculosis Infection

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Introduction
Diagnostic capacity to detect Mycobacterium avium subspecies paratuberculosis (MAP) is hampered by different stages of Johne's disease. Bison selected from one captive herd were divided into four categories based on clinical presentation and fecal qPCR test results and each category was correlated to postmortem histological and bacterial culture results.

Methods
Category 1: Clinically affected with diarrhea, weight loss, and dehydration and MAP confirmed positive via fecal qPCR (n = 6). Category 2: No clinical signs but MAP-confirmed positive via fecal qPCR (n = 2). Category 3: No clinical signs, only positive for IS900 gene in fecal qPCR (n = 4). Category 4: No clinical signs and fecal qPCR-negative (n = 1).

Results
All test results were consistent within categories, with all bison in category 1 and 2 having MAP specific lesions in histopathology, while category 3 and 4 animals lacked histological lesions. Bacterial culture, using para-JEM liquid culture system, confirmed viable MAP solely in category 1 and 2 bison. Category 3 and 4 animals were negative in culture. However, the number of tissue locations with histopathological lesions and positive tissue culture differed between category 1 and 2. In category 3, qPCR detected MAP DNA targeting IS900 gene, but all other tests were negative. Category 4 remained negative across all diagnostic methods.

Discussion
Positive fecal qPCR for IS900 and F57 genes, 100% correlated with postmortem histological and culture results. postmortem samples from IS900 fecal qPCR positive bison, did not provide any additional positive test results. Therefore, when fecal samples are available, complementary diagnostic tests have limited additional value for Johne's Disease diagnosis.
Keywords: Subclinical infection, Parallel testing, Sensitivity, Diagnostics

LAMP: A NEW WAY TO DETECT ANTIMICROBIAL RESISTANT STAPHYLOCOCCUS SPP. DIRECTLY ON THE FIELD IN 30 MIN

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Introduction

The emergence of enzymes conferring resistance to antimicrobials (AMR), encoded by genes from chromosomes or mobile genetic elements, is well investigated in human and veterinary medicine. Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-resistant Staphylococcus pseudintermedius (MRSP) can express resistance to methicillin but also to large spectrum β-lactams and other classes of antimicrobials.

Current clinical AMR diagnostic technics rely mainly on phenotypic analysis. Nevertheless, commercial means of AMR detection by PCR have become an alternative.

LAMP technology (Loop mediated isothermal AMPlification) is a Nucleic Acid Amplification Technology which can occur at constant temperature. This allows the use of this technology as a point of care test and provide results in only 30 min.

The objective is to develop a POC assay based on LAMP for the rapid detection of MRSA & MRSP in animals.

Methods

LAMP primers for S. aureus (SA) nuc, S. pseudintermedius (SP) spsL and AMR mecA and mecC genes were designed. Sensibility-specificity tests followed by evaluation of the functionality of the assay were performed by testing urine spiked with S. aureus/pseudintermedius with/without resistant genes mecA/C.

Results

Analytical tests with synthetic DNA for mecA gene in a concentration range of 1x10^9 copies/µl - 1x10^3 copies/µl selected the best primers. Sensitivity tests with genomic DNA resulted in a LoD of < 100 copies / rxn for genes mecA and nuc (SA). The LAMP assay performance was confirmed for SA and SP in urine spiked samples from dogs. No false negative or false positive results were observed.

Discussion

The test can detect specifically if the urine of the animal tested is contaminated by MRSA and MRSP. It’s a new way to detect AMR directly in veterinary clinics in only 30 min. This new tool provides a new way to detect and fight antibiotic resistance spreading in a One Health approach.

LAMP: A POWERFUL TOOL FOR DIAGNOSTIC. WHAT ARE THE ADDED VALUES TO CREATE THE BEST LAMP DIAGNOSTIC TEST?

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Introduction

LAMP technology (Loop mediated isothermal AMPlification) is a Nucleic Acid Amplification Technology which can occur at constant temperature. This allows the use of this technology as a point of care test and allow the obtention of the result in only 30 min without the necessity to use a thermal cycler. This technology can be useful to detect highly contagious pathogens causing diseases in animals.

However, in the literature, many LAMP diagnostic tests were created and different conclusions about the sensibility/specificity of these tests in comparison of PCR tests were reported.

Our goal is to explain the added value of 1) a good design of LAMP primers 2) the use of a fluorescent dye 3) a quick DNA/RNA extraction compatible with LAMP technology 4) melting curves at the end of the run.

Methods

LAMP reactions were performed with or without tools or improvement suggested (ie: quick DNA extraction) and results obtained were compared.

Results

A good design of primers is essential to have good amplification performances. Different parameters should be considered: nucleotidic sequence, variation of nucleotides between strains, mastermix and enzyme used, concentration of salts, …

The fluorescent DNA intercalator is a real added value because it detects small quantity of amplicons but also give a real time result.

Quick DNA extraction is an essential step if a complex matrix is used (ie: blood). It dramatically improves performances of LAMP tests.

Melting curves allow a verification step to confirm a specific amplicon was really synthetized.

Discussion

All LAMP tests available on the market or described in the literature are not comparable in term of performances. LAMP diagnostic test should be seen as a complete test from initial matrix to result which involve the use of good tools and extra steps as added values to obtain the best performances.

Meat authentication: the role of DNA Metabarcoding in enhancing traceability and preventing fraud

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Introduction

Food fraud is an increasing global issue, with meat products being particularly susceptible to adulteration. Fraud in the meat industry have been reported worldwide. Our aim is to implement a molecular investigation based on NGS metabarcoding approach to identify species in processed and unprocessed meat products, sold in Italy.

Method

Forty-six meat samples were collected from different companies and production batches. They were stored at -20°C prior to analysis, while DNA was obtained using a specific food extraction kit and quantified. Libraries were prepared by amplifying a region of the mitochondrial 16S gene and then sequenced on the Illumina MiSeq platform. RDADA2 and NCBI BLAST tools were used to identify sequence variants, using high similarity criteria (≥98% identity) to univocally determine species assignment.

Results

A total of 273 ASVs were identified, assigned to different species such as Gallus gallus, Bos taurus, Sus scrofa, Equus caballus, and others. DNA outcome was compared to according to declared labelling, revealing that several products (7 out of 46; 14,6%) did not match their declarations, showing fraud or adulteration. One sample, labeled as “100% beef” was totally constituted of buffalo meat, while another one, stated as ground beef, turned out to be chicken. For a sample with declared 100% horse meat, we detected a 15% pork meat.

Discussion

The results highlighted the effectiveness of DNA barcoding techniques in detecting fraud in meat products. Although limited in sample size, this work shows that meat mislabelling (malicious or occasional) is unexpectedly frequent. We plan to expand our molecular survey especially on typical Italian food containing processed meat (“pasta ripiena”) and ultra-processed products. It is then essential to standardize, regulate and promote routinary DNA-based investigation in order to improve food traceability and protect consumers, producers and retailers from fraudulent conducts.

Methicillin Resistant Staphylococcus Aureus Prevalence in in the Northern Border Regions between Pakistan and Afghanistan: A One Health Approach

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Introduction

Methicillin Resistant Staphylococcus aureus is a zoonotic pathogen of public health importance. Unrestricted cross border movement of animals and sale of raw meat at border region between Pakistan and Afghanistan presents a great risk of transmission of pathogens. The aim of this study was to investigate the prevalence of MRSA in meat shops and slaughterhouses in newly merged areas of Khyber Pakhtunkhwa Pakistan bordering Afghanistan.

Methods

Overall 300 sample- 30 each from working area, knives, hooks, meat mincers, worker hands, beef, mutton, chicken meat, and nasal and rectal swabs from animals/chickens were collected. All the samples were cultured for bacterial growth. 164 S.aureus isolates were confirmed by phenotypic identification through gram staining and biochemical tests. MRSA isolate were identified among positive isolates by performing cefoxitin disk diffusion assay. Molecular characterization of MRSA was further carried out through PCR by targeting mecA gene.

Results

Out of 164 S. aureus isolates 99 (60.36%) were resistance to cefoxitin. These isolates were also resistance to methicillin, ciprofloxacin and tetracycline. All the 99 isolates were positive for mecA gene. The prevalence of MRSA was highest in Beef 63.3% (19 /30) followed by meat mincer 53.3% (16/30), chicken meat 50% (15/30), working area 43.3% (13/30), knives 40% (12/30), Nasal swabs and hooks 23.3% (07/30), worker hands 16.6% (5/30), mutton 10% (3/30), and rectal swabs 6.66% (2/30).

Conclusions

The study revealed that MRSA is prevalent in meat as well as environment samples from northern border areas between Pakistan and Afghanistan. Due to persistent instability in area this is the first ever study on MRSA. This demand for strict measures for border control and implementation of strict hygienic measure in the meat production, processing and supply chain to prevent the contamination of environment and ultimate spread of bacteria to human population.

MOLECULAR CHARACTERIZATION OF RABIES VIRUS TRANSMITTED FROM A DOG TO A BULL IN A LIVESTOCK MARKET IN GHANA

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Introduction

Livestock rabies has significant public health, economic, and food security implications. In many rabies-endemic countries, vaccination for livestock have been adopted. Currently, Ghana do not vaccinate livestock against rabies. The devastating impact of rabies requires an expedited approach to handling outbreaks to avert transmission and death in humans and animals.

Method

A video of a suspected rabid bull at a livestock market in Ghana was shared on social media with comments of a “strange disease in cattle” on 25th September 2023. The local veterinary officer chanced on the video, leading to an on-site investigation which concluded that the bull had been bitten by a three-month-old dog four days earlier. The puppy, which was killed and buried, was subsequently exhumed and tested for RABV. The bull was humanely destroyed and the collected brain tissue was tested at the Accra Veterinary Laboratory by RT-PCR. The full genomes of the viruses from both animals were sequenced at the IAEA/FAO Animal Production and Health Laboratory, Seibersdorf, Austria.

Results

The presence of RABV was confirmed in both animals. The consensus sequences of the genomes, belonging to the Africa 2 clade, were identical, although sub-consensus variants in a subset of the sequences located in the RNA-dependent-RNA polymerase (L) gene of the bovine virus were observed.

Discussion

This case report describes the transmission of rabies virus from a puppy to a bull in a livestock market in Ghana. The case, however, highlights the inadequate knowledge of the public on bovine rabies. Thus, while rabies in the puppy was easily recognised, the virus in the bull was not, perhaps due to its rarity. The short incubation observed in the bull could be attributed to the puppy’s bite on the muzzle, which is close to the brain and possibly, high viral load.

Molecular investigation of respiratory disease-related pathogens in farm and wild animals in Southern Italy

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Introduction

As part of a project aimed at assessing, for the population of southern Italy, the health risk associated with environmental exposure to asbestiform minerals, surveys were carried out on sentinel animals in the area, such as sheep, goats, cattle and wild pigs. In case of lung lesions and respiratory symptoms, molecular screening was performed to assess the occurrence of any responsible pathogens.

Methods

A total of 321 samples were collected, consisting of 77.25% (248/321) from lung biopsies and nasal swabs from cattle, 15.57% (50/321) from lung biopsies from sheep and goats, and 7.16% (23/321) from wild boar. Tissue fragments sampled at lesions and nasal swabs were subjected to DNA/RNA extraction and analyzed by Real-Time PCR and PCR followed by Sanger sequencing, if required. Panels were standardized for molecular screening of bacterial and viral pathogens that included 15 targets for cattle, 12 for sheep and goats, and 12 for wild pigs.

Results

The screening found 69.3% positivity in cattle, 16% in sheep and goats, and 30.43% in wild pigs. The most frequent pathogens were Pasteurella multocida in cattle, Herpes Virus in sheep and goats, and Porcine Circovirus type 2 in wild pigs.

Discussion

The difference in positivity rates found in different animal species could be determined by the inclusion, for the bovine species, of nasal swabs on symptomatic animals on the farm. These results confirm the peculiar multifactorial nature of respiratory disease, a complex and often underestimated pathology, putting the spotlight on the circulation of bacterial and viral pathogens in these animal species. The results confirm the effectiveness of molecular methods, which offer advantages over traditional microbiological techniques in diagnosis.

Novel Biosafe and Scalable Detection of Influenza A (H5N1, H3N2, H1N1) for Veterinary and One Health Applications

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Introduction

Influenza A remains a global health concern due to its rapid transmission and significant morbidity. Timely, accurate, and biosafe diagnostic methods are essential for effective public health responses (Velayudhan, 2022; Uyeki, 2019; Merckx, 2017). However, U.S. regulations require influenza diagnostic samples to be processed under BSL-2 or BSL-3 conditions, limiting accessibility and delaying One Health research.

This study evaluates an integrated workflow for influenza detection outside BSL-3 facilities, combining PrimeStore® Molecular Transport Medium (MTM) — an FDA-cleared inactivation transport medium—with an automated nucleic acid extraction platform and a specialized RT-qPCR detection system. By inactivating and stabilizing viral material at the point of collection, the workflow reduces biosafety requirements, facilitating safer sample handling. We hypothesize that this workflow will maintain diagnostic accuracy while improving biosafety and accessibility.

Methods

Field samples were tested: H5N1 in dairy milk and pooled poultry oropharyngeal swabs, and H3N2/H1N1 in swine oral fluids. Samples were collected in PrimeStore® MTM (Longhorn Vaccines and Diagnostics) for inactivation and processed in a BSL-2 lab. RNA was purified using the Maxwell® RSC Pathogen Total Nucleic Acid Kit on the Maxwell® RSC 48 Instrument and detected using GoTaq® Endure RT-qPCR System with publicly available primer and probe sequences or GoTaq® Enviro Wastewater Flu A, Flu B, SC2 System (Promega). Results were compared to standard-of-care assays, and the use of positive and negative controls ensured contamination-free results.

Results

The workflow demonstrated ease-of-use, reproducibility, and strong performance outside BSL-3 facilities. Detection of a positive or negative sample matched standard PCR results with no false positives detected. Reproducible results across multiple replicates confirmed the reliability of the workflow.

Discussion

This workflow offers a biosafe, scalable, and accurate alternative for influenza detection. It expands diagnostic capabilities for clinical, veterinary, and field surveillance applications. Future work should address regulatory approval, cost-effectiveness, and validation across diverse settings.

Novel Diagnostic Assay for Haptoglobin-based Health Monitoring in Dairy Herds

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Introduction

In Germany, during the years 2016/2017, four million dairy cows guaranteed the supply reliability of milk for the entire population. At onset of milk production and in early lactation highly producing dairy cows are most susceptible for inflammatory diseases. Thus, the detection of sick animals within the dairy herds like animals with e.g. subclinic mastitis can be of economic advantage for the farmer. Intensive supervision of animals with automatable diagnostic tools is essential.

Methods

Within our innovative and multidisciplinary framework, biomarker-based veterinary diagnostics assays for cattle will have been developed. These assays are based on monoclonal antibodies against specific biomarkers capable of detecting sick animals prior the emergence of clinically detectable disease symptoms. In regards of Haptoglobin as a biomarker for diseases like mastitis the development has led to a commercialized ELISA assay.

Results

In a finalised research project different biomarkers for health monitoring in dairy cows have been identified, validated and patented. In our framework a variety of monoclonal antibodies and biobank samples for test validation as well as first assay prototypes are available and represent the basis for the development and approval of commercial diagnostic assays. Those assay would allow fast, simple and practicable detection of sick dairy cows. In regards of Haptoglobin as a biomarker for diseases like mastitis the development has already led to a commercialized assay.

Discussion

The application of novel diagnostic assays enabling early health monitoring may prevent disease dissemination and exacerbation and may support decisions in terms of therapeutic measures and prognosis. Since health and well-being of animals are closely interconnected, the development of biomarker-based health monitoring assays may contribute considerably to the principles of animals' and consumers' welfare.

Optimization of a Differential Diagnosis Method of African and Classical Swine Fevers in Field Applications

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African swine fever (ASF) and classical swine fever (CSF) are devastating viral diseases of Suidae with significant economic impacts on the global swine industry. Since the first ASF outbreak in Asia was confirmed in China in August 2018, the disease has spread rapidly across the region, sparing only Japan and Taiwan. Meanwhile, CSF (genotype 2.1) re-emerged in Japan in September 2018 after 26 years of absence, becoming endemic in most regions except the northern islands. This resurgence led to the loss of Japan's CSF-free status, as accredited by the WOAH. Compounding the challenge, CSF outbreaks have intruded into wild boar populations, complicating control and eradication efforts.

As ASF and CSF cause similar clinical signs, rapid and accurate differentiation is essential for effective containment and swift recovery of disease-free status. Given the re-emergence of CSF and the imminent risk of ASF introduction from neighboring countries, simultaneous testing for both diseases has become a priority. Until recently, local veterinary officers had to perform separate PCR and RT-PCR tests for ASF and CSF, respectively, using the same sample sets.

To streamline diagnostics, the National Agriculture and Food Research Organization (NARO) and Takara Bio Co., Ltd. jointly developed a direct multiplex real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. This innovative assay enables simultaneous detection of ASFV and CSFV from sera and tissue homogenates in a single reaction, utilizing an optimized extraction buffer (Lysis Buffer S) to process crude samples with sensitivity and specificity comparable to standard methods. Additionally, revised protocols now allow testing of whole blood with Solution N and damaged samples from decomposed carcasses with Tissue Direct Solution E.

In conclusion, our novel rRT-PCR assay provides a rapid, accurate, and cost-effective tool for ASF and CSF diagnosis, supporting early detection, field surveillance, and vaccine monitoring.

Optimized Competitive ELISA For The Detection Of H5 Antibodies Including New Clades

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Introduction

Influenza viruses, part of the Orthomyxoviridae family, are classified into four types (A, B, C, and D), with type A being the most conserved and further divided by Hemagglutinin (H) and Neuraminidase (N) antigens. Highly pathogenic H5 and H7 subtypes cause severe outbreaks in poultry, leading to significant economic losses. Since 2004, a new clade of highly pathogenic H5 has spread globally. Recently, H5N1 infections have also been detected in dairy cows in the U.S., causing respiratory symptoms and reduced milk production.
To address the need for a rapid diagnostic tool, IDvet developed the ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species ELISA. This competitive ELISA detects anti-H5 antibodies in both poultry and mammals using recombinant H5 hemagglutinin-coated plates and an HRP-conjugated anti-H5 antibody, improving detection of clade 2.3.4.4.

Methods

The ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species ELISA is a competitive assay designed to detect antibodies against the H5 of Influenza A. It utilizes plates coated with purified recombinant H5 hemagglutinin and an HRP-conjugated anti-H5 antibody for improved detection of clade 2.3.4.4. Positive and negative sample panels were tested to evaluate inclusivity, exclusivity, diagnostic sensitivity, and specificity. Results were expressed as an S/N% ratio.

Results

Inclusivity: The test detected antibodies in all nine H5-positive sera from European reference labs.
Exclusivity: Nine sera with antibodies against other Influenza A subtypes tested negative.
Diagnostic specificity: All 101 sera from free zones in France were negative.
Diagnostic sensitivity: Ducks vaccinated with recombinant H5 vaccines showed seroconversion post-vaccination.

Discussion

The optimized competitive ELISA demonstrated excellent sensitivity, specificity, inclusivity, and exclusivity. It effectively detects seroconversion induced by recombinant vaccines, making it a valuable tool for influenza monitoring in multiple species.

Optimized Nucleic Acid Extraction Protocols for Detecting Avian Influenza A virus in Extended Bovine Semen

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Introduction

In 2022, the H5N1 highly-pathogenic avian influenza (HPAI) outbreak started in the United States and spread to cattle in March 2024. HPAI Infections in lactating cattle yields high viral load in the milk, assisting in dissemination in the cattle population, and the spill back to the poultry. Specific pathogen-free semen by PCR testing is a requirement for semen exportation. While scientific evidence indicates the lack of HPAI in bull semen, the current evolving situation in the United States prompted an HPAI detection semen extraction protocol due to potential trade implications.

Methods

Reliable extraction methods were explored in 88 negative milk-based or egg-yolk-based extended semen samples, using exogenous extraction controls spiked into the lysis solution and extracted using both manufacturer-recommended and lab-developed protocols for the MagMAX CORE and IndiMag Pathogen kits. Extracts were PCR using the current NAHLN-approved Influenza A PCR assay. Next, 27 previously confirmed positive semen samples naturally infected with various pathogens were tested using in-house PCR with VetMax Fast and IndiMix JOE PCR reagents to assess the sensitivity of the refined extraction protocols. Assessment with spiked HPAI samples is in progress.

Results

After evaluating numerous MagMAX CORE and IndiMag Pathogen protocols, the standard IndiMag Pathogen kit with 75ul input effectively minimized PCR inhibitory effects. The standard MagMAX CORE protocol had inhibition with egg yolk-based semen, which improved with PK pre-treatment. Ultimately, testing of naturally infected positive semen yielded a 96.3% and 85.3% diagnostic sensitivity for the 75ul input IndiMag Pathogen protocol, and the modified MagMAX CORE protocol, respectively, with a 100% passing criteria for exogenous control for both protocols.

Discussion

This study validated an extraction protocol that efficiently removes PCR inhibitors and demonstrates high sensitivity for disease detection in milk-based and egg-yolk-based extended semen samples. This solution can be seamlessly adapted for HPAI detection in semen samples.

Outbreak investigation: exploring the means of High Pathogenic Avian Influenza virus (HPAIV) Entry into Commercial Poultry Operations in Alberta

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Introduction

Our study focuses on to understand how HPAIV enters commercial poultry flocks, track its airborne spread during barn venting after flock euthanasia, and examine the role of environmental factors in spreading the virus.

Methods

We collected samples from HPAI-affected commercial flocks in Alberta including oropharyngeal, cloacal, and dust swabs, as well as air and water samples before and after barn venting. These samples were tested for virus levels using molecular assays, sequencing, and virus isolation with the National Centre for Foreign Animal Disease (NCFAD) in Winnipeg. Preliminary results show viral DNA in air samples after venting. The Madin-Darby Canine Kidney (MDCK) cell line was used to confirm the presence of active virus in environmental samples.

Results

qPCR Ct values were used to classify infected samples into high, moderate, and low categories for Influenza A and H5 genes. Oropharyngeal, cloacal, and dust swabs showed high viral presence. Dead wild birds near the infected farm also tested highly positive. Air samples collected after venting had moderate levels of both Influenza A and H5, while pre-venting air samples were positive for Influenza A but not H5. Water samples are being analyzed to measure viral genome load, and virus propagation data will be added later to confirm active virus presence. Sequencing analysis is ongoing for further insights.

Discussion

The HPAI outbreak has heavily impacted the poultry industry. Environmental factors contribute to its spread, with air samples after venting indicating possible airborne transmission. Genome analysis of wild birds, environmental samples, and the infected farm will help trace the virus's origin.

Phenotypic and Genotypic Characterization of parainfluenza virus 5 isolated from diarrheic piglets highlights its zoonotic potential

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Background
Parainfluenza virus 5 (PIV5), is a negative-sense, non-segmented, single-stranded RNA virus a member of the Paramyxoviridae family, causes respiratory and neurological infection in several animal species. However, its role in digestive system infections is poorly understood.

Objective
This study aimed to determine the prevalence, genetic characteristics, and host range of PIV5 in diarrheic piglets in northern China and assess its potential zoonotic risk.

Method
Fecal samples from diarrheic piglets and 530 serum samples from pigs of different age groups were collected in Heilongjiang Province, China. The virus was isolated, purified, and analyzed using electron microscopy, genome sequencing, and immunofluorescent assays (IFA). A recombinant indirect ELISA (iELISA) was developed to assess seroprevalence.

Result
The PIV5 strain was identified as paramyxovirus-like particles measuring 80–200 nm in diameter through electron microscopy following four rounds of plaque purification and ultracentrifugation. Genome-wide phylogenetic analysis revealed a close relationship between the isolated strain and PIV5 strains from lesser pandas and pigs in China. The virus agglutinated guinea pig and chicken red blood cells and infected pig, human, and other mammalian cell lines, suggesting cross-species transmission potential. To assess its prevalence in swine, an indirect ELISA (iELISA) using recombinant nucleocapsid protein was developed, achieving 94.12% sensitivity, 96.4% specificity, and 95.22% agreement with IFA and no cross-reactivity with antibodies against other porcine viruses was observed. Testing 530 serum samples revealed a 75.7% PIV5-positive rate, indicating widespread infection in swine herds.
These findings emphasize the zoonotic risk of PIV5, offering critical insights into their evolution, biological characteristics, and potential impact on animal and human health.

Conclusion
PIV5 is highly prevalent in swine and has the potential for cross-species transmission, raising concerns about its zoonotic risk. Further studies are needed to clarify its pathogenic role in pigs and potential public health implications.

Poultry Vector Vaccines: Innovative Serological Assays For Vaccination Monitoring And DIVA Testing For H5 Avian Influenza A

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Introduction

Influenza viruses belong to the Orthomyxoviridae family and are classified into four types: A, B, C, and D, based on their internal nucleocapsid antigen. Type A is the most conserved and is further divided into subtypes based on its Hemagglutinin (H) and Neuraminidase (N) antigens. Some subtypes containing H5 or H7 are associated with highly pathogenic forms of the disease and high mortality rates. Since 2004, a highly pathogenic H5 lineage has been circulating globally, causing significant losses in the poultry industry. Vaccination, particularly with recombinant technology, has been crucial for disease control. In Europe, successive waves of H5 Influenza have led to a revision of vaccination strategies. To facilitate serological monitoring, IDvet has developed two indirect ELISAs: one based on the H5 protein to evaluate recombinant vaccines and another based on NP protein for differentiating infected from vaccinated animals (DIVA strategy).

Methods

Layer and broiler flocks vaccinated with different technologies (H5 RNA, r-HVT-AI(H5), and inactivated AIV-H5 vaccines) were analyzed. Antibody titers were measured using H5 iELISA, and field challenges were monitored with NP iELISA. Samples with titers above 732 for H5 iELISA and 668 for NP iELISA were considered positive.

Results

All flocks vaccinated against H5 tested positive with the H5 iELISA. Some also tested positive with the NP iELISA, indicating exposure to an HxNy field strain. The positivity in H5 iELISA without reaction in NP iELISA confirmed vaccine-induced seroconversion.

Discussion

The H5 iELISA is the only quantitative test for detecting H5 antibodies and monitoring vaccination. The NP iELISA is an effective tool for identifying wild virus presence in populations vaccinated with recombinant H5 vaccines.

Production and Quality Assurance of Primary Cells for Research, Diagnostic Purposes, and Commercial Supply.

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Introduction

At the Animal and Plant Health Agency (APHA), we produce avian and mammalian primary cells for research, diagnostics, and commercial supply. Derived directly from animal tissue, these cells retain many in vivo characteristics, making them more physiologically relevant than immortalized cell lines. They are especially useful for isolating viruses or detecting extraneous viral agents due to their sensitivity, although their preparation is labour intensive, and they have a limited in vitro lifespan.

Method

Avian primary cells are sourced from specific pathogen-free (SPF) chick embryos or chicks, depending on the required cell type. Staff with honed dissection skills carefully isolate relevant tissues, and enzymatic digestion is used to dissociate the cells into a single-cell suspension. The yield is determined by packed cell volume. Cells are then resuspended in growth medium at a known concentration, and they can be provided as a suspension or cultured in flasks. All work is carried out in a sterile environment using batch tested media.
Each production batch undergoes quality control, monitoring cell growth, viability, and contamination. Shipping conditions are validated to ensure that cells remain viable for up to 72 hours when shipped at +4°C. All processes are ISO 9001 certified, ensuring strict quality control throughout.

Results

Cell batches are monitored for up to two weeks post-production for confluence and contamination, with immediate notification to customers if any concerns arise. Feedback from customers is sought and acted upon ensuring that high standards are maintained.

Discussion

APHA consistently delivers high-quality primary cells that meet customer requirements. These cells are supplied in optimal condition within the required timeframe, supporting both research, diagnostic and commercial needs.

Production of Recombinant African Horse Sickness Virus (AHSV) Antigens for Serological Diagnosis

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Introduction

African horse sickness, caused by the African horse sickness virus (AHSV), is a serious disease of equids on the WHOA list of notifiable diseases. While Europe is currently free from the disease, the risk of introduction is rising due to climate change and animal transport. Nine serotypes of this virus have been described. Its outer capsid protein viral protein 2 (VP2) is the main target for neutralizing antibodies, determining the serotype. The virus can be detected using PCR to detect the viral genome, or ELISA to detect disease-specific antibodies targeting the VP7, protein of the intern capsid. However, there is no ELISA test to identify the serotype, only a seroneutralization taking 5-6 days for results.

Methods

This study, part of the European SPIDVAC (Safe Priority Infectious Diseases VACcines) project, aims to develop a type-specific ELISA for AHSV. Using the Modified Vaccinia Virus Ankara (MVA) expression system, a recombinant AHSV-4 VP2 antigen was produced. The VP2 coding sequence was inserted into a pVote plasmid by recombination in the Gateway® cloning system. Vaccinia virus (VacV) was used to express the recombinant VP2 in BSR cells. After production in BSR cells, recombinant VP2 was purified by immunoprecipitation using a FLAG tag fused to the viral protein sequence, providing highly purified antigens.

Results

A dual antigen sandwich ELISA was developed using the VP2 recombinant protein as both antigen for plate coating and HRP-conjugated reporter. The performance of this ELISA was evaluated with more than 600 serum samples collected from AHS negative, vaccinated or infected animals. Results showed no cross-reactivity with the other 8 AHSV serotypes and the test's specificity and sensitivity were satisfactory.

Discussion

This type of dual antigen ELISA can detect specific antibodies in any species. This ELISA could therefore be used as diagnostic tool for rapid identification of the AHSV serotype.

Quality Assurance Checks on IFAT Slides for Parasitic Testing

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Introduction

Indirect Fluorescent Antibody Test (IFAT) slides play a vital role in diagnostic, import/export and international trade testing for several parasitic diseases e.g. Leishmania (canine) and Piroplasmosis (equine) at the Animal and Plant Health Agency (APHA). The production of IFAT slides can be problematic and variable due to the nature of growing parasites and loading them onto a slide. Due to the subjective interpretation of IFAT slides, it is vital that the presentation of the parasites on the slide is optimised, therefore quality assurance is crucial for producing reliable results.

Method

IFAT Slides at APHA are commercially sourced from reputable companies whereby a small batch is obtained for initial testing. If successful, a larger batch is purchased and subject to rigorous assessment of sensitivity and consistency using controls of a known antibody titre. A standardised approach is used to assess and document all results. All work is carried out in accordance with quality standard ISO9001.

Results

In some cases, only partial batches from commercial companies have been accepted due to variation of quality between slides. This is a direct result of APHA having its own quality acceptance criteria for all IFAT slides to meet before they can be issued for testing.

Discussion

Despite companies having their own quality control system APHA has an additional standardised method which ensures consistent documentation and processes as a baseline for assessing slides. Therefore, in-house quality assurance of IFAT slides underpins the high standard of results produced at APHA.

Rapid Methods of Salmonella Detection

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Introduction

Salmonella encompasses a genus of Gram-negative, motile, rod-shaped bacilli that are subclassified into two distinct species, called bongori and enterica. Salmonella enterica is further subdivided into six subspecies, which are further classified into over 2,600 described different serotypes. Unfortunately, Salmonella organisms are responsible for over 90 million cases of illness and 155,000 deaths in humans yearly around the globe.

Methods

One of the most important aspects of controlling and understanding Salmonella outbreaks is to serotype the organism responsible for causing illness. Historically, the gold standard is culture, isolation and the Kaufmann-White (KW) classification scheme. This is laborious, time-consuming, expensive and somewhat subjective. Molecular methodologies now exist that can rapidly be used to identify the serotypes of isolates with a high degree of accuracy. We have developed methods that utilize next generation sequencing (NGS) strategies to identify serotypes and correlate those analyses with the gold standard serotyping methodologies. The novel method uses intergenic sequence ribotyping (ISR) coupled with NGS in a single PCR followed by sequencing to identify serotypes in enriched samples without requiring further culture and isolation.

Results

The molecular protocol of ISR-NGS detected more positive samples and serotypes than standard culture methodologies on carcass rinses and chick papers and was equivalent to standard methods when processing boot swabs.

Discussion

Broiler breeder vaccination programs and Salmonella surveillance have been successfully used to control serotypes of human importance in broiler chickens. Utilizing this new technology, we can reduce the time to detect serotypes of human importance from weeks to days. Streamlining Salmonella workflows allows for more efficient use of resources and targeted isolation and characterization of specific serotypes to better serve the industry, and most importantly, protect consumers.

Retrospective Analysis of Laboratory Tests on Foot And Mouth Disease in Ukraine during 2017-2024

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Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hooved animals. FMD is endemic in South America, Africa, Asia, and parts of Europe, causing large-scale economic losses. Most infected cattle produce antibodies detectable for at least 6 months after infection. The antibodies to viral nonstructural proteins (NSPs) can be used as indicators of infection.

Methods

The sera collected from different types of animals according to state surveillance and monitoring of FMD, and were tested at The State Scientific Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise. All sera were tested using ELISA for FMD NSP (ID Screen® FMD NSP Competition ELISA, France; PrioCHECK™ FMDV NS Antibody ELISA) according to the manufacturer's instructions.

Results

195 cattle from 2 regions (Sumy and Kharkiv) were investigated in 2017. 2550 cattle and 250 sheep were investigated in 6 regions of Ukraine (Donetsk, Luhansk, Mykolaiv, Sumy, Kharkiv, and Chernihiv) in 2018. The zone of active surveillance was extended to the entire territory of Ukraine in 2019-2024. 21,508 cattle, 634 sheep, 263 roe deer, and 10 deer were examined during this period. The results of all tests were negative, and no positive animals were found.

Discussion

Serologic assays that detect antibodies to NSP by ELISA have shown specificity in assessing the postinfection state and offer various advantages for use in epidemiologic surveys. The biggest advantage of this test is safe, economical, and without the need for infectious viruses. Its use as a tool for monitoring viral activity and for certification of FMDV-free animals is recommended. Serological monitoring helps provide regional and global surveillance results needed to inform FMD status as the best approach to control strategies. FMD outbreak has not been reported in Ukraine for over 30 years. However, according to international requirements, conducting laboratory tests is a mandatory condition for confirming an FMD-free country.

Review of the use of PCR for diagnosis of contagious equine metritis: the case of non-reproducible Klebsiella positive samples in France in 2024

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Introduction

Contagious equine metritis (CEM), caused by Taylorella equigenitalis, is a notifiable disease, responsible for reproductive disorders making diagnostic essential before the breeding season. Klebsiella pneumoniae and Pseudomonas aeruginosa are also often associated with equine metritis. Laboratories test for these 3 pathogens systematically. Bacteriology is the gold standard method, but qPCR is accepted by several studbooks (International code of practice, HBLB). IDvet developed a new freeze-dried quadruplex qPCR kit for detection of the 3 pathogens with direct lysis protocol and evaluated its performances.

Methods

Samples from 2024 breeding season were tested with the new kit and results were compared to those obtained with PCR and bacteriology routinely used at LABEO.
Limit of detection and exclusivity were measured for each pathogen. Subsequently, 292 DNA extracts from fresh AMIES-charcoal swabs were tested randomly among samples received at LABEO.

Results

LDPCR was determined at 3.12, 37.5, and 6.25 copies/PCR for T. equigenitalis, K. pneumoniae, and P. aeruginosa respectively. For all 3 pathogens, measured sensitivity was 100% (n=89; CI95:95.9%-100%), with good exclusivity. Out of 292 samples taken from LABEO, none was positive for T. equigenitalis and 1 sample was positive for P. aeruginosa with both PCR methods. For K. pneumoniae, 178 samples were positive with PCR usually used at LABEO, while only 21 were positive on the new PCR and 4 were confirmed in bacteriology.

Discussion

The new PCR kit gives rapid results and shows better correlation with bacteriology regarding K. pneumoniae, which significantly simplifies the workflow while increasing the results accuracy.

Salmonella Derby: Undiscovered Pathogenesis in Swine Septicemia

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Salmonella enterica subsp. enterica serovar Derby is frequently isolated from swine. However, it is not considered a significant cause of swine diseases.
We cultured multiple specimens from two grower pigs from a swine farm in Saskatchewan, Canada, where multiple sudden deaths were reported. Salmonella sp. was isolated in high abundance from heart, synovium, liver and lung from one pig and from pericardium, abdomen, lung and naval from the other.

The isolates were identified as S. Derby by whole genome sequencing, using Oxford Nanopore Technology. Comparison of draft genomes against Public Health Agency of Canada (PHAC) national Salmonella surveillance database, using whole genome Multilocus Sequence Typing (wgMLST), reveals a unique cluster distinct from other S. Derby strains in the PHAC database. This cluster differed from S. Derby isolates commonly found in Canadian swine abattoirs and farms by more than 200 loci, suggesting that the isolates recovered from the septicemia cases may represent an emerging strain of concern. To investigate the genetic factors underlying its unusual pathogenicity, the genomes from the septicemia isolates were screened against the Virulence Factor Database (VFDB). However, no unexpected virulence genes were identified when compared to other strains in the National Center for Biotechnology Information (NCBI). Interestingly, a scan for mutations in conserved S. Derby genes revealed a single point mutation in a transcriptional regulator associated with Salmonella pathogenesis. This mutation introduces a premature stop codon, causing a major truncation that likely leads to a loss of function.

Hence, we hypothesize that this transcriptional regulator likely functions as an anti-activator whose wild type function is to suppress or interfere with the activities of master positive regulators of Salmonella pathogenicity, such as hilD and hilC. Through comparative genomics, our work suggests that dysregulation of Salmonella pathogenicity pathways may transform naturally benign strains into emerging pathogens of clinical significance

Sample to Vaccine via an Integrated Cross-Functional NGS Network

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Introduction

With new vaccine platforms on the rise, such as RNA vaccines, the need for prompt and accurate sequence data from currently circulating pathogens is of paramount importance to create the next generation of “digital” vaccines. At Ceva, an integrated, cross-functional, global NGS network exists with the goals of active surveillance and characterization of relevant pathogen genes of interest (GOIs), as it pertains to vaccine production. The basic structure of the network begins with a developmental hub to develop and troubleshoot 1) wet lab protocols for capturing desired sequence data and 2) computer tools for storing, moving, analyzing, and reporting sequencing results to Ceva groups. All the developed tools are then transferred to Ceva SSIU laboratories worldwide for real-world testing and analysis. Ultimately, data is centrally stored and searchable by authorized end users for vaccine production, business needs, and on-going customer support.

Methods

Wet lab protocols include both targeted and non-targeted sequencing strategies and involve both long read (Oxford Nanopore Technologies - ONT) and short read sequencing platforms. Data for long read sequencing is analyzed in real-time via Ceva’s proprietary CevaSEQ platform, which is made possible by the unique nature of ONT’s technology. CevaSEQ not only analyzes data in real-time and is accessible from anywhere in the world with an open internet connection, but also centrally stores the data, allows for deeper characterization/analysis of data via automated workflows, and provides the generation of automated full genome analysis reports at the touch of a button.

Results and Discussion

Current methodologies and success stories that will be discussed during the presentation include IBV, PRRSV, PCV2, Influenza A, IBDV, Streptococcus suis, and Salmonella spp. For evenness, several bottlenecks and pitfalls will also be discussed for the use of NGS data as it pertains to vaccine production.

Semi-Automated DNA Extraction Workflow for Mastitic Milk Samples

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Introduction

Accurate detection of mastitis-causing pathogens in milk requires efficient DNA extraction. Staphylococcus aureus and Streptococcus agalactiae are resistant to standard chemical lysis treatment and requires additional manual steps including bead beating and centrifugation for effective lysis and DNA recovery. This study developed a semi-automated extraction protocol combining both enzymatic and chemical lysis treatment for improved DNA recovery.

Methods

The protocol integrates newly developed ExtractPro cell-wall digesting reagent with chemical lysis using the VMRD magnetic bead-based extraction kit. It was optimized using Streptococcus agalactiae-spiked milk samples and compared to standard chemical lysis. All extraction steps, including cell lysis, DNA binding, washing, and elution, were automated on a KingFisher Flex instrument. The protocol was further evaluated against another commercial DNA extraction kit using Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma bovis spiked milk samples. Extraction efficiency between methods was assessed by quantitative PCR (qPCR) analysis.

Results

The optimized workflow significantly enhanced DNA extraction efficiency for Streptococcus agalactiae with an average Ct value reduction of 12 cycles across three 10-fold dilutions representing a 4096-fold increase in DNA recovery versus chemical lysis alone. Results for Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma bovis spiked milk samples were equivalent (within 1-2 Ct values) to a similar commercial extraction kit with separate offline proprietary reagent pre-treatment step.

Discussion

The developed extraction workflow with ExtractPro reagent shows significant improvement over standard chemical lysis alone. Integration of enzymatic and chemical lysis, significantly improves DNA recovery from difficult-to-lyse Staphylococcus aureus and Streptococcus agalactiae, eliminating the need for manual steps like bead beating and improving efficiency for high-volume testing labs.

Strangles Outbreaks Management with a new Double Antigen ELISA

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Introduction

Strangles is an equine bacterial infection caused by Streptococcus equi spp. equi. It affects the upper respiratory tract and is characterised by abscessation of the lymph nodes. Due to its rapid spread, the presence of asymptomatic carriers and its ability to cause considerable economic damage, strangles represents a major health concern for equids. Serological tools are useful to confirm an outbreak after PCR testing, to assist in sanitary management, and to assess the risk of haemorrhagic purpura. IDvet has developed a new double antigen ELISA for strangles and has evaluated its performance.

Methods

Serum samples from healthy, clinically affected and infected but asymptomatic horses were collected between 2014 and 2023. Samples tested are from 5 origins (i) a first outbreak in Normandy included 24 horses, symptomatic or not that were sampled during clinical signs and two weeks later, (ii) a second outbreak was in Lyon where 24 horses were sampled, (iii) 464 sera from a longitudinal study performed in France by LABÉO. For specificity assessment, 159 horses were sampled from Iceland where strangles has never been observed. For exclusivity evaluation, 8 horses from Southern France with respiratory symptoms and PCR-positive for S. equi subsp. zooepidemicus were tested.

Results

Measured specificity of the test was 98.5% (n=159). Individual detection rate among infected herds was 77% (n=76). There was no cross-reaction with antibodies against S. equi subsp. zooepidemicus.

Discussion

The new IDvet ELISA test offers reliable diagnostic performance and is useful for sanitary management of strangles.

Study of the Presence of Cryptosporidium spp. in Bovines and Humans in Dairy Herds of the Cundi-Boyacencian Plateau – Colombia

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Introduction

Cryptosporidium spp. is a zoonotic protozoan that causes neonatal bovine enteritis with C. parvum prevalence that ranges between 3,4% and 96,6% worldwide.
It is also a leading cause of morbidity/mortality in children under 5 years old and immunocompromised humans, causing severe diarrhea that can be fatal. The zoonotic transmission occurs in people that handle parasitized calves or by ingestion of contaminated water or food.
Studying this pathogen in the Cundi-Boyacencian region is essential to protect livestock and human health relying on its milk production.

Methods

A purposive sampling of 15 dairy herds in the departments of Boyacá and Cundinarmarca was done. Herds were required to have a minimum of 20 milking cows, mechanical milking and veterinary assistance. Ninety fecal samples from calves, 70 from cows and 16 from humans were obtained. No animals or humans were dewormed within the previous month.
The modified Ziehl-Neelsen staining was done according to the protocol by Smith (2008) and for ELISA according to the instructions of the Monoscreen-Ag-ELISA BIO K-346® Kit.

Results

A positivity of 11.1% (10/90) in calves and 8.6% (6/70) in cows was determined using the modified Ziehl-Neelsen staining. By ELISA, 7.7% of calves and 1.42% of cows were positive. Only two diarrheic calves were positive, one for each technique. Humans were negative by both techniques

Discussion

The overall positivity of 10% using modified Ziehl-Neelsen is higher than the ones reported in Chiquinquirá (7%) and in the Bogotá plateau (4.9%); however, it is lower than the reported by a study done in four Colombian departments with a positivity of 26.6%. These findings confirm the Cryptosporidium spp. presence in the Cundi-Boyacencian plateau.
Given the low ELISA's sensitivity, there is the possibility of false negatives in samples with low parasite levels (<5/0,01ml sample), this may explain the low detection in the study.

Successful Inactivation of High-Consequence Pathogens, Including Ebola and Lassa virus, in PrimeStore Molecular Transport Media

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Introduction

Safe handling and transportation of cultured, human, environmental, or wildlife specimens containing high-consequence pathogens pose considerable biosafety challenges, requiring high- or maximum-containment facilities, complex logistical procedures, and substantial financial resources. Hence, this study evaluates PrimeStore Molecular Transport Medium (PS-MTM) as a suitable reagent for the inactivation of representative viruses from seven different families known to cause highly fatal diseases in humans.

Methods

Inactivation testing using PS-MTM was performed by plaque assay for 20, 30, or 60 min on human whole blood samples inoculated with Ebola virus, Lassa virus, Hendra virus, Rift Valley fever virus, eastern equine encephalitis virus, and West Nile virus. Inactivation by PS-MTM was also assessed using tissue culture infectious dose (TCID50) assay on Crimean-Congo hemorrhagic fever virus, Ebola virus, Reston virus, Marburg virus, Nipah virus, Japanese encephalitis virus, Hendra virus, and Rift Valley fever virus. For the TCID50 assay, 30 min of inactivation with PS-MTM was used, which provided 50% safety margin over the manufacturer's recommended time.

Results

Inactivation of viruses (up to 107 PFU/mL) by PS-MTM as measured by plaque assay resulted in a complete loss of viral infectivity for all tested times. Inactivation of viruses (107 TCID50/mL) by PS-MTM as assessed by TCID50 assay resulted in complete inactivation of all tested viruses. Results from the two assays confirmed that PS-MTM inactivated all tested viruses at the highest tested titers.

Discussion

This study reports comprehensive inactivation testing of high-consequence pathogens in PS-MTM. The evaluated titers (104–107 TCID50/mL or PFU/mL) were similar to or exceeded those reported in clinical samples, highlighting PS-MTM's use as a reliable tool in enabling safer workflows for molecular diagnostics, outbreak management, surveillance, and clinical research.

Surveillance of Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma bovis in Alberta Dairy Herds using qPCR on Bulk Tank Milk Samples

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Introduction
Mastitis remains a significant concern in dairy herds, affecting milk quality and causing economic losses. This study aimed to establish a surveillance program for contagious mastitis pathogens using bulk tank milk samples and determine herd-level prevalence and associations with herd characteristics across Alberta.

Methods
Bulk tank milk samples were collected from all active dairy producers in Alberta four times (Dec 2021, Apr, July, and Oct 2022) and tested for Staphylococcus aureus, Streptococcus agalactiae, and Mycoplasma bovis using real-time qPCR. Apparent herd-level prevalence was calculated for each pathogen at each timepoint, with overall prevalence estimated as the proportion of herds testing positive at least once. A mixed-effects modified Poisson regression model identified associations between pathogen detection detection and herd characteristics.

Results
Staph. aureus showed the highest prevalence, with 34%, 22%, 7%, and 27% of farms testing positive in the four sampling periods, respectively. Overall, 59% of farms tested positive for Staph. aureus at least once. Strep. agalactiae and Mycoplasma bovis prevalences remained consistently low with <2% of farms testing positive at least once for each pathogen. The prevalence of Staph. aureus-positive herds was significantly lower in central (prevalence ratio (PR)=0.80, 95% CI=0.65–0.99) and south regions (PR=0.75, 95% CI=0.60–0.93) compared to the north. Farms with automated milking systems were more frequently positive (PR=1.41, 95% CI=1.17–1.69) compared to conventional systems. Small herds were less frequently positive (PR=0.81, 95% CI=0.67–0.97) compared to medium herds.

Discussion
These findings highlight Staph. aureus as a major concern in Alberta dairy herds, while Strep. agalactiae and Mycoplasma bovis were detected at low levels but warrant continued surveillance. Routine qPCR-based surveillance is valuable for early detection and management of mastitis, contributing to improved milk quality and herd health. This study provides updated epidemiological data on contagious mastitis pathogens in Alberta, informing targeted control strategies and disease management practices.

Syndromic Surveillance of Animal Welfare using Abattoir Condemnation Data: A Rapid Scoping Review

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Introduction

Syndromic surveillance is the identification of potential health threats using non-specific health indicators that have been drawn from pre-existing data that may have been collected for other reasons. Researchers have explored applying syndromic surveillance techniques to animal welfare, including using carcass condemnation data from abattoirs as a data source. This rapid scoping review aimed to explore how syndromic surveillance techniques have been applied to monitoring and interpreting carcass condemnation data from abattoirs to understand the links between reasons for condemnation and animal welfare factors.

Methods

The Scopus database was searched from January 2004 to November 2024. In addition, a grey literature search was conducted through Google advanced search in November 2024. Following the removal of duplicates, 703 records were identified. Articles were screened and included if they involved common terrestrial farmed animal species (poultry, cattle, swine, small ruminants) and analyzed the relationship between whole carcass condemnation data collected at abattoirs and animal- or resource-based animal welfare factors.

Results

After screening, 90 records underwent a full text review. Data was extracted into an Excel sheet and analyzed descriptively. The relationships between condemnation data and welfare factors were summarized by species, geographic region, and year of data collection. Key themes, opportunities, and limitations for using condemnation data as indicators of animal welfare were synthesized and discussed.

Discussion

Surveillance of animal welfare at abattoirs using condemnation data has limitations, including inconsistent record keeping and carcass evaluation within and between abattoirs. However, it presents a valuable routinely-collected source of data that may be incorporated into farm animal welfare monitoring schemes.

Thinking outside the laboratory: 10 years of a fallen stock collection center-based farm post mortem service in the United Kingdom.

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Introduction

Farm animal pathology services in the United Kingdom have traditionally been delivered by government organizations who subsidize disease investigation and diagnosis. In 2014, reduced government funding led to the closure of several regional laboratories. Farm Post Mortems Ltd was created to investigate the feasibility of delivering a private postmortem service from a fallen-stock collection center, to complement the remaining network. This presentation will describe the operation of this service, with the challenges, benefits and outputs over the first ten years of operation.

Methods

A private farm postmortem service was set up at a fallen stock collection center in Northern England. A basic postmortem room was created, with disposal of carcasses organized via the hosting site. Farmers were able to request postmortem examinations (PMEs) directly, and all diagnostic reports were returned to the private veterinarian with the farmer copied in where possible. As data collated, they were presented at national and international conferences and published in peer-reviewed papers. The service also hosted multiple agricultural and veterinary trainees, and delivered multiple courses where case material was used for teaching purposes.

Results

From 2014-2024, an estimated 12,000 PMEs were conducted on over ten different species (predominantly cattle and sheep), from approximately 300 different farms working with over 40 veterinary practices. The service has been involved in training one board certified pathologist, four board certified bovine clinicians, around 100 vet students, four veterinary pathologists working at other organizations, and multiple veterinarians and farmers. At least ten peer-reviewed publications have been published, including reporting index cases of five new disease syndromes.

Conclusion

This farm animal postmortem service has been successful in delivering useful information to veterinarians and farmers. There are many factors here that should be considered when farm postmortem services are being reviewed or initiated.

Third-Generation Sequencing as an Efficient Approach for Investigating the Brown Hare Microbiome

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Introduction

The brown hare (Lepus europaeus Pall.) plays a crucial role in both forest and agricultural ecosystems, acting as an essential link in trophic cascades and serving as a potential reservoir for zoonotic pathogens. Its gut microbiome is vital for metabolism and nutrient digestion. However, research on the digestive microbiome of the brown hare remains limited, and the existing literature is insufficient. Next-generation sequencing (NGS) facilitates high-throughput sequencing of short DNA fragments and is widely utilized in microbiome research. Nonetheless, the short read lengths of NGS complicate the assembly of complex bacterial genomes and the differentiation of closely related taxa. In contrast, third-generation sequencing (TGS) provides long-read sequencing capabilities, allowing full-length 16S rRNA gene sequencing and enhanced taxonomic resolution.

Methods

Large intestine contents from 30 hunted brown hares were pooled into three composite samples and analyzed by third-generation sequencing 16S and 18S rRNA (Oxford Nanopore). Results were systematically compared at accuracy thresholds of 95% and 80%.

Results

The comparative analysis of taxonomic overlap reveals significant differences in microbial community composition between the 80% and 95% sequence matching thresholds. At the 80% threshold, more taxa were identified across all taxonomic levels than the 95% threshold. The study identified previously unreported microbiome components, including Spirochaetota (25.2% at 80% threshold) and Ascomycota (4.3% at 95% threshold).

Discussion

Our study represents the most thorough characterization of the brown hare microbiome to date, facilitated by the use of third-generation sequencing technology and refined analytical methods. Our findings not only include previously identified taxa but also significantly enhance our understanding of the microbial diversity associated with this important wildlife species. Additionally, our methodological approach establishes a valuable framework for future studies on wildlife microbiomes, highlighting the significance of appropriate sequence-matching thresholds and comprehensive taxonomic analysis based on full-length 16S and 18S rRNA gene sequencing.

Ultrasound Noise: A Diagnostic Tool in a Laboratory Animal Facility

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Introduction

Laboratory Animal based scientific research relies on the quality of research models to achieve credible results with a strong internal validity. There are multiple factors that can negatively impact the wellbeing of laboratory animals and compromise the quality of scientific data collected from them. Noise levels and vibrations are examples of environmental stressors that may compromise animal welfare and introduce research confounders. Laboratory animals are exposed to various noises and vibrations emanating from different sources and activities such as laboratory personnel, HVAC systems, electronic and mechanical equipment used in the laboratory, maintenance, and construction work in and around the building. Exposure to excessive noise and vibrations has detrimental effects such as hearing loss in animals subsequently affecting their ability to communicate and breed. Given this fact, it is not difficult to understand the importance of detecting and minimising such stimuli.

Method

Using Turner Scientific's Sensory Sentinel, the UCT Research Animal Facility assessed mainly rodent animal housing areas with specific attention to noise levels (in decibels, dBA) vis-à-vis species specific audiograms (frequency, Hz) of housed rodents. Although other environmental parameters were recorded (such as temperature; humidity; and light intensity) the exercise focused on noise levels during various time-points in the animal facility to include various activities such as cage changing, health checks and room cleaning. This also included recording of noise levels during the use of equipment commonly utilized for routine husbandry procedures such as cage changing stations, vacuum cleaners, and portable humidifiers.

Results

Data were collected from thirteen rooms, by species. All were suitable for housing mice. One was high risk for Hamsters.

Discussion

Findings were used to determine species specific compatibility of rooms for housing of laboratory animals and identifying sources of detrimental intrinsic and extraneous ultrasound.

Validation of a Real-time PCR Assay Targeting the RNA-dependent RNA polymerase (RdRp) Gene of Alphacoronavirus

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Introduction

Members of the Alphacoronavirus are pathogens that cause mild to severe diseases in various animal species. This group includes the porcine transmissible gastroenteritis virus (TGEV), the feline coronavirus (FCoV) which encompasses the feline infectious peritonitis virus, and the canine coronavirus (CCoV). The Molecular Diagnostic Laboratory at the Cornell Animal Health Diagnostic Center previously offered a PCR test targeting the 3-UTR of the alphacoronavirus genome, followed by High-Resolution Melting (HRM) analysis for confirmation of detection. However, the challenge of interpreting HRM output prompted us to design and validate a new real-time PCR assay utilizing the RdRp gene multiplexed with Xeno as an exogenous control.

Methods

The primers and probe were designed based on a conserved region of the RdRp gene from TGEV, FCoV, and CCoV. Primer tree analysis was performed to establish specificity for Alphacoronavirus, confirming very low similarity to the RdRp sequences of Betacoronavirus. The TaqMan™ Fast Virus 1-Step Master Mix was chosen for its superior performance against common PCR inhibitors and a rapid run time of approximately 47 minutes. The positive amplification control was sourced as a gBlock. MagMAX CORE was employed for RNA extraction with the KingFisher Flex. The multiplex assay was validated on ABI 7500 and QuantStudio 5 PCR machines.

Results

The assay's precision for repeatability, both within a single run and across multiple runs, was within the acceptable range. The analytical sensitivity, or limit of detection, was determined to be 40 copies. The assay detected reference strains of major Alphacoronavirus pathogens, except the porcine epidemic diarrhea virus (PEDV). Exclusivity was confirmed by testing negative for Betacoronavirus, parvovirus, Cryptosporidium, Lawsonia intracellularis, and various host matrices.

Discussion

This assay is suitable for detecting major Alphacoronavirus pathogens, excluding PEDV, in feces, tissues, and bodily fluids from various hosts, including ferrets, minks, and raccoon dogs.

Whole Genome Sequencing Analysis of Global Paenibacillus larvae Population

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Introduction

American foulbrood (AFB) is a fatal infectious disease of honey bee brood caused by the bacterium, Paenibacillus larvae. Infected colonies with clinical signs of AFB must be destroyed by burning. In North America, prevention of AFB outbreaks heavily relies on oxytetracycline metaphylaxis, resulting in sustained selective pressure for oxytetracycline resistance in P. larvae populations. In contrast, the use of antibiotics in prohibited in EU and New Zealand.

Method

To investigate the presence of antimicrobial resistance genes (ARGs) and genetic diversity of global P. larvae populations, publicly available P. larvae genomes (23 from Canada, 163 from New Zealand, 154 from EU) were analyzed using the Nullarbor bioinformatics pipeline to detect the presence of ARGs and virulence genes. Genetic diversity was assessed using single nucleotide polymorphism (SNP)-based phylogenetic analysis and core-genome multi-locus sequence typing.

Results

The tetL tetracycline resistance gene was identified in 13 Canadian isolates; however, multiple other ARG`s (vanF, dfrC, mgrA, norA) were found in P. larvae isolates around the world. Multiple genotype-specific virulence genes were detected; however, presence of virulence genes was not associated with either ERIC type I or ERIC type II P. larvae genotype. Phylogenetic analysis revealed a clustering of the isolates based on their respective genotype and geographic location, with distinct P. larvae populations identified in NZ, the EU and Canada.

Discussion

Overall, this pilot study highlights the suitability of WGS analysis as a part of global P. larvae surveillance for monitoring the emergence and spread of antibiotic-resistant and highly-virulent isolates and tracing the geographic origin of these P. larvae variants.