ABSTRACT BOOK

5th CONGRESS OF THE EUROPEAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS
14 – 17 OCTOBER, 2018, MCE BUSINESS AND CONFERENCE CENTRE, BRUSSELS

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KL 01 CURRENT CHALLENGES IN TUBERCULOSIS DIAGNOSIS: NEW TESTS AND THEIR APPLICATIONS IN CONTROL STRATEGIES IN WILDLIFE

Christian Gortazar Schmidt

Animal tuberculosis (TB) is caused by infection with Mycobacterium bovis and closely related members of the Mycobacterium tuberculosis complex (MTC). It is a typical multi-host infection which in Europe involves several domestic hosts including cattle, goats, sheep and pigs, as well as several wild hosts, mainly the European badger (Meles meles), the Eurasian wild boar (Sus scrofa), and cervids such as red deer (Cervus elaphus) and fallow deer (Dama dama). The host range varies among geographical regions and new hosts are steadily added to the list of animal species contributing to MTC maintenance. Moreover, M. bovis is able to survive for some time in the environment, including water and mud or even salt stones. This creates remarkable challenges for TB eradication, particularly in multi-host systems with a strong environmental component of MTC transmission.

In cattle, TB testing is traditionally based on measuring the cell-mediated immune response to more or less MTC-specific antigens, most often to M. bovis (bovine) purified protein derivative (bPPD). Additional diagnosis takes place at slaughter, based on the detection of TB-compatible lesions and on MTC-culture. Similar tests are applied to other domestic MTC hosts, too, mainly goats. In wildlife and in the context of TB control at the wildlife-livestock interface, three main needs exist: (1) in-vivo tests for use in farmed or captive wildlife (deer farms, zoos), during wildlife translocations, or for capture-test-targeted culling schemes; (2) post mortem tests used for disease surveillance, epidemiology and intervention assessment purposes; and (3) environmental DNA detection, which is increasingly used in studies on transmission routes, biosafety assessment and herd-level risk certification. New tests are increasingly developing, generating opportunities for innovation in TB control.

In situations where wildlife and the environment contribute significantly to MTC circulation, TB-control schemes addressing only cattle, or only cattle and goats, are not enough: all suitable MTC maintenance hosts need to be taken into account. The first step is performing a proper epidemiological diagnosis, i.e. a comprehensive assessment of the role of each and every host and of the environment, as well as of their interconnections, in MTC maintenance. From a diagnostic point of view, this requires combining traditional diagnostic tools (applied to livestock) with post-mortem diagnosis (for instance for game species), serology (for wildlife and pigs, if present) and environmental DNA detection. Once the main players are identified, their populations and their TB prevalence need to be monitored through time. Inexpensive diagnostic tools are needed for this so-called integrated monitoring. Only after such an integrated monitoring scheme is running does it make sense to consider intervention. Again, combined, integrated disease control schemes are more likely to succeed. Such schemes will likely make use of biosafety and prevention, population control, and vaccination. An example of such a strategy is available (in Spanish) at https://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/patubes2017_3_tcm30-378321.pdf.
KL02 - ENZOOTIC DISEASES II - KEYNOTE LECTURE

KL 02  EMERGING AND RE-EMERGING PIG DISEASES AND INFECTIONS: WHAT CAN BE EXPECTED IN THE FUTURE?

Joaquim Segalés

Emerging infectious diseases (EID) are characterized by increasing incidence following its first introduction into a new host population or in an existing one as a result of long-term changes in its underlying epidemiology. This concept also includes those diseases linked to pathogens expanding into an area in which were not previously reported, or those that changed significantly its clinical-pathological presentation. The number of novel conditions in swine included under the concept of emerging and re-emerging diseases has increased significantly during last 20–30 years. Most of them are infectious diseases, being those of viral origin of great importance. Their transmissibility and maintenance into a population is favoured by a number of phenomena, including intensive rearing practices and globalized/international trading.

Besides those novel or re-emerging pathogens able to cause disease, there are a number of newly discovered viruses for which no evidence disease association does exist. For example, from 1985 to 2010, novel pathogen species (all types) were identified at an average annual rate of 3 in pigs. The advent of modern diagnostic and research methodologies, sometimes without the need of previous knowledge about the putative pathogen (i.e., high throughput sequencing), has increased significantly the number of microorganisms that are infecting animals. In consequence, a complex scenario with novel infectious agents with unknown importance is being faced nowadays by researchers and veterinarians.

The objective of the present review is to discuss about new swine diseases or novel presentations of already known diseases, as well as newly recognized infections with an unknown pathogenic effect in pigs. Such scenario implies to play with certainties and uncertainties, since last 30 years taught us about:

• The emergence of global diseases for which there is still not a clear definitive solution (i.e., porcine reproductive and respiratory syndrome)
• The emergence of global diseases for which the pathogen existed long before, but overt disease was only recognized recently (i.e., porcine circovirus 2-systemic disease)
• The emergence of global diseases for which the pathogen has apparently varied in virulence (i.e., porcine epidemic diarrhea)
• The emergence of diseases that were not geographically expected in certain parts of the world (i.e., African swine fever in the Russian Caucasus and now in China)
• The recognition of putative novel viruses for old diseases (i.e., atypical porcine pestivirus as cause of congenital tremors type All)
• The discovery of viruses that were not novel but considered potential causes of zoonosis (i.e., hepatitis E virus)
• The discovery of viruses that were not novel with unknown outcome related with its infection, although considered to be harmless (i.e., torque teno sus viruses)
• The discovery of viruses from which we do not have idea about its disease potential, if any (i.e., porcine circovirus 3)

The list of new recognitions, identifications and discoveries is much longer and will definitively increase in the future. Can we predict the impact of these new viruses?
KL03 - STANDARDIZATION, VALIDATION AND SURVEILLANCE - KEYNOTE LECTURE

KL 03  EUROREFERENCE: SHARING AND PROMOTING REFERENCE ACTIVITIES IN ANIMAL AND PLANT HEALTH, FOOD AND DRINKING WATER SAFETY

Bruno Garin-Bastuji

Euroreference is a European online journal (www.euroreference.eu), published twice a year, in English (UK) and dealing specifically with reference activities in the areas of animal & plant health and food & drinking water safety. It aims at facilitating information dissemination and sharing among all referencing stakeholders in the area.

The Euroreference magazine, created by ANSES in 2009, has been made available in 2016, after 13 issues coordinated by ANSES, in a new format produced jointly by a group of institutions from several European Union Member States as well as by EPPO[*], making it a collective publication devoted to promoting the dissemination of knowledge at European and international levels.

It offers scientific and technical articles of interest to health protection laboratories and institutions involved in reference activities throughout Europe. Euroreference is thus designed to help enhance efficiency across a range of European reference activities.

One of its challenges is to promote the exchange of tools and methods and to encourage closer relationships between reference laboratories in different regions and countries, as well as to publish specialist knowledge, with the aim of achieving a more robust and efficient animal, plant and food health protection system in Europe. Euroreference seeks to promote this dynamic approach at the European level.

EuroReference focuses on scientific knowledge and news and deals with numerous issues concerning reference lab activities: laboratory diagnostic and analytical methods, validation studies, standard and reference materials, reagent standardisation, inter-laboratory comparisons, scientific and technical results as well as European and international projects or networking in the area, regulations governing reference activities, standardisation and quality assurance, etc. It contains news, comments, letters, dispatches and scientific and technical original or review papers.

As a common forum for the members of the networks of reference laboratories and all the players concerned, the targeted public includes scientists, engineers and technical staff of testing laboratories, reference laboratories in other Member States, national reference centres, relevant Ministries and other regulatory or standardisation bodies, as well as several operators such as animal health veterinarians, physicians or chemists involved in public health. All articles are peer-reviewed by experienced scientists, one of which belonging to a consortium institution member. However authors may come from any scientific institution throughout the world.

Recently, a DOI number has been included on each article via Zenodo, the European Research data repository, with the aim of promoting the journal indexing in international library databases.

Our aim is to convert Euroreference to a truly European journal, thus strengthening the network of EU laboratories working in these sectors and consolidate EU efficiency.
**KL04 - EPIZOOTIC, EMERGING AND VECTORBORNE DISEASES OF LIVESTOCK - KEYNOTE LECTURE**

**KL 04  AFRICAN SWINE FEVER – BETWEEN THE POLES OF THE TEXTBOOK, HISTORY AND CURRENT SITUATION**

*Sandra Blome*

African swine fever (ASF) is one of the most important epizootic diseases threatening international animal health and profitable pig production in developed and developing countries. The eponymous virus, ASF virus (ASFV), is currently the only member of the Asfarviridae family and the genus Asfivirus contained therein. In its natural distribution area, the countries of Sub-Saharan Africa, the pathogen is transmitted asymptotically between soft ticks of the genus Ornithodoros and African wild boar (warthogs and bush pigs). However, if the pathogen enters the domestic pig population, severe, mostly fatal forms of disease associated with the symptoms of a viral haemorrhagic fever prevail. European wild boar are also fully susceptible and cannot be compared with African wild suids in terms of disease progression and dynamics. Out of the sylvatic cycle, the virus can be directly and indirectly transmitted without its competent vector.

Since 2007, highly virulent ASFV strains have spread across the Trans-Caucasian countries and the Russian Federation into the European Union. In the meantime, the disease affects the Baltic EU Member States, Poland, Hungary, the Czech Republic, and Romania. In many areas, wild boar are primarily affected, which makes the fight much more difficult, especially in the absence of safe and efficacious vaccines.

Arriving in the EU, the disease has shown a dynamic that was not predicted or assumed based on historical data: there was neither a self-limitation/extinction nor a rapid spread. For the first time, the disease was able to establish itself independent of domestic pigs in the wild boar population. Many transmission pathways and the epidemiology of the disease must therefore be re-reviewed and evaluated. In detail, there is a serious lack of knowledge regarding the possible role of mechanical vectors (tabanids, mosquitoes, hard ticks), the ultimate fate of convalescent, possibly virus-bearing animals, and the full characterization of the viral strains involved. Moreover, data wild boar biology are fragmentary when it comes to home range, behavior towards carcasses, intensity of social interactions and mingling of animals of different sounders etc.

The talks will focus on areas where textbook knowledge and current disease dynamics do not match. In addition, the outcome of recent studies will be addressed and discussed.
KL05 - EPIZOOTIC, EMERGING AND VECTORBORNE DISEASES OF LIVESTOCK II - KEYNOTE LECTURE

KL 05  BLUETONGUE IN EUROPE, THE PAST, THE PRESENT AND THE FUTURE

Piet van Rijn

Bluetongue (BT) caused by the insect borne orbivirus BTV (Orbivirus, Reoviridae) is a notifiable virus disease of ruminants according to the World Organization for Animal Health (OIE). The BTV particle contains ten genome segments S1-10 of double stranded RNA encoding seven structural proteins VP1-7, present in the virus particle, and at least four non-structural proteins NS1-NS4, expressed in the infected cell. Historically, the BTV species consists of 24 BTV serotypes showing no or low cross protection. BTV transmission is almost completely dependent on competent biting Culicoides midges. Large parts of the world are endemic for BTVs associated with the presence of competent midge species. In the last decade, expansion of affected areas has been shown; northwards in Europe and the USA and southwards in Australia. In the same period, new serotypes have been discovered. Currently, >28 serotypes have been recognized based on genetic analyses of S2. More importantly, BTV and its competent midge vector seem to establish in formerly BTV-free areas, likely because of global warming and changed environmental conditions beneficial for the competent midges.

Since the end of the 20th century, several BTV serotypes have emerged in Europe (1, 2, 4, 8, 9, 16) by expansion from northern Africa and the Middle East to southern European countries. In August 2006, North-West Europe was very surprised by incursion of BTV8, a serotype never recorded in or close to Europe before. Large parts of Europe were affected in two following years and has demonstrated re-circulation after the winter (‘overwintering’) which is considered as free of active midge species. In 2008, BTV6 and 11 closely related to conventional live attenuated vaccines (LAVs) were reported in NW Europe, although use of these LAVs was prohibited in Europe. Today, BTV is still present in southern Europe. Circulation of BTVs is very dynamic over the years and intends to expand northwards, like re-emerging of BTV8 in France, a new BTV4 reassortant in South East Europe, and expansion of BTV3 from Tunis to Sicily, Italy. Because of increased alertness and surveillance programs, new variants and atypical BTV serotypes 25 and 27 has been discovered in Europe. In summary, alertness and diagnostics have reduced losses due to Bluetongue. Safe, efficacious, and preferably broad protective or tailor-made DIVA vaccines but above all accepted and affordable vaccine will be needed to further minimize economic losses eventually aiming the eradication of Bluetongue. Compatible DIVA assays will be needed to survey vaccinated livestock for BTV circulation.

Laboratories and research institutions have developed and incorporated diagnostics for BTV and BTV antibodies. Proficiency testing well organized by the EURL for >10 years demonstrates that EU member states are well equipped to diagnose BT. Generally, serogroup specific real time RT-PCR tests and commercially available ELISAs are used for frontline diagnostics.

BTV detection by virus isolation (VI) as diagnostic test has been completely replaced by high throughput PCR diagnostics. VI is however still operational to culture virus from index cases aiming fundamental virological research. In general, panBTV PCR tests are used to confirm BTV infection followed by serotyping of the virus with serotype specific PCR assays or sequencing of S2. PanBTV PCR tests detect BTV as early as 1-2 days after experimental infection, irrespective of the serotype. Further, PCR positivity lasts much longer than the infectious period, e.g. up to 200 days post infection in cattle. Thus, PCR tests are suitable to diagnose BTV infected animals.

BTV PCR tests target BTV specific regions in several highly conserved genome segments, like S1 and S7, but also in highly targets in variable S10. Obviously, serotype specific PCR tests target highly variable S2 encoding serotype dominant VP2 protein. Serotype identification is important for epidemiological studies aiming to trace the source of a BTV outbreak. In addition, the serotype must be determined to vaccinate with the appropriate vaccine. Further, to detect wild...
type BTV in vaccinated livestock, DIVA PCR tests could be used compatible with used BT DIVA vaccine. Finally, a tremendous progress in the field of next generation sequencing will shorten the time between detection through frontline panBTV PCR testing and detailed like full genome information. Full genome sequences of all kind of BTV variants will become massively available in the future. This genetic information is important for in silico validation of panBTV frontline diagnostics and serotype specific PCR tests.

Serogroup specific ELISAs detecting antibodies against immunodominant VP7 protein of BTV are commercially available. Several setups of VP7-based high throughput ELISAs such as competition -, indirect -, and sandwich ELISAs have been developed. All assays are sensitive and specific and detect animals as early as one week after experimental infection. VP7 antibodies can also be detected in milk samples. Milk samples of professional dairy cattle farms are often available as these are collected for surveillance programs of other bovine diseases. Detection of BTV-antibodies is very sensitive, and a very low percentage of seropositive lactating cows can be detected by testing one single sample of bulk milk. VP7-based ELISAs cannot be used as DIVA assays, since applied BT vaccines are traditionally virus-based BT vaccines such as LAV or inactivated BTV vaccine inducing high antibody titres against VP7. Neutralizing antibodies are serotype specific but are less sensitive and are only detected later after infection. Anyway, neutralization assays are also time consuming, laborious and therefore expensive. There is a need for serotype specific ELISAs, Elispot assays or other serological tools to determine the serotype specific immune response. At the moment, there is a strong preference for serotype specific PCR assays or next generation sequencing to determine the serotype of circulating BTV.

Expectedly, Bluetongue will maintain and re-emerge in Europe and other parts of the world in the coming decades. Quality controlled, high throughput, rapid BT diagnostics has significantly improved disease control but rapid serotype specific diagnosis and detailed identification of emerging BTVs should be improved. Further, safe, efficacious, affordable and generally accepted BT DIVA vaccines with will reduce losses. For this, highly specific and sensitive DIVA assays that are compatible with the used vaccine must be developed and validated, primarily by comparison with frontline diagnostics for Bluetongue.
KL06 - ONE HEALTH: FOODBORNE PATHOGENS, ZOONOSIS AND ANTIBIORESISTANCE - KEYNOTE LECTURE

KL 06  IT'S TIME TO ACT: THE EU ENGAGEMENT AGAINST ANTIMICROBIAL RESISTANCE

Antonia Ricci

Antimicrobial resistance is a wide and complex concept, which involves different competences in the framework of veterinary public health.

Monitoring and surveillance of antimicrobial resistance have the following main objectives:

- detect emergence, and understand dissemination of AMR.
- provide data relevant for risk assessment
- plan interventions and measure their effects.

According to this strategy, at European level the three agencies competent in this sector (EFSA – European Food Safety Authority, ECDC – European Center for Disease prevention and Control and EMA – European Medicines Agency) are carrying out several activities, some of them summarised below.

Analysis of antimicrobial use and resistance (JACRA)

Following the publication of the first report in 2015, the second ECDC/EFSA/EMA joint report on the integrated analysis of antimicrobial consumption (AMC) and antimicrobial resistance (AMR) in bacteria from humans and food-producing animals addressed data obtained by the Agencies’ EU-wide surveillance networks for 2013–2015 (EFSA Journal 2017;15(7):4872,135 pp. doi:10.2903/j.efsa.2017.4872). AMC in both sectors, expressed in mg/kg of estimated biomass, were compared at country and European level. Substantial variations between countries were observed in both sectors.

Univariate and multivariate analyses were applied to study associations between AMC and AMR. In 2014, the average AMC was higher in animals (152 mg/kg) than in humans (124 mg/kg), but the opposite applied to the median AMC (67 and 118 mg/kg, respectively). In 18 of 28 countries, AMC was lower in animals than in humans. Univariate analysis showed statistically-significant associations between AMC and AMR for fluoroquinolones and Escherichia coli in both sectors, for 3rd and 4th-generation cephalosporins and E. coli in humans, and tetracyclines and polymyxins and E. coli in animals. In humans, there was a statistically-significant association between AMC and AMR for carbapenems and polymyxins in Klebsiella pneumoniae. Consumption of macrolides in animals was significantly associated with macrolide resistance in Campylobacter coli in animals and humans.

Multivariate analyses demonstrated that 3rd- and 4th-generation cephalosporin and fluoroquinolone resistance in E. coli from humans was associated with corresponding AMC in humans, whereas resistance to fluoroquinolones in Salmonella spp. and Campylobacter spp. from humans was related to consumption of fluoroquinolones in animals. These results suggest that from a ‘One-health’ perspective, there is potential in both sectors to further develop prudent use of antimicrobials and thereby reduce AMR.

Joint EFSA-EMA opinion on the reduction of the need to use antimicrobials in animal husbandry (RONAFA)

This Scientific Opinion (EFSA Journal 2017;15(1):4666, 245 pp. doi:10.2903/j.efsa.2017.4666) was published by EFSA and EMA following the request of the European Commission to review measures taken in the EU to reduce the need for and use of antimicrobials in food-producing animals, and the resultant impacts on antimicrobial resistance (AMR).
Reduction strategies have been implemented successfully in some Member States. Such strategies include national reduction targets, benchmarking of antimicrobial use, controls on prescribing and restrictions on use of specific critically important antimicrobials, together with improvements to animal husbandry and disease prevention and control measures. Due to the multiplicity of factors contributing to AMR, the impact of any single measure is difficult to quantify, although there is evidence of an association between reduction in antimicrobial use and reduced AMR. To minimise antimicrobial use, a multifaceted integrated approach should be implemented, adapted to local circumstances.

Recommended options include: development of national strategies; harmonised systems for monitoring antimicrobial use and AMR development; establishing national targets for antimicrobial use reduction; use of on-farm health plans; increasing the responsibility of veterinarians for antimicrobial prescribing; training, education and raising public awareness; increasing the availability of rapid and reliable diagnostics; improving husbandry and management procedures for disease prevention and control; rethinking livestock production systems to reduce inherent disease risk.

**Joint ECDC-EFSA-EMA opinion on outcome indicators on surveillance of AMR and use of antimicrobials**

This is also a joint opinion by ECDC, EFSA and EMA, which have established a list of harmonised outcome indicators to assist European Union Member States in assessing their progress in reducing the use of antimicrobials and antimicrobial resistance (AMR) in both humans and food-producing animals (EFSA Journal 2017;15(10):5017, 70 pp. https://doi.org/10.2903/j.efsa.2017.5017 ISSN:1831-4732).

For humans, the indicators for antimicrobial consumption include: total consumption of all antimicrobials for systemic use, ratio of community consumption of certain classes of broad-spectrum to narrow-spectrum antimicrobials, and consumption of a series of both broad- and narrow-spectrum antimicrobials frequently used in healthcare settings. Proposed indicators for AMR in humans are: meticillin-resistant Staphylococcus aureus and 3rd-generation cephalosporin-resistant Escherichia coli, Klebsiella pneumoniae resistant to several important antimicrobials, penicillin- and macrolide-resistant Streptococcus pneumoniae, and emerging carbapenem-resistant Klebsiella pneumoniae. For food-producing animals, indicators for antimicrobial consumption include: overall sales of veterinary antimicrobials, sales of 3rd- and 4th-generation cephalosporins, sales of quinolones, and sales of polymyxins. Finally, indicators for AMR in food-producing animals are proposed: full susceptibility to a predefined panel of antimicrobials, proportion of samples containing ESBL-/AmpC-producing E. coli, multi-drug resistance, and resistance to ciprofloxacin, in indicator E. coli. For all sectors the chosen indicators, which should be reconsidered at least every five years, are expected to be valid tools in monitoring antimicrobial consumption and AMR.
Infectious livestock diseases pose important threats to sustainable food production, with viral diseases such as foot-and-mouth disease (FMD) amongst the most important. Accurate and rapid diagnostic tests are an essential component of contingency plans to detect, control and eradicate these threats. Diagnosis currently involves a pipeline that starts with clinical suspicion, followed by collection of samples, transport of specimens to a centralised laboratory setting (e.g. national / international reference laboratories), analysis of samples using a range of diagnostic tests and reporting of results. However, the transport of specimens from the field to the laboratory can be a lengthy process that can delay critical decision-making and severely affect the quality of the samples. Furthermore, many diagnostic tests require well-equipped laboratories, often problematic for endemic countries which lack infrastructure and financial resources for disease surveillance and diagnostics. These important limitations of centralised diagnostic testing have motivated the development of prototype tools for the rapid, simple detection of livestock pathogens, based on advancements in the development of technologies for personalised human medicine. However, many of these tests are not yet routinely used or commercially available. In this talk, I will discuss the most promising examples of such assays, and highlight the challenges that remain to transition these tests from applied research and development into routine use.
ORAL PRESENTATIONS
O 01 MYCOBACTERIUM AVIUM PARATUBERCULOSIS ANTIBODIES IN MILK AS A PROGNOSTIC INDICATOR OF JOHNES DISEASE IN DAIRY COWS

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Introduction:
Milk antibody testing by elisa is commonly used as part of health management programmes for the control of Johnes disease in dairy herds to define cows that are likely to be shedding mycobacterium avium paratuberculosis (MAP). Regular testing provides a testing history for individual animals which can be used as a prognostic indicator for clinical or subclinical Johnes disease.

Materials and Methods:
The individual milk test results for over 3000 dairy cows which were tested as part of commercial Johnes control programmes were examined. Cows with at least four high elisa results in their lifetime were studied to determine their health and survival beyond the first positive test result. Each animal was categorised into one of three groups according to the testing history, and the prognosis correlated to each of the three patterns of test results:

Results:
Cows with consistely high MAP elisa results are shown to be most likely to be suffering from health problems associated with subclinical Johnes disease, and are likely to succumb to clinical Johnes disease if retained in the herd. Cows with rising MAP elisa results are the most likely to have a poor prognosis.

Discussion and Conclusion:
The lifetime history of MAP elisa tests on milk can be used to determine the prognosis of cows when used as part of a MAP control programme, enabling the selection of cows for culling to minimise the economic losses from poor health and clinical Johnes disease in affected cattle.
OP01 - ENZOOTIC DISEASES - ORAL PRESENTATIONS

O 02 DETECTION OF ACTIVE INFECTION OF NEW-BORN CALVES BY MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) IN FIRST DAYS OF LIFE

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Introduction:
In herds monitored for paratuberculosis, it is essential to detect active infection in new-born calves (either transmitted by the mother or other calves) as early as possible to exclude them from the breeding herd.

Current diagnostic tools allow reliable detection from only 18-24 months and are not able to distinguish between active and passive infection. Therefore, it is essential to develop new diagnostic tools, easily used by routine testing laboratories, to detect low levels of MAP potentially present in blood of new-born calves.

Materials and Methods:
Three dairy cattle herds were selected in terms of prevalence and clinical expression (Table 1).

Table 1: Specifications of herds selected for the study

<table>
<thead>
<tr>
<th>Herd</th>
<th>Percent of MAP prevalence (cattle MAP-POSITIVE by ELISA, BioLisa® Kit MAP Ab or by PCR, Bio-T kit® MAP on faeces)</th>
<th>Clinical expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19%</td>
<td>No clinical expression</td>
</tr>
<tr>
<td>B</td>
<td>47%</td>
<td>No to mild clinical expression</td>
</tr>
<tr>
<td>C</td>
<td>17%</td>
<td>Severe clinical expression leading to death</td>
</tr>
</tbody>
</table>

We pre-selected 24 MAP-Positive and 38 MAP-Negative females, calving at the same time, to create a cohort of calves born from either shedding and non-shedding cows. The status of the females was confirmed by PCR (Bio-T kit® MAP, BioSellal) on faecal samples before and after calving.

Each calf was monitored monthly from birth, over a six-month period, using Actiphage-PCR on whole blood (Actiphage™Rapid kit, PBD Biotech and Bio-T kit® MAP) and also PCR on faecal samples.

Results:
Natural, active infection was identified by detection of MAP in blood samples using Actiphage-PCR from the first day of life, but bacteraemia was transient (Table 2).
Discussion and Conclusion:
Using an optimized, easy-to-use Actiphage-PCR protocol, it was possible to detect active infection by MAP in blood of calves with good reproducibility of results. The early detection of MAP infection by Actiphage-PCR will be very useful to monitor and improve preventive measures to limit the spread of paratuberculosis on farms.

Table 2: Results of MAP detection on calves followed monthly from birth until 6 months by Actiphage-PCR on blood samples (active infection) and PCR on faeces samples (passive infection)

<table>
<thead>
<tr>
<th>Herd</th>
<th>Separation of calving boxes according to the MAP status of cows before calving</th>
<th>Calves born from MAP-POSITIVE cows before and/or after calving</th>
<th>Calves born from MAP-Negative cows before and after calving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females removed from mothers within 15 minutes of birth, nursery in individual boxes for 2 weeks, then collective boxes by age group until weaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colostrum intake of the mother even for MAP-POSITIVE cows, mother milk for 3 days, then milk powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd A</td>
<td></td>
<td>Total No. of calves</td>
<td>Actiphage-PCR POSITIVE on blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Herd B</td>
<td></td>
<td>Total No. of calves</td>
<td>Actiphage-PCR POSITIVE on blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 calves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 calves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>Female - at 1 day of life - Ct 33.86 - Negative after</td>
</tr>
<tr>
<td>3 calves</td>
<td></td>
<td></td>
<td>Male - at 15 days of life - Ct 38.72 - Negative after</td>
</tr>
<tr>
<td>Herd C</td>
<td></td>
<td>Total No. of calves</td>
<td>Actiphage-PCR POSITIVE on blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 calf</td>
<td></td>
<td></td>
<td>Male - at 84 days of life - Ct 36.82 - Negative before and after</td>
</tr>
</tbody>
</table>
O 03 CONSTRUCTION OF THE REFERENCE STANDARD FOR THE DETECTION AND QUANTIFICATION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS BY QUANTITATIVE PCR IN FAECES

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Introduction:
Nowadays, quantitative PCR (qPCR) becomes a frequent method for the direct detection of Mycobacterium avium subsp. paratuberculosis (MAP). Qualitative interpretation of qPCR data is not sufficient for the determination of infectious status of the animal due to the effect of passive shedding. Therefore, precise quantification of MAP is essential. However, the quantity of MAP assessed by qPCR is dependent on the type of the qPCR quantification standard. In practise this means that quantities of MAP determined by the different qPCR assays in different laboratories are not comparable with each other. The idea of reference standard should bring the unification of the DNA standards in qPCR quantification of MAP to enable to compare quantitative data.

The aim of this study was to prepare a reference quantification standard for the determination of MAP quantity in faeces by qPCR, choose the suitable storage matrix and test the stability and repeatability over the time.

Materials and Methods:
Supernatant of the faecal suspension was artificially contaminated with MAP reference strain, mixed with the matrix and lyophilized. Different types of matrices were tested. Repeatability and stability of the MAP reference standard in time were tested by qPCR.

Results:
We have selected the most suitable lyophilization matrix and tested stability of the reference standards in time.

Discussion and Conclusion:
The initial batch if reference standard is now available for the testing in routine laboratories. As OIE RL for paratuberculosis we informed OIE Biological Standards Commission to include qPCR standard among OIE-Approved Reference Standards.

This work was supported by projects Vl20152020044 and RO0518.
**OP01 - ENZOOTIC DISEASES - ORAL PRESENTATIONS**

**O 04 EARLY DIAGNOSIS OF MYCOPLASMA HYOPNEUMONIAE: COMPARISON OF THREE DIFFERENT LIVE PIG SAMPLING TECHNIQUES**

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2 Swine Veterinary Center, Dept. Swine Medicine, St. Peter- MN, USA
3 Minnesota University, Veterinary Population Medicine Department & Veterinary Diagnostic Laboratory, St. Paul- MN, USA
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**Introduction:**
Mycoplasma hyopneumoniae diagnosis in live pigs remains a challenging issue under field conditions. Previous studies have shown that serology only results in seroconversion after at least 21 days, whereas under field conditions, an earlier diagnosis would assist an earlier intervention in terms of preventive vaccination or treatment aiming to reduce the use of antimicrobials. Therefore, the objective of the current study was to evaluate the sensitivity of three different live pig sampling techniques towards the recovery of *Mycoplasma hyopneumoniae* under field conditions.

**Materials and Methods:**
Three different live pig sampling techniques in order of sampling, tracheo-bronchial swabs sampling (TBS), laryngeal swab sampling (LS) and collection of pharyngeal mucus by a spoon method (SP) were compared under practical conditions in a farm with clinical respiratory problems related to *Mycoplasma hyopneumoniae*. The samples were collected from the same pig (TBS>LS>SP) and put on ice until analysis in the laboratory using a qPCR.

**Results:**
The mean outcomes of the Ct values of the three methods were statistically tested for equality. Overall F-test revealed significant differences (P < 0.05) between TBS and LS/SP. No significant differences were observed between LS and SP.

**Discussion and Conclusion:**
TBS sampling revealed to be the most sensitive sampling technique in live piglets related to the obtained Ct values, followed by LS and SP, although the latter were not significantly different. In conclusion, live pig sampling for the detection of *Mycoplasma hyopneumoniae* could be most optimally performed using TBS, although other techniques such as LS/SP could also prove effective depending on the resources available.
OP02 - ENZOOTIC DISEASES II - ORAL PRESENTATIONS

O 05 EXPERIMENTAL INOCULATION OF DOGS WITH CANINE CIRCOVIRUS-1 (CANINE CV): CLINICAL SIGNS, VIRUS SHEDDING AND TISSUE DISTRIBUTION

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3 Michigan State University, Veterinary Diagnostic Laboratory, Lansing, USA

Introduction:
CanineCV has been detected in several countries, mostly associated with enteric disease and often in combination with other pathogens. We previously reported the involvement of CanineCV and CPV-2 in recurrent outbreaks of fatal bloody diarrhea and granulomatous inflammation in a breeding colony. The goal of this study was to reproduce infection and potentially disease in dogs infected with CanineCV only.

Materials and Methods:
Dogs were experimentally inoculated (n=2) with CanineCV-positive serum, which tested negative by PCR for other canine viral pathogens, or sham-inoculated (n=1). Feces, whole blood and serum were collected at defined intervals and tested by PCR for CanineCV. Dogs were euthanized at 30 dpi. Collected tissues were examined histologically, by in situ hybridization (ISH) and transmission electron microscopy (TEM). Tissues were tested by PCR.

Results:
Both CanineCV inoculated dogs developed grade 3 of 4 diarrhea that lasted for several days. Very high levels of CanineCV DNA were detected by PCR in various tissues. ISH demonstrated abundant amounts of CanineCV DNA in the cytoplasm and nuclei of histiocytic infiltrates in all lymphoid organs and intracytoplasmic inclusion were detected by TEM. Significant levels of CanineCV DNA were detected in blood and serum throughout the study.

Discussion and Conclusion:
To our knowledge, this is the first experimental inoculation of dogs with CanineCV. Infected dogs developed diarrhea, concomitant with fecal shedding. High level viremia of both cell-free and cell-associated virus were detected. The presence of characteristic lesions, linked to viral replication, support the pathogenic potential of CanineCV.
O 06 DETECTION OF ANTIBODIES TO VARIOUS EUROPEAN AND ASIAN STRAINS OF VARIANT INFECTION BRONCHITIS VIRUS USING COMMERCIAL ANTIBODY ELISA KITS

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² Zoetis, Diagnostics, Lyon, France
³ GD Animal Health, R&D Department, Deventer, Netherlands

Introduction:
Infectious bronchitis virus (IBV) infection is usually a mild respiratory disease in young birds but may also result in drop in egg production in layers and airsacculitis in broilers. It remains economically important due to the performance and economic losses to producers. The virus easily mutates producing new variant strains and serotypes that may cause disease despite vaccination. The rapid detection of these new variant strains is critical in controlling the disease. A series of tests was conducted to demonstrate the ability of antibody ELISA tests to monitor immune status of vaccinated flocks and detect new disease challenge from variant IBV serotypes.

Materials and Methods:
The first part of the study monitored the serologic immune response of birds at various time points post vaccination. The second part compared sera from IBV-vaccinated broilers before and after experimental challenge with 793B, Q1 or Variant 2 IBV serotypes. The third part of the study tested a panel of 10 different monospecific sera to demonstrate detection of antibodies to the variant IBV serotypes.

Results:
Some ELISA kits detected the immune response to various IBV vaccination programs. Immune response to infection was detected as early as 5 days post-challenge even in vaccinated birds. ProFLOK® IBV Ab ELISA kits detected all IBV monospecific serotypes tested while some kits did not detect all serotypes.

Discussion and Conclusion:
Detection of immune response to variant IBV serotypes differs between ELISA tests. ProFLOK IBV Ab ELISA was able to detect antibodies to common and all variant IBV serotypes tested, as early as 5 days post-challenge.
O 07 MOLECULAR IDENTIFICATION OF PCV3 INFECTION IN SEVERAL SPANISH PIG FARMS

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Introduction:
Two species of PCV are well studied at date; PCV1 which is considered non-pathogenic and PCV2 that causes significant economic impact in swine industry worldwide. Recently, a novel PCV3 has been identified in sows affected by PDNS and reproductive failure; however, there is still sparse information about this agent. This study describes a qPCR assay for detection of PCV3 and preliminary survey for this virus in Spain.

Materials and Methods:
A qPCR assay targeting the cap gene was developed for identification of PCV3. A total of 97 clinical cases (abortion=28; respiratory=69) from Spain (n=91) and Portugal (n=6) were evaluated. Samples included fetal tissue, lung, oral fluid, swabs and BALF; and were also tested for PCV2, PRRSV and SIV. Nucleotide sequences of cap gene from six Spanish PCV3 samples were obtained.

Results:
The qPCR assay showed specific detection of PCV3 and this agent was identified in 10% of clinical cases, in 5 of 17 Spanish provinces and mainly in animals with respiratory disease (9/69). PCV3 was detected in all different kind of samples, but always in co-infection with other pathogens. Sequences of Spanish-PCV3 showed 99%-100% homology to sequences available in the GenBank.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Total</th>
<th>PCV3 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal tissue</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>Respiratory swabs</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Oral fluid</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>BALF</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>
Discussion and Conclusion:
Here in, we describe PCV3 infection on animals from several pig farms in Spain, but also from different provinces suggesting a remarkable distribution of this virus in our country. Although most data associate PCV3 with PDNS and reproductive failure, we found most positive cases in pigs with respiratory disease. However, further studies are necessary to investigate pathogenicity and epidemiology of this agent.
OP02 - ENZOOTIC DISEASES II - ORAL PRESENTATIONS

08 COMPARISON OF DIFFERENT SEROLOGICAL AND MOLECULAR TESTS FOR THE DETECTION OF SMALL RUMINANT LENTIVIRUSES (SRLVs) IN BELGIAN SHEEP AND GOATS

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2 DGZ, Dierenarts Gezondheidszorg- Begeleiding en Ontwikkeling, Lier, Belgium
3 Arsia, Laboratoire & Diagnostic, Ciney, Belgium
4 Sciensano, Coordination of Veterinary Activities, Brussels, Belgium

Introduction:
Small ruminant lentiviruses (SRLVs) cause animal health problems and economic losses in small ruminants worldwide. Since no therapies exists, good diagnostic tools are crucial for the control of the disease. Here, we compared the performance of different diagnostic tests.

Materials and Methods:
5 commercial ELISAs, 2 AGIDs and one inhouse qPCR were used to analyze (i) sera and PBMCs of 553 sheep and 394 goats coming from a seroprevalence study; (ii) 50 sera from known SRLV positive sheep and goats; (iii) sequential sera and PBMCs from experimentally infected sheep and goat.

Results:
Unexpectedly, combining both AGIDs resulted in 100 % sensitivity and specificity in both sheep and goat samples. An overall good performance was also found for the ELISAs with some differences between the 5 kits. The highest sensitivities in the sheep study were observed in the Hyphen (98.0 %) and IDVet (100 %) kits. In goats, IDVet, LSI and IN3 kits showed a sensitivity of 100 %. Where the Idexx ELISA was the least sensitive kit, it showed to have the highest specificity in both sheep (99.6 %) and goats (100 %).

The qPCR showed to be highly specific in both species (100 %), but the sensitivity remained sub-optimal with 87.5 % in sheep and 83.3 % in goats.

Almost all tests allowed detection of SRLV infection within 28 dpi, with first positive detection via qPCR at 14 dpi.

Discussion and Conclusion:
A combination of sensitive and specific ELISAs seems most efficient for SRLV detection in control programs. AGIDs and qPCR remain useful to confirm the true infection status of an animal.
OP02 - ENZOOTIC DISEASES II - ORAL PRESENTATIONS

O 09 VALIDATION ACCORDING TO OIE CRITERIA OF THE SALMONELLA ABORTUSOVIS TEST

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2 Diatheva srl, Production, Cartoceto, Italy
3 Diatheva srl, Sale, Cartoceto, Italy
4 University of Urbino “Carlo Bo”, Biomolecular Sciences, Urbino, Italy
5 Zooprofilattico Sperimentale dell’Abruzzo e del Molise, Reparto di Sierologia Centro di Referenza Nazionale per le Brucellosi, Teramo, Italy
6 Instituto Nacional de Investigación Agraria y Veterinaria- I.P. INAV- IP, Oeiras, Portugal
7 Soclab laboratório Lda, Ribeira São João, Portugal
8 Istituto Zooprofilattico Sperimentale della Sardegna “G. Pegreffi”, Laboratorio di Sierologia e Virologia diagnostica, Cagliari, Italy

Introduction:
Salmonella abortusovis is host-adapted to sheep causing infections characterized by abortion. The high incidence represents a major threat to the flocks resulting in important economic losses. It is among the diseases listed by OIE and, in several countries, included in Regulations defining the sanitary conditions for sheep trade and movement. The aim of the study was to validate the Salmonella abortusovis Test (Diatheva) to ensure that such test can establish the disease with a high confidence level.

Materials and Methods:
The test was validated according to OIE criteria. 321 sheep sera samples (reference, naturally infected, vaccinated and negative) were examined to determine: cut-off, analytical and diagnostic characteristics, reproducibility. Precision and accuracy were also defined using results examined in an inter-laboratory trial.

Results:
The cut-off was calculated and expressed as SP ratio ≤0.350. Analytical sensitivity was determined to be 1:12800 by endpoint titration. Analytical specificity was evaluated by testing seven sera from sheep exposed to other pathogens. No cross reactions were observed, with exception for Chlamyphila psittaci and Brucella melitensis which may produce faint response. The positive and negative Likelihood ratio were 48 and 0.02 respectively. Repeatability inter/intra assay and Reproducibility inter laboratories results are reported in Tab 1A , 1B. The diagnostic accuracy was considered high as the area under the curve index was 0.99. Diagnostic characteristics are detailed in Tab 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CV Intro-assay</th>
<th>CV Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>2%- 9%</td>
<td>10%</td>
</tr>
<tr>
<td>Ctr-</td>
<td>5%- 15%</td>
<td>15%</td>
</tr>
<tr>
<td>Negative</td>
<td>5%-10%</td>
<td>15%</td>
</tr>
<tr>
<td>Low titre</td>
<td>4%-15%</td>
<td>15%</td>
</tr>
<tr>
<td>Medium titre</td>
<td>1%-6%</td>
<td>15%</td>
</tr>
<tr>
<td>High titre</td>
<td>1%-7%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Table 1A: Repeatability Study

<table>
<thead>
<tr>
<th>Sample</th>
<th>CV Intro-assay</th>
<th>CV Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr+</td>
<td>0.2%-3%</td>
<td>1%</td>
</tr>
<tr>
<td>Ctr-</td>
<td>0%-11%</td>
<td>11%</td>
</tr>
<tr>
<td>Negative</td>
<td>1.5%-21%</td>
<td>21%</td>
</tr>
<tr>
<td>Low</td>
<td>2%-10%</td>
<td>21%</td>
</tr>
<tr>
<td>High</td>
<td>1%-15%</td>
<td>21%</td>
</tr>
</tbody>
</table>

Table 1B: Inter laboratories Reproducibility Study

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Efficiency</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Youden Index</th>
<th>Odds ratio</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>96%</td>
<td>97%</td>
<td>96.5%</td>
<td>9%</td>
<td>96%</td>
<td>0.94</td>
<td>996</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 2: Diagnostic characteristics
Discussion and Conclusion:
Salmonella abortusovis Test revealed good performances, demonstrating its suitability to determine the immune status in animals post vaccination, to certify freedom from infection and for confirmatory diagnosis of suspect or clinical cases.
O 10 DIAGNOSTIC STRUCTURE AND PROCESS WITHIN A NATIONAL REFERENCE LABORATORY

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Introduction:
Wageningen Bioveterinary Research (WBVR) is the national reference laboratory for notifiable animal diseases in the Netherlands. Before 2004, research and diagnostics were performed within specialized disease groups (swine fever, avian diseases group etc). In 2004, a diagnostic department was formed to separate diagnostic activities from research activities. The reasons for this were a) to further intensify and improve research by increased focus on research and better allocation of personnel and funding within the specialized disease groups, b) to further professionalize the diagnostics by standardizing and harmonizing quality management and logistics, thus improving the efficiency and c) to be better prepared for notifiable animal disease crises. Now, almost 15 years later, the advantages and disadvantages of the separation of diagnostics within a specialized department are discussed.

Materials and Methods:
The diagnostic department is logistically setup to handle routinely large numbers of samples (> 300,000 samples/year). This setup does not change during disease crisis.

Results:
The track and trace of samples, the logistic process and the crisis organisation of the diagnostic department will be shown in more detail at the conference.

Discussion and Conclusion:
During outbreaks the past 15 years, WBVR was capable to scale up quickly and to handle large numbers of samples. Furthermore, the diagnostic department was capable to further professionalize the diagnostics and accredit many tests for ISO17025. The disadvantage of the separation is that constant attention is needed for a close contact between the research labs and diagnostic labs to guarantee advise on lab results, use of new technologies, changes in epidemiological situations etc.
OP03 - STANDARDIZATION, VALIDATION AND SURVEILLANCE - ORAL PRESENTATIONS

O 11 VERIFICATION OF QUANTIFICATION STANDARDS USED IN QUANTITATIVE PCR BY DROPLET DIGITAL PCR

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Introduction:
Currently, quantitative PCR (qPCR) is one of the most widely used molecular biological methods for the detection of microbial pathogens. Moreover, qPCR allows to quantify microbial load according to the standard calibration curve. Therefore, precise determination of the DNA quantity in the standard is essential. Spectrophotometry is used most often for this purpose, but the spectrophotometric measurement of the nucleic acid absorbance is affected by its purity, which may lead to incorrect dilution of the standard. A promising tool for accurate quantification of these standards is the recently developed droplet digital PCR (ddPCR) method, which provides absolute quantification without the need for a calibration curve.

The aim of this study was to assess suitability of ddPCR for the independent verification of qPCR quantification standards.

Materials and Methods:
We have selected 4 qPCR assays for the quantification of microbial pathogens and analyzed linearized and circular form of plasmids by qPCR and ddPCR. The effect of repeated plasmid isolation and usage of different plasmid purification kits was assessed as well.

Results:
Spectrophotometric measurement of the absorbance of the nucleic acid overestimated by 30–50 %. The results show that the linearized plasmid was more suitable for the standard than the circular, since amplification of the target nucleic acid sequence is better accessible.

Discussion and Conclusion:
Using ddPCR, we have verified the four selected quantification standards used in our laboratory. Due to the variability in plasmid batch-to-batch preparation, ddPCR can provide a very powerful tool for the control of plasmid production in time.

This work was supported by projects RO0518 a QK1810212.
O 12 DEVELOPMENT AND VALIDATION OF A MULTIPLEX-TANDEM (MT) PCR FOR THE DIAGNOSIS OF BOVINE RESPIRATORY DISEASE COMPLEX

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Introduction:
Bovine Respiratory Disease Complex is a multifactorial syndrome responsible for substantial economic losses to the cattle industry worldwide. Environmental, housing, nutritional and stress factors increase susceptibility to viral insult and secondary bacterial infection. Rapid and multitargeted diagnostic tests would allow appropriate and timely interventions, reducing welfare implications and costs. We have thus developed and validate a multiplex-tandem (MT) respiratory PCR targeting the nine major pathogens associated to this syndrome.

Materials and Methods:
Nucleic acid were extracted from 196 archived Bovine Respiratory Samples (tissues, swabs, BALs) previously tested positive in virus specific TaqMan PCRs. Samples were assayed in parallel with the MT-PCR and the respiratory PCRs and discordant events identified. After optimisation of cycle conditions discordant samples were re-assayed and those still discordant sequenced.

Results:
Following optimisation the majority of the samples tested gave comparable results, with the exception of two virus targets which are still under investigation. Sensitivity and specificity values are reported in table 1.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BoHV1</td>
<td>74.6</td>
<td>98.4</td>
</tr>
<tr>
<td>Bpi3</td>
<td>92.3</td>
<td>100.0</td>
</tr>
<tr>
<td>BRSV</td>
<td>100.0</td>
<td>99.4</td>
</tr>
<tr>
<td>BCoV</td>
<td>76.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>M. bovis</td>
<td>100.0</td>
<td>97.7</td>
</tr>
<tr>
<td>Histophilus somni</td>
<td>100.0</td>
<td>92.2</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mannheimia haemolytica</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
We have concluded the initial laboratory validation and are currently performing the field validation of the respiratory MT-PCR. Field validation is carried out to assign clinical relevance to CT values. We are also re-assessing the test performance for the two viral targets with low sensitivity values by increasing the number of samples tested. This test will be soon integrated in the Scottish Disease Veterinary Surveillance Scheme.
**OP04 - STANDARDIZATION, VALIDATION AND SURVEILLANCE II - ORAL PRESENTATIONS**

**O 13 THE USE OF WEB BASED TOOLS TO SUPPORT INTERPRETATION OF IDEXX MILK ELISA JOHNE’S RESULTS IN THE UK**

P. Orpin1

1 Park Veterinary Group, Farm Department, Leicester, United Kingdom

**Introduction:**
Over 2500 dairy herds in the UK are tested every 3 months for Johne’s ELISA results as part of a National Johne’s Management Plan (NJMP). Fluctuation of ELISA results is normal and can trigger confusion for both vets and farmers and is a common reason for the lack of progress with Johnes disease testing. Web based tools (Herdwise, Myhealthyherd) were developed to aid in the interpretation and impact of the test results.

**Materials and Methods:**
National Milk Laboratories developed a web based tool (Herdwise) for the display and interpretation of Johne’s milk ELISA results. Cows were categorised into 3 risk groups using a red, amber and green coding system using an algorithm within the Herdwise program.

Myhealthyherd developed a parallel development which allowed test prevalence data to be converted into true herd prevalence based on 30 cow targeted and whole herd screens. Predicted future true herd prevalence is graphically illustrated through integration of biosecurity and biocontainment risk scores of disease entry and spread for the herd.

**Results:**
The commercially successful quarterly milk testing program supported by education of vets and farmers and web based tools has been central to the success of the NJMP in the UK.

**Discussion and Conclusion:**
The advent of web based tools allows for improved reporting and interpretation of laboratory results using more nuanced and sophisticated methodologies. Opportunities exist to improve the uptake of surveillance with greater emphasis on herd specific display of results delivering more value to both the vet and farmer.
Introduction:
Infections due to bovine viral diarrhoea virus (BVDV) are endemic in most cattle-producing countries throughout the world and bovine viral diarrhoea is considered as a transboundary disease. The key elements of a BVDV control programme are vaccination, biosecurity, elimination of persistently infected (PI) animals and surveillance. The aim of this study was to assess the sensitivity (Se) and the specificity (Sp) of two commercial competitive enzyme-linked immunosorbent assay (cELISA) tests based on selected immunodominant BVDV proteins, respectively the non-structural protein NS3 (p80) and the recombinant envelope glycoprotein E0 (Erns).

Materials and Methods:
Both tests were used on individual serum samples randomly sampled from young bovines in Southern Belgium in order to detect specific BVDV antibodies. Se and Sp were assessed using a Bayesian approach.

Results:
The Se and Sp were estimated respectively at 97.2% (with 95% credibility interval [Cr I]): 95.1–99.8) and 98.7% (95% Cr I: 96.6–99.9) for the first test and 95.8% (95% Cr I: 91.1–99.7) and 96.1% (95% Cr I: 95.1–97.7) for the second test. The results obtained with the two tests were not significantly different. In addition using both cELISAs, the current BVDV exposure among young bovines in Southern Belgium was estimated at 23.3% (95% Cr I: 20.6–26.2).

Discussion and Conclusion:
This study supports the use of a cELISA test based on NS3 or E0 protein as an efficient diagnostic tool to be applied in the surveillance of BVDV. Its regular use combined with virological testing of all new-borns to detect PI animals would be recommended in the surveillance of BVDV.
OP04 - STANDARDIZATION, VALIDATION AND SURVEILLANCE II - ORAL PRESENTATIONS

O 15 COMPARISON OF PTS RESULTS BETWEEN ROBUST AND TRADITIONAL METHOD

W. Swart

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Introduction:
GD Animal Health organizes international PTS’s since 2002. Nowadays PTS’s are held for 25 diseases. For most diseases 8 samples to be measured in duplo, are send to the participants. The within lab sr and between lab reproducibility sR are calculated in a simple and straightforward way, after the data has been cleared of outliers through the Cox and Grubbs tests. Aim of this project was to investigate the effect of the introduction of the calculation of sr and sR by a robust method. The expectation was that values would become slightly higher because outliers are not discarded totally.

Materials and Methods:
The data of 12 different ELISA tests was used for 6 poultry diseases: AIV, IBDV, IBV, NDV, Mycoplasma-MG and Mycoplasma-MR. For each disease a pool of samples is available from which 8 samples are chosen randomly every year. Years vary from 2006 to 2015. For each of the samples, if chosen in that year, the means and sr and sR are calculated the classical way with outliers removed and the robust way.

Results:
Both the sr and sR standard deviations were higher on average if calculated with the robust method. The average ratio between both methods for sr was 1.30, the average ratio of sR was 1.13. The results were similar for all tests and all years.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p5</th>
<th>p25</th>
<th>p50</th>
<th>p75</th>
<th>p95</th>
<th>mean</th>
<th>sd</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>sr</td>
<td>0.88</td>
<td>1.04</td>
<td>1.18</td>
<td>1.42</td>
<td>2.00</td>
<td>1.30</td>
<td>0.48</td>
<td>943</td>
</tr>
<tr>
<td>sR</td>
<td>0.85</td>
<td>0.95</td>
<td>1.04</td>
<td>1.18</td>
<td>1.55</td>
<td>1.13</td>
<td>0.48</td>
<td>943</td>
</tr>
</tbody>
</table>
Discussion and Conclusion:

PTS results calculated by the robust method, contrary to expectation, lead to smaller $s_r$ and $s_R$ compared to traditional method. The reason for this is that the robust method treats more data points as outliers compared to the classical outlier Cochran and Grubbs tests.
OP04 - STANDARDIZATION, VALIDATION AND SURVEILLANCE II - ORAL PRESENTATIONS

O 16 AN INTERLABORATORY TRIAL TO EVALUATE THE RELIABILITY OF PCR METHODS FOR MYCOPLASMA BOVIS DIAGNOSIS IN SIX EUROPEAN COUNTRIES

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Introduction:
Several species-specific PCRs based on a variety of target genes are currently used for the diagnosis of Mycoplasma bovis infections in cattle herds with respiratory diseases and/or mastitis. Because of the diversity of methods, regular performance comparisons are required to ascertain the quality of diagnosis. The present study proposes an interlaboratory trial of PCR assays currently in use in six national institutes dealing with animal health in Europe.

Materials and Methods:
Three different assays to assess the specificity, sensitivity and comparability of the PCR assays were conducted. Results were compared, when appropriate, to those obtained through isolation by culture. The sensitivity and comparability assays were conducted using bronchoalveolar fluids of veal calves, artificially contaminated or naturally infected.

Results:
A total of five different DNA extraction methods, seven different real-time and/or end-point PCR methods targeting four different genes and six different real-time PCR platforms were used. Only one commercial kit was assessed, all other PCR assays were in-house tests. The analytical specificity of the PCR methods was comparable. The limit of detection varied from 10 to 10^3 CFU/ml to 10^6 CFU/ml for the real-time and end-point assays, respectively. Cultures were also shown to detect down to 10 CFU/ml. Although Ct values obtained from naturally infected samples were highly variable, the final categorization by the different laboratories was essentially the same.

Discussion and Conclusion:
With a few exceptions, all methods run routinely in the participating laboratories showed comparable performance, which leaves a lot of possibilities for a good quality diagnosis.
O 17 FIRST REPORT OF LINEAGE 5A VELOGENIC AVIAN PARAMYXOVIRUS IN THE NORTH-WEST EUROPE, BELGIUM

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Introduction:
Belgium has been free of velogenic avian paramyxovirus type 1 since the implementation of compulsory vaccination in 1993.
In the spring of 2018 this changed with the detection of lineage 5a velogenic APMV-1 in belgian hobby chicken.

Materials and Methods:
Diagnostic samples from Belgium (April 2018) and the Grand Duchy of Luxembourg, purchased on a belgian live bird market, (Mai 2018) were evaluated for APMV-1-presence.
First line diagnostic testing for the presence of avian paramyxovirus type 1 is performed by generic realtime RT-PCR.
Subsequently, positive APMV-1 samples were inoculated onto embryonated specific pathogen free chicken eggs.
By cleavage site sequencing the pathotype was identified, who was confirmed by classical hemagglutination inhibition test.

Results:
Belgium:
After detection of APMV-1 in realtime RT-PCR, virological isolation was initiated to allow further characterisation of the strain.
The velogenic pathotype was demonstrated by cleavage site sequencing (RRQ*KRF) and confirmed by the obtained HI-profile.
Sequence analyses grouped the Belgian isolate together with 2016-Pakistani isolates, a 2013 poultry Bulgarian isolate and a 2016 wild bird isolate of Cyprus, of lineage 5a.

Grand Duchy of Luxembourg:
Also for the Grand Duchy of Luxembourg velogenic APMV-1 presence was confirmed by RRT-PCR detection, followed by cleavage site sequencing pathotyping and HI-identification after viral isolation. Phylogenetic analysis of sequences demonstrated grouping with the lineage 5a APMV-1 viruses.
Next to the epidemiological link of purchase, sequence analyses confirmed a close relationship between the Belgian and Luxembourg cases.

Discussion and Conclusion:
This was the first detection of velogenic APMV-1 in Belgium and Luxembourg since 1993, and the first detection of lineage 5a in the North West of Europe.
OP05 - EPIZOOTIC, EMERGING AND VECTORBORNE DISEASES OF LIVESTOCK - ORAL PRESENTATIONS

O 18 DEVELOPMENT AND EVALUATION OF A MULTIPLEX CLASSICAL RT-PCR FOR SIMULTANEOUS DETECTION AND TYPING OF FMDV IN WEST AFRICA

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Introduction:
Foot-and-Mouth Disease, (FMD) caused by Foot-and–Mouth Disease Virus (FMDV) is one of the most economically devastating diseases affecting cloven-hoofed animals. There are seven immunologically and genetically distinct serotypes (O/A/C/Asia1/SAT1-3) further subdivided into different topotypes and genotypes. The West African territories are considered as regions with continuous FMDV circulation of serotypes O, A, SAT1 and SAT2. An early diagnosis of FMD is crucial to implement adequate outbreak management. This study describes the development of a multiplex conventional RT-PCR for both detection and typing of FMD virus circulating in this region.

Materials and Methods:
The RT-PCR reactions were developed with primer sets targeting the 3D coding region, the VP1 coding region (O/A/SAT1/SAT2-specific) and the b-actin gene to produce amplicons with sizes easily distinguishable by agarose electrophoresis. A 6-plex prototype (O/A/SAT1/SAT2/3D/ b-actin) was finally developed using a panel of reference strains and positive field samples from Benin and the corresponding isolates. This test is currently evaluated on a larger panel of field samples collected in Nigeria and Senegal.

Results:
Using 6-plex protocol, 37 samples from Benin were positive for the 3D target and were correctly serotyped by 6-plex (33) or by triplex and simplex RT-PCR (4). 39/40 isolates from Nigeria were properly serotyped using 6-plex (30) or simplex (9). The corresponding field samples as well as 39 clinical positive samples from Senegal are under investigation.

Discussion and Conclusion:
We have developed and evaluated a 6-plex RT-PCR that could be easily implemented in diagnostic laboratories in endemic countries, providing an improvement for rapid detection and typing of FMDV strains.
OP05 - EPIZOOTIC, EMERGING AND VECTORBORNE DISEASES OF LIVESTOCK - ORAL PRESENTATIONS

O 19 DO COMMERCIALLY AVAILABLE LYSIS BUFFERS INACTIVATE FOOT-AND- MOUTH DISEASE VIRUS?

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Introduction:
Laboratories working with live foot-and-mouth disease virus (FMDV) must maintain a high-level of biocontainment. However, if infectious virus is reliably inactivated during processing, low-risk molecular testing can be performed in standard laboratories.

Materials and Methods:
Nine commercial lysis buffers (AL, AVL, MagMAX, MagMAX CORE, NM1, RAV1, RLT, TRIzol, and VL) were tested for their inactivation efficacy against different FMDV serotypes, infectious doses and/or different sample matrices. Residual infectivity after the addition of lysis buffer was evaluated by inoculating susceptible cell cultures and observing for cytopathic effect.

Results:
Preliminary results suggest that FMDV inactivation can vary by lysis buffer and sample matrix, and that a secondary heat inactivation step may not always eliminate residual infectivity.

Discussion and Conclusion:
These results highlight the need for validation experiments to be conducted when selecting a lysis buffer and/or before removing samples from high containment.
O 20 IDENTIFICATION AND MOLECULAR CHARACTERISATION OF A NOVEL GROUP A ROTAVIRUS IN PIGEONS DURING THE 2016–2017 OUTBREAKS IN AUSTRALIA

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Introduction:
Group A rotavirus is a major cause of diarrhoeal illness in humans, as well in mammalian and avian animal species worldwide. Since mid-2016, there has been a highly infectious disease with high level mortalities (up to 30 %) in kept pigeons (racing and fancy) in lofts across Australia. In a joint investigation with Australian state veterinary laboratories, samples from affected birds from different geographic locations were submitted to AAHL for laboratory testing since late December 2016. Here we report identification and characterisation of a novel group A rotavirus from the disease outbreak.

Materials and Methods:
Liver and cloacal swab specimen from pigeons with clinical symptoms collected during 12/2016–05/2017 were analysed. Next Generation Sequencing (NGS) was conducted using MiSeq (Illumina) system. A qRT-PCR assay was developed and validated.

Results:
Complete genome sequence of a group A rotavirus was discovered from all samples tested. The virus genome had high sequence identity to a recently-identified group A virus from a fox and an avian strain PO13 from a pigeon. Phylogenetic analysis demonstrated that the virus is genotype G18P[17] in the group A avian rotavirus lineage.

Discussion and Conclusion:
A previously unknown group A rotavirus was detected and identified during the disease investigation. This is the first demonstration of the group A rotavirus in pigeons with systemic diseases in Australia. A qRT-PCR assay was developed and transferred to the Laboratories for Emergency Animal Disease Diagnosis and Response network in Australia. This diagnostic assay has facilitated rapid and specific diagnosis of this disease during the outbreaks.
O 21 RT-PCR DECENTRALIZATION FOR BTV-4 DIAGNOSIS IN REGIONAL LABORATORIES IN THE MANAGEMENT OF THE EMERGENCE OF BTV-4 IN FRANCE IN 2017

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Introduction:
Bluetongue virus (BTV) emerged in 2006 in mainland France. After several vaccination campaigns of cattle, sheep and goats, France obtained an OIE/EU recognized BTV-free status in December 2012. In 2015, BTV-8 re-emerged in central France. In December 2016, BTV-4 was detected in South of Corsica. At the beginning of November 2017, BTV-4 was detected in our laboratory. This presentation describes the detection of this case and the decentralization of BTV-4 RT-PCR methods in regional laboratories.

Materials and Methods:
A 15-day-old calf which was born in Haute-Savoie moved to an assembly center in Loire prior to being exported to Spain. Blood sample was taken and rtRT-PCR analyses and isolation assays on KC, embryonated eggs and BSR cells were carried out. BTV-4 was fully sequenced. BTV-4 real-time RT-PCR assays were developed and decentralized to regional laboratories. To evaluate the spread of BTV-4, blood cattle samples were collected in Haute-Savoie and neighboring departments in November and December 2017 (45 farms/department and 20 animals sampled/farm).

Results:
The calf was detected BTV positive with pan-BTV rtRT-PCR kit and positive with a specific BTV-4 rtRT-PCR. Sequence analysis showed a close relationship with the BTV-4 isolated in Corsica (2016). 84 BTV-4 outbreaks were detected in mainland France.

Discussion and Conclusion:
A compulsory vaccination campaign was decided in November 2017. However, the limited availability of vaccines led to a change in the BTV control strategy. On the first of January 2018, the whole of mainland France switched to BTV-4/BTV-8 restriction zone.
**O 22 INVESTIGATION OF THE ROLE OF RACING PIGEON IN THE DISPERSION PROFILE OF CLADE 2.3.4.4.B. HPAI**

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**Introduction:**
Pigeons have the reputation of being poorly susceptible to avian influenza infection. However, natural and experimental pigeon infections with the highly pathogenic Asian lineage H5N1 and clade 2.3.4.4.b H5N8 viruses suggest some degree of susceptibility, with limited clinical signs, ineffective propagation and poor dissemination.

The clade 2.3.4.4.b HP H5N8 viruses introduced into Europe during the 2016 autumn wild bird migration season demonstrated an increased wild bird range and continued circulating far into summer 2017. The prolonged circulation plus the risk for new arrivals during the fall of 2017, raises the fear for an enzootic situation in Europe, increasing the risk of pigeon contact with AI-infected environments.

As past pigeon sensitivity studies are somewhat contradictory and are mostly performed on young feral pigeon, the relevance of these data for adult racing pigeon, participating in international long distance contests, remain uncertain. To evaluate the susceptibility of this category of racing pigeon to the evolved H5N8 HPAI virus a dose-evaluation, infectivity and transmissibility study will be performed. To study the transmissibility contact pathogen free chicken will be added and evaluated.

**Materials and Methods:**
In vivo infection studies with two Belgian clade 2.3.4.4.b 2017 strains and one clade 2.3.2.1.c Indonesian strain were performed.

**Results:**
Different sensitivity patterns have been demonstrated for the HP H5 strains under evaluation.

**Discussion and Conclusion:**
This knowledge on susceptibility, clinical impact and transmission potential are important to determine the precaution measures necessary to implement by pigeon fanciers to protect their flocks, and to evaluate the dispersion risk towards commercial poultry.
O 23 FEATHERS AS SAMPLE FOR EFFICIENT DETECTION OF CLADE 2.3.4.4. H5N8 HPAI VIRUSES IN DUCKS

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Introduction:
Highly Pathogenicity Avian influenza viruses (HPAIVs) display a tissular pantropism, which implies that they could spread in feather pulp (FP). We investigated on field and experimental H5N8 HPAI the suitability of FP to detect clade 2.3.4.4, H5N8 HPAIV in ducks.

Materials and Methods:
Six duck flocks, confirmed H5N8 positive, were included from January to March, 2017: on each flock, at least 10 non-clinically affected birds were sampled: tracheal (TS), cloacal swabs (CS) were taken, and immature wing feathers. RNA was extracted from swabs or feather pulp and real-time RT-PCR was performed for M and H5 genes. M or H5 RNA copies was quantitated and the distribution of viral RNA loads was statistically analyzed using bayesian approaches. An experimental H5N8 infection was performed on 10-week-old mule ducks.

Results:
In all flocks included in the field study, loads of viral RNA detected in FP were at least equivalent and in most cases up to 103 higher than those detected in either TS or CS. At the flock level, detection performances of feather pulp samples were significantly much better than TS or CS. IHC assays performed on feather follicles confirmed an intense viral staining. The elective tropism of clade 2.3.4.4. HPAIVs in FP was confirmed on experimentally-infected ducks, from Day-1 to day-10 post-infection.

Discussion and Conclusion:
These data, based on both spontaneous and experimental H5N8 infections, suggest that FP should be considered as suitable sample for detection of clade 2.3.4.4 HPAI viruses. Further investigations are needed to clarify their relevance in the protocols of HPAI surveillance.
O 24 DETECTION AND IDENTIFICATION OF YEAST IN MILK SAMPLED FROM COW WITH SUBCLINICAL MASTITIS

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Introduction:
Mastitis are among the most important and most common diseases of dairy cows. Although, most control measurements are focused on detection and elimination bacterial pathogens, there is growing interest in non-bacterial agents causing mastitis such as yeast.

Materials and Methods:
In total, 910 randomly selected milk samples from cow with subclinical mastitis were collected from 17 dairy farms in the Czech Republic. Samples were cultivated on Sabouraud agar with glucose and chloramphenicol and DRBC agar. Isolates were identified by using biochemical assay and by molecular analysis (sequencing of D1/D2 and ITS region).

Results:
Out of 910 milk samples, 67 yeast isolates from 64 (7.0 %) milk samples were obtained. Candida spp. and Trichosporon spp. were most frequently identified species. Nevertheless, biochemical analysis was usually not able to differentiate individual species in comparison to molecular methods. Using molecular identification, 54 % (36) of isolates belong to genus Candida with the most common species C. krusei, C. kefyr and C. famata. Trichosporon sp. with the most common species T. cutaneum and T. lactis was identified in 19 % (13) of isolates.

Discussion and Conclusion:
In different countries, the frequency of yeast isolation from cow varies considerably, with reported rates less than 2 % in countries with good hygiene condition and with elevated rates more than 15 % in countries with poor hygienic condition. It is demonstrated that Candida spp. are the most frequently isolated species, which is in agreement to our study. In addition, C. albicans common human pathogen was not isolated in our study.

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**OP07 - ONE HEALTH: FOODBORNE PATHOGENS, ZOONOSES AND ANTIBIOTIC RESISTANCE - ORAL PRESENTATIONS**

**O 25 CAMPYLOBACTER JEJUNI/COLI DETECTION RATES IN FRESH BROILER MEAT BY CULTURAL ENUMERATION AND QUANTITATIVE REAL-TIME PCR**

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**Introduction:**
In 2017 a quantitative process hygiene criteria (PHC) for Campylobacter on broiler skin sample at slaughterhouse were introduced in the European Union. With implementation of these criteria improvement of slaughterhouse hygiene is intended, which should lead subsequently to a reduction of Campylobacter on fresh poultry meat.

**Materials and Methods:**
Fresh poultry meat without skin were analyzed quantitatively and qualitatively for Campylobacter jejuni/coli according to ISO 10272. In parallel, the recently by Haas et al. (2017) developed qPCR was tested as an alternative quantitative approach.

**Results:**
A total of 301 fresh broiler meat samples were analyzed. Two samples (1 % (95 %CI 0.2 – 2.0)) showed a Campylobacter jejuni/coli contamination >10 cfu/g by cultural enumeration. By analyzing the samples with qPCR 22 samples showed a positive result (7 % (95 %CI 5 – 12)), with Ct-value of 35 – 40, indicating a low level contamination. In 14 samples the qPCR was inhibited, and 265 were negative (Ct values > 40). In contrast, when analyzed by enrichment 159 samples (53 % (95 %CI 47 – 58)) turned out to be positive for Campylobacter jejuni/coli.

**Discussion and Conclusion:**
The results show, that already before implementing the new PHC, only a few fresh broiler meat samples (1 %) exhibit enumerable amounts of Campylobacter jejuni/coli. Even by qPCR only 7 % of the samples were positive at a low contamination level. In contrast, a remarkable percentage (53 %) of meat samples was positive for Campylobacter jejuni/coli when enrichment was used. It is questionable, if the newly introduced quantitative criterium will be effective enough to substantially decrease the very high percentages of low level contaminated fresh broiler meat.
O 26 FIRST LINE TYPING FOR BRUCELLA ABORTUS AND BRUCELLA MELITENSI S

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Introduction:
Brucella abortus and B. melitensis are genetically monomorphic and, therefore, an accurate comparison of isolates within one species using molecular typing can often be challenging. Nonetheless, MLVA is considered a reliable tool for identification and investigation of brucellosis outbreaks. To date, 9 different MLVA schemes utilizing 34 VNTRs have been proposed. Our aim was to design new MLVA panels in order to simplify the typing strategy without compromising accuracy.

Materials and Methods:
A set of 202 Brucella isolates from Italy, Portugal, Egypt and Greece were typed using 34 VNTRs. To select the best typing markers, we calculated their discriminatory capacity, the linkage disequilibrium and the index of association. The performance and the convenience criteria were evaluated and compared with the previous methodologies. The data obtained were deposited in the MLVA web repository of the BrucMedNet project.

Results:
Based on the MLVA analysis, we selected two different panels dedicated to B. abortus and B. melitensis (Table 1).
Each was composed of 7 VNTRs, which had 100% of typeability, a good association index and a medium or high Simpson diversity index (SDI). These panels were in good concordance with the published panels and resulted in a similar or higher number of genotypes (Figure 1).

Discussion and Conclusion:
Although WGS is revolutionizing the molecular epidemiology tools, MLVA is still an effective first line typing array due to its rapid turnaround time and the lower cost. The new schemes aim to replace the previous MLVA panels and will contribute to an efficient, fast and cost-effective routine tests for the surveillance of brucellosis.
OP07 - ONE HEALTH: FOODBORNE PATHOGENS, ZOONOSIS AND ANTIBIORESISTANCE - ORAL PRESENTATIONS

O 27 GENOTYPING OF BACILLUS ANTHRACIS STRAINS CIRCULATING IN ITALY BASED ON SNPS AND 31-LOCI MULTI LOCUS VNTR ANALYSIS

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Introduction:
In Italy, anthrax is considered an endemic disease affecting ruminants with sporadic zoonotic occurrences in humans. In this investigation, we analyzed 222 B. anthracis strains (195 from animal species and 27 from environment) isolated in different Italian anthrax outbreaks from 1954 to 2017. The canonical SNPs assay (CanSNPs) and the multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) were used to differentiate the strains.

Materials and Methods:
The phylogenetic identity was determined through the research of polymorphisms for CanSNPs, with 14 PCR assays for allelic discrimination. A 31-loci MLVA assay was performed to determine B. anthracis genotypes. Eleven PCRs reactions were performed (2 singleplex and 9 multiplex) to amplify VNTRs.

Results:
The analysis of 14 CanSNPs, allowed to identify four main lineages: A.Br.011/009, A.Br. 008/011, A.Br. 005/006 and B. Br. CNEVA. However, the lineage A major subgroup A.Br.011/009 (Trans-Eurasian or TEA stains), represents the dominant population of B. anthracis in Italy, particularly in southern part of the country. The MLVA with 31 VNTRs analysis, demonstrated 55 different genotypes (Fig.1).
Discussion and Conclusion:
The data obtained by 31 loci MLVA showed an increasing number of genotypes circulating in Italy, compared to the MLVA test at 15 loci by which we obtained just 30 genotypes. Most of them are genetically very similar to each other, confirming the hypothesis that all of them are the result of the evolution of a local common ancestral strain. The genotyping analysis with methods such as CanSNPs and MLVA, is a very valuable tool for studying the diversity, evolution, and molecular epidemiology of B. anthracis.
OP07 - ONE HEALTH: FOODBORNE PATHOGENS, ZOONOSIS AND ANTIBIORESISTANCE - ORAL PRESENTATIONS

O 28 PREVALENCE OF VEROCYTOTOXIGENIC ESCHERICHIA COLI STRAINS IN RAW BEEF IN SOUTHERN ITALY

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Introduction:
Verocytotoxin (VT)-producing Escherichia coli (VTEC) are a significant foodborne public health hazard. The consumption of VTEC-contaminated food is an important transmission route. The aim of this study was to investigate the prevalence of Verocytotoxin (VT)-producing Escherichia coli (VTEC) in raw beef samples and products thereof from retail markets of Apulia (southern Italy).

Materials and Methods:
In 2017, 270 meat samples (100 beef hamburger, 100 raw ground beef, 50 beef carpaccio and 20 dried sausages) were collected from retail markets in Apulia (Italy). The samples were analyzed according to ISO 13136:2012. According to the EURL for E. coli procedures, O104 and O45, O55, O91, O113, O121, O128, and O146 serogroups identification was performed. In addition, the isolates were characterized by PCR for stx gene subtypes, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and antimicrobial resistance pattern.

Results:
VTEC strains were isolated from 3.3% of overall meat analysed samples. Data about isolates were reported in Table 1. The isolates displayed a multi-drug resistance phenotype. In addition, the restriction profiles of PFGE showed that only STEC isolated from two carpaccio samples resulted to be highly correlated (88.2%), also confirmed by MLST (ST101).

<table>
<thead>
<tr>
<th>K.</th>
<th>Meat type</th>
<th>Virulence genes</th>
<th>Serogroup</th>
<th>Subtype</th>
<th>MLST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raw ground beef 1</td>
<td>-</td>
<td>-</td>
<td>O.N.T.*</td>
<td>ver3g</td>
</tr>
<tr>
<td>2</td>
<td>Raw ground beef 2</td>
<td>-</td>
<td>-</td>
<td>O.N.T.</td>
<td>ver2c</td>
</tr>
<tr>
<td>3</td>
<td>Raw ground beef 3</td>
<td>-</td>
<td>-</td>
<td>O.N.T.</td>
<td>ver3c</td>
</tr>
<tr>
<td>4</td>
<td>Hamburger</td>
<td>-</td>
<td>-</td>
<td>O.N.T.</td>
<td>ver3c</td>
</tr>
<tr>
<td>5</td>
<td>Beef carpaccio 1</td>
<td>-</td>
<td>-</td>
<td>O.N.T.</td>
<td>ver3c</td>
</tr>
<tr>
<td>6</td>
<td>Beef carpaccio 2</td>
<td>-</td>
<td>-</td>
<td>O55</td>
<td>ver3c</td>
</tr>
<tr>
<td>7</td>
<td>Beef carpaccio 3</td>
<td>-</td>
<td>-</td>
<td>O55</td>
<td>ver3c</td>
</tr>
<tr>
<td>8</td>
<td>Beef carpaccio 4</td>
<td>-</td>
<td>-</td>
<td>O.N.T.</td>
<td>ver3c, ver3a, ver3e</td>
</tr>
<tr>
<td>9</td>
<td>Dried sausage</td>
<td>-</td>
<td>-</td>
<td>O113</td>
<td>ver3c, ver3d</td>
</tr>
</tbody>
</table>

*O.N.T., O not typeable.
Discussion and Conclusion:
Although there is a low prevalence rate of VTEC, their occurrence in raw beef and in ready to eat beef products as carpaccio represents an important public health risk. The presence of VTEC points to the need for proper hygiene during meat production to reduce the risk of food-borne illness and transmission of multi-drug resistant organisms via foods to humans.
OP08 - ONE HEALTH: FOODBORNE PATHOGENS, ZOONOSIS AND ANTIBIORESISTANCE II - ORAL PRESENTATIONS

O 29 ANTIMICROBIAL SUSCEPTIBILITY TESTING: EUCAST VERSUS CLSI?

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Introduction:
Antimicrobial Susceptibility Testing (AST) is crucial for a targeted antimicrobial therapy and its interpretation is based on Clinical Breakpoints (CBPs). Currently, two independent organization providing CBPs for both, human and veterinary medicine, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the American Clinical Laboratory and Standards Institute (CLSI).

Materials and Methods:
Minimum inhibitory concentrations (MICs) were tested for a total of 97 S. uberis, 33 S. suis, 78 T. pyogenes, 81 P. multocida and 37 M. haemolytica by broth microdilution. Histograms of MIC distributions were visually inspected and grouped into three categories: presumptive WT bacterial populations, presumptive WT/non-WT bacterial populations, and truncated MIC distributions.

The relations of CLSI CBPs to the MIC distributions were grouped into three categories: CBPs separate WT from non-WT populations, CBPs split WT populations, and CBPs that did not separate WT from non-WT populations.

Results:
Comparison of CLSI CBPs with MIC distributions revealed three different scenarios. First, the CLSI CBPs were situated between WT and non-WT populations. In the second scenario, CBPs were situated within the putative WT population. Third, the CBP was not situated between the presumptive WT and non-WT populations.

Discussion and Conclusion:
Our results demonstrated that veterinary CBPs of the CLSI guidelines do not always correspond to a clear separation of the WT/non-WT population. As long as CBPs are not available from EUCAST and consistent with CLSI, our data support the recommendation by EUCAST to determine whether the MIC of the isolate is consistent with the WT population for the species or not.
**O 30  NEW APPROACH FOR THE DETECTION OF TRIChinELLA SPIRALIS IN SLAUGHTERED PIGS**

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**Introduction:**

*Trichinella spiralis* (*T. spiralis*) is a foodborne pathogen that is transmitted to humans by consuming undercooked meat of infected pigs. To prevent human infection, meat inspection is mandatory in slaughterhouses of many countries. Consequently, a fast, cost-effective and simple method to prepare meat samples by mechanical shredding followed by an immunoassay to detect specific *T. spiralis* antigens was developed.

**Materials and Methods:**

100 x 1 g diaphragm crus from infected and non-infected pigs were shredded with buffer in a high-quality knife mill for 9 minutes (Fig. 1). Sample collection and subsequent particle size measurement occurred at different time points. After a centrifugation step, the tissue extract samples were diluted and used in a *Trichinella* specific bead-based antigen detection immunoassay.

**Results:**

During the first minutes of shredding over 90% of the meat particles had a diameter of about 600 µm, but after 6 minutes the particles were already smaller than 20 µm. This means that every encapsulated *Trichinella* larva was destroyed by the knife of the mill and excretory and secretory antigens could be released. The smaller the particles the more *Trichinella* antigens could be detected by the immunoassay.

**Discussion and Conclusion:**

A specific, sensitive and simple diagnostic tool with a detection limit of less than 3 ng/ml antigen was developed. At the moment, optimization, validation and further studies are ongoing to estimate the suitability of the test for routine diagnostics of *T. spiralis* in slaughtered pigs.
O31 CHARACTERIZATION OF MRSA ISOLATED FROM VETS AND FARMERS BY WHOLE GENOME SEQUENCING

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Introduction:
Over the last decade an increasing prevalence of methicillin resistant Staphylococcus aureus (MRSA) in Swiss pigs was noted. These isolates mostly belong to the livestock-associated sequence type (ST) 398-complex. However, ST398 can be transmitted to humans, where it can also cause infections. Therefore, we analyzed MRSA isolates from veterinarians and farmers.

Materials and Methods:
Swiss veterinarians and farmers were screened for the presence of MRSA. The obtained strains (15 from veterinarians and 8 from farmers) as well as selected isolates from pig noses (n=12), pork (n=2) and poultry meat (n=3) were characterized by whole genome sequencing (Illumina Next-Seq, v2, 2x150 bp). Core genome MLST was performed and a minimum spanning tree created with Bionumerics 7.6. The genomes were also screened for virulence factors.

Results:
In total eight of 15 isolates from veterinarians and six of eight strains from farmers belonged to the livestock associated ST398; all of the veterinarians reported treating large animals. In small animal veterinarians only non-ST398 MRSA were found - indicating a non-animal derived colonization.

Figure 1: Minimum spanning tree from cgMLST data
Genes encoding staphylococcal enterotoxins, toxic shock syndrome toxin-1 or leucocidins were only detected in non-ST398 isolates

Discussion and Conclusion:
Human ST398, especially those from farmers and large animal veterinarians, did indeed cluster together with pig isolates indicating an epidemiological relationship. Since however no enterotoxins or leucocidins were detected in these strains their pathogenic potential might be lower compared to human associated types.
**O 32** SURVEY ON NOROVIRUS AND HAV PRESENCE IN SHELLFISH DURING WINTER SEASON 2017–2018 IN APULIA REGION (SE ITALY)

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**Introduction:**
Shellfish are an important vehicle for transmission of food-borne pathogens including norovirus (NoV) and hepatitis A virus (HAV). The risks related with consumption of shellfish are greater if these products are eaten raw or slightly cooked. The aim of this study was to collect data on the prevalence of HAV and NoV in shellfish sold in Apulia region (Southern Italy).

**Materials and Methods:**
From October 2017 to April 2018, a total of 121 samples of different shellfish species sold in Apulia region were analyzed. The samples were subjected to viral analysis for determination of HAV and NoV GI and GII using the molecular method described by International Standard ISO/TS 15216-2:2013.

**Results:**
Of the 121 analyzed samples, the nucleic acid of NoV was detected in 34 samples (28.1 %) and in one sample was detected also HAV (0.8 %). Out of 34 NoV-positive samples, 20 were NoV GII (58.8 %), 14 samples contained both genogroups GI and GII (41.2 %).

**Discussion and Conclusion:**
The low presence of HAV confirms the low prevalence of this virus in shellfish in Italy. The prevalence of NoV in shellfish revealed here is similar to what observed in other studies. With regard to the prevalence of the genogroups of NoV, the results confirm the higher circulation of NoV GII with respect to GI. The results confirm the higher frequency of NoVs from December to March, in the cold months. The findings confirm that NoVs can be easily detected in mussels, thus confirming the potential role of this food for transmission of viral gastroenteritis.
O 33 PERFORMANCE OF A SYNTHETIC OPS ANTIGEN-BASED DIVA ASSAY FOR THE DIAGNOSIS OF BRUCELLA ABORTUS IN CATTLE

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Introduction:
Brucella abortus is one of the causative agents of brucellosis, a zoonotic disease found worldwide in cattle, sheep, goats and swine that results in billions of dollars in economic loss, particularly in endemic areas. For diagnosis of B. abortus, the sLPS antigen ELISA is one of the OIE recommended tests, and is very sensitive but has a significant number of false positive serum reactors (FPSR). Synthetic antigens OPS-derived show considerable promise to decrease this false positive rate. A previously published study using a synthetic antigen-based ELISA demonstrated 100 % specificity and sensitivity with 125 negative and 45 positive samples defined by culture. The synthetic antigen ELISA correctly identified 32 of 125 sLPS ELISA FPSR as negative, resulting in a 25 % improvement in specificity for FPSR.

Materials and Methods:
The new VMRD Brucella abortus sAg antibody assay designed with synthetic antigen technology was evaluated using 256 negative samples derived from multiple US cattle herds and 31 defined samples obtained from the USDA-NVSL.

Results:
The VMRD assay demonstrated 100 % specificity and correctly identified 26/31 positive samples for a sensitivity of 84 %. Based on these sample sets, the reference cELISA and FPA assays showed a relative sensitivity of 77.4 %. The assay appears to not detect antibodies in RB51 vaccinated animals but does detect antibodies in animals vaccinated with B. abortus strain 19, similar to the cELISA.

Discussion and Conclusion:
The VMRD Brucella abortus sAg antibody assay is more sensitive than FPA or the cELISA (84 % vs 77 %) with 100 % specificity in non-infected animals.
OP09 - ADVANCES IN DIAGNOSTICS - ORAL PRESENTATIONS

O 34 MALDI-TOF MASS SPECTROMETRY IDENTIFICATION OF FUNGAL STRAINS: VALIDATION FOR THE VETERINARY PRACTICE

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Introduction:
In clinical laboratories, MALDI-TOF MS is generally recognized as a simple, reliable and cost-effective alternative for the identification of microorganisms. The method is routinely used to identify bacteria and yeasts and can successfully be implemented for molds. However, numerous reports underline the requirement of a robust database.

In this frame, our teams collaborated to develop the most extended MALDI-TOF reference spectra database for fungi. Since this database was thoroughly tested with medical isolates, the goal of this study is to evaluate whether it could also be applied effectively on veterinary isolates.

Materials and Methods:
During six months, 285 isolates from various Belgian veterinary settings were collected and analyzed. The dataset included 177 yeasts, 96 molds and 12 dermatophytes. The MALDI-TOF MS result were compared to the identifications given by the partner, and in case of discrepancy, identification was confirmed by DNA sequencing.

Results:
Using MALDI-TOF MS, 258 strains (90 %) were correctly identified up to the species level. Eighteen and 9 isolates were not or incorrectly identified, respectively, mainly due to confusions with closely related species or the absence of corresponding reference spectra in the database.

In comparison, identifications provided by the partners were as follows: 200 isolates (70 %) were correctly identified up to the species level, 39 and 46 were not or incorrectly identified, respectively.

Discussion and Conclusion:
In conclusion, MALDI-TOF MS can be a powerful and accurate method for fungal identification in the veterinary practice. Moreover, most of our database is freely available online for the scientific community.
**OP09 - ADVANCES IN DIAGNOSTICS - ORAL PRESENTATIONS**

**O 35 DEVELOPMENT OF MULTIPLEX TOOL FOR DETECTION OF PATHOGENIC AGENTS IN FOOD AND ENVIRONMENT**

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**Introduction:**
Microbiological safety of food and environment is currently a highly discussed topic. Detection of pathogenic agents in most cases relies on protocols using real time PCR (qPCR). However, qPCR has a significant drawback. There is not enough fluorescent dyes to create sufficiently robust systems for detection of larger number of targets. For this purpose, the xMAP® technology based method is being developed.

**Materials and Methods:**
The method uses two DNA probes for every target. One of them carries specific TAG sequence. These probes are linked together in presence of target DNA. Universal primer binding sequences at the ends of probes allow amplification of ligated probes. One of the two primers used for amplification is labelled. Detection of amplified probes uses sets of magnetic beads with unique absorption spectrum which are covered by anti-TAG sequences. Amplified probes are thus bound by their specific TAG sequence to complementary anti-TAG sequence on appropriate beads. Signal from beads is eventually read by MAGPIX® instrument.

**Results:**
Until now, several systems comprising human and veterinary pathogens have been developed (e.g. Campylobacter jejuni, Listeria monocytogenes, Salmonella enterica, Escherichia coli, Giardia lamblia, Taenia saginata, Norovirus, hepatitis A virus, hepatitis E virus). Validation of those systems and development of other targets is underway.

**Discussion and Conclusion:**
The developed method provides unique tool with potential to make routine analysis of various samples much more robust (up to 50 targets in one reaction), less laborious, less expensive and time consuming.

**References:**
The work was supported by Ministry of the Interior of the Czech Republic (VI20152020044).
OP09 - ADVANCES IN DIAGNOSTICS - ORAL PRESENTATIONS

O 36  NEXT GENERATION SEQUENCING TO STUDY MULTIDRUG RESISTANT SALMONELLA STRAINS

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Introduction:
Salmonella enterica is one of the most common causes of bacterial foodborne illness worldwide. The majority of Salmonella infections are attributed to consumption of contaminated food. Recently, the occurrence of Salmonella isolates resistant has increased among food and human isolates.

Materials and Methods:
The susceptibility to various antibiotics molecules was evaluated according to the guidelines of the Clinical Laboratory Standards Institute. The library pools were subjected to sequencing on a MiSeq platform (Illumina). Reads were assembled using a local installation of Spades software, a de novo assemblers that use short read sets as input and apply the de Bruijn graphs algorithm for generating longer contigs for following elaboration.

Results:
The analysis pipeline involved the whole genome sequencing by means of Illumina Miseq NGS platform and a bioinformatic analysis pipeline developed by Center for Genomic Epidemiology of Danmark CGE - DTU. A detail of resistance genes are shown in table 1. The point mutation found in almost samples of Salmonella species is shown in Table 1.

Discussion and Conclusion:
The phenotypic MDR pattern of Salmonella showed good correlation with the genotypic analysis. Many resistance genes were present and correlate positively with the resistance phenotype. The presence of a resistance gene in a bacterial strain doesn’t mean that it’s biological active (e.g. may not be transcribed or translated) but it can be very indicative of the presence of phenotypic resistance in a particular strain.

References:
O 37 ACTINOBACILLUS PLEUROPNEUMONIAE SEROTYPING BY NOVEL QPCR SYSTEM

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Introduction:
A total of 16 serotypes of Actinobacillus pleuropneumoniae (APP) have been reported so far; nevertheless, data indicating which serotypes are more virulent is still lacking for most countries. Thus, updated information related to a particular geographical area is relevant. Current techniques of APP serotyping present certain methodological limitations; cross reactions when using serological tests and inaccurate identification through Apx genes. The aim of this work was to develop a complete set of APP typing qPCR reactions to conduct a preliminary survey regarding the current situation of APP in Spain.

Materials and Methods:
One real time PCR for detecting APP (all serotypes) and 14 qPCR multiplex assays were designed to unequivocally detect serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9/11, 10, 12, 14, 15 and 16. A collection of reference strains containing every single serotype was gently provided by University of Montreal (Canada) to conduct the validation. After that, respective isolations from 76 pleuropneumonia compatible cases collected in Spain from 2015 to date were analyzed.

Results:
The proposed qPCR assays detected the required serotype only confirming the specificity of the tests. Microbiological isolations resulted: ser7 (1 %), ser8(9 %), ser 9/11(14 %), ser2(22 %), ser4(25 %) and ser13(27 %).

Discussion and Conclusion:
These results agree partially with a former Spanish report to the extent of high prevalence of ser2, ser4 and ser9/11. However, contrary to what was previously described, we found the ser13 as the most prevalent. Considering the advantages of qPCR technique, further studies are planned to validate the simultaneous detection of every serotype when analyzing directly the tonsils.

We conclude that these sets of qPCR assays are a valuable tool to serotype APP strains.
**O 38  NOVEL IVD TOOLS FOR DIAGNOSING FILARIASIS IN HUMANS AND ANIMALS**

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**Introduction:**

Filariasis is a parasitic disease caused by an infection with roundworms. These are spread by blood-feeding black flies and mosquitoes. Eight known filarial nematodes use humans as their definitive hosts. In addition there are many species known to infect animals. Most of these parasites are causing zoonotic diseases and can infect both, humans and animals. Therefore tools for monitoring acute and past diseases in animals and humans are needed to efficiently manage the disease.

**Materials and Methods:**

The aim of this work was to develop ELISAs to detect anti-Filariasis antibodies (total antibodies and IgG4) and Filariasis antigen in human as well as veterinary specimens. Predefined human samples from acute as well as past infections as well as veterinary samples have been used for the development and validation of the test systems.

**Results:**

The human and veterinary total antibody detection assay as well as the human IgG4 detection assay show a sensitivity and specificity of over 95%. The human and veterinary antigen detection assay shows a sensitivity and specificity of over 98%. The human total antibody assay has already been certified according to Directive 98/79/EC of the European Parliament.

**Discussion and Conclusion:**

With the newly developed ELISA system it is possible to diagnose Filariasis infections in both, humans and animals. This will be extremely helpful in diagnosing acute infections and monitoring treatment success in the human as well as animal population (life stock, pets and wild life). Serological surveys in the animal field will help to improve the risk assessment for the human population in certain endemic regions.
O 39 BEAD-BASED MICROARRAY FOR THE SIMULTANEOUS DETECTION OF ANTIBODIES TO ASFV AND CSFV

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Introduction:
African swine fever (ASF) and Classical swine fever (CSF) are both highly contagious diseases of domestic pigs and wild boar. In the last years, several outbreaks have been detected in the Caucasus, Russian Federation and Eastern Europe. The probability of finding these two viruses in the same area is increasing, and since differentiation between the two diseases cannot be done by clinical or post-mortem examination, a laboratory diagnosis is required.

Materials and Methods:
A bead-based microarray employing some of the most immunogenic antigens of these viruses has been developed for the simultaneous detection of antibodies against ASFV and CSFV. For ASFV the viral antigens VP72 and VP32 were used. Both proteins contain immunogenic epitopes that induce an antibody response in swine serum. For CSFV the E2 glycoprotein was used, since it is known to be the most immunogenic viral antigen and widely used in diagnosis. A triplex assay, using VP72, VP32, and E2 proteins, was set up and optimized using a panel of 352 well characterized sera from animals experimentally infected with ASFV or CSFV.

Results:
Preliminary results show a good sensitivity, and no cross-reactivity between CSF and ASF samples, neither with related infectious agents such as BDV or BVDV. Further studies will be done using field samples.

Discussion and Conclusion:
This multiplex assay shows a good tool for differential diagnosis between ASF and CSF. This method is rather versatile, offering the possibility of increasing the panel of antigens from other viruses that could be of interest for a differential diagnosis along with ASF and CSF.
OP10 - ADVANCES IN DIAGNOSTICS II - ORAL PRESENTATIONS

O 40 NANOPORE SEQUENCING IN CLINICAL VETERINARY VIROLOGY: PROOF-OF-CONCEPT STUDIES AND PERSPECTIVES

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Introduction:
Diagnostics in veterinary medicine is undergoing a revolution with the implementation of cutting-edge technologies. Oxford Nanopore Technologies (ONT) is revolutionizing the field of Next Generation Sequencing (NGS) with its MinION, a portable NGS device for high throughput sequencing. This technology is based on the reading of the DNA strand during its passage through a pore. Moreover, there is theoretically no read size limitation, allowing a full viral genome sequencing with just a few reads.

Materials and Methods:
MinION sequencing was applied to the identification of pathogens involved in three cases of avian pox in laying hens in 2016 and 2017, and one case of viral pancreatitis in guinea fowl in 2017, putatively associated with an adenovirus. Total DNA was directly extracted from tissues or swabs using phenol:chloroform and DNA was then submitted to library preparation before sequencing. Bioinformatics analysis consisted of sequence assembly using a reference and/or de novo assembly.

Results:
We obtained whole genomes of avian poxviruses (n=3) and adenoviruses (n=1) from clinical material, without any isolation or PCR-based enrichment. The quantity of viral reads ranged from 2234 to 3905 for the poxvirus and we obtained 4781 adenovirus reads.

Discussion and Conclusion:
The MinION device is obviously suitable for the sequencing of veterinary viruses from clinical material. The error rate of the reads generated by the MinION is quite high regarding other technologies such as Illumina. However, for projects that focus on the identification of a virus based on its whole genome, the long reads generated by ONT remain essential.
O 41 DEVELOPING A MICROARRAY-ELISA SANDWICH IMMUNOASSAY FOR THE DIAGNOSIS OF SWINE ENTERIC DISEASES IN FECAL SAMPLES

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Introduction:
Porcine epidemic diarrhea virus (PEDV) and porcine rotavirus (PR) are the main causative agents of acute diarrhea in piglets in Spain. PEDV is closely related to the transmissible gastroenteritis virus (TGEV) and both induce similar clinical signs and lesions. We have developed a microarray-ELISA immunoassay that allows specific, differential and simultaneous detection of antigenic markers of these swine pathogens in fecal samples.

Materials and Methods:
Microarray-ELISA immunoassays were carried out in a sandwich format and colorimetric images were analyzed and measured using CLAIR ArrayReader (Sensovation). Capture antibodies were printed onto sciPLEXPLATES 96 microplates (Scienion) using NanoPlotter microarrayer (GeSim). All mAb and antigenic protein markers were developed by Ingenasa. 41 fecal samples infected with PEDV and/or rotavirus (no TGEV positive cases were available so far) were provided by University of Córdoba and CRESA, all of them tested by RT-PCR.

Results:
Five capture and three detector mAbs were selected out of 10 by testing in checkerboard mELISA experiments. LOD of 10 and 5 ng/mL was determined for American and European types of PEDV S proteins, respectively. LOD of TGEV virus spiked in feces was only 2-3 times higher than that using diluent buffer. Taking RT-PCR as reference, mELISA rendered 76 % and 63 % of sensitivity for PEDV and rotavirus diseases. No false positive results were obtained from any of the three diseases.

Discussion and Conclusion:
Further analysis will be done to improve mELISA diagnostic parameters by running a higher number of natural samples not only tested by RT-PCR but by other immunological method, that is more fairly comparable to mELISA.
O 42 DETECTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS PRRSV USING AN APTAMER-ANTIBODY SANDWICH ASSAY (ELAAS).

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Introduction:
Porcine Reproductive Respiratory Syndrome (PRRSV) is a disease caused by an enveloped virus of the family Arteriviridae. The presence of this disease generates great economic losses. We have developed a sandwich enzymatic method based on an immobilizing capture aptamer against the N protein, and a monoclonal antibody (mAb) for the detection of PRRSV in sera and fluids (ELAAS).

Materials and Methods:
Aptamers against the recombinant N protein were selected by the SELEX technique and used, in combination with monoclonal antibodies, to develop an ELAAS. The IN3 # 6,8R aptamer conjugated to Biotin was captured on Streptavidin plates in carbonate buffer pH = 9.6, overnight at 4°C. After washing and stabilization steps, samples were incubated one hour at 37°C and then incubated with the mAb-1AC7 (anti-N) conjugated to Peroxidase (HRP). The reaction was reveal with TMB for 10 minutes before being stopped with 0.5M H2SO4. Enzyme activity was measured in an absorbance reader at 450 nm.

Results:
The ELAAS was able to detect 75pg/ml of both European and American recombinant protein. Field samples evaluated by RT-PCR, correlated perfectly with the newly developed ELAAS. The ELAAS showed a sensitivity of 91.3% and Specificity of 88.9%.

Discussion and Conclusion:
The ELAAS assay can be used as an alternative method for the rapid and accurate detection of PRRSV. This new method does not require any complicated or expensive equipment additional to those currently used in laboratories for the diagnosis of this disease, so it can be a very useful tool in the control of the disease.

References:
E-Multi-DETECT
EUROSTARS
POSTER PRESENTATIONS
P 01 A NEW REAL TIME RT-PCR FOR DETECTION AND DIFFERENTIATION OF AVIAN BORNAVIRUSES

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Introduction:
Avian bornaviruses (ABV) are suspected to be the causative agent of proventricular dilatation disease (PDD), which has been diagnosed mainly in psittacine birds worldwide, but was also detected in other avian species. Today, ABV comprise 13 genotypes divided into 5 phylogenetic groups. Post mortem diagnosis of PDD is usually done by necropsy and histology. Confirmation of ABV is achieved by immunohistochemistry or reverse transcriptase polymerase chain reaction (RT-PCR). Various conventional RT-PCR methods have been developed. Some of them cover a broad range of ABV but none of them is able to detect all ABV genotypes. Furthermore, real-time RT-PCRs were shown to be more sensitive than conventional RT-PCR but they did not improve coverage of ABV genotypes. We developed a real-time RT-PCR, which can be used to detect and differentiate the five known phylogenetic groups of ABV.

Materials and Methods:
Degenerate primers (two forward and one reverse primer) were designed to detect ABV from all phylogenetic groups. Three probes with different labels were designed to differentiate between the three of the five phylogenetic ABV groups passeriformes (CaBV, EsBV), psittaciformes 1 and waterbirds.

Results:
First the primers were tested in a conventional RT-PCR. A 125 bp band was detected for 9 different ABV genotypes but not for mammalian bornavirus (BoDV-2). In the real-time RT-PCR the three probes resulted in detection of the ABV RNA of the respective phylogenetic group.

Discussion and Conclusion:
Further experiments will reveal more detailed data concerning sensitivity and selectivity of the new real-time RT-PCR assay.
PS 01 - POSTER SESSION

P 02 UNDIAGNOSED Q FEVER ABORTION IN CATTLE?

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Introduction:
Pathogenesis of Q fever abortion in cattle is still poorly understood. *Coxiella burnetii* (Cb) is detected in abortions but at normal parturitions, too. Two unusual cases of abortions are presented which might have been missed by conventional diagnosis.

Materials and Methods:
Two dairy cow herds with >10% abortions were analysed. In one herd (A) abortions were restricted to heifers; in the second herd (B) all age-groups were affected. Case animals and age-matched controls were tested for phase I- and phase II-antibodies by ELISA. Surveillance included qPCR-testing of vaginal swabs collected post-partum. Herd B was vaccinated with Coxevac® and cases/controls were reanalysed for phase II-antibodies.

Results:
In both herds Cb was detected at normal parturitions. In herd A heifers with abortion had no phase II-antibodies while controls tested positive. In herd B serological data were inconclusive: both groups were positive, but only three case-cows (one primiparous!) had low phase II-titers (<40). Cb was not detected in foetuses/placentas at post-mortems. However, after vaccination case cows showed significantly higher phase II-titers compared to controls.

Discussion and Conclusion:
Cb-detection in swabs and phase II-antibodies in primiparous cows confirmed infection. Absent or low phase II-titers (undetectable in CFT!) might be associated with cellular immunity, which is a classical situation for intracellular pathogens! The vigorous antibody response after vaccination indicated a boostered immune response in case cows and suggested Cb as the cause of abortion. Abortion is explained by re-infection of immune or rather hyperreactive animals. The failure to detect Cb in foetus/placenta is explained by the short time for multiplication.
P 03 MASTITIS DIAGNOSIS UTILIZING A TURNKEY SOLUTION FROM THERMO FISHER SCIENTIFIC’S ANIMAL HEALTH GROUP

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Introduction:
Identifying pathogens in mastitic milk traditionally required culturing, which is labor-intensive and time-consuming. The advent of qPCR testing provides a faster and much more convenient alternative. The Animal Health group at Applied Biosystems™ (AB) Thermo Fisher Scientific now offers a complete mastitis solution.

Materials and Methods:
The solution includes a new module to be use with the MagMAX™ CORE Nucleic Acid Purification Kit for DNA isolation protocol. This kit offers benefits such as ambient-temperature shipping and storage, inclusion of all reagents in one box, and verified automated processing on the Thermo Fisher™ King Fisher™ magnetic particle processor. The extracted DNA can then be tested with three different 4-plexed VetMAX™ MastiType PCR kits, on either the 7500 or QuantStudio 5 (QS5) series of AB Real-Time PCR Systems. Data analysis for each of these uses a new cloud-based software with a user-friendly graphical interface.

Results:
The MagMAX™ CORE Nucleic Acid Purification Kit has been validated on a broad range of mastitic milk field samples containing each of the target organisms. Extracts have been validated on the three different 4-plexed VetMAX™ MastiType PCR kits (Micro4, Myco8 and Multi panels) allowing a total detection of 19 species. This entire workflow takes about 3 hours and provides equivalent results on the 7500 and QS5 Real-Time PCR Systems.

Discussion and Conclusion:
Detection of mastitic pathogens infections needs diagnostic tools that are easy to handle and can provide rapid and accurate results in order to ensure the efficacy of surveillance and control programs. The Thermo Fisher Scientific mastitis solution is designed to meet these expectations.
**P 04 VALIDATION OF THE ID GENETM PRRSV QPCR KIT**

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1 IDvet, Genetics, Grabels, France

**Introduction:**
Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious porcine viral disease, causes economic losses in the pig industry worldwide. PRRS viruses (PRRSV) is classified into two distinct genotypes: European (EU) and North American (NA). In 2006, a highly pathogenic NA strain (HP-PRRSV), characterized by high fever, morbidity and mortality, emerged in China.

The ID Gene™ PRRSV kit is a triplex real time PCR assay that allows the detection and differentiation of both types of PRRSV. It simultaneously amplifies target RNAs of the European and North American types (including HP-PRRSV strain) and an exogenous internal control.

**Materials and Methods:**
The qPCR kit was validated in collaboration with the FLI Reference Laboratory in Germany (Friedrich-Loeffler-Institut). Panels used to test the inclusivity study:

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>14</td>
</tr>
<tr>
<td>Field</td>
<td>82</td>
</tr>
<tr>
<td>Ring trial</td>
<td>7</td>
</tr>
<tr>
<td>Vaccinate</td>
<td>5</td>
</tr>
</tbody>
</table>

Diagnostic sensitivity was evaluated through the analysis of samples previously analyzed with FLI in-house method:
- 107 field infected or exposed samples, including 10 field samples of chinese HP-PRRSV strain (Organs, Sera, Tissues homogenates, Oropharyngeal fluids)
- 25 negative samples

**Results:**
The inclusivity panels of strains were reliably detected, regardless of the genotype.

<table>
<thead>
<tr>
<th>Analytical sensitivity of the multiplex PRRSV RT-qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU type</td>
</tr>
<tr>
<td>10 copies per PCR</td>
</tr>
<tr>
<td>NA type</td>
</tr>
<tr>
<td>5 copies per PCR</td>
</tr>
<tr>
<td>100% of positive samples from both types were found positive</td>
</tr>
</tbody>
</table>

The IDPRRSV kit showing a high diagnostic sensitivity.

**Discussion and Conclusion:**
The ID Gene™ PRRSV Triplex PCR kit demonstrates high specificity and sensitivity on all matrices used for the detection of PRRSV by RT-qPCR. It efficiently detects positive animals in the field and correctly identifies all strains. Combined with magnetic beads extraction kit (MAGFAST™, 20 minutes) and the IDEAL™ automate, results may be obtained in less than 2 hours. The IDPRRSV kit is a ready-to-use, reliable tool for the detection and typing of PRRSV.
P 05 NEW APPROACH FOR SMALL RUMINANT LENTIVIRUS FULL GENOME CHARACTERIZATION REVEALED THE CIRCULATION OF DIVERGENT STRAINS

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2 Istituto Zooprofilattico Sperimentale della Sardegna, Virology, Sassari, Italy

Introduction:
Small Ruminant Lentiviruses (SRLV) include at least 4 viral genotypes highly divergent from each other. New phylogroups have been identified targeting conserved regions. However this approach suffers from the potential risk to misamplify highly divergent strains. Pathogenic strains are frequently adapted to fibroblastic cells but putative non-pathogenic strains have been recently detected and their isolation require a different approach.

Materials and Methods:
We developed a fast and effective method for SRLV full genome characterization after cell culture isolation. Spleen explants from SRLV serological positive goats and sheep from northwestern Italy were collected. Spleen derived macrophage culture was monitored for Reverse Transcriptase Activity. The RNA was extracted from the supernatant of positive culture and full genomes were obtained using Illumina MiSeq platform.

Results:
A total of 42 samples were collected from 16 sheep and 26 goats. Thirty-three were antibody positive and 26 were serotyped. Twenty-six RT activity positive cultures were obtained. Nineteen isolates were readily available after the first collection time, while additional 7 isolates showed RT activity after 3–4 weeks p.c. Half of the strains showed CPE on fibroblastic cells. Full genome was obtained by 22 isolates belonging to A and B genotypes.

Discussion and Conclusion:
The success of this approach is based on the following features: spleen is one of the main target for SRLV persistence; red pulp is a reserve of resident macrophages, the main target for SRLV replication in vivo; RTA is a sensitive assay for any replicating retrovirus; de novo sequencing do not require genetic knowledge in advance.
EVALUATION OF A NEW DOUBLE-ANTIGEN INDIRECT ELISA FOR THE SPECIFIC DIAGNOSIS OF PORCINE BRUCELLOSIS

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² Acscidiate / LNCR, a, Maisons Alfort, France
³ Anses, uzb, MaisonsAlfort, France

Introduction:
Current serological tools (complement fixation test –CFT- or Rose-Bengal test –RBT-) do not offer satisfactory results for swine brucellosis diagnosis. A recently described indirect ELISA (iELISA) for the detection of anti-\textit{B. suis} antibodies, based on two antigens derived from rough (r-) and smooth (s-) \textit{Brucella} LPS (McGiven, 2012), was shown to limit false positive serological reactions (FPSR).

Materials and Methods:
The ID Screen® Brucella suis Indirect is a double-well iELISA using s- and r-LPS antigens. Test performance was assessed by testing 3 groups of swine sera (origin: France): infected herds positive reactors (n=286; positive in CFT and RBT); free herd FPRS (n=202; positive in RBT); free herds negative reactors free herds (n=720, RBT negative).

Results:
Results were expressed as S/P % and interpreted as per manufacturer’s instructions. Animals are positive for \textit{B. suis} when positive with both s- and r-LPS antigens. FPRS should only react with s-LPS, and are considered as negative.

Measured sensitivity was 72.7 % (67.5–77.9 %, n=286); specificity was 99.9 % (99.6–100 %, n=720). Out of 202 FPRS, 197 were found negative with the ELISA, drastically reducing the FPRS rate: measured Sp on FPRS was 97.5 % (95.3–99.7 %, n=202). Different cut-off values were tested; A ROC curve analysis was performed. It is possible to increase the \textit{B. suis} sensitivity without affecting specificity. Increasing sensitivity could be interesting when testing herds where the presence of \textit{B. suis} has been confirmed.

Discussion and Conclusion:
This iELISA could improve surveillance and control programs of porcine brucellosis by reducing the impact of FPRS in herds historically negative for \textit{Brucella} infections.
P 07 A NEW ELISA ASSAY FOR GLANDERS DIAGNOSIS

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2 ANSES, uzb, Maisons-Alfort, France
3 ANSES, uzb, Maisons Alfort, France
4 Lanagro, m, Recifir, Brazil
5 Faculty of Veterinary Science-, University of Agriculture, Faisalabad, Pakistan

Introduction:
Glanders is a zoonotic disease caused by Burkholderia mallei, a gram-negative bacterium. The internationally mandatory complement fixation test (CFT) for testing of equine sera is repeatedly leading to discrepant results. While false positive results pose a problem for diagnosticians, animal health authorities and owners, false-negative results can turn a risk into a possible threat.

Materials and Methods:
The ID Screen® Glanders ELISA is an indirect enzyme-linked immunosorbent assay (ELISA) based on a native semi-purified antigenic fraction. Specificity was evaluated with sera from disease-free and non-vaccinated regions (n=640). Sensitivity was evaluated with sera coming from endemic areas (Brazil, Pakistan; n=253 and 49, respectively). ELISA results were compared with CFT, Western-Blot and/or bacteriology.

Results:
The measured specificity was 99.7 % (CI95 %: 98.9–99.9) considering doubtful samples as negative and 99.4 % (CI95 %: 98.4–99.8) considering doubtful samples as positive. Out of 302 sera collected in a farm with an ongoing outbreak of glanders, the ID Screen® picked up 176 samples whereas the CFT identified 27 samples as positive and 46 as doubtful. Out of 108 sera CFT negative/ELISA positive, 60 were confirmed positive by Western-Blot, 33 were negative and 15 gave inconclusive results.

Discussion and Conclusion:
Validation results showed an excellent specificity and a good sensitivity, equivalent or greater compared to techniques currently used. However, more extensive validation of the test is needed. This ELISA technique could be a good alternative to actual methods for the diagnosis of glanders.
P 08  EXTENDED VALIDATION DATA OF ELISAS ON WILD BOAR, ORAL FLUID ELISA PROTOTYPE, AND QPCR FOR AFRICAN SWINE FEVER DIAGNOSIS

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1 IDvet, R&D, GRABELS, France

Introduction:
IDvet offers ASF diagnostic solutions for antibody detection (indirect and competitive ELISAs) in serum, blood filter paper and meat juice samples, and qPCR. This study shows complementary data for ELISAs performance on wild boar sample - that can possibly raise specificity issues -, results of an oral fluid (OF) ELISA prototype positive, and qPCR validation data.

Materials and Methods:
Diagnostic specificity of the IDScreen® ASF ELISAs on wild boar was evaluated through the analysis of 255 wild boar samples coming from disease-free areas. 85 OF coming from experimental infections and 56 negative OF were tested on the OF ELISA prototype. Diagnostic sensitivity of the IDGene TM qPCR was evaluated through the analysis 162 field infected or exposed samples (tissues or blood) and 303 field samples (blood, organs, soft tick homogenates) from recent outbreaks (Eastern Europe, Africa). Theses sample were also tested with the UPL-qPCR and with the OIE qPCR.

Results:
The measured specificity on the 255 wild boar samples was 100 % for the Indirect ELISA and 98.4 % for the competitive ELISA. The specificity of the OF ELISA was 100 % and the correlation with the OIE modified ELISA protocol was excellent (k=0.953). For the IDGene qPCR, 95 out of 162 field infected or exposed samples (tissues or blood) tested were positive (UPL-qPCR : 91 % and OIE qPCR : 91 %), 303 field samples (blood, organs, soft tick homogenates) from recent outbreaks (Eastern Europe, Africa) were tested; the measured sensitivity was 98.6 %.

Discussion and Conclusion:
IDvet offers a full range of ELISAs and qPCR tools for accurate and rapid diagnosis of ASF.
P 09 NEW VALIDATION DATA FOR THE ID SCREEN® PRRS INDIRECT ELISA

L. Comtet¹, L. Olagnon¹, P. Pourquier¹
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Introduction:
The ID Screen® PRRS Indirect ELISA is designed to detect antibodies directed against Porcine reproductive and respiratory syndrome (PRRS) in porcine serum and plasma. It may be used with individual porcine serum or plasma. The kit includes microplates coated with PPRSV recombinant type 1 and type 2 proteins and an anti-porcine IgG horseradish peroxidase (HRP) conjugate. This poster presents updated validation data.

Materials and Methods:
Diagnostic specificity was evaluated on 1844 samples: 950 fattening pigs sera from herds known as PRRS negative for years (Brittany, France); 894 breeding swine sera from France and others European countries. 648 sera from negative and infected herds were tested in parallel using the ID Screen® PRRS Indirect ELISA and a commercially available and widely used ELISA (Kit A).

Results:
The measured specificity was 99.9 % (CI95 %: 99.8 % - 100.0), n=1844. Of 640 samples tested, 648 gave identical results on both tests. The percentage of correlation was 98.8 % and the k coefficient (0.974; CI95 % 0.955–0.992) indicated very high correlation with Kit A. In the range 0–2.5, S/P values were equivalent to kit A. (r²=0.85; y=0.93x+0.1)

Discussion and Conclusion:
The ID Screen® PRRS Indirect ELISA kit demonstrates high specificity, efficiently detects positive animals in the field, correctly identified all strains, and demonstrates excellent correlation with another commercial ELISA. Results are obtained in just 75 minutes. Filter paper samples might be used with this kit, making sample collection easier and cheaper.

The ID Screen® PRRS Indirect ELISA is a reliable tool for the detection of anti-PPRSV antibodies.
P 10 REFINEMENT OF THE RAPID PASSAGE PROTOCOL FOR ISOLATION OF AVIAN INFLUENZA VIRUS FROM SIX DAYS TO TWO DAYS

V. Coward1, R. Hansen1, H. Everest1, V. Ceeraz1, C. Russell1, J. Cooper1, E. Agyeman-Dua1, A. Holland1, S.M. Reid1, J. Mynn1, T. Dorsett1, S.M. Brookes1, I.H. Brown1

1 Animal and Plant Health Agency, Department of Virology, Addlestone, United Kingdom

Introduction:
Virus isolation by rapid passage in embryonated fowl eggs (EFEs) is a gold standard technique for avian influenza virus (AIV) detection. At day two post-inoculation into the allantoic cavity, the chorio-allantoic fluid (CAF) from one egg is tested for HA activity; if positive the CAF is characterised to identify the virus. If negative, it is further passaged in EFEs. At day six, remaining eggs are tested by haemagglutination assay (HA). In this study, clinical samples positive for H5N6 HPAIV were inoculated into EFEs to determine whether the currently-adopted rapid passage protocol can be reduced from six days to a single two–day passage. This would support a reduction in the length of time businesses/premises remain under official restrictions when investigating notifiable disease suspicions, reducing costs to the government or industry through earlier detection of infection.

Materials and Methods:
Nine oropharyngeal or cloacal swabs from “found-dead” wild birds were inoculated into EFEs and incubated at 37°C for six days. CAF was harvested after two days (passage-one) and six days (passage-two), and tested by HA and the matrix-gene assay for generic AIV detection (Nagy et al., 2010).

Results:
Passage-one harvest had no HA titre, but was weakly-positive by RRT-PCR. However, virus replicated quickly during passage-two: killing the eggs within one day.

Discussion and Conclusion:
These preliminary data will lead to further investigation to confirm whether the current six-day rapid passage protocol can be reduced to a single two–day passage (or an alternative protocol using rapid passage) to isolate AIV.
P 11 THE RESEARCH OF SEXED SEMEN OF BULLS-PRODUCERS

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2 Moscow State Academy of Veterinary Medicine and Biotechnology Named K.I. Skryabin

Introduction:
Artificial insemination is a successful technique that is used for the breeding of cattle. Sex-sorted semen is produced using physical and chemical methods, so it is important to control semen quality.

Materials and Methods:
Sex-sorted frozen semen samples from 20 bulls were studied. Forward motility (FM), presence of abnormal forms, acrosome integrity and sperm DNA fragmentation index were examined.

Results:
1. The percentage of the FM in all studied samples was lower than 40 %.
2. In 80 % of these samples the content of abnormal forms exceeds 18 %.

Table 1. The content of pathological forms in the samples

<table>
<thead>
<tr>
<th>Bulls-producers</th>
<th>the number of the studied sperm samples</th>
<th>The content of pathological forms in the samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Separated head</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>23%</td>
</tr>
</tbody>
</table>

Fig. 1. Pathological forms of sperm (a - separated head, b - twisted tail, c - bent tail, d - abnormal head)

3. The average number of sperm with intact akosome was 74 % in all samples studied, and from one bull the content of the number of sperm with intact akosome was less than 50 %.
4. It was revealed that in 4 bulls the index of DNA fragmentation was more than 30 % in the sperm head and in 6 bulls with fragmented mtDNA the glow of the tail part was found, i.e. mitochondrial dysfunction. At 6 bulls the glow of the tail part was revealed, i.e. mitochondrial dysfunction.
Discussion and Conclusion:
The low mobility, a large number of abnormal forms were recorded and mitochondrial dysfunction was detected in sperm of bulls-producers. Thus, the expanded spermogram of samples of sex-sorted semen showed a close relationship between the structure of mitochondrial (mtDNA) and nuclear DNA and the biological fullness of sperm. The content of sperm with abnormal morphology, especially with an anomaly of the flagellum, has a close relationship with the violation of mtDNA, and has a direct impact on the FM sperm.
P 12 A MOLECULAR METHOD AS REPLACEMENT FOR CLASSICAL SEROTYPING OF THE MOST COMMON SALMONELLA FROM PORK AND POULTRY SECTORS IN BELGIUM

M. Gand1, W. Mattheus1, A. Saltykova2, N. Roosens2, K. Dierick1, K. Marchal3, S. De Keersmaecker2, S. Bertrand1

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2 Sciensano, Platform Biotechnology and Molecular Biology, Ixelles, Belgium
3 Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium

Introduction:
The combat against zoonotic Salmonella is crucial to rapidly identify serotypes that may contaminate the food chain. Despite its worldwide use, the serotyping by slide agglutination, the classical technique for the characterization of Salmonella, is expensive, not always objective and requires trained personnel. Molecular methods have proven to be more suitable tools for a fast and accurate geno-serotyping. Here, based on the scientific literature and our own genomic study using Whole Genome Sequencing data, we developed a Multiplex Oligonucleotide Ligation – Polymerase Chain Reaction (MOL-PCR) method using the Luminex technology for the identification of the Salmonella serotypes isolated from poultry and pork.

Materials and Methods:
Specific molecular markers for different Salmonella serotypes and/or serogroups are detected by specific ligation probes during the MOL-PCR. The amplified ligation fragments created this way are hybridized to microspheres and detected via fluorescence measurements on a Luminex instrument.

Results:
The developed multiplex is composed of 20 markers. When detected together, the specific combination of markers allows the identification of the targeted serotypes. These combinations are converted into a barcode system using a Decision Support System (DSS) developed in-house, easily giving a clear and objective result. In case of partial identification, the system clusters the samples into one of the most common serogroups if it belongs to one of them.

Discussion and Conclusion:
The MOL-PCR linked to the Luminex technology allows the fast geno-serotyping of the most common Salmonella encountered in Belgium. The DSS helps to interpret the data, yielding a clear, rapid and objective result in contrast to the classical serotyping technique.
P 13 VALIDATION OF A NEW SEROLOGY TOOL FOR JOHNE’S DISEASE IN CATTLE, INCLUDING PRELIMINARY VALIDATION FOR BOVINE MILK AND CAPRINE SERUM

S. Hines¹, J. Rzepka¹

¹ VMRD- Inc., VMRD- Inc., Pullman, USA

Introduction:
Johne’s disease is a chronic and contagious wasting disease of all ruminants caused by Mycobacterium avium ssp. paratuberculosis (MAP). A new indirect ELISA has been validated for the detection of anti-MAP antibodies in bovine serum, with preliminary validation in bovine milk and caprine serum.

Materials and Methods:
This assay was developed with antigen processed from whole MAP, and anti-ruminant secondary antibody to detect cattle, sheep, and goat anti-MAP antibodies. Bovine serum (negative n=243, positive n=61) and milk (negative n=98, positive n=91) samples were acquired from the US Johne’s Disease Integrated Program, with additional positive sera from the University of Wisconsin (n=329) and NVSL (n=43). All were characterized by fecal culture and PCR and tested in the VMRD MAP Ab ELISA kit. For caprine serum, negative samples were confirmed by either culture (n=76) or necropsy (n=10). Positive samples (n=71) were obtained from a Johne’s vaccination study and confirmed by necropsy.

Results:
Sensitivity and specificity for bovine serum were 93.1 % and 90 %, respectively. Two other commercial ELISAs demonstrated sensitivity of 91 % with 85.4 % specificity and 86.6 % with 89.3 % specificity on this sample set. For milk, sensitivity and specificity were 72.5 % and 88.8 % respectively, compared to 64.8 % and 86.7 % for another commercial ELISA. In caprine serum, Se was 72.5 % with specificity of 100 %. In two other commercial ELISAs, this sample set showed sensitivity of 62.9 % and 52.9 % at 100 % specificity.

Discussion and Conclusion:
This indirect ELISA has been submitted for USDA licensure for bovine serum based on this validation performance, and validation is ongoing for bovine milk and caprine serum.
PS 01 - POSTER SESSION

P 14 NOVEL DIAGNOSTIC ASSAYS FOR BIOMARKER-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. Milk is a decisive economic factor for the producers, which can only be sustained through the maintenance of animal’s health. Thus, the detection of sick animals within the dairy herds can be of economic advantage for the farmer.

Materials and Methods:
Within our innovative and multidisciplinary framework, biomarker-based veterinary diagnostics’ sensitive diagnostic assays for cattle will be developed. These assays will be based on monoclonal antibodies against specific biomarkers capable of detecting sick animals prior the emergence of clinically detectable disease symptoms.

Results:
The department ‘Therapy Validation’ of the Fraunhofer Institute for Cell Therapy and Immunology Leipzig exhibits excellent expertise and technical requirements for the development of immunoassays. 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 may 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.

Discussion and Conclusion:
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.
PS 01 - POSTER SESSION

P 15 INVESTIGATION OF VIRULENCE GENES USING MULTIPLEX PCR AND ANTIBIOTIC RESISTANCE AGAINST ENTEROCOCCUS SPP. ISOLATED FROM BROILER CHICKEN IN KOREA

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Introduction:
Enterococcus spp. are opportunistic pathogens causing lameness in broiler chickens, and result in serious economic losses worldwide. The virulence of Enterococcus spp. is associated to be several potential virulence genes including fsr, efm, esp, cylA, cad1, ace, gelE and asa1. We developed a multiplex PCR for the simultaneous detection of virulence genes in Enterococcus spp. and report the prevalence of virulence genes and antimicrobial resistance in Enterococcus isolates.

Materials and Methods:
The multiplex PCR with 8 pairs of primers was performed with 80 Enterococcus isolates from broiler chickens with lameness. We confirmed the detection limits of the multiplex PCR. And also we tested antibiotic susceptibility against 49 Enterococcus spp. with 16 antimicrobials.

Results:
The detection limits of the multiplex PCR for E. faecium, E. faecalis and E. hirae were 64.0 pg/ul, 320.0 pg/ul and 1.6 ng/ul DNA, respectively. The efm and cad1 genes were detected in all 26 E. faecium, and only cad1 gene was observed in E. hirae. No virulence genes were found in E. gallinarum isolates. In antibiotic susceptibility test, all the 49 isolates were resistant to tigecycline. The most frequent resistance properties were resistant to ciprofloxacin and the next is quinupristin/dalfopristin.

Discussion and Conclusion:
This is the first multiplex PCR assay to simultaneously detect 8 virulence genes in Enterococcus spp., and this method provides diagnostic value for the accurate, rapid and convenient detection of virulence genes. The majority of isolates are resistant to tigecycline, ciprofloxacin, quinupristin/dalfopristin and tetracycline.

References:
PS 01 - POSTER SESSION

P 16 A SEVERE OUTBREAK OF INCLUSION BODY RHINITIS IN PRE-WEANED PIGS IN THE UK

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⁴ APHA, Veterinary Surveillance, Bury St Edmunds, United Kingdom

Introduction:
Porcine cytomegalovirus (PCMV) infection is widely prevalent among pigs, mostly being sub-clinical and clinical disease, known as inclusion body rhinitis (IB), tends to be mild, although neonates can develop fatal systemic disease. An ongoing problem of sneezing, nasal discharges, malaise and reduced growth with high morbidity in litters from three weeks of age into the post-weaning period was investigated. Submitted pigs showed marked accumulations of mucopurulent material around the turbinates and significant occlusion of nasal passages. Disease occurred in litters from all sow ages.

Materials and Methods:
DNA was extracted from tissues from three piglets and screened initially by a Pan-herpes PCR (Ehlers et al. 1998) followed by a PCMV qPCR (Vladimir et al. 2016). Histopathology and bacteriology were undertaken on turbinates and lung.

Results:
Histopathology revealed lesions in the nasal cavity pathognomonic for IB. All samples were positive in the Pan-herpes PCR with sequences similar to those of PCMV and porcine lymphotropic herpesvirus (PLHV). PCMV qPCR further confirmed PCMV nucleic acid in all samples with the highest load in turbinates. Testing showed no evidence of concurrent swine influenza.

Discussion and Conclusion:
Primary PCMV infection was confirmed as the cause of this significant IB outbreak with secondary Pasteurella multocida infection. A large intake of replacement gilts had been sourced several months earlier. Whether this disrupted endemic immunity to PCMV in the resident herd is unclear. The role of the immunosuppressive PLHV is also uncertain in this outbreak. Gilt acclimatisation procedures were improved and piglet vaccination for PRRS was delayed to avoid exacerbating disease.
PS 01 - POSTER SESSION

P 17 SVANOVIR® A. SUUM-AB ELISA IS AN EFFECTIVE SCREENING TOOL FOR DETERMINING THE INFECTION LEVEL OF ASCARIS SUUM IN FATTENING PIGS

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² Ghent University, Laboratory of Parasitology - Faculty of Veterinary Medicine, Ghent, Belgium

Introduction:
Ascariasis is a chronic disease in pigs caused by the nematode Ascaris suum. It is highly prevalent in industrialized pig farms as well as in traditionally managed indoor herds and causes significant production loss. Ascariasis is subclinical and effective monitoring is warranted.

SVANOVIR® A. suum-Ab ELISA is a diagnostic tool to assess the exposure levels to A. suum. A serological test is a more practical and accurate alternative to standard test methods.

Materials and Methods:
The SVANOVIR® A. suum-Ab is based on the assay from the University of Ghent and optimized for commercial use. It is a solid phase indirect ELISA coated with Ascaris suum Haemoglobin antigen, detecting antibodies to A. suum. SVANOVIR® A. suum-Ab has been validated on serum from experimentally infected pigs and on samples from a field study. The precision of assay (repeatability and reproducibility) has been studied. All development steps and production of SVANOVIR® A. suum-Ab follow ISO certified processes.

Results:
Results show that the SVANOVIR® A. suum-Ab has a strong agreement with the assay from Ghent University on the experimentally infected animals and the field samples. Validation studies also show that the assay is robust and results were reproducible.

Discussion and Conclusion:
The results from the validation studies showed that SVANOVIR® A. suum-Ab is a robust and sensitive diagnostic tool that can be used to assess exposure of fattening pigs to A. suum. This allows for an estimation of the economic losses due to this parasite and monitors the efficacy of anthelmintic treatment programs.
PS 01 - POSTER SESSION

P 18 DEVELOPMENT OF AN BARTONELLA HENSELAE SPECIFIC ELISA

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² Research and Development, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany
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Introduction:
Bartonella henselae causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. 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.
The aim of this study is to design an ELISA for detection of B. henselae to improve the shortcomings of the currently used immunofluorescent test (IFT), e.g., objective and reproducible results and less hands-on time.

Materials and Methods:
Test development is based on different B. henselae strains and quality assured patient 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 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 and Conclusion:
We show a strategy for antigen testing and selection from B. henselae protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for B. henselae is possible.
P 19  PFGE ANALYSES OF SHEWANELLA SPP. COLLECTED FROM FRESHWATER FISH

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Introduction:
Pulsed-field gel electrophoresis (PFGE) is considered as a gold standard for typing of many bacterial pathogens. The usefulness of this technique for differentiation of Shewanella spp., a causative agent of shewanelliosis has not yet been described.

Materials and Methods:
Selected isolates of Shewanella putrefaciens, S. xiamenensis, S. oneidensis collected from different freshwater fish species were used in our study. PFGE was performed using in-house adapted Standard Operating Procedure for PulseNet PFGE of Escherichia coli O157:H7, Escherichia coli non-O157 (STEC), Salmonella serotypes, Shigella sonnei and Shigella flexneri (https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). Agarose embedded genomic DNA samples were digested with several enzymes (FseI, SgrDI, XbaI, Smal, Apal) selected based on in silico analysis of reference strains genome.

Results:
Each of the applied enzyme produced PFGE profiles which were inadequate for BioNumerics analysis. The main reason was a large number of the bands located too close for reliable analysis. Often, the size of DNA fragments was much lower than requested 33.3 kb. Laboratory results significantly differed from theoretical scheme generated in silico.

Discussion and Conclusion:
The study indicates that contemporary PFGE method is not suitable diagnostic tool for typing of S. putrefaciens, S. xiamenensis and S. oneidensis.

References:
This work was supported by the Polish National Science Centre project “Studies on genotypic characterisation of Shewanella putrefaciens group isolates from freshwater fish in Poland” (Grant No 2015/19/N/Z7/01687).
P 20 DIVERSITY OF SHEWANELLA SPP. ISOLATED FROM BALTI

C CODE (GADUS MORHUA)

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Introduction:
Shewanella spp. is group of halophilic bacteria isolated mainly from marine and brackish wa-
ters, but also from the intestine of sea fish. Our study of Baltic codes showed a large number of
microorganisms isolated from the skin and kidney, among which bacteria biochemically identi-
fied as Shewanella putrefaciens group were dominant. Due to the fact that the biochemical
analysis was not able to properly determine the bacterial species, molecular studies of the col-
clected isolates were performed for this purpose.

Materials and Methods:
Total of 45 isolates of Shewanella spp. collected from Baltic code were analyzed based on
sequencing of the conservative region of the 16S rRNA gene. The purified PCR-products about
1600 bp were sequenced using DNA Analyzer. Obtained results were alimented using Molecular
Evolutionary Genetics Analysis (MEGA) version 7.0 and compared with GenBank sequences.

Results:
Phylogenetic analysis revealed Shewanella baltica as a dominant species (41 isolates - 91 %),
with similarity level varied from 99.52 % to 100 %. Only two isolates were identified as S. putrefa-
ciens (4 %) with conformity of 99.35–99.72 %. One isolate was classified as Shewanella denitrifi-
cansat a similarity level 99.72 %. Classification of one isolate to the species level was impossible.

Discussion and Conclusion:
Our results indicate predominant role of Shewanella baltica among bacteria isolated from Bal-
tic code belonging to Shewanella putrefaciens group. This is significant difference compare to
Shewanella species isolated from freshwater fish, where S. putrefaciens are dominant.

References:
This research was supported by The National Centre for Research and Development under
the Strategic Program Biostrateg II [grant no 296211/4/NCBR/2016].
ERIC-PCR ANALYSES OF SHEWANELLA SPP. COLLECTED FROM FRESHWATER FISH

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Introduction:
Different species of bacteria Shewanella cause health disorders in freshwater fish. Based on 16 S rRNA gene sequence differentiation between species were observed, however it does not provide insight into genetic diversity needed for characterization of these bacteria.

Materials and Methods:
To overcome this gap we analyzed 56 Shewanella spp. isolates (including 51 filed isolates from different freshwater fish species and 5 reference strains) with ERIC-PCR assay. The results were calculated with BioNumerics software (version 7.5).

Results:
Analysis of 56 samples revealed 49 different genotype patterns (UPGMA, Dice, 1 % position tolerance). With an arbitrary threshold of 55 % similarity, the profiles were clustered into 17 distinct groups. Eleven isolates (20 %) were classified to two predominant groups (6 and 13). Six groups (1, 2, 4, 8, 16, 17) were represented by single isolates. Two reference strains (ATCC 8073 and JCM16212) were clustered into the same group with similarity level 76.9 % while the remaining strains (ATCC 700550, ATCC 8071, ATCC BAA) represented unique profiles.

Discussion and Conclusion:
Our study confirmed the genetic diversity of Shewanella spp. and indicates that ERIC-PCR might be useful tool for characterization of closely related Shewanella isolates.

References:
This work was supported by the Polish National Science Centre project “Studies on genotypic characterisation of Shewanella putrefaciens group isolates from freshwater fish in Poland” (Grant No 2015/19/N/NZ7/01687).
**P 22 FIRST ISOLATION OF BLANDM-5 HARBOURING ESCHERICHIA COLI FROM TWO CLINICAL CASES IN DOGS IN SWITZERLAND**

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**Introduction:**
Carbapenem-resistant Enterobacteriaceae (CRE) are a threatened public health issue due to limited therapeutic treatment options. The number of CRE reports are increasing and they have been isolated from humans, livestock, environmental and food sources. Here we report the occurrence of two carbapenem-resistant E. coli, isolated from a wound infection (A) and a urinary catheter tip (B), respectively.

**Materials and Methods:**
Clinical specimens were obtained from the animal hospital. The two isolates (A and B) were identified using MALDI-TOF MS. Furthermore, antibiotic susceptibility testing and microarray analysis were performed. Strain A was further analysed by whole genome sequencing (WGS).

**Results:**
Both isolates were identified as Escherichia coli and showed resistance against twelve of the sixteen tested antibiotics, including imipenem, ertrapenem and meropenem. Microarray analysis revealed the presence of a NDM-type carbapenemase gene in strain A and a NDM-5 carbapenemase gene in strain B. WGS showed the carbapenemase resistance gene NDM-5.

**Discussion and Conclusion:**
Dissemination of CRE in veterinary medicine and especially in companion animals is an emerging problem. Various cases of E. coli isolates harbouring the OXA-48 gene have already been reported all over the world. Class B New-Dehli metallo-beta-lactamase resistances in E. coli isolates have been reported from China (NDM-1) as well as Algeria and Finland (NDM-5)¹, but never in Switzerland. A systematic monitoring to assess the dissemination of CRE not only in human, but also in companion animals and veterinary clinics due to the close contact of humans and companion animals is urgently needed.

**References:**
PS 01 - POSTER SESSION

P 23 FIRST DETECTION “IN-FIELD” OF LYME BORRELLIA IN TICKS BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TECHNIQUE

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Introduction:
The Loop-mediated isothermal amplification (LAMP) technique can be applied in-field. We report the results related to *Borrelia burgdorferi* sensu-latino detection in ticks by LAMP.

Materials and Methods:
The experiment was carried out in June, 2017 in a protected area where previously ticks were found positive for Lyme disease agents. Ticks were collected in an one-day campaign by flagging and morphologically identified using a portable stereomicroscope. Each specimens was crushed in a mortar using a sterile pestle and DNA extracted using commercial Kit. In order to applied the isothermal reaction in the field, the LAMP assay was set up by the Isothemal Master-Mix, which contained a DNA binding fluorescent dye. Reactions were run on the portable instrument Genie-III and results visualized in real time. For the determination of *B. burgdorferi* s.l. species, the tick DNA was examined in laboratory the next day by a PCR targeting the groEL gene. The gathered amplicon was purified and sequenced.

Results:
Five collected ticks were identified as *Ixodes ricinus* nymphs. One specimen (1/5) resulted positive to *B. burgdorferi* s.l. by LAMP. The species determination obtained by the analysis of the nucleotide sequence of the groEL gene amplicon showed 100 % identity with the corresponding portion of *B. garinii*.

Discussion and Conclusion:
Our experience demonstrated for the first time the in-field detection of Lyme Borrelia by LAMP approach directly on the spot. It suggests that this unconventional technique could be used in-field as a concrete screening strategy because it provides useful and immediate information in areas where Lyme disease agents occur.
P 24 MALDI-TOF MS ANALYSIS REVEALS DIVERSITY OF AESCULIN-POSITIVE KATALASE-NEGATIVE GRAMPOSITIVE-COCCI IN BOVINE MASTITIS MILK SAMPLES.

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Introduction:
Escculin-positive streptococci play an important role in acute and chronic intramammary infections (IMI). Correct diagnosis to the species level has been difficult, time consuming and was expensive. Diagnosis with Maldi-Tof ms is quick and affordable and facilitates the diagnosis of mastitis pathogens to the species level.

Materials and Methods:
Milk samples from cows with IMI (n=2230) were directly cultivated on Esculine-Blood agar and Chromogenic media (BioMérieux, CPSE). After 1 day of incubation the cultures were analysed due to their colony morphology and esculin reaction by ultraviolet light. Escculin positive colonies were further investigated by Maldi-TOF ms (bruker).

Results:
1043 (46.7 %) of all milk samples contained esculin positive streptococci. The most prevalent among these was Sc. uberis (n=786; 35 %) making it the most prevalent single mastitis pathogen. Other Streptococci were Sc. gallolyticus (0.3 %) and Sc. mitis (0.1 %). Other Escculin-positive cocci can not be distinguished from Streptococci with culture methods alone. We found Aerococcus viridans (4 %), E. saccharolyticus (3.1 %), E. faecalis (1.3 %), E. faecium (1.7 %), Lactococcus lactis (0.6 %), and Lactococcus garvieae (0.4 %).

Discussion and Conclusion:
It is well known that Sc. uberis plays a very important role in bovine IMI. The introduction of Maldi-Tof ms allows for further typing and reveals a plethora of species that were possibly misdiagnosed. The correct diagnosis to the species level is important for antibiotic treatment and epidemiologic understanding.
PS 01 - POSTER SESSION

P 25  DIFFERENTIATION BETWEEN SALMONELLA ENTERITIDIS VACCINE 441/014 AND SE FIELD STRAINS AND BETWEEN ENTERITIDIS AND TYPHIMURIUM IN ONE REAL-TIME PCR REACTION

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Introduction:
Salmonella is causing gastrointestinal diseases in human. Because animals and eggs are the main source of Salmonella infections it is necessary to restrict outbreaks in primary production stage (PPS). An important strategy to reduce infections is the vaccination. Therefore the control of Salmonella contamination, but also the differentiation of pathogenic field strain from non-pathogenic vaccine is essential for decisions of resulting consequences.
BIOTECON Diagnostics has developed two lyophilized real-time PCR Kits: The first one enables the detection of Salmonella spp. The second make the detection and differentiation between Salmonella Enteritidis (SE) field and SE vaccine strain 441/014 and between of SE and STM (Salmonella Typhimurium) in one reaction possible. Analysis needs less than 24 h instead of 5 days with microbiological methods.

Materials and Methods:
Validation study of vetproof SE Vaccine Detection 1 Kit in combination with DNA extraction Kit foodproof StarPrep Three.

Results:
Inclusivity: 100 % specificity was reached with 66 tested Vaccine 441/014, 63 SE field and 59 STM strains. Additionally 198 Salmonella field strains were analyzed as field strains, except of one. Exclusivity: 37 non target species were not detected, whereas integrated internal amplification control was positive.
The kit has analyzed concentrations of 100 cfu/ml SE/STM/SE vaccine enrichment cultures with amplification and melting curve differentiation, even if the concentration of the background flora is 100000 fold higher.
More than 150 naturally contaminated PPS samples were analyzed accurately.

Discussion and Conclusion:
The validation data demonstrate: With the vetproof SE Vaccine Detection 1 Kit contamination controls of PPS can be conducted with high sensitivity, specificity and convenience.
P 26 AUTOMATIC MAGNETIC BEAD-BASED EXTRACTION OF 1 TO 48 SAMPLES USING THE NEW INDI MAG 48

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Introduction:
Plastic waste is an issue in automated molecular biology protocols with high minimum sample numbers. A common choice for the automation of magnetic bead-based nucleic acid extraction from animal samples are 96-well platforms, which require plastic ware for 96 samples, regardless of the actual sample number.

The IndiMag 48 is a new platform from INDICAL BIOSCIENCE, designed to be as fast and reliable as currently available solutions, but with greater flexibility. The IndiMag 48 accepts 1 to 48 samples and only requires plastic ware for the desired number of samples.

In this study, we evaluated the reliability of IndiMag 48 extraction protocols to show that there is a viable option for maintaining or improving result quality.

Materials and Methods:
We compared the 5-step extraction protocol for the KingFisher Flex System and the 4-step protocol for the IndiMag 48. Nucleic acids were extracted from serum, blood, tissue, fecal and milk samples using the MagAttract 96 cador Pathogen Kit and the MagAttract Mastitis Kit. The isolates were tested using different virotype and bactotype PCR assays from INDICAL.

Results:
In terms of reliability, comparable results were obtained for BVDV- and SBV-positive samples with both protocols. MAP-positive fecal samples showed better results with the IndiMag 48 protocol. The results for Gram-positive and Gram-negative bacteria were also comparable.

Discussion and Conclusion:
IndiMag 48 supports cost-efficient nucleic acid extraction with reduced plastic waste thanks to its flexible sample size acceptance. It is highly user friendly. The run time and result reliability are comparable to or better than those obtained with the 96-well platform assessed here.
P 27 DEVELOPMENT OF AN ALTERNATIVE MULTIPLEXED BEAD-BASED AVIAN INFLUENZA H5 PATHOTYPING ASSAY

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Introduction:
Early warning avian influenza (AI) surveillance and rapid diagnosis are necessary to contain highly pathogenic H5 AI (HPAI) outbreaks. In this context, fast pathotyping is indispensable to allow the prompt implementation of restriction measures to limit virus spread. Currently, distinction of low and highly pathogenic AI (LPAI/HPAI) is mostly determined by sequencing the haemagglutinin gene cleavage site (CS).

Materials and Methods:
Here, an alternative specific multiplex bead-based luminex fast assay for H5 pathotyping is developed. Specific SNP (single-nucleotide polymorphism)-based primers for LPAI and HPAI CS were designed to determine the sample’s molecular pathotype.

Results:
An efficient and specific multiplex bead-based H5 pathotyping luminex assay was put in place and optimised.

Discussion and Conclusion:
This alternative strategy may be considered for implementation in routine diagnostics and may be extended for AI neuraminidase (NA) subtyping.
P 28 PRELIMINARY RESULTS OF POLYMERASE CROSS-LINKING SPIRAL REACTION (PCLSR) OPTIMIZATION FOR DETECTION OF MYCOPLASMA BOVIS IN CATTLE

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Introduction:
Mycoplasma bovis infects cattle, leading to respiratory tract disease, arthritis, mastitis and other diseases. A reliable diagnosis of M. bovis in cattle should be the combination of various laboratory methods because clinical signs are not pathognomonic.

Materials and Methods:
In the present study polymerase cross-linking spiral reaction (PCLSR) to detect M. bovis DNA in cattle was developed and optimized. This method relies on isothermal amplification of M. bovis DNA. Two outer-spiral primers and additionally cross-linking primer were specifically designed. The 3' sequence of outer-spiral and cross-linking primers were complementary to oppD/F gene of M. bovis, the 5’sequence – to DNA of black widow alpha-latrotoksin. The reaction temperature and duration were optimized. The results were visible by color change.

Results:
The test was able to detect all M. bovis strains analyzed and did not cross-react with other Mycoplasma species or other microorganisms.

Discussion and Conclusion:
Effective diagnosis of M. bovis in cattle should not be time consuming and expensive. Previous PCR methods were not sensitive enough and some isothermal loop-mediated methods have poor specificity. The test optimized in present study is specific due to combination of outer and cross-linking primers, sensitive and additionally little time and cost consuming. In conclusion, the new PCLRS test was optimized and can be used for M. bovis detection in cattle.

References:
Funded by KNOW (Leading National Research Centre) Scientific Consortium “Healthy Animal – Safe Food”, decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.
P 29 DETERMINATION OF EXOSOMES INFECTIVITY IN CATTLE INFECTED WITH BOVINE LEUKAEMIA VIRUS (BLV)

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Introduction:
Exosomes are small membranous microvesicles, extracellularly released from variety of mammalian cells. The aim of the study was to isolate and determine infectivity of exosomes in samples from BLV infected cattle.

Materials and Methods:
Exosomes were isolated from the blood sera and supernatant of dendritic cells (DCs) from BLV infected cell cultures and healthy control cows. Immunological status of animals was determined by ELISA and qPCR. Exosomes were isolated by differential centrifugation. Filtered supernatant was ultracentrifuged at 120,000 x g for 3 h, at 4 C. The pellet of EVs was resuspended in PBS. Purified protein from each sample was used for Western Blot. Proteins separated on SDS-PAGE were transferred to an Immobilon-P PVDF membrane. The presence of BLV proteins was detected with the use of monoclonal antibodies specific BLV gp51 and a BLV p24. The lysosomal markers: CD63, CD9 and flotillin-1 were determined. The visualization was performed with chemiluminescence method.

Results:
The presence of viral proteins: gp51 and protein p24 was detected in exosomes isolated from the sera and supernatants of DCs culture infected with BLV, but they were absent in exosomes isolated from healthy cattle. The cellular markers: CD63, CD9 and flotillin-1 were detected in infected with BLV and non infected samples.

Discussion and Conclusion:
The viral proteins: gp51 and p24 were present in exosomes isolated from the sera and supernatants of the BLV infected dendritic cell culture, what suggested that BLV proteins were released into exosomes in sera and supernatant, and could be transferred into recipient cells as an alternative route not requiring virus infection.
P 30 EVALUATION OF THE PERFORMANCES OF A MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS AB ELISA IN YOUNG ADULT COWS THROUGH A LONGITUDINAL APPROACH

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Introduction:
The objective of this study was to assess sensitivity and specificity of an ELISA test for detection of paratuberculosis in cows younger than 5 years-old through a longitudinal approach.

Materials and Methods:
The study is based on individual results (ELISA and PCR) of 3660 cattle coming from 82 herds registered in a voluntary program for the control of paratuberculosis which were at least tested once with ELISA between 2 and 4 years old and on which at least one PCR test was performed after 5 years old. Based on results obtained after the age of 5 years, cows with at least 1 positive ELISA and 1 positive PCR test results was classified as “infected” (n=150) and cows with at least 3 negative ELISA and 3 negative PCR, as “free” (n=119). The results obtained with the ELISA test (ID Screen® Paratuberculosis Indirect, IDVet®, Montpellier, France) between year 2 and year 4 of age on “infected” and “free” animals were used to assess respectively the sensitivity and the specificity of the test.

Results:
On « infected » animals, 251 ELISA were performed before the age of 5 years whose 24 with a positive result which corresponds to a sensitivity of 9.6 % (IC 95 %: 5.9 – 13.2). Within the « free » animals, all of the 204 ELISA tests performed before the age of 5 years were negative indicating a specificity of 100 %.

Discussion and Conclusion:
Our study confirms the poor sensitivity of serology approach for detecting paratuberculosis in cows younger than 5 years-old.
P 31 SEROPREVALENCE OF BORDER DISEASE VIRUS AND OTHER PESTIVIRUSES IN SHEEP IN ALGERIA AND ASSOCIATED RISK FACTORS

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Introduction:
BDV is a Pestivirus responsible for significant economic losses in sheep industry. The present study was conducted between 2015 and 2016 to determine the flock seroprevalence of the disease in Algeria and to identify associated risk factors.

Materials and Methods:
56 flocks from nine departments were visited and 689 blood samples were collected from adult sheep between 6 and 24 months of age and from lambs younger than 6 months. 576 serum samples were tested by Ab ELISA, to detect specific antibodies against Pestivirus and 197 of them were further characterized by VNT (virus neutralization test) for the detection of BDV, BVDV-1 and BVDV-2 specific neutralizing antibodies.

Results:
144/197 sera were positive in VNT for BDV, and 2 sera were strongly positive BVDV-2. All samples were tested by RT-PCR for detecting RNA virus as well as by Ag ELISA, but no Persistently Infected (PI) animals were found. Fifty-five flocks (98 %) had at least one positive animal and the apparent within-flock seroprevalence was estimated to be 60.17 % (95 % C.I.: 52.96 – 66.96). The true prevalence based on sensitivity and specificity of the ELISA was 68.20 % (95 % C.I.: 60.2 – 76.3). Several risk factors were identified as linked to BDV such as climate, landscape, flock management and presence of other species in the farm.

Discussion and Conclusion:
These high seroprevalence rates suggest that BDV is widespread and is probably endemic all over the country. Further studies are needed to detect and isolate the virus circulating in the country and understand the distribution and impact of Pestiviruses in the Algerian livestock.
PS 01 - POSTER SESSION

P 33 PESTI VIRUS ANTIBODY PREVALENCE IN SEMI DOMESTICATED REINDEER IN NORTHERN SCANDINAVIA AND FINLAND

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4 Natural Resources Institute, Green Technology, Myllytie, Finland
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Introduction:
The aim of this study was to investigate change over time of pestivirus prevalence in Scandinavian reindeer herds. Serum samples were collected from nine Fennoscandian reindeer herds, three from Sweden, Norway and Finland, respectively, and assessed for the prevalence of pestivirus IgG by ELISA.

Materials and Methods:
During slaughter blood samples was collected, centrifuged and sera were collected. 273 sera were analyzed for BVDV/BVD antibodies (Synbiotics SERELISA BVD p80Ab Mono Blocking Kit). Negative samples were analyzed with pestivirus qPCR (Hoffman et al 2006).

Results:
Sweden: the seroprevalence was 48 % (n=132).
Norway: the seroprevalence was 23 % (n=60), in one heard of three no positive samples were found i.e. the prevalence in the positive herds was 35 % (n=40).
No seropositive samples were found in the Finnish samples (n=81).

Discussion and Conclusion:
Serology show that the BVDV/BVD antibody prevalence varies between herds and between countries. The trend is that seroprevalence have increased in northern Sweden while it is more similar over the last decade in Norway. Also, the prevalence may differ within a country as shown in Norway with a prevalence ranging from 0–45 %. A striking result is that pestiviruses appears to be absent in the Finnish reindeer population.
THE IMPORTANCE OF MONITORING NEOSPOROSIS STATUS IN DAIRY FARMS – CASE STUDY

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Introduction:
Bovine neosporosis control programs are based on serodiagnosis and herd management. On dairy farms adhering to Bovicontrol® all animals present, born and purchase are tested for Neospora antibodies.

Materials and Methods:
The farm joined the program in 2012. The animals are distributed by 2 units, in different villages; the 1st unit includes the adult cows and calves until weaning; in the 2nd unit we have calves and heifers up to 7 months of gestation. Every 6 months the animals after weaning are tested for N. caninum as well as all aborted animals. For Neospora antibody testing we use the ELISA Test kit CIVTEST® BOVIS NEOSPORA. Results are expressed in two groups, positive and negative, according to the manufacturer.

Results:
In December 2012, the prevalence of N. caninum was 6.7% (12/180). The farmer was advised to implement the appropriate measures. Between January 2013 and April 2017, 183 females born were tested and only 2 were positive and were culled. There were only two seropositive cows on the farm in April 2017. In November 2017, 6 positive calves appeared in 31 tested. All remaining animals from unit 2, previously tested negative, were retested and 50 new infections (61.7% 50/81) were observed. Twenty of the fifty new animals were already culled.

Discussion and Conclusion:
The results demonstrate the importance of individual testing for N. caninum for monitoring and early detection but also the intensification of biosecurity measures; in this case, the entrance of a dog, which proved to be N. caninum positive.
P 35  SEROPREVALENCE OF NEOSPORA CANINUM IN PORTUGUESE DAIRY FARMS

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Introduction:
Neospora caninum is a protozoan parasite of animals, responsible for neosporosis with a worldwide distribution. To control the infection, it is important to identify seropositive animals and implement appropriate measures. In Portugal, since 2009, under a voluntary control program (Bovicontrol®) all the animals present in the farm are tested to neospora antibody.

Materials and Methods:
All the animals (35933) present in the 403 dairy farms under Bovicontrol® program were tested for neospora antibody, between March 2009 and April 2018. The majority of samples were analysed using the ELISA Test kit CIVTEST® BOVIS NEOSPORA, considered one of the best-adjusted ELISA with Se and Sp >95%. Results are expressed in positive and negative, according the manufacturer.

Results:
The proportion of positive samples was 16.3% (5840/35933). All samples tested negative on 52 (12.9%) farms. Until 5% of seropositive animals we found 66 (16.4%) farms, between 5% and 10%, 92 farms (22.8%), in the range 10%–20%, 91 (22.6%), in the range 20%–50%, 89 (22.1%) and with more than 50% of positive animals 13 (3.2%) farms. The average percentage of seropositive animals in the different ranges was 3.1% (0–5%), 7.5% (5%–10%), 14.6% (10%–20%), 30.4% (20%–50%) and 60.2% (<50%).

Discussion and Conclusion:
The results confirm the widespread of Neosporosis among portuguese dairy farms, with the majority of farms being infected. In a study carried out in 2010, using the same ELISA Test, but in BMT, they found 60.2% positive farms.
PS 01 - POSTER SESSION

P 36 DETECTION OF VARIANTS OF BOVINE LEUKEMIA VIRUS WITH MUTATIONS ASSOCIATED WITH LOW NUMBER OF PROVIRUS COPIES

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Introduction:
Bovine leukemia virus (BLV) is the etiological agent of enzootic bovine leucosis (EBL) and mandatory screening for EBL includes detection of specific antibodies by ELISA and AGID tests. Quantitative real-time PCR (qPCR) and nested PCR have been also approved for detection of proviral DNA, especially for confirmation of inconclusive serological test results. BLV variants characterized by low provirus load can hamper molecular diagnosis of BLV infection. The aim of the study was to identify the BLV variants with mutations in regulatory regions of Long Terminal Repeat (LTR) and to assess the association of these mutations with proviral load.

Materials and Methods:
DNA was isolated from PBLs of 94 cattle infected with BLV and LTR sequences were generated and analyzed for the presence of mutations. Number of provirus copies per 1000 cells was measured using TaqMan qPCR. Chi-square test was used to analyze the association between mutation frequency and copy number.

Results:
Presence of the following mutations: T(-41)A, T(-37)A in TATA box and TC(+191/+192)del, C(+190)T in DAS region of LTR in animals with low copy number (0-550) was significantly frequent than in group of cattle with high copy number (>550) (p<0.1, p<0.018, p<0.031 and p<0.00002).

Discussion and Conclusion:
Four types of mutations in regulatory elements of BLV LTR were found in infected cattle. The presence of these mutations was associated with low number of proviral copies. This can lead to possible limits of successful application of qPCR for molecular diagnosis of infection with BLV.
**Development of Tools for Diagnosis and Prevention of Nodavirus Outbreaks**

*P. 37*

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¹ INGENASA, Research & Development, Madrid, Spain

**Introduction:**
Viral nervous necrosis (VNN) is a highly infective neuropathological disease caused by piscine Betanodaviruses with significant economic impact in marine aquaculture. Disease surveillance rely on RT-qPCR tests that are being applied in our laboratory. In addition, we aim to develop on-site diagnostic tests that would help to implement early management measures. Formation of betanodavirus virus-like particles (VLPs) has been reported and their use as prospective vaccines is promising. Furthermore, they could be ideal antigens to raise specific conformational antibodies that can be of use in lateral flow chromatographic devices.

**Materials and Methods:**
Nodavirus capsid protein cDNA from two nodavirus genogroups (RGNNV and SJNNV) were expressed using baculovirus expression system. VLPs were purified by ammonium sulphate precipitation and sucrose gradient centrifugation and characterized by electron microscopy and thermal stability assays. Purified particles were used to immunize mice and rabbits to obtain monoclonal and polyclonal antibodies, respectively.

**Results:**
We report the generation of betanodavirus VLPs of two nodavirus genotypes and its application to generate antibodies from which different immunoassays were developed, including immunofluorescence, a sandwich ELISA and immunochromatography. The assays were tested using infectious viruses, recombinant VLPs and field samples of seabass brain.

**Discussion and Conclusion:**
Baculovirus expression of nodavirus capsid protein lead to the formation of VLPs resembling the parental viruses that were applied to the development of specific antibodies and immuno-diagnostic assays. The assays could be useful for disease verification of symptomatic individuals. Furthermore, the yield and purity of the obtained VLPs could make them suitable vaccine candidates.
P 38  EVALUATION OF THE VETMAX™-GOLD AIV DETECTION KIT AND VETMAX™-GOLD SIV DETECTION KIT FOR DETECTION OF AVIAN AND SWINE INFLUENZA VIRUSES

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Introduction:
It has long been recognized that real-time reverse-transcription polymerase chain reaction (RRT-PCR) assays play an important role in the rapid identification of avian and swine influenza A viruses (AIV and SIV, respectively). The aim of this study is to evaluate the performance of the VetMAX™-GOLD AIV Detection Kit (AIV Kit) and VetMAX™-GOLD SIV Detection Kit (SIV Kit) to detect AIV and SIV in clinical samples.

Materials and Methods:
The performance of the AIV Kit was compared with the matrix (M)-gene RRT-PCR assay for generic influenza A virus detection (Nagy et al., 2010) by parallel testing of AIV-positive and -negative representing eurasian AIV subtypes, original clinical samples (oropharyngeal and cloacal swabs) from a 2015 UK outbreak of H7N7 low pathogenicity AI (LPAI), and samples containing H5N6 highly pathogenic AI (HPAIV) 2018. The AIV Kit was similarly compared with the “perfect match” influenza A virus M-gene RRT-PCR assay for generic detection of SIV (Slomka et al., 2010).

Results:
There was complete concordance between the AIV Kit and M-gene influenza A RRT-PCR assay results. The AIV Kit was on average 2.48 Cts lower than the Nagy M-gene assay. However, 15 genuine additional RNA samples from the 2015 H7N7 LPAIV incursion and seven samples from H5N6-infected wild birds were positive by the AIV Kit but negative by the M-gene (Nagy) test using a Ct value of 36.0 as the positive/negative threshold.

Discussion and Conclusion:
These data illustrate the high diagnostic sensitivity and specificity of the AIV Kit for the detection of AIV and SIV in archival and currently-circulating strains.
P 39 MOLECULAR EPIDEMIOLOGY OF CANINE ADENOVIRUS TYPE 1 (CAdV-1) IN FREE-RANGING SMALL CARNIVORES IN BERLIN AND BRANDENBURG, GERMANY

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Introduction:
To date, there is no knowledge about the molecular epidemiology of CAdV-1 in free-ranging small carnivores in the federal states of Berlin and Brandenburg. However, serological data collected in this region (1991–1995) indicate that CAdV was at least endemic in red foxes (Vulpes vulpes) with a seroprevalence of 3.5%.

Materials and Methods:
In our preliminary study, we examined relevant tissues (liver, kidney, brain) from 46 red foxes and 15 raccoons (Procyon lotor) submitted during the year 2017 in the framework of the national rabies monitoring program by real time PCR, histopathology and immunohistochemistry.

Results:
CAdV-1 specific genome sequences were detected in the tissues of six red foxes (13%) with ct-values ranging between 16.2 and 35.8. Immunohistochemically, CAdV-1 specific antigen was demonstrated in the brain of two foxes with nonsuppurative meningoencephalitis, mainly in endothelial cells. All raccoons were CAdV-1 negative.

Discussion and Conclusion:
Infection of red foxes with CAdV-1 leads to virus excretion via exudates and urine for up to nine months post-infectionem in reconvalescent animals resulting in contamination of the environment. Consequently, knowledge about the occurrence and infection rate of CAdV-1 in free-ranging small carnivores is of crucial importance for small animal veterinary health purposes, since the decision to include the virus in vaccination schemes for domestic animals has to take the infection pressure in account. Our study confirmed the enzootic occurrence of CAdV-1 in red foxes. The detection rate by PCR was considerably higher than by serology. However, the relevance of this finding has to be confirmed by future serological and molecular investigations.
PS 01 - POSTER SESSION

P 40 PREVALENCE OF VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM PIGLETS WITH POST-WEANING DIARRHOEA IN BELGIUM AND THE NETHERLANDS

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Introduction:
Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea and is caused primarily by entero-toxigenic E. coli (ETEC). The most common fimbriae in PWD are F4 and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the study was to determine the prevalence ETEC subtypes causing PWD in Belgium and The Netherlands.

Materials and Methods:
A total of 504 pig herds distributed in the Benelux showing clinical signs of PWD were sampled between January 2014 and December 2016. Rectal swab samples (n=5) from diarrheic pigs were collected and submitted to IZSLER (Brescia, Italy) to analyze the presence of virulence factors – adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e).

Results:
In total, 526 non-hemolytic and 784 hemolytic E. coli strains were isolated and subsequently tested by PCR. The prevalence of the different ETEC subtypes was as follows: F4-ETEC (24.4 %) and F18-ETEC (19.2 %). On a herd level, the prevalence of the different ETEC subtypes was as follows: F4-ETEC (45.8 %) and F18-ETEC (37.5 %). Besides ETEC, 22 isolates (1.7 %) were classified as Shiga toxin-producing E. coli (STEC).

Discussion and Conclusion:
This study confirms that fimbriae type F4 was slightly more prevalent than F18 among E. coli isolates from PWD cases in Belgium and The Netherlands. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive and control measures such as live oral vaccination.
PS 01 - POSTER SESSION

P 41 WHERE TO DETECT SALMONELLA SSP. IN BPW?

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Introduction:
The detection of Salmonella spp. according to ISO 6579 (2017) yield buffered peptone water (BPW) as pre-enrichment medium. The samples are diluted 1/10 and incubated at 36 °C ± 2 °C for 18 h ± 2 h. After incubation, only 0.1 ml is transferred to the selective enrichment Modified Semi-solid Rappaport Vassiliadis (MSRV). The location in the BPW where this volume is taken, is of major importance for a correct result.

Materials and Methods:
10 inoculated samples (faeces, swabs and boot cover swabs) were analysed according to ISO 6579 using MSRV. The volume BPW needed to incubate in the selective enrichment was taken from the upper (1) and lower (2) 10 %, and after stomachering (3). The MSRV was incubated for 24 and 48 h.

Results:
After 24 h incubation, no location presented a recovery of 100 % (8/10 in the upper and 6/10 in the lower 10 % BPW, and 9/10 after stomachering). When the MSRV was incubated for 48 h, the recovery increased to 100 % in the upper 10 % and after stomachering, but only 90 % was recovered from the lower 10 % of the incubated BPW.

Discussion and Conclusion:
When isolating Salmonella spp. according to ISO 6579, the volume BPW used to inoculate the selective enrichment should be taken from the upper part of the BPW or after stomachering. This is not well described in this ISO method and should be taken into account.

References:
ISO 6579:2017: Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella.
P 42 DETECTION AND PHYLOGENETIC ANALYSIS OF PORCINE GROUP A ROTAVIRUS FROM KOREAN WILD BOAR AND DOMESTIC PIGS

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Introduction:
Group A rotaviruses, a member of the Reoviridae family, are the cause of acute diarrhea in young animals and children. Porcine Rotavirus (PRoV) infections are prevalent and are commonly associated with diarrhea from sucking and weaned pigs. In the present study, PRoVs were detected from diarrheic feces of domestic pigs and wild boar in Republic of Korea and phylogenetic characterization conducted for the VP7 and VP4 genome segments of PRoVs.

Materials and Methods:
Fecal samples from domestic pig farms from 2014 to 2017 and from captured wild boar (2016–2017) were collected 352 and 449, respectively, and identified PRoV VP7 and VP4 gene using the PCR.

Results:

Discussion and Conclusion:
There is no PRoV detection in the wild boar living in wildlife ecosystem in Republic of Korea, but it is confirmed that various types of PRoV group A strains found in domestic pigs. The vaccine development for current Korean circulating strains may require for more effective virus control.
P 43 DETECTION AND GENETIC EVOLUTION OF BOVINE CORONAVIRUS IN VIETNAM

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Introduction:
Bovine coronavirus (BCoV) was first identified during an outbreak of diarrhea among neonatal calves in the 1970s. Later, it occurred in association with winter dysentery in adult cattle and with respiratory tract infection in calves and cattle. Here, we examined the prevalence of BCoV infection among calves in Vietnam and genetically characterized Vietnamese BCoVs through phylogenetic and evolutionary analyses of the S and HE proteins.

Materials and Methods:
Between January and November 2017, diarrheic fecal samples (n=125) were collected from cows on farms located in northern Vietnam. BCoV-positive feces were diagnosed using a Rapid BoviD-5 antigen kit and PCR analysis. BCV sequences were used to generate a BEAST input file using BEAUti within BEAST package v1.8.1.

Results:
Vietnam BCoV-positive feces were identified to 5.6% (7 of 125) and belonged within C1 cluster on phylogenetic tree analysis. The evolutionary rate of C1 was estimated to be $7.232 \times 10^{-4}$ substitutions/site/year, and the most recent common ancestor (tMRCA) of 48 C1 was estimated to date back to 1968 (95% HPD 1962–1974). Molecular clock analysis of S gene sequences estimated that the time of divergence from a common ancestor of C1 and C2 was estimated to be 1962. The effective population sizes for the BCoV C1 and C2 clusters fell rapidly from 2000 and 2004, respectively.

Discussion and Conclusion:
Vietnam BCoV sequences might share a common ancestor with the Cuban BCoV strain (high nucleotide sequence similarity within the same cluster, (C1)).
P 44  GENETIC CHARACTERIZATION OF PORCINE EPIDEMIC DIARRHEA VIRUS FROM KOREAN PIGS: 2013–2018

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Introduction:
Porcine epidemic diarrhea (PED) virus belongs to Coronaviridae cause severe and highly contagious swine disease characterized by watery diarrhea and dehydration in piglets with high mortality. PEDV was first detected in United Kingdom in 1978, and since has been identified in worldwide including United States, Hungary, Italy, China, Korea and Vietnam. Since 2013, a newly emergency PED infection caused huge economic loss in Korea. In this study, we examined the molecular characterization of Spike (S) gene of PEDV strains isolated from Korea between 2013 and 2018.

Materials and Methods:
PED-positive intestine and fecal samples from pig farms were collected 116 from 16 different regions for 6 years. PCR for the PEDV S gene was performed using the previous published primers.

Results:
The 106 PEDV strains collected from pig farms had same S gene length (4,161 nucleotide (nt)) but the S gene length of the 10 strains revealed 4,152–4,201 nt resulted from gene deletion and insertion. The 114 of 116 stains including 8 strains with gene deletion and insertion were belonged to group G2b, but the remaining two strains contained in group INDELs. High sequence homology in nucleotide (96.5–99.8 %) and amino acid (97.0–99.7 %) were observed among the 114 Korean PEDV strains.

Discussion and Conclusion:
Most S gene of Korean PEDV strains except the two strain (US INDELs group) between 2013 and 2018 showed similar nt length and belonged to G2b group contained US and China PEDV strains. The data showed that the genetic variation of Korean PEDV strains seems to be progressing slowly.
PS 01 - POSTER SESSION

P 45 RAPID, ON SITE, DIAGNOSIS OF FMD AND SAFE AND COST-EFFECTIVE SHIPMENT OF SAMPLES USING LATERAL FLOW DEVICES FOR LABORATORY DIAGNOSTICS

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Introduction:
Foot-and-mouth disease (FMD), caused by infection with foot-and-mouth disease virus (FMDV), is one of the most economically devastating diseases affecting cloven-hoofed animals. Identification of circulating strains is important for efficient FMD control. However, due to shipping requirements of potentially infected samples, the cost of sample submission to reference laboratories remains a major obstacle. A cost-effective and safe method for shipment of samples from FMD-suspected cases, based on the inactivation of FMDV on lateral flow devices (LFDs) has been developed and validated in the laboratory which allows subsequent detection and typing of FMDV by RT-PCR and virus rescue using RNA transfection (Romey et al. 2017). The present study aims to further evaluate this protocol on freshly collected clinical samples through collaboration in endemic countries in order to test the performance and safety of the entire process directly in the field.

Materials and Methods:
Epithelium or vesicular fluid samples will be collected from suspect clinical cases of FMD in Nigeria, Turkey and Pakistan and will be tested in the field using LFDs. The selected positive inactivated (or not) LFDs will be submitted to reference laboratories (France, Denmark) for molecular detection and virus rescue.

Results:
Sample collection and inactivation on LFDs are in progress. Transfections are currently being optimized to ensure virus rescue from RNA genomes recovered from inactivated LFDs.

Discussion and Conclusion:
This study will contribute to demonstrate that using LFDs is a safe way for room-temperature, dry-transport of inactivated FMDV samples from endemic areas. It will substantially decrease the shipping cost thus increasing field sample submission.
PS 01 - POSTER SESSION

P 46 DEVELOPMENT AND VALIDATION OF A NEW ONE-WELL ELISA FOR THE DIAGNOSIS OF BESNOITIA BESNOITI ANTIBODIES ON BOVINE SERUM SAMPLES

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2 Agrivalys71, Serology, Mâcon, France
3 BioDev, Scientific Direction, Chasselas, France
4 Biosellal, Direction, Dardilly, France

Introduction:
Besnoitia besnoiti is a vector-borne protozoan parasite which can affect ruminants, especially bovine. Clinical signs of bovine besnoitiosis are mostly skin thickening, weight loss, alopecia and decrease of performances.

The disease spreading and its impact on the livestock production leads to an increase of screening programs. Serological antibodies detection by Western-Blot remains the reference method for diagnosis, however ELISA testing is currently highly used for its low cost, convenience, automatization and rapidity.

As parasite ELISA tests showing substantial unspecific background, a two-well ELISA tool is currently widely used in France for B. besnoiti diagnosis. To increase analytical capacity and reduce cost, a new one-well ELISA kit was developed using highly purified antigen.

Materials and Methods:
A panel of 800 bovine serum samples, consisting of field samples and experimentally infected animals, were tested on both ELISAs, as well as inter and intra-assay reproducibility.

The cross-reaction with antibodies to another parasite (Neospora caninum) was also evaluated.

Results:
The correlation between the two ELISA was very high (Kappa = 0.97).

Coefficients of variation of our kit demonstrated high level of reproducibility (CV repeatability = 3.9 % and CV reproducibility 9.5 %).

The absence of cross-reaction was proved without any false positive sample within N. caninum positive population.

In addition, those assays highlighted the nearly absence of reaction in the two-well ELISA control wells (control wells mean OD = 0.095), questioning the two-well technic relevance.

Discussion and Conclusion:
The developed one-well ELISA revealed reliable technical performances equivalent to the two-well technic, indicating its potential used for besnoitiosis diagnostic and control within cattle herds.
EMERGENT PROPERTIES AND PROPAGATION PATHWAYS OF THE PEDV FIELD ISOLATE IN THE MODEL EXPERIMENT

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Introduction:
Most susceptible to PEDV are newborn piglets under 10 days, they is no immunity, and this is the main reason for the high susceptibility to PEDV infection. The main method of infecting animals with PED causative agent is oral.

Materials and Methods:
Two groups of non-colostrum piglets were arbitrarily selected in the herd free from PED. The piglets from the experimental group were challenged with the PEDV orally in a dose of virions of 1-10 GE. The extraction of NA with using the kit «BioExtract Column» («Biosellal», France), RT-PCR with using a kit «Bio-T kit®PEDV all-TGEV» («Biosellal», France) and CFX 96 Real-Time System («Bio Rad», USA).

Results:
One of the likely ways of spreading the PEDV in the piglets could be the transfer of the virus by insects. In all farm, M.domestica are extremely common and in the conditions of the experiment were also present in the vivarium in sufficient quantities. It was the M.domestica that could be an effective PEDV carrier, that was confirmed by the results of RT-PCR analysis of the PEDV on the surface and in the body of M.domestica. The results of studies of M.domestica showed a value of Ct=31.44±0.68, which roughly corresponds to the of 10²-10³ GE of virions of the PED causative agent in 1 ml of flushing.

Discussion and Conclusion:
That one of the most probable mechanisms of infection of piglets PEDV is the spread of the causative agent with M.domestica
PS 01 - POSTER SESSION

P 48 INVESTIGATION OF FUR ANIMAL BOTULISM OUTBREAK IN POLAND

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Introduction:
The reservoirs of botulinum neurotoxin-producing Clostridia are fishes, birds, mammals and their slaughter by-products, what pose potential threat for carnivores. As yet, the fur animal botulism has been reported due to botulinum toxin (BoNT) type A, B, C, CD and E. The present paper reports the investigation of mink botulism outbreak.

Materials and Methods:
In summer 2016 botulism was suspected in five farms in Poland. All minks were immunized against BoNT type C and all of these farms were supplied with feed by the same feed processing plant. The feed contained poultry slaughter by-products, fish by-products, extruded cereals, dried haemoglobin, fishmeal, meal of pork, acidifier and preservative. The mink feed as a ready-to-use product was unpasteurised. Laboratory tests included animal sera, internal organs and implicated feed.

Results:
Presence of BoNT was confirmed by mouse bioassay in serum (farm A and B), liver extract (farm A, B, C and D) as well as feed extract (farm B and E). The serotyping of positive serum revealed presence of BoNT type E and F in farm A and BoNT type C and F in farm B. Moreover, the presence of BoNT protein in pooled serum from farm B was detected by LC-MS/MS method, where non-toxic non-haemagglutinin protein of C. botulinum was revealed.

Discussion and Conclusion:
The animal botulism has been diagnosed in Poland since 10 years, but these mink botulism were the largest laboratory confirmed outbreaks so far. To the best of our knowledge, this is the first reported mink botulism outbreak due to BoNT type F.
P 49  A HUGE OUTBREAK OF BOVINE BOTULISM – TRICKY WAY TO THE DIAGNOSIS

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Introduction:
Animal botulism is life-threatening flaccid paralysis caused by ingestion of botulinum neurotoxin (BoNT) or botulinum neurotoxin-producing Clostridia spores. The reservoirs of the pathogen are fishes, birds and mammals as well as decaying carcasses occasionally occurred in poultry litter. Crops fertilization with poultry litter can induce botulism of animals due to contaminated grass, hay or silage. This paper reports the diagnosis of two cattle botulism outbreaks, where totally more than 350 animals have died.

Materials and Methods:
In summer 2017 the disease was suspected in two farms (A and B) in the Mazovia region of central Poland. Animals were not immunized against botulism and fields of corn crops were fertilized with poultry litter, which was both utilised in farm biogas plant and obtained digestate was repeatedly applied on fields with silage plants. The animals were fed with corn silage, rapeseed meal, protein concentrate as well as mineral-vitamin premix. For laboratory diagnostics were collected animal serum, liver, spleen, kidney, lumen content of small intestine, rumen content as well as implicated feeds. Official veterinary control involved milk powder, cream powder, butter and swabs from dairy plant processing implicated milk.

Results:
Presence of BoNT was confirmed by mouse bioassay in serum and liver extract of farm A animals and culture of intestinal content of farm A and B animals. The positive samples revealed presence of BoNT type C and D.

Discussion and Conclusion:
The animal botulism has been diagnosed in Poland since 10 years, but these bovine botulism were the largest laboratory confirmed outbreaks so far.
P 50  VALIDATION OF REAL-TIME PCR KITS FOR SPECIFIC DETECTION OF CLASSICAL AND AFRICAN SWINE FEVER VIRUS

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Introduction:
Classical Swine Fever (CSF) and African Swine Fever (ASF) have similar clinical signs and are present in many parts of the world. Moreover, ASF is reported in European Union, with recent dissemination in Hungary. This emphasizes the need for rapid, reliable and effective differential diagnosis tools for these diseases.

Materials and Methods:
In this context, BioSellal decides to develop three ready-to-use real-time PCR kits:

<table>
<thead>
<tr>
<th>Name of the kit</th>
<th>Level of multiplexing</th>
<th>CSF (FAM)</th>
<th>ASFV (VIC)</th>
<th>Internal positive control (Cy5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-T kit® CSFV</td>
<td>Duplex</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-T kit® ASFV</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Bio-T kit® CSFV &amp; ASFV</td>
<td>Triplex</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Validations of each Bio-T kit® were performed according to the specifications of the French National Reference Laboratory (NRL) for CSF and ASF (ANSES Ploufragan-Plouzané, France) which include the general guidelines defined by the French AFNOR standard NF U47-600-2. Sample type that could be used for analysis were whole blood (on EDTA), serum, plasma, cell culture supernatant, spleen, tonsil, lymph nodes for either CSFV and ASFV and blood swabs for ASFV.
Results:
The results of the validation of the three Bio-T kit® were presented in the table below.

<table>
<thead>
<tr>
<th>PCR Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio-T kit® CSFV</td>
</tr>
<tr>
<td><strong>Exclusivity</strong></td>
<td>Specific detection of CSFV, on different genotype such as 1.1 to 1.3, 2.1 to 2.3, 3.1 to 3.4 (NRL and VI, VIII, IX, X, XVII and XXIII)</td>
</tr>
<tr>
<td><strong>Inclusivity/Exclusivity on classical and African swine fever viruses</strong></td>
<td>CSFV samples</td>
</tr>
<tr>
<td></td>
<td>ASFV samples</td>
</tr>
<tr>
<td><strong>Limit of Detection</strong></td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td><strong>Efficiency</strong></td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td><strong>Complete Method Characteristics</strong></td>
<td>Results with BioExtract&lt;sup&gt;®&lt;/sup&gt; SuperBall® (magnetic beads extraction of nucleic acids)</td>
</tr>
<tr>
<td>French NRL Required level of Detection on EDTA whole blood, serum, plasma, cell supernatant</td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td>French NRL Required level of detection or limit of detection on spleen</td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td>French NRL Required level of Detection on tonsil, ganglia</td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td>French NRL Required level of Detection on swabs</td>
<td>CSFV</td>
</tr>
<tr>
<td></td>
<td>ASFV</td>
</tr>
<tr>
<td><strong>Diagnostic sensitivity</strong></td>
<td>CSFV on 0 blood, 2 tonsils, 1 ganglia and 15 spleens</td>
</tr>
<tr>
<td></td>
<td>ASFV on 0 blood, 2 tonsils, 1 ganglia and 15 spleens</td>
</tr>
<tr>
<td><strong>Diagnostic specificity</strong></td>
<td>CSFV on 1 blood, 2 tonsils, and 15 spleens</td>
</tr>
<tr>
<td></td>
<td>ASFV on 1 blood, 2 tonsils, and 15 spleens</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
The Bio-T kit<sup>®</sup> CSFV, Bio-T kit<sup>®</sup> ASFV and Bio-T kit<sup>®</sup> CSFV & ASFV are three effective tools allowing the differential diagnosis of CSF and ASF. Moreover, the ready-to-use triplex one-step Master Mix could be an interesting tool to survey both diseases into regions where CSFV and ASFV are presents.
PS 01 - POSTER SESSION

P 51 DIAGNOSIS AND CHARACTERISATION OF FATAL CANINE LOUPING-ILL

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1 Moredun Research Institute, Diagnostic, Penicuik, United Kingdom
2 University of Glasgow, Centre for Virus Research, Glasgow, United Kingdom

Introduction:
Louping-ill (LI) is a tick-transmitted, frequently fatal, encephalitis primarily affecting sheep and red grouse; LI is caused by louping ill virus (LIV) a tick-transmitted, positive sense, single stranded Flavivirus of the genus Flaviviridae. The disease is rare in non-ruminant mammals such as pigs and generally induces non-fatal cases in horses, hares and humans. To date, there is a single report of one presumed and one confirmed case of fatal louping-ill in dogs (both post-parturient working Collies) as well as a case in which the dog recovered.

Materials and Methods:
Here we describe a new fatal case of canine LI were we had the opportunity to determine the morphology and distribution of histological lesions in the canine brain, the usefulness in dogs of IHC and PCR used routinely for definitive diagnosis in sheep and if the virus responsible for this case differed significantly at the genome level through PCR and sequencing from viruses recovered from clinically affected sheep.

Results:
Serological and molecular (Real-time PCR) tests showed presence of LIV-specific IgM and RNA respectively. Histological examination demonstrated characteristic hallmarks of flavivirus infection; immunohistochemistry confirmed presence of LIV antigen in neurons and Purkinje cells. Sequence and phylogenetic analysis indicated close similarity of the dog LIV strain to British ovine strains.

Discussion and Conclusion:
We offer, for the first time a full description of a fatal case of canine Louping ill, confirmed at serological, histological, molecular and phylogenetic level. The LIV canine strain did not differ significantly from ovine strains indicating probable inter-species transmission.
P 52 HOW MANY ASTROVIRUSES INFECT WILD BOAR?

S. Šalamúnová1, A. Jackova1, S. Vilcek1

1 The University of Veterinary Medicine and Pharmacy in Košice, Department of Epizootiology and Parasitology, Košice, Slovakia

Introduction:
The family Astroviridae consists of two genera, Avastrovirus and Mamastrovirus, whose members infect avian and mammalian hosts, and occasionally may cause gastroenteritis. Studies have demonstrated that divergent astroviruses (AstV) can infect the same species and interspecies transmission events have occurred between wild and domestic species. The aim of this study was reported the presence and genetic characterization of AstV in wild boars.

Materials and Methods:
Organ homogenates (n=200) originated from wild boars from whole Slovakia were collected. Total RNA was isolated using TRIZol Reagent according to manufacturer’s instructions. The cDNA was prepared using random primers and Revert Aid Premium reverse transcriptase. A semi-nested PCR was used for AstV detection. The amplicons (n=13) were sequenced and the phylogenetic tree was constructed by MEGA 6.

Results:
PCR assay confirmed 30 samples (15 %) positive for AstV. Phylogenetic tree indicated that 10 nucleotide sequences belong to porcine AstV, two sequences belong to avian AstV and one sequence belong to bat AstV. The most represented lineage was PAstV-2 (n=8), followed by lineage PastV-4 (n=2). Two avian AstV clustered on phylogenetic tree with chicken AstV-2 sequences.

Discussion and Conclusion:
For the first time the presence of astroviruses in organ homogenates in wild boars in Slovakia was detected. Except porcine AstV, we have identified two avian AstV and one bat AstV. The presence of avian and bat astroviruses in wild boars confirms the ability of AstV of interspecies transmission, not only between mammalian, but also between avian and mammalian species. Supported by project No APVV-15-0415.
PS 01 - POSTER SESSION

P 53 NEW PORCINE CIRCOVIRUS IN GERMANY?

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² Kansas State University, College of Veterinary Medicine, Manhattan- Kansas, USA
³ Vaxxinova GmbH, Deutscher Platz 5e, 04103 Leipzig, Germany

Introduction:
In 2016, a novel circovirus, designated porcine circovirus 3 (PCV3) was first identified in US swine. Several studies have recently demonstrated the existence of PCV3 in Asia, Brazil and Europe, associated with different clinical syndromes and diseases in pigs of different ages. For simultaneous detection of PCV2 and PCV3 a new multiplex qPCR assay was developed and validated.

Materials and Methods:
DNA was extracted from different sample material using QIAamp cador Pathogen Mini Kit or MagAttract 96 cador Pathogen Kit (INDICAL). For qPCR detection virotype PCV2/PCV3 Primers/Probes combined with virotype Mix +IC-DNA (INDICAL) was used. DNA of US and German derived field samples was tested and a complete German PCV3 genome was sequenced.

Results:
The new virotype PCV2/PCV3 qPCR assay shows a high analytical sensitivity. Testing a panel of DNA from PCV2 and PCV3 field samples resulted in a sensitivity of 99 % and a specificity of 100 % compared to the reference tests. The sequence homology to two US strains was determined as 99.4 % (US/SD2016) and 99.06 % (US/MN2016) and as 99 % to full genome sequences of 15 German strains.

Discussion and Conclusion:
Using the virotype PCV2/PCV3 Primers/Probes in combination with virotype Mix +IC-DNA, PCV2 and PCV3 could be identified in both German and US porcine samples simultaneously. PCV3 was detected in samples with clinical signs of PMWS, PDNS and respiratory diseases. Further studies are recommended to investigate the pathogenic role of PCV3. PCV3 could be identified in German samples as early as 2008, showing that this is not a newly emerging virus in Germany.
PS 01 - POSTER SESSION

P 54 SURVEY OF ZOONOTIC PATHOGENS IN WILD RODENTS IN SOUTHERN BELGIUM: DETECTION OF TULA VIRUS IN WOOD MICE

V. Suin¹, L. Lempereur², A. Dobly³, P. Maes⁴, S. Lamoral¹, A. Hamouda¹, B. Lossom², M. Lahaut⁵, S. VanGucht¹, B. Brochier¹

¹ Sciensano, Viral Diseases, Brussels, Belgium
² University of Liège, Laboratory of Parasitology and Parasitic Diseases, Liege, Belgium
³ Sciensano, Biological Standardisation, Brussels, Belgium
⁴ Rega Institute, National Reference Center for Hantavirus, Leuven, Belgium
⁵ University of Liège, Laboratory of Parasitology and Parasitic Diseases, Liege, Belgium

Introduction:
Rodents are important hosts for ticks, especially Ixodes ricinus which represents the predominant tick of rodents and humans in Europe. Rodents can act as reservoirs of pathogens which are potentially zoonotic such as Borrelia spp., Babesia spp., Anaplasma phagocytophilum, Rickettsia spp, tick-borne encephalitis virus (TBEV) and Hantavirus.

Materials and Methods:
We investigated the presence of these viral, bacterial and parasitic zoonotic pathogens in a risk-based convenience sample of bank voles (Myodes glareolus) and wood mice (Apodemus sylvaticus) in southern Belgium. In total, 260 bank voles and 47 wood mice were trapped in 2014 and 2015 from 5 locations in the Walloon Region (Tinlot, Marche-en-Famenne, Forrière, Vaux-sur-Sûre and Gouvy). Trapping locations were selected based on the presence of cattle previously found seropositive for TBEV.

Results:
Five out of 191 bank voles and 1/124 wood mice were seropositive for TBEV antibodies. TBEV RNA was however not detected in any rodent. We did find rodents positive for DNA of Babesia microti, Anaplasma phagocytophilum, Neoehrlichia mikurensis, Rickettsia spp., Hantavirus. Moreover, we report the detection of Tula virus in wood mice in Belgium.

Discussion and Conclusion:
TBEV antibodies were already found in sentinel species as cattle, roe deers and wild boar but the virus was never isolated from rodents in Belgium. There is a real need to better screen rodents and also ticks in the future in Belgium. Tula virus is usually carried by voles from the Microtus genus, mainly M. arvalis and M. levis, but not A. sylvaticus. Hantavirus host specificity merits further investigation, especially among wild rodents.
PS 01 - POSTER SESSION

P 55  PCR DETECTION OF INFECTION AGENTS IN FROZEN BULL SEMEN IN RUSSIA

S. Yatsentyuk1, A. Kozlova1, N. Gorbacheva1
1 The Russian State Center for Animal Feed and Drug Standardization and Quality VGNKI, Biotechnology, Moscow, Russian Federation

Introduction:
Artificial insemination (AI) is a successful technique that is used for the breeding of cattle around the world. However, there are some potential risks of the spread of microbial agents through the semen used for AI. Some of microbial agents may be non-pathogenic, other can cause serious diseases in recipient farm animals. We studied frequency of different agents in frozen bull semen samples of Russian origin and imported frozen semen straws.

Materials and Methods:
Nucleic acids from semen samples were extracted with RIBO-prep («Amplisens», Russia). Pathogens detection was performed using PCR-kits (Table 1). Positive samples were confirmed by nucleotide sequencing.

<table>
<thead>
<tr>
<th>No</th>
<th>PCR-KIT</th>
<th>INFECTION AGENT</th>
<th>DETECTION</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LSI VetMAX™ Neospora caninum</td>
<td>Neospora caninum</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>2</td>
<td>LSI VetMAX™ Bovine Herpes Virus Type 4</td>
<td>Bovine herpes virus 1</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>3</td>
<td>LSI VetMAX™ Histophilus somni</td>
<td>Histophilus somni</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>4</td>
<td>LSI VetMAX™ Campylobacter spp.</td>
<td>Campylobacter spp.</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>5</td>
<td>LSI VetMAX™ Campylobacter fetus</td>
<td>Campylobacter fetus</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>6</td>
<td>LSI VetMAX™ Mycoplasma bovis</td>
<td>Mycoplasma bovis</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>7</td>
<td>LSI VetMAX™ C. burnetii and Chlamydia spp</td>
<td>C. burnetii and Chlamydia spp</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>8</td>
<td>LSI VetMAX™ BVDV 4ALL</td>
<td>Bovine viral diarrhea virus</td>
<td>Real-Time</td>
<td>Life Technologies Corporation (France)</td>
</tr>
<tr>
<td>9</td>
<td>PCR-kit “MIK-KOM”</td>
<td>Mycoplasma spp</td>
<td>Conventional PCR</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>10</td>
<td>PCR-kit “CHLA-KOM”</td>
<td>Chlamydia spp</td>
<td>Conventional PCR</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>11</td>
<td>PCR-kit “RHINO-KOR”</td>
<td>Bovine herpes virus 1</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>12</td>
<td>PCR-kit “LEUKOSIS”</td>
<td>Bovine leukemia virus</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>13</td>
<td>PCR-kit “CAM-BAK”</td>
<td>Campylobacter jejuni</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>14</td>
<td>PCR-kit “SBV”</td>
<td>Schmallenberg virus</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>15</td>
<td>PCR-kit “LP89”</td>
<td>Pathogenic Leptospira spp</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>16</td>
<td>PCR-kit “VD”</td>
<td>Bovine viral diarrhea virus</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>17</td>
<td>PCR-kit “BRU-KOM”</td>
<td>Brucella spp</td>
<td>Real-Time</td>
<td>Amplisens (Russia)</td>
</tr>
<tr>
<td>18</td>
<td>PCR-kit for detection DNA Lumpy skin disease virus</td>
<td>Lumpy skin disease virus</td>
<td>Real-Time</td>
<td>FractalBio (Russia)</td>
</tr>
</tbody>
</table>
Results:
281 frozen bull semen samples from local and foreign AI centers were tested by PCR kits published in Table 1.

Pathogenic Leptospira, Chlamydia spp., Neospora caninum, Brucella spp., Campylobacter fetus, Bovine leukemia virus, Schmallenberg virus, Lumpy skin disease virus were not detected. But 3 viral and 6 bacterial targets were revealed.

Positive results listed in Table 2.

Table 2. Results of the study of bull’s semen in PCR

<table>
<thead>
<tr>
<th>The positive samples</th>
<th>Research samples</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local AI centers</td>
<td>Foreign AI centers</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=113</td>
<td>N=188</td>
<td>N=281</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive samples</td>
<td>%</td>
<td>Positive samples</td>
<td>%</td>
</tr>
<tr>
<td>BHV1</td>
<td>2</td>
<td>1.77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BHV4</td>
<td>1</td>
<td>0.88</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>BVDV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>C. burnetii</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>7.14</td>
</tr>
<tr>
<td>H. somni</td>
<td>103</td>
<td>91.15</td>
<td>104</td>
<td>61.9</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>96</td>
<td>84.96</td>
<td>102</td>
<td>60.71</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>19</td>
<td>16.81</td>
<td>4</td>
<td>2.38</td>
</tr>
<tr>
<td>Mycoplasma spp.</td>
<td>103</td>
<td>91.15</td>
<td>95</td>
<td>56.55</td>
</tr>
<tr>
<td>Mycoplasma bovis</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
H. somni and some Campylobacter and Mycoplasma species are common inhabitants of the genital tract of bulls. Some strains of Histophilus somni and Mycoplasma species may cause a wide variety of diseases in cattle. Detection of Mycoplasma in bull semen requires additional species differentiation (M.bovigenitalium, M.californicum, U.diversum). Examination of bull semen for AI seems to be necessary for the control and prevention of the pathogens distribution.
PS 01 - POSTER SESSION

P 56 DEVELOPMENT OF THE REAL-TIME PCR FOR THE DETECTION AND DIFFERENTIATION OF MYCOPLASMA CALIFORNICUM, M.BOVIGENITALIUM, M.BOVIS AND U. DIVERSUM

A. Kozlova1, N. Gorbacheva1, S. Yatsentyuk1

1 The Russian State Center for Animal Feed and Drug Standardization and Quality, Biotechnology, Moscow, Russian Federation

Introduction:
Mycoplasma is one of the main causes of mastitis, pneumonia and reproductive disorders in cattle. Differentiation of pathogenic Mycoplasma (M.bovis, M.californicum, M.bovigenitalium) and Ureaplasma diversum is an important task.

Materials and Methods:
DNA from bull semen samples was extracted with RIBO-prep. Mycoplasma spp. detection was performed using conventional PCR-kit «MIK-KOM» («Amplisens», Russia). Detection of M.bovis, M. bovigenitalium, M.californicum and U.diversum was carried out on Rotor Gene Q (Qiagene). Primers and DNA-probes were designed on sequences of the UvrC gene for M.bovis, 16S rRNA for M.bovigenitalium and U.diversum. Primers and DNA-probes for M.californicum rpoB gene described in [1].

Results:
The exogenous Internal positive control was added to control successful DNA extraction. The detection limit of all PCR assays was from $10^3$ to $5 \times 10^3$ copies/ml of targeted DNA. DNA samples of 5 different Mycoplasma species, 20 heterologous bacteria, 6 viruses and bovine genomic DNA were tested. Specific primers and DNA-probes showed no cross-amplification with heterologous Mycoplasma species, bacteria and viruses.

281 frozen semen samples for Artificial insemination were tested. The results listed in table 1.

<table>
<thead>
<tr>
<th>Pathogen co-infection</th>
<th>Local AI centers</th>
<th>Foreign AI centers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=113</td>
<td>N=168</td>
<td>N=281</td>
</tr>
<tr>
<td>Positive samples</td>
<td>Positive samples</td>
<td>Positive samples</td>
<td>Positive samples</td>
</tr>
<tr>
<td>Mycoplasma spp.</td>
<td>103</td>
<td>91.2</td>
<td>95</td>
</tr>
<tr>
<td>M. bovis</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>M. bovigenitalium</td>
<td>58</td>
<td>51.3</td>
<td>36</td>
</tr>
<tr>
<td>M. californicum</td>
<td>73</td>
<td>64.6</td>
<td>36</td>
</tr>
<tr>
<td>U. diversum</td>
<td>62</td>
<td>54.9</td>
<td>21</td>
</tr>
</tbody>
</table>

Pathogen co-infection was detected in 89 (31.6 %) samples with M. californicum / M. bovigenitalium (55 cases, 19.6 %) and M. bovigenitalium/ U. diversum (49 cases, 17.4 %) being the most dominating combinations.

Discussion and Conclusion:
RT-PCR assays can be used for monitoring of semen quality as well as control and prevention of the pathogens distribution.

References:
P 57  EVOLUTION OF ANTIBIOTIC RESISTANCES IN TWO BACTERIAL GROUPS OVER TIME

J. Blanco, T. Martin-Muñoz, C. Martín, M. García

1 Universidad Complutense de Madrid, Animal Health, Madrid, Spain

Introduction:
Bacterial antibiotic resistance constitutes a first level health threat. This is not a problem that affects only Human Medicine, but within the concept “one world, one health” must also take into account Animal Medicine and Environmental aspects.

Materials and Methods:
Two types of bacterial groups were used: E. coli isolated from dog urine, and positive coagulase staphylococci isolated from canine otitis. And for each of them, two isolation periods: One between 1998 and 2008, and another from 2017. For each of these groups 20 strains were tested, giving a total of 80 strains studied.

The antibiotic sensitivity of the strains was determined with the use of AST-GN65 cards for sensitivity to 19 antibiotics in gram-negative bacteria, in addition to the detection of ESBL, and AST-GP76 for sensitivity to 21 antibiotics in gram-positive. Both use the automated reading system Vitek2.

Results:
When comparing the results between the isolates in the two periods of time, a clear increase in antibiotic resistance was observed.

Discussion and Conclusion:
It is evident that the abuse and misuse of antibiotics contributes to a remarkable increase of the antibioresistance. That is why health education measures must be promoted at all levels, in order to achieve a more rational use of antibiotics.

We thank Biomerieux for the free supply of the material used in this study.
PS 01 - POSTER SESSION

P 58 ENVIRONMENTAL SAMPLING FOR THE DETECTION OF ANTIBIORESISTENT BACTERIA IN THE HOSPITALIZATION AREA OF A VETERINARY HOSPITAL

J. Blanco1, S. Santos-Ocaña1, C. Martin1, M. García1

1 Universidad Complutense de Madrid, Animal Health, Madrid, Spain

Introduction:
The realization of this research work focused on the maintenance area of the hospitalized animals. Regarding bacteria, the work focused on the determination of Carbapenemase producing Enterobacteria (CPE) and Extended Spectrum Betalactamase producing Enterobacteria (ESBL).

Materials and Methods:
Sampling was carried out in 24 different points of the Hospitalization area. To do this, sponges were used to take samples soaked in peptone water and kept in sterile bags. An enrichment was performed by incubation 24 h at 37 °C. Later it was inoculated in two culture media: ChromID CARBA (to detection of CPE) and ChromID ESBL (to detection of ESBL).

Results:
CPE were isolated in 6 of the 24 points: In 4 different wheels of the stretchers that bring patients from the operating room (4 Klebsiella and 1 E. coli), in the hands of a resident (Klebsiella) and in the sole of footwear of one facultative (Klebsiella). ESBL were isolated in 17 of the 24 sampling points, in most of them Klebsiella (16) and also E. coli (7). Also in this case it was isolated from different parts of the floor of the hospitalization area, including the floor of boxes and animal cages, staff hands and sole of the footwear of the staff, in addition to the wheels of the stretchers.

Discussion and Conclusion:
The isolation of antibiotic resistant bacteria in an area of hospitalization is worrisome, and affects the need for extreme hygiene measures in order to avoid nosocomial infections.
P 59 OCCURRENCE OF ATYPICAL MYCOBACTERIOSIS IN AFRICAN CICHLIDS REARED IN A PRIVATE AQUARIUM

E. Bozzetta¹, D. Mugetti², K. Varello¹, M. Righetti², D.R. Francese¹, M. Prearo²

¹ Istituto Zooprofilattico Sperimentale del Piemonte- Liguria e Valle d’Aosta, SC Istopatologia e Test Rapidi Centro di Referenza Nazionale Indagini Biologiche Anabolizzanti Animali - CIBA, Torino, Italy
² Istituto Zooprofilattico Sperimentale del Piemonte- Liguria e Valle d’Aosta, SS Laboratorio Specialistico Ilttiopatologia, Torino, Italy

Introduction:
Piscine Mycobacteriosis is a chronic progressive infectious disease caused by microorganisms belonging to the genus Mycobacterium. Clinical signs often appear at advanced stages of the disease and are non-specific such as emaciation, abdominal swelling, haemorrhagic and dermal lesions. Mycobacteria are also associated with human infections. Aim of the study was to describe a case of mycobacterial infection in African cichlids from Lake Malawi held in a private aquarium in Italy.

Materials and Methods:
During fish health monitoring activities 54 cichlids were examined. The animals were euthanized and subjected to necropsy. Samples from liver, spleen and gut were collected for parasitological, virological and bacteriological investigations, including mycobacterial culture, and for histology. The tissues for histopathology were processed by standard paraffin wax techniques and stained with haematoxylin-eosin (HE) and Ziehl–Neelsen stain (ZN). For culture the decontaminated homogenate was inoculated on Stonebrink e Löwenstein-Jensen Medium at 28° and 37°C. The purified isolates were characterised by molecular identification.

Results:
At necropsy, no visible lesions in the organs were observed. Microscopically, nine fish revealed single or multiple granulomas and 5 were positive at ZN. Twenty-two fish (prevalence 40.7 %) resulted positive and the isolates identified as M. peregrinum (12), M. fortuitum (3), M. chelonae (3), M. gordonae (2), M. abscessus (1), Mycobacterium spp (1).

Discussion and Conclusion:
Piscine mycobacteriosis is often underestimated, monitoring activities are an important tool to preserve the welfare of ornamental fish and to safeguard public health. This study showed that ornamental fish may be source of infection for risk groups, such as fishery professionals or ornamental fish hobbyists.
PS 01 - POSTER SESSION

P 60 PRELIMINARY STUDY OF IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF BACILLUS ANTHRACIS STRAINS ISOLATED IN ITALY FROM 2001 TO 2017

V. Manzulli1, M. Caruso1, L. Serrecchia1, D. Galante1, A. Donatiello1, V. Rondinone1, S. Zange2, A. Tschene2, A. Parisi1, D. Cipolletta1, A. Fasanella1

1 Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Anthrax Reference Institute of Italy, Foggia, Italy
2 Bundeswehr Institute of Microbiology, Central Diagnostic Unit, Munich, Germany

Introduction:
Anthrax, whose causative agent is Bacillus anthracis, is a zoonotic disease that affects a wide range of animal species (primarily ruminant herbivores) and can be transmitted to humans through consumption or handling of contaminated animal products. In Italy, animal anthrax is endemic and is characterized by sporadic outbreaks occurring mainly during summer. Due to the often fatal outcome of human cases, the quick administration of definitely effective antimicrobials is crucial either in prophylaxis, after presumptive exposure, or for the therapy of clinical cases.

Materials and Methods:
A total of 100 Bacillus anthracis strains, temporally, geographically and genetically different isolated during anthrax outbreaks in Italy from 1984 to 2017, were screened for their susceptibility to 16 clinically relevant antimicrobial agents by using the broth microdilution method. All the strains are representative of 30 different MLVA genotypes. The following antibiotics were tested: gentamicin, ceftriaxone, streptomycin, penicillin G, clindamycin, chloramphenicol, vancomycin, linezolid, cefotaxime, tetracycline, erythromycin, rifampicin, amoxicillin, ciprofloxacin, doxycycline and trimethoprim.

Results:
All the isolates were susceptible towards most of the tested antimicrobial with the exception of trimethoprim for which all of them resulted resistant and ceftriaxone and cefotaxime for which an intermediate level was recorded as reported in Table 1.

Table 1: Antibiotic susceptibilities of 100 isolates of Bacillus anthracis

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>MIC Breakpoints* (µg/ml)</th>
<th>% of isolates with the following interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%*</td>
<td>S(+)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.008-16</td>
<td>0.125</td>
<td>4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.25-512</td>
<td>0.8</td>
<td>16</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.005-32</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.001-2</td>
<td>0.015</td>
<td>0.5</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.008-16</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.06-128</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.06-64</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.06-64</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.25-512</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.008-16</td>
<td>0.125</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.008-16</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.004-8</td>
<td>0.125</td>
<td>1</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.008-16</td>
<td>0.06-0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.004-8</td>
<td>0.03-0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.004-1</td>
<td>0.015</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.125-128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

* S, susceptible; I, intermediate; R, resistant.
* MIC at which 50% or 90% of tested isolates are inhibited.
Discussion and Conclusion:
Although the Centers for Disease Control and Prevention recommend the use of doxycycline, ciprofloxacin, penicillin G and amoxicillin for the treatment of human cases and for post exposure prophylaxis to anthrax spores, this study suggests also a high degree of susceptibility in vitro of B.anthracis to many other antimicrobials.
MOLECULAR ANALYSIS OF PLASMIDS FROM MULTI-DRUG RESISTANT SALMONELLA ENTERICA SER. INFANTIS FROM POULTRY

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**Introduction:**

S. Infantis is a serovar found in multiple animal hosts and products of animal origin. S. Infantis infections were reported to increase in many countries and often is complicated by multiple resistance to medically and veterinary important antimicrobials.

**Materials and Methods:**

Three multiresistant S. Infantis isolates were obtained from chicken sources during drug-resistance monitoring in Russia. DNA was extracted and then tagmentated by the Nextera XT. The library was prepared and sequenced on a Miseq 250 base paired-end run. Illumina reads were trimmed with removing of sequencing adapters and than assembled using SPAdes. The assembled sequences were annotated with the RAST. ResFinder, ARG-ANNOT and CARD tools were used too to identify resistance determinants.

**Results:**

All isolates had a gyrA-p.D87Y or gyrA-p.S83Y mutations and harbored 8–10 resistance genes ([blaCTX-M-14, aadA1, aac6-lac, dfrA14, sul1, tetA, tetR, tetM, mdsABC, mdtk, sdiA, golS]) per one isolate located at large plasmids. The plasmids with size 309270_bp, 314737_bp, 333679_bp correspondently were similar to those described for S. Infantis human isolate from Italy and for different isolates from USA [1]. Analysis of the complete sequences confirmed the presence of an IncFIB-like incompatibility group replicon for these presumably conjugative plasmids. Drug-resistance genes are grouped together at two distinct sites of plasmids.

**Discussion and Conclusion:**

The discovery of S. Infantis plasmids similar to megaplasmids which have been discovered early is excellent example of how easily resistant bacteria can spread internationally. The example illustrates the importance of applying a global “One Health human and animal” perspective to combat antimicrobial resistance.

**References:**

A NEW REAL-TIME PCR METHOD FOR DETECTION OF BARTONELLA SPECIES, IN SEROLOGICALLY POSITIVE CATS

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Teramo, Italy
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Molecular Biology and Omics Technologies, Teramo, Italy

Introduction:
Bartonella henselae is the principal etiologic agent of the zoonosis Cat Scratch Disease (CSD), transmitted to humans by bite or scratch of cat, both persistently bacteremic and healthy reservoir of Bartonella, infected by vector flea Ctenocephalides felis.

The aim of this study was to develop a novel specific molecular method to detect Bartonella spp. in blood samples of cats.

Materials and Methods:
Fifty-two blood samples of cats, collected random from Abruzzo region, were examined for Bartonella infection by Real-time PCR and IFAT. Total genomic DNA, extracted from whole blood samples, were subjected to a novel Real-time PCR method that amplifies a specific region of nuoG gene. Subsequently, a partial region of 23S gene was sequenced in positive samples. Sera were tested by IFAT to detect IgG and IgM antibodies against Bartonella spp, using slides containing B. henselae (ATCC 49882), cultured in L929 cell line.

Results:
Seven blood samples out of fifty-two (13.4 %) resulted positive for Bartonella spp. in both tests. The sequence analysis of Real-time PCR positive samples identified B. henselae in four samples and B. clarridgeiae in two samples. One positive sample wasn’t identified (low amount of DNA). IFAT revealed IgG antibodies against Bartonella spp. in all positive PCR cats, while IgM antibodies were identified in two cats (Table1).

Table 1 Serological and Real-time PCR results

<table>
<thead>
<tr>
<th>No. of Cats</th>
<th>IgG</th>
<th>IgM</th>
<th>Real-time PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/160</td>
<td>neg</td>
<td>POS</td>
</tr>
<tr>
<td>2</td>
<td>1/130</td>
<td>neg</td>
<td>POS</td>
</tr>
<tr>
<td>3</td>
<td>1/160</td>
<td>neg</td>
<td>POS</td>
</tr>
<tr>
<td>4</td>
<td>1/130</td>
<td>neg</td>
<td>POS</td>
</tr>
<tr>
<td>5</td>
<td>1/140</td>
<td>2/80</td>
<td>POS</td>
</tr>
<tr>
<td>6</td>
<td>1/140</td>
<td>2/80</td>
<td>POS</td>
</tr>
<tr>
<td>7</td>
<td>1/160</td>
<td>1/64</td>
<td>POS</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
This study showed a good correlation between positive antibody titers and Real-time PCR results. This molecular method could be considered useful to detect the Bartonella infected cats that represent a risk for the transmission of CSD.
PS 01 - POSTER SESSION

P 63  EPIDEMIOLOGICAL INVESTIGATION ON ERYSIPELOTHRIX SPP. IN WILD BOARS AND POTENTIAL RISKS AT THE WILDLIFE-DOMESTIC-HUMAN INTERFACE

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1 Istituto Zooprofilattico Sperimentale della Lombardia e dell’ Emilia Romagna “Bruno Ubertini”, Brescia, Italy
2 National Veterinary Institute, Uppsala, Sweden
3 Aarhus University, Tjele, Denmark
4 Swedish University of Agricultural Sciences, Uppsala, Sweden

Introduction:
Erysipelothrix rhusiopathiae (ER) and E. tonsillarum (ET) are the two main species of the genus Erysipelothrix. As ER may pose zoonotic and economic concerns while ET is thought to be avirulent for swine, their different epidemiological role emerges. Wild boar (Sus scrofa) have been suggested as potential reservoir of Erysipelothrix spp. and the little information available warrants investigation of a possible risk of infection at the wildlife-domestic-human interface. The ER seroprevalence and the prevalence of Erysipelothrix spp. isolates were evaluated in wild boars from Northwest Italy to disclose factors associated with the epidemiology of the infection.

Materials and Methods:
During the 2015/2016 and 2016/2017 hunting seasons 971 sera were collected from 8 macro-areas with different density of pig farms. Additionally, 223 tonsils were gathered during 2016/2017. Results were analysed by multivariate logistic regression.

Results:
An overall ER seroprevalence of 59.63% emerged. Subjects from high density pig farms macro-area showed the highest seroprevalence (p<0.0001). Erysipelothrix spp. was isolated from 67 boars. Until now, partial 16S sequencing was performed on 10 isolates: BLAST analysis identified 7 as ER and 3 as ET.

Discussion and Conclusion:
The increased seroprevalence in the high density pig farms macro-area could support a relation between seroprevalence and the presence of farms, although in this mechanism an effect of wild boar density cannot be ruled out. Healthy wild boars carrying ER in their tonsils may serve as a source of infection for domestic pigs and pose zoonotic and economic risks. Further sequencing will provide clues to better evaluate the potential risks related to wild boars.
P 64 BOVINE TUBERCULOSIS AND ITS DANGER TO HUMANS

A. Gritly¹

¹ Military Veterinary Service, General Directorate of Military Health, Tunis, Tunisia

Introduction:
In Tunisia, despite the implementation of a National Program Against Bovine Tuberculosis, this disease is still widespread, particularly among dairy herds. It remains a constant threat not only for livestock but also for humans.

Materials and Methods:
In this study, we assessed 400 cattle reacting to tuberculin, that were slaughtered in the National Program Against Bovine Tuberculosis. A macroscopic study was performed on all organs. To confirm the diagnosis of tuberculosis, samples were processed for histological and bacteriological analyzes.

Results:
Among animals reacting to the tuberculin test, only 320 (80 %) had macroscopic lesions and 70 (17.5 %) had microscopic lesions. The lesions were mainly localized in the respiratory system (370 cases, 92.5 %). They were dominated by the classic nodular caseous shape, especially in lymph nodes (320 cases, 80 %) and secondary lesions in lungs (80 cases, 20 %). The microscopic lesions were pathognomonic of tuberculosis and consisted mainly in KÖSTER follicles. The Ziehl-Neelsen method confirmed the presence of Acid Fast Bacilli in 280 cases (70 %). The absence of Acid Fast Bacilli does not exclude the diagnosis of tuberculosis, hence the interest of bacterial cultures. Indeed, cultures revealed the presence of Mycobacterium bovis in 110 cases that were negative for the Ziehl-Neelsen staining.

Discussion and Conclusion:
In Tunisia, bovine tuberculosis remains common. The disease is a major public health problem with entailing considerable economic losses and a relatively high morbidity in humans.
**P 65 COMPARISON OF BRUCELLA MELITENSI S AND BRUCELLA OVIS BY PROTEOMIC APPROACH**

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2 Istituto Zooprofilattico Sperimentale Abruzzo e Molise, Bioinformatics, Teramo, Italy

**Introduction:**
In this work the proteome of two *Brucella* species, *B. melitensis* type strain 16M and *B. ovis* REO198, showing different phenotypes, pathophysiology and host-pathogen interactions were compared, in order to identify proteins that may correlate to different phenotypic characteristics and to search virulence or immunogenic factors for a better characterization of *Brucella* pathogenicity.

**Materials and Methods:**
*B. melitensis* and *B. ovis* were grown in Tryptose soy agar plates for 48–72 h (5–10 % CO2, 37 °C) and sonicated. Proteins were then separated by SDS-PAGE and 5 areas were analysed by nLC-MS/MS (Figure 1). The identified proteins were annotated by bioinformatics softwares (SignIP, TMHMM, LipoP, PSORTb).

Figure 1. SDS-PAGE and Comassie stain of *B. melitensis* 16M (lane 1) and *B. ovis* REO198 (lane 2). The highlighted areas were analysed by nLC-MS/MS. Lane M: molecular weight marker.

**Results:**
A total of 273 proteins were identified by nLC-MS/MS for *B. melitensis* 16M and 606 proteins for *B. ovis* REO198 (peptide thresholds: >95,0 %; protein thresholds: >99,0 %; minimum three peptides/protein). The subcellular localization of identified proteins is shown in Table 1.
Table 1. Subcellular localization of the identified Brucella proteins

<table>
<thead>
<tr>
<th>Localization</th>
<th>B. melitensis 16M</th>
<th>B. ovis REO198</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic proteins</td>
<td>175</td>
<td>383</td>
</tr>
<tr>
<td>Cytoplasmic Membrane</td>
<td>24</td>
<td>57</td>
</tr>
<tr>
<td>Periplasmic</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Outer Membrane</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Extracellular</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>44</td>
<td>116</td>
</tr>
<tr>
<td>Total</td>
<td>273</td>
<td>606</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
A greater number of proteins were identified for B. ovis REO198 in comparison with B. melitensis 16M. After further analysis, some of these proteins could provide the scientific explanation to the phenotypic and pathophysiology differences observed among the species of the genus Brucella. In the next step outer and periplasmatic proteins will be screened for predictive virulence and immunogenic attributes.
P 66 DEVELOPMENT OF REAL-TIME PCR KIT FOR SPECIFIC DETECTION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND TYPE 3 (PCV3)

F. Pez1, J. Ventosa1, C. Pelletier1, E. Sellal1

1 BioSellal, R and D, Dardilly, France

Introduction:
While PCV2 is associated with different symptoms in pigs collectively named porcine circovirus disease, PCV3 is an emerging virus with unknown pathogenicity. High levels of viremia were reported in sick animals with severe respiratory, skin disease or reproductive disorders. Quantitative and specific diagnostic tool for Porcine Circovirus are essential for distinction between PCV2 and PCV3 and could play an important role to clarify PCV3 pathogenicity.

Materials and Methods:
A triplex ready-to-use real-time PCR kit was developed. It allows the specific detection of PCV2 and PCV3 with an internal positive control. Bio-T kit® validations were done according to the AFNOR standard NF U47-600-2. For blood sample, a relative quantification of the viral load of each target is allowed thanks to a reference material set at the interpretation threshold (MRSI) that could be provided by BioSellal. For PCV2, this threshold was set at 106 GE/ml according to previous publication and was extrapolated at the same level for PCV3.

Results:
Bio-T kit® PCV2 & PCV3 validation results was presented in the table below.

<table>
<thead>
<tr>
<th>PCR Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion</td>
<td></td>
</tr>
<tr>
<td>PCV2</td>
<td>Specific detection of PCV2 validated on 25 PCV2 positives samples provided by GD Animal Health (Ring Tilburg, Netherlands)</td>
</tr>
<tr>
<td>PCV3</td>
<td>Specific detection of PCV3 validated on 6 blood samples confirmed by SANGER sequencing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Verified on viruses and bacteria present in the same ecological niche or genetically related to PCV2 and PCV3 such as PCV2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Limit of Detection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV2</td>
<td>10 copies/PCR</td>
</tr>
<tr>
<td>PCV3</td>
<td>10 copies/PCR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Efficiency</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV2</td>
<td>102%</td>
</tr>
<tr>
<td>PCV3</td>
<td>110%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linearity range</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV2</td>
<td>10^6 to 10 copies/PCR</td>
</tr>
<tr>
<td>PCV3</td>
<td>10^6 to 10 copies/PCR</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
The Bio-T kit® PCV2 & PCV3 enables rapid and automated differential detection of PCV2 and PCV3. Moreover, the use of a common and ready-to-use MRSI will improve the accuracy of PCV2 and PCV3 viral loads quantification, regardless of the extraction method and used thermocycler, and thus the overall consistency of these measurements between different laboratories. Finally, the Bio-T® PCV2&PCV3 kit could be a very interesting tool to investigate more precisely the PCV3 pathogenicity.

References:
P 67 THE IMPROVEMENT OF ISOLATION TECHNIQUES IN THE RESEARCH OF ESCHERICHIA COLI STEC

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Introduction:
Escherichia coli STEC are defined verocytotoxic as producers, due to a phenomenon of lysogenic convergence, of two powerful cytotoxins: verocytotoxin 1 or SLT1 (or Shiga-like toxin 1), verocytotoxin 2 or SLT2 often associated to the first one from which it differs at least from the antigenic point of view. The aim of this study is the improvement of cultivation isolation techniques in the research of serogroups O157, O111, O26, O103, O145.

Materials and Methods:
65 samples were analyzed among meat, vegetables, dairy products, as part of the national food control plan, in the period from September 2017 to March 2018 with a real time PCR method according to ISO / TS 13136 : 2012.

Results:
Out of 65 analyzed samples, 2 meat samples were positive, in particular a positive bovine burger for the O103 serogroup and a positive sheep burger for the O145 and O26 serogroups, whereas they were negative for pathogenicity genes on culture typical colonies as indicated in ISO 13136. The result was a presumptive presence of STEC. For the same samples, non-typical colonies, although not expected by the standard, were also tested for serum agglutination, which instead showed a positive result both for pathogenic genes and for serogroups.

Discussion and Conclusion:
The obtained results suggest the need to examine, with real time PCR, all the serum agglutination positive colonies for the research of pathogenicity genes even if they do not have a typical appearance.

References:
P 68  FIRST RECORDS OF THE PRESENCE OF LYSSAVIRUSES IN BATS IN BELGIUM

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¹ Sciensano, SD Infectious Diseases in Humans- Viral Diseases, Brussels, Belgium

Introduction:
Rabies is a zoonotic infectious disease caused by an infection with a virus belonging to the Genus Lyssavirus. The majority of the infections are the result of a bite from a dog infected with the classical rabies virus, but most of the different lyssaviruses also circulate in bats. Currently, 4 of the 12 lyssaviruses species are known to circulate in European bats. Since the first report in 1954, over 1000 positive bat cases have been detected in Europe. The majority of them were infected with European Bat Lyssavirus-1 or -2.

Materials and Methods:
Both samples were diagnosed using FAT and qRT-PCR.

Results:
In 2016, the first endemic case of European Bat Lyssavirus-1 in Belgium was detected in the province of Luxemburg. The serotine bat, unable to fly, was submitted to the national reference laboratory for rabies for testing, where the diagnosis was confirmed using direct fluorescent antigen and PCR testing. In 2017 the national reference laboratory for rabies confirmed a second case, again in a serotine bat coming from the same province.

Discussion and Conclusion:
These two local cases confirm that bat lyssaviruses, more particularly European bat lyssavirus-1, circulate in Belgian bats. Based on the limited historical surveillance data, it is not possible to assess whether these case are exemplary of a recent incursion of this virus in Belgian bats or longstanding unrecognized endemic circulation. Further surveys targeting bat species, which are known reservoirs for European bat lyssavirus, are ongoing and will help to map the spread of this virus in Belgium.
MONITORING THE SPREAD OF ESBL GENES AND OTHER CRITICAL ANTIMICROBIAL RESISTANCE DETERMINANTS IN GRAM-NEGATIVE BACTERIA WITH A HOME-MADE BEAD ARRAY

M. Timmermans¹, P. Wattiaux¹, C. Boland¹

¹ Sciensano, Animal infection, Uccle, Belgium

Introduction:
Molecular characterization of antimicrobial resistance (AMR) determinants is recommended to monitor mechanisms underlying AMR in indicator bacteria like E. coli. An affordable all-in-one alternative to whole genome sequencing is needed to conduct this monitoring in large collections of Gram-negative bacteria in a one-health context.

Materials and Methods:
Phenotypic resistance profiles obtained by micro-dilution analysis are compared with genotypic resistance profiles obtained with a home-made multiplex Ligase Chain Reaction assay analyzed on a bead-array hybridization platform (Luminex®). In case of non-concordance between profiles, sanger sequencing is realized to determine AMR causes. 185 E. coli strains isolated in 2015 in Belgium were assayed with this array.

Results:
The assay gave a correspondence of 91.9% between the AMR genetic determinant and the phenotypic resistance profile. Most (13/15) of the discordances were due to a presumptive AmpC phenotype for which no plasmidic AmpC gene (CMY-1/CMY-2 group or ACC-1 like) were detected by the array. Sequencing of the chromosomal ampC promoter, attenuator and 5'-end regions in these strains identified mutations causing the chromosomal overexpression of ampC explaining the AmpC activity of 11 of these isolates, thereby relieving the apparent discordance.

Discussion and Conclusion:
An extended version of this array is under development, aiming to target the mutations upstream of ampC and key genetic determinants involved in the resistance to fluoroquinolones, colistin and other critical antibiotics used against Enterobacteriaceae. Thanks to its open design, this kind of multiplex assay is easily updatable if additional targets are needed.
PS 01 - POSTER SESSION

P 70 VETERINARY EVALUATION OF CHROMID® COLISTIN R AGAR, A NEW CHROMOGENIC MEDIUM FOR SCREENING OF ENTEROBACTERIACEAE WITH ACQUIRED RESISTANCE TO COLISTIN

M. Treilles¹, A. Colin², S. Marchand³, Y. Bala⁴

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² Qualys, Veterinary Bacteriology, Champdeniers, France
³ Biomerieux, Global Microbiology Solutions Marketing, Marcy l’Etoile, France
⁴ Biomerieux, Clinical Affairs Microbiology, Marcy l’Etoile, France

Introduction:
Plasmid-mediated and, therefore, transferable bacterial polymyxin resistance was discovered from both humans and animal strains in 2015 in China. In this context, French Food and Agriculture Ministry decided to focus on colistin resistance detection in its second National Action Plan “EcoAntibio²” for the reduction of the risks of antimicrobial resistance in veterinary medicine. One of the objectives of this plan is to decrease the use of colistin of 50% over 5 years on cattle, pig and poultry farm and to monitor its resistance rate in some sentinel strains. Culture-based testing constitutes a key tool for screening veterinary specimens and fulfilling that monitoring purpose.

Materials and Methods:
Specificity was assessed based on the testing of 30 fresh caecal content samples balanced among poultry (n=10), swine (n=10) and calves (n=10). For the Sensitivity, because of the low prevalence of resistant strains, 30 characterized pure strains of Escherichia coli and Salmonella sp. from veterinary isolates with an acquired resistance to colistin were used to contaminate the 30 fresh negative caecal samples recruited for the specificity study.

Results:
The overall specificity and sensitivity evaluated during this external trial was excellent and the ChromID colistin R media enabled the selective recovery of specific enterobacteriaceae resistant to colistin without false positive results.

Discussion and Conclusion:
Designed to culture selectively and differentiate with chromogenic properties the Colistin resistant Enterobacteriaceae species, this media is an ideal tool for large screening trials as well as confirmation of colistin resistance in dominant or subdominant flora.
PS 01 - POSTER SESSION

P 71 EVALUATION OF MYCOPLASMA BOVIS ANTIBODY ELISA FOR INDIVIDUAL MILK AND BULK MILK SAMPLES

M. Aalberts¹, C. Smits¹, M. Holzhauer²

¹ GD Animal Health, Research and Development, Deventer, Netherlands
² GD Animal Health, Ruminant Health, Deventer, Netherlands

Introduction:
Mycoplasma bovis (M. bovis) can cause severe infections in cattle, that are mainly characterized by arthritis and mastitis in adult cows and bronchopneumonia young stock. Infections can be detected by antibody ELISA in serum and milk. In this study, individual milk and bulk milk testing in a commercially available M. bovis antibody ELISA was evaluated.

Materials and Methods:
Samples were taken at twenty dairy farms that reported an outbreak of M. bovis infections, at one, three, six, nine and twelve weeks after the outbreak. 397 paired milk/serum samples were tested in an M. bovis antibody ELISA (BioX Diagnostics), that classifies results into five categories (0 to 5+). Furthermore, in 53 bulk milk samples Mycoplasma culturing was performed as well as antibody testing.

Results:
Considering a test result of 2+ or higher as positive, relative sensitivity and specificity of the ELISA in individual milk samples as compared to serum were 55 % and 92 % respectively. Sensitivity could not be improved by adjusting cut-offs without severely affecting specificity. Bulk milk ELISA results showed a perfect agreement with results of bulk milk Mycoplasma culturing.

Discussion and Conclusion:
As the level of M. bovis antibodies in individual milk samples does not sufficiently reflect M. bovis serum antibody levels, testing individual milk samples by ELISA is not a preferred method. The use of bulk milk samples to identify herds with M. bovis outbreaks seems promising.
P 72 COMPARISON OF SENSITIVITY AND SPECIFICITY OF CIVTEST® SUIS PRRS A/S WHEN DETECTING ANTIBODIES AGAINST GENOTYPE II PRRSV IN PIG SERA

M. Domenech¹, M. Ortiz¹, E. Coma², L. Porquet¹, X. Rebordosa²

¹ HIPRA, Girona, Amer, Spain
² HIPRA Scientific, Girona, Amer, Spain

Introduction:
PRRSV is a major source of economic losses in pigs, causing reproductive failure in sows and respiratory disease in piglets. Serological diagnosis is considered a reliable tool for herd monitoring.

The objective of the study is to compare CIVTEST® SUIS PRRS A/S with another commercial indirect ELISA (ELISA-I) and with the known expected results.

Materials and Methods:
The study analyses 41 negative and positive reference sera (vaccinated or infected) from GD (Netherlands) with known reactivity to the different genotypes of the PRRSV using 3 batches of CIVTEST® and 1 batch of ELISA-I.

Results:
The results of Sensitivity and Specificity of CIVTEST compared with the known results are shown in Table I:

Table I. (95% Confidence Int.)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97.2</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>85.83-99.51</td>
<td>56.65-100.00</td>
</tr>
</tbody>
</table>

All known negative sera showed IPRC values lower than 20 (cut-off) so the Specificity of CIVTEST SUIS PRRS A/S is 100%. All positive sera except one showed IRPC values ≥ 20 when tested. The Sensitivity of CIVTEST SUIS PRRS A/S is 97.2%. The results obtained with the three batches of the kit were qualitatively equal and so was the result obtained with the ELISA-I kit.
Discussion and Conclusion:

The results obtained with both kits for all sera except one were in agreement with those expected. For this serum, both kits scored negative probably because the serum sample was taken in the acute phase of the disease. The results obtained show 100% of agreement between both kits. CIVTEST® shows identical qualitative scores with all batches, therefore, the reproducibility of the kit has been proved satisfactory.

Figure I. Results obtained when testing the same serum samples with three different batches of CIVTEST SUIS PRRS A/S.
P 73 PROVED DETECTION OF SPECIFIC ANTIBODIES AGAINST VIRULENT GENOTYPE II STRAIN OF PRRS VIRUS WITH CIVTEST® SUIS PRRS A/S

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2 HIPRA Scientific, Girona, Amer, Spain

Introduction:
As widely known, there are two main genotypes of PRRS virus, which may circulate concurrently or either one of them prevail in a particular geographical area: genotype I and II.

The predominant genotype of PRRSV in the Asian pig-producing countries is type II. The highly pathogenic strain (HP-PRRS) that has been spreading in the Southeast region since it emerged in China in 2006, has been taken into account in this study.

In order to demonstrate the suitability of CIVTEST® SUIS PRRS A/S indirect ELISA kit to detect specific antibodies against HP-PRRS, serum samples were withdrawn at different time-points from piglets challenged with this strain. All samples were analysed with the kit.

Materials and Methods:
The tested sera belong to eight 3-week-old unvaccinated piglets that had been challenged with highly virulent genotype II strain. Blood samples were taken at days 0, 3, 7, 10, 14 and 21 post-infection and antibody level determined by using CIVTEST® SUIS PRRS A/S.

Results:
The results of IRPC obtained from each serum sample have been tabulated next:
Table 1.

<table>
<thead>
<tr>
<th>Pig ref</th>
<th>D0</th>
<th>D3</th>
<th>D7</th>
<th>D10</th>
<th>D14</th>
<th>D21</th>
</tr>
</thead>
<tbody>
<tr>
<td>311</td>
<td>0.4</td>
<td>-1.1</td>
<td>-0.7</td>
<td>5.2</td>
<td>44.7</td>
<td>†</td>
</tr>
<tr>
<td>321</td>
<td>9.8</td>
<td>2.3</td>
<td>0.1</td>
<td>5.2</td>
<td>46.1</td>
<td>104.0</td>
</tr>
<tr>
<td>328</td>
<td>0.7</td>
<td>0.3</td>
<td>12.8</td>
<td>116.1</td>
<td>215.9</td>
<td>†</td>
</tr>
<tr>
<td>333</td>
<td>3.3</td>
<td>-1.0</td>
<td>0.8</td>
<td>30.6</td>
<td>118.0</td>
<td>210.1</td>
</tr>
<tr>
<td>334</td>
<td>-0.6</td>
<td>2.1</td>
<td>4.6</td>
<td>50.7</td>
<td>165.9</td>
<td>204.5</td>
</tr>
<tr>
<td>336</td>
<td>4.2</td>
<td>0.5</td>
<td>10.0</td>
<td>82.6</td>
<td>129.9</td>
<td>214.2</td>
</tr>
<tr>
<td>340</td>
<td>0.4</td>
<td>-1.7</td>
<td>1.2</td>
<td>9.9</td>
<td>31.8</td>
<td>146.4</td>
</tr>
<tr>
<td>359</td>
<td>0.1</td>
<td>-1.2</td>
<td>3.1</td>
<td>19.0</td>
<td>138.6</td>
<td>224.9</td>
</tr>
</tbody>
</table>

Table 1: IRPC results obtained for each sample at the different time-points post infection. The cut-off value of the kit is ≥20 positive and <20 negative.

All the piglets included in the study were seronegative at the beginning of the study. 50% of the animals were seropositive at day 10 post-infection and all animals (100%) were seropositive at day 14 post-infection.
Discussion and Conclusion:
The results obtained were totally in agreement with those expected as the suitability of CIVTEST® SUIS PRRS A/S to detect specific antibodies against the Highly Pathogenic genotype II strain of Porcine Reproductive and Respiratory Syndrome Virus (HP-PRRS) was proved.
PS 01 - POSTER SESSION

P 75 STORAGE OF BOVINE EAR NOTCHES FOR GENETIC PURPOSES: EVALUATION OF THE DNA COLLECTED WITH DIFFERENT PROTOCOLS

I. Alexandre¹, P. Cuvelier¹, G. Fabien¹

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Introduction:
In 2017, ARSIA (Regional Association for Animal Identification and Health) considered storing DNA from ear notches sampled in the frame of the BVD program with the idea of creating a large DNA bank of the livestock in Wallonia (Southern Belgium). Several options of storage have been evaluated, taking into account parameters such as the quantity/quality of the collected DNA, the financial aspect and logistics/easy handling.

Materials and Methods:
Five storing/processing protocols were evaluated: (1) Storage of the unprocessed biopsies at -20 °C. (2) Allflex eluant in which the ear notches have incubated at -20 °C. (3) Lyzed ear notches à -20 °C. (4) Storage on blotting paper (Ahstrom-Munksjö GenSaver™ Color 2.0 96 spots) of the Allflex eluant in which the ear notches have incubated. (5) Lyzed earnotches on blotting paper. Quantity of the extracted DNA was assessed with Quantus (Promega). Quality was assessed through SNP Bovine Truseq profiling (Miseq, Illumina) and hybridisation on 10K illumina Beadship.

Results:
Mean DNA quantities (ng/µl) were respectively for each protocol: 87, 7.91, 5.52, 0.48 and 0.03. Mean call rates for hybridisation ranged from 98.5 % (protocol 1) to 94.80 % (protocol 4). Mean call rates for SNP profiling ranged from 96.0 % (protocol 2) to 48.0 % (protocol 4).

Discussion and Conclusion:
Quantity of DNA available for genetic analyses is strongly dependant on the system of storage. Genetic applications on the ear notches or the eluants stored at -20 °C showed the best results. Protocol 2 appears to be the cheapest whereas storage on blotting paper is the most easy handling and does not require energy source.
PS 01 - POSTER SESSION

P 76 BOVINE TUBERCULOSIS: COMBINATION OF TOOLS TO MAINTAIN FREE STATUS IN BELGIUM

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Introduction:
Since 2003, Belgium obtained the officially bovine tuberculosis (bTB) free status (Decision 2003/467/CE). However, surveillance remains important because almost every year one or more outbreaks are detected. In this context of very low bTB prevalence, the efficacy of skin test is less performant.

The aim of this study was to evaluate the performance of two tests: The IFN-γ assay and an ELISA for detection of antibodies against the M. tuberculosis complex.

Materials and Methods:
After stimulation of complete blood with PPD bovine and PPD avium, the IFN-γ level is measured using the ID Screen® Ruminant IFN-g (ID-vet, Montpellier, France). For the detection of antibodies against the M. tuberculosis complex, the IDEXX Mycobacterium bovis antibody kit (IDEXX, Westbrook, Maine) was used. Veterinary inspectors (Federal Agency for the Safety of the Food Chain) performed tuberculin skin tests.

Results:
Between 2016 and 2018, three positive farms were studied. In total 283 animals were tested with skin test and IFN-γ assay. The agreement between the two tests is 64% and increased till 78% when doubtful results in skin test were classified as positive. Seroprevalence in the three farms are respectively 7%, 20% and 6%. Overall, agreement between ELISA and skin test is 48%.

Discussion and Conclusion:
In conclusion, the IFN test showed the same ability to confirm bTB outbreaks as skin test. In combination with the ELISA, the two tests allowed the evaluation of the spread of infection in the positive farms.
P 77 MONITORING OF BHV-1 VACCINATED CALVES COMPARING AN INDIRECT AND A BLOCKING ELISA WITH THE NEUTRALIZATION ASSAY

O. Boix Mas¹, M. Baratelli¹, E. Coma Oliva², M. Blanch Freixa¹, S. Gascon Torrens¹

¹ HIPRA, Amer, Girona, Spain
² HIPRA Scientific, Amer, Girona, Spain

Introduction:
The aim of this study was to compare antibody response in vaccinated calves using an indirect ELISA (CIVTEST® BOVIS IBR), a blocking ELISA (CIVTEST® BOVIS IBRgB) and a neutralization assay (SN).

Materials and Methods:
Fifty four calves between 6 and 7 months of age were selected for the study. Forty four animals were divided into 3 groups (A, B and C) and vaccinated with 3 different BHV-1 live vaccines, and twelve (group D) were not vaccinated. All the animals were tested at 42 d.p.v. and 178 d.p.v. Comparisons between assays were performed using Spearman’s rho statistics.

Results:
The results from each of the techniques for each group are shown in Figure 1.

![Figure 1](image-url)

Figure 1. Quantitative results divided into four groups: blue, green, yellow and red corresponding to groups A, B, C and D.

Using Spearman’s rho statistics, indirect ELISA showed a very strong correlation and it was described by a linear function. Blocking ELISA showed a less strong correlation and it was described by a polynomial function (Figure 2).
Discussion and Conclusion:

In Figure 2, we can see that the titre of the indirect ELISA increased proportionally to the value of SN. In the case of blocking ELISA, it saturated at low positive values of SN, always showing the same titre and not offering a differentiation between low and high positives.

In conclusion, the ELISA kits tested can be divided into two categories: CIVTEST® BOVIS IBR, which is better at quantifying antibody levels; CIVTEST® BOVIS IBRgB, which is better at distinguishing positive animals. Depending on the purpose of your serological study, you should choose the better kit: indirect ELISA for quantifying antibodies and blocking ELISA for epidemiological studies.
PS 01 - POSTER SESSION

P 78 DESIGNS OF SPECIFIC RT-PCR FOR DETECTION VARIANT 2 GENOTYPE OF AVIAN INFECTIONS BRONCHITIS VIRUS

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¹ Faculty of Veterinary Medicine- University of Tehran-, Department of Microbiology & Immunology, Tehran, Islamic Republic of Iran
² Islamic Azad University- Faculty of Veterinary Medicine, Department of Clinical Sciences, Karaj- Alborz, Islamic Republic of Iran
³ Razi Vaccine and Serum Research and Institute, Research, Karaj, Islamic Republic of Iran
⁴ Iranian Veterinary Organization, Research, Tehran, Islamic Republic of Iran
⁵ University of Tehran- Faculty of Veterinary Medicine, Department of Microbiology and Immunology, Tehran, Islamic Republic of Iran

Introduction:
Infectious bronchitis (IB) is always involved in avian respiratory complex and is included in almost all cases. To detect this virus, several diagnostics such as serology and molecular assays are used. Molecular RT-PCR based tests are the best approaches to trace and detect the genome of the virus and are extensively used around the world. Poultry in the Middle East suffer from frequent respiratory disease outbreaks associated with IBV variant 2 strains, and recently variant 2 have been reported from Europe. Detection the variant 2 is so important for the lab without using the genotyping based on S1 gene.

Materials and Methods:
In this study after the bioinformatics analysis on the S1 gene, we designed the primers for detection of Variant 2 IBV. For checking the specificity of the primers, we run RT-PCR on different variant 2 positive controls and other IBV genotypes (Mass, 793/B, QX, Q1, D274) and some positive controls and also other avian RNA viruses (Avian Influenza, Newcastle Disease, Avian Penumo virus,...). For finding the sensitivity of the primers, we did RT-PCR on different concentrations of RNA.

Results:
Specificity results shows that the specific primers detect variant 2 and can detect the IBV genome in 0.1 ng/ul.

Discussion and Conclusion:
The results show this specific RT-PCR can be detected variant 2 IBV in mixed infections in the flock without using sequencing and.
P 79 VALIDATION OF THE USE OF POOLED SERUM SAMPLES FOR THE SEROLOGICAL DETECTION BY ELISA OF PRRS VIRUS

F. Smeets¹, C. Quinet¹, I. Alexandre¹, P. Gotalle¹, F. Gregoire¹
¹ ARSIA, Laboratory and Diagnostics, Ciney, Belgium

Introduction:
Monitoring of porcine reproductive and respiratory syndrome (PRRS) in negative finishing herds is usually performed by testing for the presence of antibodies against PRRS virus in individual serum with ELISA test. Pooled 5 sample testing can be a strategy to increase the power and reduce the cost. The objective of this study was to validate the pooled serum samples for detection of PPRS virus antibodies by ELISA.

Materials and Methods:
Samples of sera were collected in 2016 from Walloon farms and stored at -20 °C. An indirect ELISA test (Idexx PRRS X3 Ab Test, USA) was performed, using the positivity cut-off (0.4) prescribed by the supplier. Testing was first performed on individual samples (n=1315), then on the same samples pooled by 5, with minimum 1 pool per farm (total of 263 pools tested).

Results:
Results allow to assess a relative sensitivity for the ELISA PRRS on pools of 5 sera of 94.6 % (IC95 %: 91–98 %) and a relative specificity of 100 %. The calculated Cohen's kappa coefficient is 0.939. Eight pools were found negative despite the presence of one or 2 positive constitutive sera; the mean S/P value of these sera was 0.59 +/-0.19.

Discussion and Conclusion:
The pooling of sera for ELISA detection of SDRP virus antibodies resulted in our study in a slight loss of sensitivity, particularly for animals with very low antibody titres. This method should only be used for monitoring negative commercial farms (not for selection and multiplication breeding herds), provided that a regular monitoring is carried out.
PS 01 - POSTER SESSION

P 80  EUROPEAN VALIDATION OF A NEW ELISA FOR DETECTION OF BOVINE VIRAL DIARRHEA VIRUS ERNS ANTIGEN IN EAR NOTCHES

S. Guillossou¹, A. Kenworthy², J.K. Tena², W. Campbell², P. Meeus², A. Airault¹, E. Michel³, C. Lucas², C. Levesque Ninio³, E. Le Drean³

¹ Zoetis, Zoetis France SAS, Lyon, France
² Zoetis, Zoetis LLC, Kalamazoo- MI, USA
³ Labocea, Fougeres site, Fougeres, France

Introduction:
The objective of the study was to evaluate performances of a new ELISA for the detection of Bovine Viral Diarrhea Virus (BVDV) Erns antigen in bovine ear tissue samples and to demonstrate its suitability for PI detection and BVD control.

Materials and Methods:
A total of 219 ear notch samples from two collections which originated from France and Belgium were used to estimate diagnostic sensitivity (n=89) and specificity (n=130) in comparison to RT-PCR. Samples were selected to estimate inclusive (n=10) and exclusive (n=9) analytical specificity. One sample was serially diluted to assess the limit of detection (LOD). All tests were performed blindly at Labocea, France. Test kits evaluated were two ELISA (Kit A: SERELISA® BVDV Erns Ag Capture, Zoetis LLC; Kit B: IDEXX BVDV Ag/Serum Plus Test, IDEXX Laboratories Inc.). The extraction methods and assay protocols were performed per kit specific directional inserts.

Results:
With all samples combined, estimates of diagnostic specificity for both kits were 100 % (95 %CI:[98.1 – 100 %]). The estimate of diagnostic sensitivity for Kit A was 98.9 % (95 % CI:[94.9 – 99.9 %]) and for kit B was 100 % (95 % CI:[97.2 – 100 %]). Analytical specificities, inclusive and exclusive, were 100 %. No cross-reaction was observed. Final dilutions which repeatedly tested positive were 1/150 for kit A and 1/40 for kit B.

Discussion and Conclusion:
Using positive and negative ear notch samples sourced from France and Belgium, performances achieved with SERELISA BVDV Erns Ag Capture demonstrate the assay is suitable for PI detection and BVD control.

References:
We thank Dr C. Quinet, ARSIA, for access to the samples from Belgium.
P 81 OPTIMISATION AND VALIDATION OF A RT-QPCR DIAGNOSTIC TOOL FOR THE DETECTION OF ATYypical PORCINE PESTIVIRUS

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1 Moredun Research Institute, Diagnostic, Edinburgh, United Kingdom
2 Moredun Research Institute, Vaccines, Edinburgh, United Kingdom
3 Animal and Plant Health Agency, Pig Disease Surveillance, Bury St Edmunds, United Kingdom
4 Moredun Scientific, Animal Health, Edinburgh, United Kingdom
5 University of Edinburgh, The Royal Dick School of Veterinary Studies, Edinburgh, United Kingdom
6 Moredun Research Institute, Disease Control, Edinburgh, United Kingdom
7 The Roslin Institute, Infection and Immunity, Edinburgh, United Kingdom

Introduction:
A novel pestivirus, Atypical Porcine Pestivirus (APPV) has been associated with clinical cases of Congenital Tremors Type AII in piglets in Europe, Asia and North America. Diagnostic tools are needed for detection and quantification of the virus. Here we present the optimisation and validation of a RT-qPCR and use of the assay in an experimental APPV infection.

Materials and Methods:
A one-step TaqMan RT-qPCR detecting the NS3 gene of APPV was optimised. Specificity validation was performed by melt curve analysis, sequencing, and testing against other porcine pathogens and pestiviruses. Sensitivity was evaluated by determining the assay’s limit of detection and the effect of different matrices. The qPCR was employed to quantify APPV in an experimental infection in which Landrace piglets (n=3) were challenged with APPV-containing tissue homogenates.

Results:
The optimised RT-PCR assay amplified a single PCR product which sequencing confirmed as the NS3 gene of APPV, with a limit of detection of 3.2 x 10^3 gene copies/μl. No amplification of sequences from six porcine pathogens and four non-APPV pestiviruses was observed. The PCR detected APPV in serum, mucosal swabs and tissue homogenates. In APPV-challenged piglets peak serum viremia between 7 and 9 days post-infection (DPI). APPV was detected from 7 DPI in oropharyngeal and rectal swabs and 9 DPI in nasal swabs.

Discussion and Conclusion:
An optimised RT-qPCR was shown to have analytical sensitivity and specificity for APPV. This assay detected APPV in swabs and serum of APPV-infected piglets and will be useful in both research and clinical settings.
PS 01 - POSTER SESSION

P 82 VALIDATION OF REAL-TIME PCR ASSAY FOR THE DETECTION OF ECHINOCOCCUS CANADENSIS IN WOLF (CANIS LUPUS)

V. Hirvelä-Koski1, P. Heikkinen1, M. Isomursu1

1 Finnish Food Safety Authority Evira, Research and Laboratory Services Department, Oulu, Finland

Introduction:
Zoonotic parasite Echinococcus canadensis (a.k.a. E. granulosus G10) occurs in a sylvatic cycle involving wolf (Canis lupus), reindeer (Rangifer tarandus) and wild cervids. Wolf is the definitive host of this parasite in Finland. Isaksson et al. (2014) described a magnetic capture probe DNA extraction method and real time hydrolysis probe PCR assay (MC-PCR) for the detection of E. multilocularis in faecal samples of red fox (Vulpes vulpes). This assay was modified to detect E. canadensis DNA in faecal samples of wolves and validated this test against the golden standard method, sedimentation and counting technique.

Materials and Methods:
Intestines of 69 wolves were examined for Echinococcus canadensis worms using the sedimentation and counting technique. The faecal samples of these wolves were examined for the presence of E. canadensis DNA by modified MC-PCR.

Results:
Analytical sensitivity of MC-PCR was 3 eggs/3 ml. Diagnostic sensitivity was 76 % (95 % C.I. 56.5–89.7) and diagnostic specificity 95 % (95 % C.I. 83.1–99.4). The predictive values of the positive and negative result were 92 % and 84 %, respectively.

Discussion and Conclusion:
Sedimentation and counting technique is time-consuming but it is the most sensitive method for the detection of E.c. infection in wild canid populations. MC-PCR is a more cost effective method for large sample sizes with a possibility to sample live animals.

References:
PS 01 - POSTER SESSION

P 83 EPIDEMIOLOGICAL SURVEY OF ENTERIC VIRUSES OF MALLARDS (ANAS PLATYRHYNCHOS) IN THE CZECH REPUBLIC

R. Moutelíková1, J. Prodělalová1, K. Mikšová2, J. Kamler3

1 Veterinary Research Institute, Virology, Brno, Czechia
2 Masaryk University - Faculty of Science, Department of Experimental Biology, Brno, Czechia
3 Mendel University - Faculty of Forestry and Wood Technology, Department of Forest Protection and Wildlife Management, Brno, Czechia

Introduction:
Wild birds including mallard ducks are generally known as important reservoirs of many pathogenic viruses including economically important diseases, which can be spread to poultry.

Materials and Methods:
Gut content samples from 209 mallard ducks (approx. 10 individuals from each of 21 sampling places) obtained from August to September 2016 were screened for the presence of avian rotaviruses (RVA, RVD, RVF, and RVG), coronaviruses, Influenza A virus, and Anatid herpesvirus 1 (also known as duck plague virus or duck enteritis virus) by reverse transcription PCR/qPCR or PCR. Both RNA and DNA were extracted using TRI Reagent®. Positive results of PCR were confirmed by direct sequencing of obtained amplicons.

Results:
Within sample collection, only Influenza A virus was detected by one-step RT-qPCR in 4.8% of samples (n = 10/209). Positive samples were classified as low pathogenic avian influenza strains belonging to the subtypes H3, H4, and H6. Moreover, samples were tested for the presence of rotaviruses, coronaviruses, and Anatid herpesvirus 1 with negative results, although Anatid herpesvirus 1 has worldwide distribution in migratory waterfowl.

Discussion and Conclusion:
With the exception of avian influenza virus, the presence of other avian viruses in the Czech Republic has not been surveyed yet. As mallards are well known as natural reservoir of LPAI strains, obtained results are not surprising. However, there is continuous risk of spillover into domestic bird populations.

The study was supported by the Ministry of Agriculture of the Czech Republic (grants number QJ1630210 and RO0518).
P 84 CONTROLLING THE CONTROLS – EXPLORING THE RELATIONSHIP BETWEEN B-ACTIN AND PATHOGEN PCR RESULTS

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Introduction:
Beta-actin is a commonly used RT-PCR “housekeeper” control to evaluate sample quality/extraction and to prevent false negative results for the interest RT-PCR. This study examines the semi-quantitative relationship between the results of concurrent β-actin and Bovine Viral Diarrhoea RT-PCR’s.

Materials and Methods:
Blood (n=1631) and tissue (n=649) samples (national eradication) were tested using concurrent BVD (McGoldrick 1999) and B-actin RT-PCR tests (Toussaint 2007). To enable visualisation, results were banded together and various patterns/correlations tested. All statistics were performed using Excel 2010 (Microsoft, USA).

Results:
Figure 1 demonstrates for BVD blood samples that the simple correlation between B-actin and the interest pathogen (BVD) RT-PCR CT values is not strongly quantitative (r=-0.200) but it is relatively consistent & plausibly sequential. The coefficient of variations are 16.1, 10.0 and 116.4 for B-actin bands <25, 25-30 and 30-35 respectively. With higher levels of virus (lower BVD CT results) a greater proportion of samples score B-actin CT values between 30-35 than any other band. Only when BVD RT-PCRs are CT=25, could the corresponding B-actin PCR result also be a technical FAIL (5.4% of samples).

Discussion and Conclusion:
Internal controls are critical inclusions in all laboratory tests - particularly PCR. Every internal control should be carefully matched/monitored for fitness of purpose in context with the interest assay.
References:
**P 85 DEVELOPMENT OF THREE REAL-TIME RT-PCR KITS FOR SPECIFIC DETECTION OF AVIAN INFLUENZA VIRUS AND H5, H7, H9 TYPING**

**F. Pez**, A. Meunier, C. Pelletier, E. Sellal

1 BioSellal, R and D, Dardilly, France  
2 BioSellal, Direction, Dardilly, France

**Introduction:**
Avian influenza viruses type A (AIV) are endemic in wild birds and their introduction and conversion to highly pathogenic AIV in domestic farm birds is a cause of serious economic losses as well as a risk for potential transmission to humans. This emphasizes the need for rapid, reliable and effective differential diagnosis tools for AIV detection and furthermore for HA typing such as H5, H7 and H9.

**Materials and Methods:**
In this context, BioSellal decides to develop three ready-to-use real-time PCR kits:

<table>
<thead>
<tr>
<th>Name of the kit</th>
<th>Level of multiplexing</th>
<th>FAM</th>
<th>VIC</th>
<th>Cy5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-T kit® Avian &amp; Swine Influenza Virus</td>
<td>Triplex</td>
<td>Avian &amp; Swine Influenza Virus</td>
<td>Exogenous Internal positive Control (IC)</td>
<td>Endogenous Internal Positive Control (IPC)</td>
</tr>
<tr>
<td>Bio-T kit® AIV genotyping H5 &amp; H7</td>
<td></td>
<td>Avian Influenza Virus type H5</td>
<td>Avian Influenza Virus type H7</td>
<td>Endogenous Internal Positive Control (IPC)</td>
</tr>
<tr>
<td>Bio-T kit® AIV genotyping H9</td>
<td>Duplex</td>
<td>Avian Influenza Virus type H9</td>
<td>n/a</td>
<td>Endogenous internal positive Control (IC)</td>
</tr>
</tbody>
</table>

Validations of each Bio-T kit® were performed according to the specifications of the French National Reference Laboratory (NRL) for AIV (ANSES Ploufragan-Plouzané, France) which include the general guidelines defined by the French AFNOR standard NF U47-600-2. Sample type that could be used for analysis were tracheal, oropharyngeal, cloacal swabs and organs. The analysis could be performed individually or by pool up to 10, according to local regulations.

**Results:**
The results of the validation of the three Bio-T kits® were presented in the table below and compared to the AIV French reference methods for Bio-T kit® Avian & Swine Influenza Virus and Bio-T kit® AIV genotyping H5 & H7 or H9.
Discussion and Conclusion:
The Bio-T kit® Avian & Swine Influenza Virus, Bio-T kit® AIV genotyping H5 & H7 and Bio-T kit® AIV genotyping H9 are highly specific, sensitive and reproducible. Thus, these three effective tools allow the rapid and reliable diagnosis of AIV and the genotyping of H5, H7 and H9 strains.
P 86  DETECTION OF MULTIPLE VIRAL INFECTION IN DRONE EJACULATE USED FOR ARTIFICIAL INSEMINATION

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Introduction:
Instrumental insemination has been widely used in honeybee breeding programs. It represents primary method of controlling mating in the case there is no other option to isolate effectively breeding population. In general, instrumental insemination provides numerous benefits including the ability to store and ship semen.

Materials and Methods:
Between May and July 2016, nineteen pooled semen doses of ten drones per each apparently healthy colony prepared for use in artificial insemination were sampled. Nucleic acids were isolated from 3 μl of pooled ejaculate using Chemagic Viral DNA/RNA Kit (Perkin Elmer Chemagen, Germany). Ejaculates were examined by RT-PCR or PCR assays for the presence of the DWV, ABPV, BQCV and AmFV.

Results:
Pooled ejaculates collected in season 2016 in Czechia (n = 9), Austria (n = 2), Germany (n = 5), and Hungary (n = 3) showed 84.2% positivity for at least one of the tested viruses and multiple viral infection was found in 63% of tested samples (n = 12/19). The most prevalent AmFV was detected in 78.9% of samples. DWV was the second most abundant virus in the study (68.4%) and ABPV was present in 15.8% of samples.

Discussion and Conclusion:
The presence of co-infecting viruses in drone semen, which are common in honeybee colonies and known to be able to provoke overt infection, indicates that venereal way of transmission could be underestimated. Thus, the need of collected semen testing is obvious.

The study was supported by Ministry of Agriculture of the Czech Republic (grant number QJ1510113).
P 87  DEVELOPMENT AND VALIDATION OF THE METHOD OF THE FISH SPECIES IDENTIFICATION USING NEXT GENERATION SEQUENCING TECHNOLOGIES

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Introduction:
For the investigation of raw meat, fish and single-component products we developed the technique of the species identification based on Sanger sequencing in 2015.
This study aimed at the development of a technique based on the Next-generation sequencing of four fragments of the mitochondrial genome that allows to identify species of fish in multi-component products.

Materials and Methods:
DNA isolation by silica-based methods, PCR, DNA library preparation by Nextera XT DNA Library Prep Kit, NGS (MiSeq System, Illumina).

Results:
In this study was used amplicon resequencing approach. To amplify fragments of COI, 16SrRNA, cyt b and 12SrRNA genes of different species of fish the degenerate primers were designed. Primers to amplify cyt b and 12SrRNA genes were modified using the overhang adapter sequence according to 16S Metagenomic Sequencing Library Preparation Protocol. The optimal PCR conditions were selected.
For DNA library preparation Nextera XT protocol (for COI and 16SrRNA amplicons) and 16S Library Prep protocol (for cyt b and 12SrRNA) were applied. The database of sequences of the mitochondrial genome of 130 fish was created to mapping reads to the reference genome.
To assess the possibility of fish identification the control panel contained single- and multi-component samples, including artificially created mixtures, canned fish and feeds, was prepared.
In most cases valid results were obtained. Amplification of the short fragment of the 12SrRNA gene allows to identify a species when testing high-processed products.

Discussion and Conclusion:
The identification technique using NGS have been developed. It was found that the technique is appropriate for species identification of fish in multi-component products.
PS 01 - POSTER SESSION

P 88 HOW TO STANDARDIZE BVDV-BULK-MILK-TESTING

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Introduction:
Brucellosis, bovine enzootic leucosis and IBR are controlled serologically by bulk-milk-testing. The required sensitivity of tests is defined by a weak positive sample which has to be detected at a dilution reflecting the number of pooled lactating cows. A weak-positive standard and the minimum seroprevalence to be detected are not yet defined for BVDV.

Materials and Methods:
Paired blood/milk-samples were collected from all lactating cows in 7 herds (313 cows), in which BVDV was detected in 2017 (ear-notch-testing) and which were not yet vaccinated. Neutralisation test (NADL) and 4 commercially available ELISA were performed. The history of BVDV was assessed for each herd.

Results:
In 4 herds BVDV was detected only in 2017, seroprevalences (NT≥5) were 15 %, 30 %, 50 %, and 60 %. In 3 herds BVDV was detected for 2, 3 and 4 years, seroprevalences were 35 %, 60 %, and 95 %, respectively.

The distribution of NT-positive samples was analysed: Titers (CI95 %) for the 1st decile (D1), 1st quartile (Q1) and median (M) were 40(40-80), 113(93-113), and 160(160-226). The rates of samples which scored positive in milk-tests and test specificities are shown in the table.

Specificity and detection rate (%) of milk-samples from NT-positive animals in BVDV-ELISA A, B, C and D.

<table>
<thead>
<tr>
<th>NT-class</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;NT5*</td>
<td>98.8</td>
<td>98.5</td>
<td>96.3</td>
<td>96.2</td>
</tr>
<tr>
<td>D1 (&gt;40)</td>
<td>92.9</td>
<td>98.7</td>
<td>88.6</td>
<td>95.0</td>
</tr>
<tr>
<td>Q1 (&gt;113)</td>
<td>96.7</td>
<td>100.0</td>
<td>92.5</td>
<td>97.5</td>
</tr>
<tr>
<td>M (&gt;160)</td>
<td>95.9</td>
<td>100.0</td>
<td>97.9</td>
<td>97.9</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
BVDV-antibody tests are less sensitive compared to e.g. IBR-tests. Therefore milk samples from weak-positive animals are inappropriate to define the limits of detection. We are suggesting to define a positive reference sample by the distribution parameters of the neutralization titers in positive herds. Data of intra-herd seroprevalence will define the dilution to be detected positive.
PS 01 - POSTER SESSION

P 89  COMPARISON OF 3 DIFFERENT ELISA KITS FOR SCREENING OF NEOSPORA CANINUM IN CATTLE

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Introduction:
Neospora caninum is a protozoan parasite with worldwide distribution, recognized as major cause of spontaneous abortion in cattle. In Belgium, antibodies against Neospora are detected on >60 % of the herds and >10 % of individual animals. Moreover, antibodies are present in 20 % of cows with spontaneous abortions. A Neospora-seropositive cow has a threefold greater risk of abortion as compared to a Neospora-seronegative cow. The study objective was to compare three commercially available ELISA kits under routine laboratory conditions.

Materials and Methods:
44 serum samples were randomly selected from bovine samples submitted to the laboratory for routine analyses. A reference serum was also included. The samples were tested using three different Neospora ELISA tests. ELISA 3 was run with a short and long protocol. ELISA 1 served as ‘golden standard’ within the current laboratory diagnostics.

Results:
ELISA test 1 and 3 demonstrate a 100 % agreement, including the short protocol of ELISA 3. In ELISA 2, 2 samples were categorized, at least once, as ‘doubtful’, being positive in the other ELISA protocols. Looking at the result on negative and non-negative level (not all tests describe a doubtful zone), there is also a 100 % agreement for ELISA kit 2.

Discussion and Conclusion:
The comparison of 3 ELISA tests detecting antibodies against Neospora caninum resulted in 100 % agreement, as compared to ELISA 1 that was routinely used within the laboratory. Therefore, the 3 ELISA kits are practically interchangeably within the routine diagnostic testings for Neospora caninum.
P 90  COMPARISON OF REAL-TIME PCR KITS AND DIRECT LYsis BUFFERS FOR DETECTION OF BVD VIRAL RNA IN BOVINE EARNOTCHES

L. Allais1, E. Van Driessche1, G. Vanantwerpen1, M. Vanrobaeys1

1 Animal Health Care Flanders, Laboratory, Torhout, Belgium

Introduction:
Real-time PCR is a commonly used technique to detect the Bovine Viral Diarrhea Virus (BVDV) in bovine earnotches. A raw extraction method with direct lysis buffer is used to purify the RNA. Preceding the validation of a real-time PCR kit and lysis buffer for the BVD earnotch program, we performed a pre-validation to choose a real-time PCR kit and lysis buffer.

Materials and Methods:
We compared two Real-Time PCR kits (alphabetically): VetmaxTM BVD4ALL (LSI) and Virotype BVDV RT-PCR kit (Qiagen); and three direct lysis buffers (alphabetically): ID GeneTM EZNOTCH (IDvet), RealPCR Rapid Lysis Buffer (IDEXX) and Virotype Tissue Lysis Reagent (Qiagen). We performed tests on earnotches of different persistently infected (IPI) calves.

Results:
We examined the BVDV RNA level up to five days after extraction with direct lysis buffer. In addition, we compared storage at 4°C, -20°C and -80°C. Table 1 shows the comparison between PCR kit A and B, using three different lysis buffers. We observed that kit B detects lower levels of BVD RNA. Also, the BVD RNA degrades upon storage at 4°C. We performed the same test (Table 2) with PCR kit B and lysis buffer X and Y with samples of two different IPI calves, storing the samples at -20°C and -80°C. Up to five days, no RNA degradation occurs.
Discussion and Conclusion:
We conclude that the PCR kit B is more sensitive than kit A. The best storage temperatures for
earnotch extracts are -20°C or -80°C. The best results were obtained with PCR kit B combined
with the lysis buffer Y.

### Sample storage at 4°C

<table>
<thead>
<tr>
<th>PCR KIT A</th>
<th>BUFFER X</th>
<th>BUFFER Y</th>
<th>BUFFER Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 1</td>
<td>Day 0</td>
<td>Undetermined</td>
<td>32.44</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Undetermined</td>
<td>35.06</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>Undetermined</td>
<td>37.22</td>
</tr>
<tr>
<td>Calf 2</td>
<td>Day 0</td>
<td>35.78</td>
<td>38.91</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>35.01</td>
<td>37.79</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>37.32</td>
<td>30.89</td>
</tr>
<tr>
<td>Calf 3</td>
<td>Day 0</td>
<td>Undetermined</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Undetermined</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>Undetermined</td>
<td>/</td>
</tr>
<tr>
<td>Calf 5</td>
<td>Day 0</td>
<td>31.07</td>
<td>25.42</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>31.10</td>
<td>27.97</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>35.48</td>
<td>31.03</td>
</tr>
<tr>
<td>Calf 9</td>
<td>Day 0</td>
<td>Undetermined</td>
<td>29.23</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>41</td>
<td>31.88</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>35.62</td>
<td>31.78</td>
</tr>
</tbody>
</table>

### PCR KIT B

<table>
<thead>
<tr>
<th>BUFFER X</th>
<th>BUFFER Y</th>
<th>BUFFER Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 1</td>
<td>Day 0</td>
<td>35.98</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>36.94</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>38.47</td>
</tr>
<tr>
<td>Calf 2</td>
<td>Day 0</td>
<td>25.25</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>28.64</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>29.17</td>
</tr>
<tr>
<td>Calf 3</td>
<td>Day 0</td>
<td>35.06</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>38.86</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Calf 5</td>
<td>Day 0</td>
<td>25.15</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>28.89</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>29.67</td>
</tr>
<tr>
<td>Calf 9</td>
<td>Day 0</td>
<td>24.55</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>28.68</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>29.56</td>
</tr>
</tbody>
</table>

Table 1. Comparison PCR kit A versus B. / degraded sample. Day 0: PCR and extraction on the same
day. Day 1: PCR 24h after extraction, sample stored at 4°C. Day 2: PCR 48h after extraction, sample
stored at 4°C.

### STORAGE AT -20°C AND -80°C

<table>
<thead>
<tr>
<th>BUFFER Y</th>
<th>-20°C</th>
<th>-80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Earnotch 013</td>
<td>Day 0</td>
<td>23.35</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>22.91</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>22.92</td>
</tr>
<tr>
<td>Calf 5 Earnotch 063</td>
<td>Day 0</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>25.69</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>24.97</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>25.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BUFFER X</th>
<th>-20°C</th>
<th>-80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Earnotch 014</td>
<td>Day 0</td>
<td>27.72</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>28.36</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>28.19</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>28.72</td>
</tr>
<tr>
<td>Calf 5 Earnotch 065</td>
<td>Day 0</td>
<td>24.51</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>24.62</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>27.29</td>
</tr>
</tbody>
</table>

Table 2. Storage BUFFER X versus Y. Day 0: PCR and extraction on the same day. Day 1: PCR 24h after
extraction, sample stored at -20°C or -80°C. Day 2: PCR 48h after extraction, sample stored at -20°C or
-80°C. Day 5: PCR 144h after extraction, sample stored at -20°C or -80°C.
PS 01 - POSTER SESSION

P 91 NEW, HIGHLY EFFECTIVE METHOD FOR DETECTING NEMATODE EGGS (ASCARIS SPP., TOXOCARA SPP., TRICHURIS SPP.) IN DEHYDRATED SEWAGE SLUDGE

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Introduction:
The objective of this study was to estimate the effectiveness of the own method (OM) in detecting nematode eggs in sewage sludge samples containing flocculants.

Materials and Methods:
The study was performed on sewage sludge samples naturally contaminated (NC) or spiked (S) with eggs of Ascaris suum, Toxocara canis and Trichuris vulpis. The effectiveness of the OM was compared to 4 other methods by testing of NC samples. Next, method standardisation was performed using spiked sewage sludge samples.

Results:
The study demonstrated that OM efficiency was 6 to 65 times greater than other methods, depending on the method and type of detected eggs. Limit of detection (LOD) of OM were calculated as 10, 5 and 3 eggs/50 g of sample for A. suum, T. vulpis and T. canis eggs, respectively. The limits of quantification (LOQ) of the OM were established as 200 eggs/50 g of sample for A. suum and T. vulpis eggs and 50 eggs/50 g of sample for T. canis eggs. The rectilinear regression functions, which determined the relationship between the number of eggs detected in OM measurements and the number of eggs contained in the samples, were characterised by high and statistically significant coefficients of determination (r²). The slopes of the trend lines were 0.3188, 0.3821 and 0.3276, and the intercepts were -11.223, -9.0261 and -23.15 for A. suum, T. canis and T. vulpis eggs, respectively.

Discussion and Conclusion:
The study confirmed that the OM may be applied to quantify parasite eggs in dehydrated sewage sludge containing polyelectrolytes.
P 92 THE USE OF DIGITAL COLORIMETRIC ANALYSIS FOR VIABILITY ASSESSMENT OF ASCARIS SUUM EGGS

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Introduction:
The aim of the study was to develop a method for the colorimetric evaluation of nematode eggs using appropriate instruments, which would eliminate the stages based on subjective assessments by analysts.

Materials and Methods:
The materials for the study were live and dead (inactivated) eggs of the Ascaris suum. Viability of the eggs was assessed using four different kits for fluorescent staining (for each technique, a series of photos were taken). Images of stained eggs were analysed using graphic software with RGB (red-green-blue) function. The viability of the eggs was assessed according to the relative positions of the distributions of colour intensities of live or dead eggs - distributions area’s overlap index (DAOI), and distributions area’s separation index (DASI) were calculated.

Results:
Computer analysis of the intensity of green colour was not satisfactory. However, analysis of images in the spectrum of red colour proved useful for the effective differentiation between live or dead eggs. The best parameters were observed using the Annexin V FITC Apoptosis Detection Kit (DASI = 41 and 67).

Discussion and Conclusion:
The investigation confirmed the usefulness of fluorescent dyes used in conjunction with digital analysis for the assessment of the viability of A. suum eggs. The use of computer software allowed a better objectivity of the assessment, especially in the case of doubtful staining.
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