ID-01

The impact of bovine viral diarrhea virus infection on milk production of Dutch dairy herds

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Objectives: Bovine viral diarrhea virus (BVDV) is endemic in many countries and can have a major impact on cattle health. BVDV can among others cause diarrhea, fever, pneumonia, growth retardation, immunosuppression, and reproductive disorders, thereby reducing milk production and consequently causing economic losses. Research on the effect of BVDV on milk production is relatively outdated and hardly investigates changes in milk production before and after a new BVDV infection in the herd. Moreover, it is unclear whether the impact of BVDV changes when an increased proportion of farms participates in a BVDV surveillance program. The objectives of this study are to determine the loss in milk production as a result of a new BVDV infection in dairy herds participating in the Dutch BVDV-free program between 2007 and 2017.

Material & Methods: Longitudinal herd-level BVDV surveillance data of 4,334 dairy herds participating in the BVDV-free program were combined with monthly test-day milk production data on herd level from 2007-2017. This combined dataset consisted of 3,126 herds, of which 2,486 herds were BVDV-free during the whole study period and were defined as free-herds. 640 herds lost their BVDV-free status at a certain moment during the study period and were defined as breakdown-herds. To estimate the impact of BVDV infection within breakdown-herd, milk production before and after the infection was compared. Since milk production fluctuates over time, the milk production data of the free-herds was used to correct for that. A linear mixed regression model was used to estimate changes in milk production before and after BVDV infection on an annual and quarterly basis. The model included the fixed variables BVD status, breakdown-herd or free herd, year, season, and a random herd-effect that takes the repeated measurements within the herd into account. The dependent variable was the average daily milk production on the test-day. In our analyses, we assumed that the risk period for milk production loss began with the BVDV infection. The moment of BVDV infection is difficult to determine accurately. Therefore, four scenarios were developed to reflect different possible moments of BVDV infection. In the default scenario, the breakdown date was assumed to be the start of BVDV infection, and the years/quarters after the breakdown date were included as a risk period for milk production loss. Three additional scenarios were studied in which it was assumed that the breakdown is caused by the birth of a persistently infected (PI) calf, being born 4, 6 or 9 months before the breakdown date. In these scenarios, the risk period for milk production loss started when the PI calf was born.

Results: Results for the default scenario showed that in the first year after the breakdown date, the average milk production loss per cow per day was 0.04 kg. In the first to fourth quarters of the first year after infection, the average milk production loss per cow per day were 0.13, 0.08, 0.01 and 0.07 kg, respectively. Overall, in the first year after BVDV infection of the default scenario, milk production losses ranged from 570 to 1,603 kg at a Dutch herd level, with an average of 1,086 kg per herd per year or 13 kg per cow per year. In the results of scenarios 1, 2 and 3, the negative effects of BVDV introduction were mainly in the second year after BVDV introduction, and the average milk production decreased by 0.05, 0.06, 0.05 kg/cow/day, respectively. Results show that the risk period for milk production loss due to BVDV introduction is mostly in the first two years after BVDV introduction, especially in the first quarter.

Conclusion: This study shows that the BVDV infection occurring in herds in a BVDV-free surveillance program has a limited but negative impact on milk production of dairy herds, mainly in the first year after infection. The main reason why a new introduction of BVDV caused such a small loss of milk production on dairy farms is probably due to the participation in the BVDV-free program. This BVD surveillance program enables participating farmers to identify and remove the PI animals very quickly after the infection, which may limit extensive transmission of the virus to the lactating herd and avoid large milk production losses.

Keywords: Bovine viral diarrhea, dairy, milk production.

ID-02

Case reports - *Mannheimia haemolytica* in dairy cows

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Objectives: *Mannheimia* (*M.*) *haemolytica* is one of the most important pathogens of respiratory disease in young cattle (calves and feedlot cattle). In recent years, however, cases of severe pneumonia caused by *M. haemolytica* in lactating dairy cows, have been reported. By means of two clinical cases in Germany, the wide range of clinical symptoms and diagnostic as well as treatment options are discussed to raise the awareness of this disease in dairy cows.

Material and Methods: The first case: an increased number of deaths occurred on a dairy farm with 500 lactating cows in Mecklenburg-Western Pomerania. When the veterinarian was consulted, five animals had already died, and more than 15 cows showed severe fever and an increased respiratory rate. Cows of all ages and in different stages of lactation were affected.

As a new silo had been opened preceding illness, Clostridia infection was suspected. Samples were taken from the affected animals for further diagnosis. One moribund cow was euthanized and sent to the state laboratory for postmortem examination.

The second case: the course of disease was more prolonged on a dairy farm with 110 lactating cows. One cow showed unspecific symptoms such as drop in milk in produc-

tion, anorexia and a slightly elevated body temperature. As metal particles were found by the metal detector of the feed mixer acute traumatic reticuloperitonitis was diagnosed. In the following days, 9 other cows fell ill with similar symptoms One of them developed severe disease (recumbency, bloody nasal discharge) and was euthanized for postmortem examination. On inquiry, the farmer said that three new heifers had entered the farm 12 days before.

Results: In both cases severe acute pneumonia was diagnosed on autopsy. On bacteriological testing, *M. haemolytica* was detected in lung tissue of both cows. *M. haemolytica* Serotype A1 was also found in other organs of the cow from case 1 and in nasal swabs and as well as in lung fluid taken via transtracheal aspiration (TTA) taken from diseased cows. Nasal swabs taken from diseased cows on farm 2 were also positive for *M. haemolytica*.

On farm two, paired serum samples were taken from all diseased cows and antibodies against BRSV, PI3-V, M*ycoplasma bovis* and *M. haemolytica* were measured. Only the titers of *M. haemolytica* antibodies showed an increase over time and, in six of ten animals, this increase could be valued as seroconversion.

Diseased animals were treated with antibiotics and nonsteroidal anti-inflammatory drugs (NSAID) after *M. haemolytica* was confirmed as infective agent and all cows responded well to this treatment. On farm 1, clinically healthy animals were vaccinated with a trivalent vaccine (Bovilis® Bovipast® RSP, MSD Animal Health). A few days after the first vaccination, the disease was already beginning to subside. After the second vaccination 4 weeks later, no further disease or death were observed in the dairy cows.

Conclusion: Both cases show that *M. haemolytica* can infect dairy cows and cause severe economic losses due to substantial drop in milk production or even sudden death of diseased animals.

Clinical symptoms may vary considerably and make it difficult to make the right diagnosis. To confirm an infection with *M. haemolytica*, either detection of bacteria in samples from the respiratory tract or proven seroconversion shown by an increase in antibodies can be used.

Vaccination can protect a herd, especially if new animals are entering the farm regularly.

Keywords: Mannheimia haemolytica, dairy cows, serology, vaccination.

ID-03

Different etiological agent associations detected in bovine respiratory disease (BRD) outbreaks in unweaned and fattening calves

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Objective: Bovine Respiratory Disease (BRD) is a leading cause of economic loss, hampering animal welfare and intensive antimicrobial use in cattle operations. BRD is a multifactorial infectious disease that usually affects a group of animals and it is caused by a complex interaction between different viral and bacterial pathogens in single or mixed infections, the immune status of the host and environmental and management factors. The aim of this study was to investigate the frequency of detection of the main etiological agents involved in BRD and their association with BRD outbreaks.

Material and methods: One hundred fifty six (n= 156) outbreaks of BRD that were sent for diagnosis at the Laboratory of diagnosis EXOPOL between January 2020 and November 2021 were included in the study. Inclusion criteria were geographical localization of the outbreaks in Spain and complete test results for the detection of Parainfluenza 3 virus (PI-3), Bovine respiratory syncytial virus (BRSV), Bovine coronavirus (BCov), Bovine viral diarrhea virus (BVDV) and Bovine herpesvirus 1 (BoH-1v), and the bacteria *Mannheimia haemolytica (Mh)*, *Pasteurella multocida (Pm)*, *Histophilus somni (Hs)* and *Mycoplasma bovis (Mb)*. Diagnosis was performed in pools of 5 samples from different animals of the same outbreak by quantitative PCRs (qPCRs). Clinical specimens were bronchoalveolar lavages (n= 96), lung (n= 35), nasopharyngeal swabs (n= 8) and tracheal scrapes (n= 2) or mixtures of them (n= 15). Outbreaks were from unweaned (n=32), fattening (n= 107), replacement (n=8) and adult (n= 1) animals or unknown (n= 8).

A cluster analysis of categorical variables was performed using the hierarchical clustering method. The Boolean variables (presence/absence) included as active variables to define clusters were detection of PI-3, BRSV, BCov, BVDV, BoH-1v, *Mh, Pm, Hs* and *Mb*. Associations between cluster and categorical variables were analyzed using the chi-square test and significance level set at $P < 0.05$.

Results: The most frequently detected virus were BCov (39.7%), followed by PI3 (26.3%), BRSV (19.9%) and BVDV (17.7%) and the less frequent BoH-1v (3.2 %). The most frequently detected bacteria were *Pm* (85.9%), followed by *Mb* (77.6%), *Mh* (64.1%) and *Hs* (42.3%). Cluster analysis grouped outbreaks into two clusters. Cluster 1 included outbreaks were the detection of the PI3 (4.7 %), BRSV (4.7 %), BCov (24.5 %) and BVDV (6.6 %) viruses and *Mb* (72.6 %) was significantly lower and detection of *Hs* (51.9 %) was significantly higher than in the total of outbreaks. Opposite, cluster 2 included outbreaks were the detection of the PI3 (72.0%), BRSV (52.0 %), BCov (72.0 %) and BVDV (40.0 %) viruses and *Mb* (88.0 %) was significantly higher, and detection of *Hs* (22.0 %) was significantly lower than in the total of outbreaks. In cluster 1 and cluster 2, no statistically significant differences were observed in the frequencies of detection of *Pm* (84.0 and 90.0 %, respectively) and *Mh* (60.4 and 72.0%, respectively). It was found an association between both clusters and the productive stage of the animals ($P<0.05$). Thus, the frequency of outbreaks from fattening animals were significantly higher (OR

3.5; CI95% (1.5-7.8) in cluster 1 than cluster 2 (78.8% versus 59.2%, respectively), whereas the frequency of outbreaks from unweaned animals was significantly lower in cluster 1 than cluster 2 (14.1 versus 36.7%, respectively). There was no association between clusters and the geographic area of outbreaks.

Conclusion: These results suggest that in unweaned animals the respiratory viruses or *Mb* would have a greater clinical significance playing a role either as responsible for clinical processes or cooperating as predisposing agents for bacterial infections. On the contrary, in fattening animals, other predisposing factors such as stress associated with transport of animals or mixing of animals of different origins in feedlots would have a greater relevance. Consequently, the management and sanitary measures carried out for an efficient control of BRD in calves should be adapted to the different productive stages. In this regard, possible measures to consider could be the vaccination of unweaned animals against respiratory viruses or the incorporation of *Pm*, together with *Mh*, to the vaccines used in cattle which could help to substantially reduce the incidence of BRD.

Keywords: Bovine respiratory disease, outbreaks, cattle, BRD asociated infectious agents.

ID-04

Four years of mandatory BoHV1 (IBR) control programme in dairy herds in the Netherlands

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Objectives: Bovine herpesvirus-1 (BoHV1), the causative agent of Infectious Bovine Rhinotracheitis (IBR) was first reported in the Netherlands in 1973. In 1997, a national eradication programme was initiated, with at the time an estimated nationwide herd prevalence of 84%. Only the use of gE-deleted markervaccines was permitted enabling testing for antibodies against wildtype BoHV1 with gE-ELISA.

In 1999, the national programme was abruptly suspended when a BVDV2-contaminated batch of IBR-vaccine resulted in severe clinical illness and death. After this disappointing end, the IBR-certification programme was continued voluntary for almost two decades. In 2015, when discussion on a new national programme for IBR-eradication started, 43% of dairy herds participated in IBR-certification routes (28% IBR-free, 15% IBR-unsuspected). The herd prevalence in dairy herds had decreased to 15.6% by that time.

In April 2018, a mandatory IBR-control programme only for dairy herds was introduced by the dairy industry and is carried out by Royal GD. The goal is to stimulate more herds to become IBR-free, to keep them IBR-free and mitigate the risk posed by infected herds through vaccination. Non-dairy cattle herds can voluntarily participate. The objective is to describe the progress of IBR control.

Materials & methods: Dairy herds are obliged to participate in one of the three major routes that can lead to a BoHV1-free herd status:

- IBR-free certification
- IBR-unsuspected certification
- IBR-vaccination certification

IBR-free certification starts with individual serum gE-antibodies screening of the herd, subsequent monitoring of the free status is performed by monthly bulk milk IBRgE testing.

IBR-unsuspected certification starts with a negative bulk milk gE-antibodies screening, subsequent monitoring of the unsuspected status is performed by monthly bulk milk IBRgE testing. After at least two years of IBR-unsuspected status, herds can qualify for an IBR-free status by individual serum gE-antibodies screening of cows older than 6 years.

IBR-vaccination certification is granted after the first whole herd vaccination with gE-deleted markervaccine (all cattle over 3 months of age) and is prolonged when the herd is vaccinated every six months thereafter. This route is mandatory for infected herds (positive bulk milk gE-antibodies screening).

In addition to the monthly bulk milk surveillance, other risk-based monitoring tools are applied. After purchase, cows from non-free herds are automatically noted in IBR-free and IBR-unsuspected herds and require mandatory testing for gE-antibodies. All cows that have aborted are mandatorily tested for gE-antibodies. In both cases, when cows test positive they need to be culled. Furthermore, when clinical signs of IBR are noticed in IBR-free or IBR-unsuspected herds it is mandatory to submit nasal swabs for PCR testing on IBR-virus.

The progress of the nationwide IBR-control programme is monitored by combining diagnostic test results with cattle movement data. Semi-annual analyses of the following key figures is performed: (1) the percentage of IBR-free, (2) IBR-unsuspected and (3) IBR-vaccinated herds, (4) incidence of new IBR-infections detected by bulk milk screening and (5) virus detection in nasal swabs.

Results: The percentage of dairy herds with a favourable IBR-situation almost doubled since the start in 2018. At the end of 2021, 55% of dairy herds were certified IBR-free, 27% IBR-unsuspected and 18% IBR-vaccinated. After four years of new IBR-regulation, the incidence of new IBR-infections detected by bulk milk screening (often subclinical outbreaks) decreased from 0.53% in 2018 to 0.26% in 2021. Annually, nasal swabs from around 200 herds are submitted for PCR testing and in 2018 BoHV1 was detected in 12.9% of herds. This figure dropped to 6.6% in 2021. Since the start of the new mandatory phase and up to the end of 2021, in total 109 IBR-free dairy herds had an outbreak (out of on average 15.304 dairy herds).

Amongst non-dairy herds, 22% is certified IBR-free on a voluntary basis. Even without mandatory control in these herds, the herd prevalence in non-dairy herds declined over the period 2016 to 2020.

Conclusion: Even though the Dutch IBR-programme is only mandatory for dairy herds, much progress is made in the

Dutch cattle sector. National implementation of IBR-eradication for all bovine herds by the government is discussed by the cattle industry and the Ministry of Agriculture. With regular monitoring, thorough and timely insight on the progress of IBR-eradication is obtained and evidence-based decisions can be made for further developments in the eradication programme.

Keywords: BoHV1; IBR; eradication; gE-antibodies; bulk milk.

ID-05

A synergy between influenza D virus and *Mycoplasma bovis* in bovine respiratory disease

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Objectives: Since its discovery in 2011 in the United States, the novel influenza D virus (IDV) of the *Orthomyxoviridae* family was found spread among swine and ruminants on four continents so far, confirming a worldwide distribution. Cattle were suggested to be the main host. Experimental infections in naïve calves showed that IDV infects both the upper (URT) and lower (LRT) respiratory tracts, with a moderate pathogenicity and a high level of transmission (Salem et al. 2019). In addition, IDV detection during bovine respiratory disease (BRD) field outbreaks suggests that this virus can be considered at least as predisposing or co-factor of BRD (Mitra et al., 2016). To study the influence of IDV on other respiratory pathogens and to confirm its role as a co-factor of BRD, we performed experimental co-infections of calves with both IDV and *Mycoplasma bovis* (*M.bovis*). A frequent association between IDV and *M.bovis* was indeed found in 42% of animals with respiratory signs in veal calf units in France.

Material & Methods: Experimentation was performed under EEC guidelines (86/609/CEE) and official French ethical agreement. Twenty-nine calves (free of IDV and classical respiratory pathogens) were distributed into four separate pens. In three pens, 8 calves per group were intranasally nebulized at day 0 (D0) through a mask, with 10^7 $TCID_{50}$ per calf with a French IDV strain D/bovine/France/5920/2014, with 10^{10} colony forming unit per calf of the French *M.bovis* strain RM16, with both pathogens, respectively. Five non infected calves made up the fourth group. All calves were examined from 3 days before challenge (D-3) to the end of experimentation (D21) for clinical signs, gross and microscopic lesions (D6 for 3 infected calves per group and D21). IDV and *M.bovis* quantification was measured by qRT-PCR and qPCR in both URT (nasal swabs (NS) from D1 to D21) and LRT (bronchoalveolar lavages (BAL) at D-1, D2, D7, D14 and D21) and tissues (nasal turbinate, trachea and lung at D6 and D21). Gross lesions, histopathology, cell identification and counting from BALs and blood were investigated to assess pathology. The immune response was assessed for antibody seroconversion (IHA, ELISA) and cellular responses.

Results: Clinical examination confirmed that IDV induced a mild respiratory disease between D5 and D10 characterized by coughing, tachypnea, and dyspnea. Calves infected by *M. bovis* started showing moderate clinical signs later, from D7-D8 to D19. These calves mainly showed tachypnea, muco-purulent discharge, a more frequent and bad cough and sometimes labored breathing with abnormal lung sounds (wheezing). In the co-infected group, clinical signs were similar to those of the *M. bovis* group except that they occured earlier, starting fom D4, and were more severe. The mean clinical scores indicated significant differences between the co-infected groups and other groups from D6 to D8. The severity of the clinical manifestations, especially in the co-infected group, was correlated with severe gross and microscopic lesions in respiratory tissues (nasal turbinates, trachea and lungs), mainly characterized by loss of ciliature, necrosis of the respiratory epithelium, and mononuclear cells and neutrophils infiltrations in the lungs and BALs. No statistical differences were observed between groups for IDV replication in URT or LRT, except that IDV replication was longer in the monoinfected group. On the other hand the earlier replication of *M. bovis* in NS and respiratory tissues of the co-infected group correlated with the clinical differences observed between co-and mono *M.bovis* infected groups. After D10, only *M.bovis* was detected in NS, BALs and tissues in co-infected group while IDV was observed in NS, BALs and lungs in the IDV group until D21. Finally calves seroconverted against IDV as early as at D7 for IDV mono and co-infected groups while antibody response against *M.bovis* occured later. First results of cellular immune response examination indicate that IDV modulates the innate immunity against *M. bovis,* leading to more severe pathology.

Conclusion: Altogether, these results suggest a synergy between IDV and *M. bovis* respiratory infections in young calves with implications for the control of respiratory diseases. Indeed, IDV increases the severity of *M.bovis* infection leading to severe respiratory signs. First results of cellular immune response examination indicate that IDV modulate the immune response against *M. bovis* and confirm that IDV acts as an initiator pathogen for BRD.

Keywords: Bovine-Influenza D-Mycoplasma bovis-respiratory-BRD.

ID-06

Cross perceptions of farmers, veterinarians and physicians on Q fever: "one health" approach

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Objectives: The objective of this study was to describe the knowledge and assess the perceptions, in a cross-sectional manner, of ruminant farmers, rural vet practitioners and physicians practising in rural areas. Under a "one-health" approach, the goal was to identify, if necessary, areas for improvement in terms of awareness of the disease and exchanges between these professions.

Materials and Methods: To this end, an opinion poll was conducted with the help of an independent polling institute (Via Voice). Hundred physicians and 100 veterinarians practising in areas < 20,000 inhabitants (rural areas) and 374 farmers geographically matched with both vets and physicians (respectively 134 sheep farmers, 100 goat farmers and 198 cattle farmers) were interviewed. All participants were randomly selected in the selected areas. The questionnaire was built around 3 main parts: (i) basic knowledge about Q fever in humans and ruminants, (ii) perception of the risk (including zoonotic risk) and (iii) knowledge about control measures both in humans and animals.

Results: Among farmers, 76% declared knowing the disease with difference depending on the species: 64% for cattle (57% for beef farmers and 76% for dairy farmers), 84% for sheep and 90% for goat farmers. For the rest of the survey, only slight differences were observed between goat, sheep and cattle farmers leading us to gather their results. The following table displays some (not exhaustive) important results. Abortion was the main clinical sign attributable to Q fever (45% for farmers and 70% for vets). Reproductive disorders were mentioned only by 7% of cattle farmers and 19% of vets. More than 29% of cattle farmers and 32% of small ruminants farmers and 21% of vets did not know about the clinical signs of Q fever highlighting the need to promote knowledge about the disease, particularly the negative impact on reproduction besides abortion. For physicians, the clinical signs the most frequently reported as leading to suspect Q fever was fever (67%) followed by joint pain (29%), pneumonia (24%) and fatigue syndrome (22%). Among all zoonotic diseases offered to the respondents, Q fever was ranked among the most at risk one as 2nd, 4th and 5th respectively for farmers, vets and physician in line with low perception for the zoonotic risk (despite recent and regular human outbreaks). However, awareness among farmers is higher among those who receive the public. For vets, the perception of farmers of the impact for their herd or their health was considered as non-sufficient in 62 and 80% respectively. Regarding the farmer's knowledge about prevention measures for their animals or their own health, vets considered it as non-sufficient in 77 and 82% respectively. Among barriers to tackle the disease, vets stipulated the lack of abortion reporting by farmer (65%), the funding's of ancillary test to confirm the disease (70%) and the choice of animals to be sampled (40%). Among farmers experiencing Q fever 53% did not implemented control measures. For those who implemented control measures vaccination was implemented in only 25% of the cases.

Table. Some examples of answers to the opinion survey.

Questions	Farmers (% respondants)	Vets (% respondants)	Physicians (% respondants)
I have been exposed to Q fever in my work	12%	49%	25%
I know this disease is zoonotic	65%	66%	83%
Introduction of Q fever is at risk for animal and/or human health	10%	30%	27%
Isolation of affected animals when Q fever is detected	55%	54%	35%
Vaccination considered as control action	31%	35%	14%

Conclusion: All together, these results support a very heterogeneous level of knowledge about the disease in any population. The perception of the risk, particularly the zoonotic one, also appears to be fairly low. The implementation of appropriate control measures needs to be improved, as does the dialogue between the breeder-veterinarian-doctor trio

Keywords: Q fever; Perception survey, Zoonosis, One health.

ID-07

Tracing the spread of bovine respiratory syncytial virus (BRSV) between herds

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Objectives: Bovine respiratory syncytial virus (BRSV) causes respiratory disease in cattle and has been diagnosed in 12% to 83% of respiratory disease outbreaks in Europe [1,2]. In the north of Scandinavia, the control of BRSV relies on biosecurity rather than on vaccination despite that the epi-

demiological cycle and the modes of transmission of this virus are poorly understood. The objective of this study was to deepen this knowledge, at the national, regional and between-herd level, with the final goal to improve the control of BRSV.

Material and methods: Upsurges of outbreaks of respiratory disease occurred in 2016 and 2020, and whole BRSV-genome sequences were obtained from 54 cattle in 34 herds throughout Sweden. Partial genome sequences were additionally generated from the G-gene. In 2020, clusters of outbreaks were identified in the three counties: Dalarna, Uppland, and Jämtland, from which complete sequences were obtained from 5/5, 7/9 and 15/25 herds with diagnosed outbreaks. In three of the herds in Uppland, whole-genome data was additionally generated from BRSV collected in 2016. Phylogenetic analyses were performed to determine genetic relationships between different circulating viruses. Possible routes of introduction 3 to 4 weeks before the report of the outbreak were investigated, such as purchase or contact with animals from other holdings, visits, interactions with animal professionals, as well as contact with animal transport vehicles. Based on epidemiological and phylogenetic information, an attempt was made to identify how BRSV was introduced in some of the farms. One to two months after the last detected outbreak, bulk tank milk was collected from 30 herds in Jämtland, 23 of which without recent history of respiratory disease. This milk was analysed for BRSV-specific IgG1.

Results: Despite that BRSV is an RNA virus, few genomic changes were detected within counties. Nevertheless, there was a spatial clustering of virus genome sequences between counties, with rare introductions from one county into another. The viruses obtained in Uppland 2016 were more closely related to some of the viruses from the same region in 2020 than to viruses from other regions, indicating that the virus had remained in the area. Viruses collected in one herd in 2016 and 2020 had only 15 mutations difference, whereas viruses collected in different counties in 2020 differed with approximately ten times more changes. This data suggests that when a different lineage is detected in an area, it is the result of an introduction from another area rather than due to the rapid genetic evolution of strains.

BRSV-specific antibodies were detected in bulk tank milk from 18/30 herds in Jämtland after the wave of outbreaks in 2020. Based on sequencing data and epidemiological investigations, human, animal, material or transport contacts were suspected to be the origin of transmission of BRSV between some of the herds. Such contacts occurred 10-26 days before the first observed clinical signs.

Conclusions: The stability of the BRSV genome impedes the tracing of this virus. Nevertheless, the epidemiological information and preliminary phylogenetic data suggested that BRSV is introduced 2-3 weeks before outbreaks are reported, possibly sometimes by professionals or transports in contact with cattle on the recipient farms. The Swedish context is characterised by high biosecurity measures and scarce animal exchanges. These findings can be used as the basis to design and implement effective biosecurity recommendations to stop the spread of BRSV during an epidemic.

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Keywords: Bovine respiratory syncytial virus, transmission, tracing, sequencing, epidemiological investigation.

ID-08

Prevalence, biosecurity and risk management of bovine coronavirus infections on dairy farms in Europe

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Objectives: The objective of this cross-sectional field study is to obtain an estimate for the farm prevalence of Bovine Coronavirus (BCoV) in dairy production in Europe, and to characterize farm-level risk factors in management and biosecurity that are linked to BCoV infection in neonatal and weaned dairy calves.

Material & Methods: A convenience sample of 130 European Union (EU) dairy farms with at least 100 lactating cows each were enrolled in this study. The farm enrolment was based upon the country's relative magnitude of the national dairy production. Nasal and f aecal swabs were collected for BCoV detection, blood and bulk tank milk samples were collected for specific BCoV antibody detection. Depending on the farm size, 10-20 samples from neonatal calves under 3 weeks of age, 10-20 samples from the most recently weaned calves and 5-10 samples from fresh cows, and one bulk tank milk sample were collected. All samples were shipped and analysed at the same laboratory. BCoV presence was determined in nasal and faecal swabs using semi-quantitative Real Time PCR (RT-PCR). Bulk tank milk and serum samples were tested for the presence of BCoV antibodies using ELISA. On each farm an extensive questionnaire was performed to determine various husbandry (i.a. vaccination of mother cows against bovine coronavirus) and biosecurity management factors. The Biocheck survey (https://biocheck.ugent.be, Ugent, Belgium) was used to score the biosecurity system. Correlations between the results from testing of nasal and faecal samples, as well as blood and bulk tank milk samples were determined. Prevalence estimates for samples within farms and countries were calculated. Multivariable analysis was used with dichotomous outcomes (logistic models) or ordinal outcomes (cumulative logistic or linear models) to determine risks for the presence of BCoV and BCoV antibodies in neonatal calves, weaned calves, fresh cows, and bulk tank milk. Random effect models were used where appropriate.

Results: The study is ongoing. To date we have partial or complete results from 45 dairy farms in Belgium, Czech

Republic, Denmark, France, Italy, Netherlands, Portugal, and Sweden. Some preliminary results and data analysis are presented in this abstract, pending further enrolment. Antibody levels in bulk milk samples, measured as % inhibition, were on average 86 (++++) and all tested farms had animals with levels above 54 (+++). Mean antibody levels were 56 in preweaned calves, 39 in weaned calves and 62 in fresh cows. Approx. 50% of herds were using fresh cow vaccinations against bovine coronavirus during the dry period to boost colostral immunoglobulins, and there was a non-significant trend for increased antibody levels in neonatal calves and fresh cows in those herds. There was poor correlation (Pearson's correlation coefficient r=0,41) between bulk tank milk antibody levels and serum antibody levels. Presence of BCoV was confirmed in 17% of nasal and faecal samples from 27 herds. BCoV was found in nasal and/or faecal samples from 74% of herds. Fifty percent of herds had one or more animals that were BCoV positive on nasal swabs. Presence of BCoV was demonstrated in 23% of nasal samples from neonatal calves, 20% of samples from weaned calves, and 8% of samples from fresh cows. There was no significant difference in recovery of viral nucleic acid from nasal versus faecal swabs. There was poor correlation between animal antibody levels and virus shedding in animals (r=0,48). The biosecurity on 41 dairies was scored with overall score of 58%, external biosecurity score of 69% and internal biosecurity score of 41%. The biosecurity scores recorded were slightly above the world average score usually recorded for dairies that have used the Biocheck survey. The virus shedding in cattle tended to be higher in herds with overall higher biosecurity scores. However, antibody levels in cattle were non-significantly lower in herds with a higher biosecurity score.

Conclusion: The preliminary results from this study indicate that BCoV is commonly present in both the respiratory and enteric pathway in the dairy cattle population in the EU, with all herds being seropositive to the virus, and the virus present in numerous herds. The farm management and biosecurity measures associated with BCoV will be further investigated.

Keywords: Bovine coronavirus, prevalence, biosecurity, risk management, Europe.

ID-09

Pathogen-specific prevalence and pathogen associations during outbreaks of Bovine Respiratory Disease in calves in Flanders

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Objectives: Bovine respiratory disease (BRD) is a major health problem during calf rearing in many farms. The objective of this study was to obtain further insights into the importance of different pathogens involved and possible pathogen associations during outbreaks of BRD in calves in Flanders.

Materials & Methods: A cross-sectional study was performed from January 2019 until December 2021. The target population consisted of cattle herds from the northern part of Belgium (Flanders) with a current acute outbreak of BRD. Respiratory samples, consisting of either nasopharyngeal swabs, broncho-alveolar lavage fluid or lung tissue, were collected from affected calves in those herds and submitted for pathogen detection. Pathogen detection was performed using semi-quantitative real-time PCR test targeting seven bovine respiratory pathogens: bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus type 3 (PI3V), bovine coronavirus (BCoV), *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, and *Histophilus somni*. The results were analyzed using R software (R Core Team, 2017). Multivariable logistic regression models were constructed for each of the seven respiratory pathogens. As predictors, the PCR results of the other six pathogens besides the outcome pathogen were considered for the analysis. Additional predictors in the models were season of outbreak occurrence and sample type. Statistical significance was set at $p < 0.05$.

Results: In total 245 outbreaks of BRD were assessed. At least one pathogen was detected in 185 (75.5%) of those outbreaks. Single as well as multiple viral infections were detected in 31.8 and 43.7% of outbreaks, respectively. BRSV was the most frequently isolated virus (38 positive/245 outbreaks, 15.5%). In 73.7% of outbreaks where BRSV was detected, it was the only viral agent detected. BCoV was detected in 31 out of 245 outbreaks (12.7%) and was the only virus detected in 21 of those outbreaks (67.7%). PI3V was only detected in 3.7% of outbreaks and these were predominantly multiple viral infections (88.9%). *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis*, and *Histophilus somni* were detected in, respectively 58.8, 25.3, 21.6, and 20.8 % of the outbreaks.

In the present study, a PCR positive result for BRSV was associated with an increased detection rate of *Mycoplasma bovis* (OR 2.65, $CI_{95\%}$ 1.17-6.01) and PI3V (OR 9.63, $CI_{95\%}$ 2.14-52.5). *Mannheimia haemolytica* was associated with an increased detection rate of PI3V (OR 7.36, $CI_{95\%}$ 1.51-53.9), *Mycoplasma bovis* (OR 2.21, $CI_{95\%}$ 1.07-4.55), and *Pasteurella multocida* (OR 2.19, $CI_{95\%}$ 1.09-4.57). Detection of BCoV during an outbreak of BRD was associated with a higher risk for the detection of PI3V (OR 5.96, $CI_{95\%}$ 1.24-29.8). Besides the association of *Mycoplasma bovis* with *Mannheimia haemolytica* and BRSV, *Mycoplasma bovis* detection was also associated with a higher risk for the detection of *Pasteurella multocida* (OR 2.80, $CI_{95\%}$ 1.35-6.17), and *Histophilus somni* (OR 2.96, $CI_{95\%}$ 1.46-5.96). A seasonal effect was shown for BRSV (OR 4.85, $CI9_{5\%}$ 2.09-12.70) and *Mannheimia haemolytica* (OR 3.09, $CI_{95\%}$ 1.54-6.37) isolation, with a higher prevalence in winter and spring compared to summer and autumn. Sample type was only observed to be associated with the isolation rate of *Mannheimia haemolytica, with a* more frequent isolation from broncho-alveolar lavage fluid than deep nasopharyngeal swabs (OR 7.9, $CI_{95\%}$ 1.29-1.53).

Conclusion: The use of PCR as a diagnostic tool during BRD outbreaks is very valuable since in more than 75% of cases an etiological diagnosis could be established. BRSV and BCoV are the most frequently involved viral pathogens and acted predominantly as single viral agents. We demonstrated a seasonal influence on the occurrence of BRSV and

Mannheimia haemolytica with higher risk of disease in winter and spring. Finally, we found multiple interactions between pathogens responsible for BRD outbreaks in calves. This observation could be useful for the implementation of specific combined preventive measures at the farm level.

Keywords: Bovine respiratory disease, calves, diagnosis, PCR.

ID-11

Genomics-based epidemiology and antimicrobial susceptibility of *Mycoplasma bovis* isolates from veal, dairy and beef herds

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Objectives: *Mycoplasma bovis* associated pneumonia is feared around the world, in particular because of its poor response to antimicrobial therapy. A rise in antimicrobial resistance of *M. bovis* is reported worldwide over the last two decades. Whether different, more resistant strains exist in industries such as the veal industry (more intensive antimicrobial use) compared to conventional dairy and beef farms, is currently unknown. Therefore, the objectives of this study were to compare strains originating from the veal, dairy and beef industry both on their genetic relatedness and antimicrobial susceptibility.

Materials and methods: MIC-values were determined for macrolides, tetracyclines, florfenicol, gentamicin, enrofloxacin and tiamulin with microbroth dilution on 144 epidemiologically independent Belgian *M. bovis* isolates (31 dairy, 70 beef, 11 dairy-beef mixed and 32 veal farms), mostly obtained from the respiratory tract. For a selection of 100 of these isolates (29 dairy, 41 beef, and 30 veal), the whole genome was sequenced by MinION Nanopore sequencing. The reference strain *M. bovis* PG45 was used as quality control in all experiments. Antimicrobial susceptibility data were analyzed using the epidemiological cut-off estimated by the visual eye-ball method to distinguish between wild type (WT) and non-wild type (nWT). Single Nucleotide Polymorphism (SNP) analysis was performed to type *M. bovis* strains, but also to pinpoint specific genetic markers in targeted genes, which were shown to associate with the observed phenotypic susceptibility results. Binary logistic regression (0: WT; 1: nWT) was performed on different sectors to compare antimicrobial susceptibility between sectors. In addition, a phylogenetic tree was developed using CSI Phylogeny (Center for Genomic Epidemiology) for SNP calling on consensus sequences to compare strains between sectors.

Results: Highest MIC-values were observed for macrolides, where almost all strains showed acquired resistance against 16-membered macrolides (tilmicosin and tylosin), and about 50% against the 15-membered macrolide, gamithromycin. A limited number of isolates showed acquired resistance against gentamicin, florfenicol, enrofloxacin and tiamulin. Almost all strains belonged to the wild type population for the tetracyclines (oxytetracycline and doxycycline). A remarkable difference between sectors was observed for gamithromycin, showing that beef herds (59% nWT) had a three times higher odds (95%CI: 1.23-7.35) for gamithromycin resistant *M. bovis* than dairy herds (32% nWT)($P = 0.02$), whereas veal herds did not significantly differ from both sectors (47% nWT). The phylogenetic tree showed different clusters, although strains could not be associated with certain sectors. Specific genetic markers could be linked to acquired resistance for most strains.

Conclusions: This study shows that acquired resistance in Belgian *M. bovis* isolates is highest against macrolides, and minimal for tetracyclines. Secondly, no clear difference in acquired resistance (with the exception of gamithromycin) or strains between sectors were observed. This information could contribute to recommendations on antimicrobial therapy in case of *M. bovis* outbreaks and to further understanding of the epidemiology of this pathogen.

Keywords: Belgium, epidemiological cutoff, gamithromycin, tetracyclines.

ID-12

Intranasal Bacterial Therapeutics Reduce Colonization by the Respiratory Pathogen *Mannheimia haemolytica* in Dairy Calves

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Background/Objectives: Six *Lactobacillus* strains originating from the nasopharyngeal microbiota of cattle were previously characterized *in vitro* and identified as candidate bacterial therapeutics (BTs) for mitigating the bovine respiratory pathogen *Mannheimia haemolytica*. In the present study, these BT strains were evaluated for their potential to (i) reduce nasal colonization by *M. haemolytica,* (ii) modulate the nasal microbiota, and (iii) stimulate an immune response in calves experimentally challenged with *M. haemolytica.*

Materials and Methods: Twenty-four Holstein bull calves (1 to 3 weeks old) received either an intra-nasal BT cocktail containing 6 *Lactobacillus* strains (3 x 109 CFU per strain; BT + Mh group) 24 h prior to intranasal *M. haemolytica* challenge (3 x 108 CFU) or no BTs prior to challenge (Mh, control group). Nasal swab, blood, and transtracheal aspiration samples were collected over the course of 16 days after BT inoculation. Counts of *M. haemolytica* were determined by culturing, and

the nasal and tracheal microbiotas were evaluated using 16S rRNA gene sequencing. Serum cytokines (interleukin-6 [IL-6], IL-8, and IL-10) were quantified by enzyme-linked immunosorbent assay (ELISA).

Results: Administration of BT reduced nasal colonization by *M. haemolytica* (*P* = 0.02), modified the composition and diversity of the nasal microbiota, and altered interbacterial relationships among the 10 most relatively abundant genera. The BT + Mh calves also had a lower relative abundance of *Mannheimia* in the trachea (*P* <0.01) but similar cytokine levels as Mh calves.

Conclusion: This study demonstrated that intranasal BTs developed from the bovine nasopharyngeal *Lactobacillus* spp. were effective in reducing nasal colonization by *M. haemolytica* in dairy calves.

Keywords: Bovine respiratory disease, Lactobacillus spp., respiratory microbiota, dairy calves, probiotics.

ID-13

Prevalence of different pathogens of bovine respiratory disease (BRD) in feedlot cattle in Spain

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Objectives: Bovine respiratory disease (BRD) is a common endemic disease among Spanish feedlot cattle. BRD is the major cause of morbidity and mortality in feedlot cattle, resulting in significant economic losses due to treatment costs as well as reduced feed efficiency and animal product quality. Multiple factors such as the feedlot environment, co-infection with viruses, and stress related to transportation and mixing of cattle may contribute to the development of BRD. The disease complex is caused by one or more primary pathogens, including respiratory viruses and *Mycoplasma* spp., commonly complicated by a secondary bacterial infection, or by bacteria alone. Important bacteria associated with bronchopneumonia in feedlot cattle include *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis.*

The studies on the prevalence of different pathogens in Spain are very few and there is no study that covers the prevalence of virus and bacteria and that focuses on the entire Spanish territory so far. The aim of the current study was to obtain real data on the prevalence of viral and bacterial pathogens in feedlot cattle in Spain in the two main production management systems, suckling calves and yearlings calves.

Materials and methods: The study was carried out in Spain in 2017. We collected samples from 58 feedlots to determine the prevalence of the major viral and bacterial pathogens. Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza Virus type 3 (PI-3) and the bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis* are considered to be major primary and secondary pathogens in BRD.

The typical size of batches of cattle entering Spanish feedlots ranges between 80 and 120 animals. Serum samples were collected from at least 10 randomly selected animals at 30-40 days on feed (DOF), representing approximately 10% of the animals in a batch. Serum samples were collected from 58 different feedlots: 22 units feeding suckled calves and 36 units feeding yearling calves with an annual production of 270.000 calves, representing around 17 % of the total feedlot production in Spain. The feedlots were located in the main cattle feedlot regions of Spain: Catalonia, Andalusia, Castilla la Mancha, Castilla León and Galicia. A total of 723 serum samples were collected. Refrigerated serum samples were sent to a Spanish commercial laboratory for assay of viral and *M. bovis* antibodies using ELISA kits.

Deep nasopharyngeal swabs or trans-traqueal aspirations samples were collected for bacteriology from 21 feedlots from calves requiring BRD treatment during the feeding period. A total of 188 samples were analyzed in the laboratory. Upon arrival, the swabs were suspended and seeded onto a range of plates containing suitable culture media. The bacterial colonies were identified according to morphology, Gram staining, and biochemical and growth characteristics. Identification of *M. bovis*, *M. haemolytica* and *P. multocida* was confirmed by polymerase chain reaction test.

Results: The results obtained revealed the presence of *M. bovis*, *M. haemolytica* and *P. multocida* in BRD cases in 100 %, 53 % and 88 % of the feedlots respectively. The culture individual prevalence in the positive feedlots was 77 % for *M. bovis*, 42 % for *P. multocida*, 25 % for *M. haemolytica* and 0 % for *T. pyogenes* and *H. somni.*

A high seroprevalence for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* was found in both suckling and yearling calves feedlots. The seroprevalence for suckling calves feedlots was 100 %, 91 %, 82 %, 77 % and 91 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. The seroprevalence for yearling calves feedlots was 82 %, 94 %, 94 %, 97 % and 100 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. A high individual seroprevalence ranging from 30 to 70 % was also found at 30-40 DOF.

Conclusions: The prevalence of the major BRD pathogens in Spanish feedlots is high in both types of feedlot production units. The data indicate that contagion of the pathogen is rapid in Spanish feedlots. The results underscore the importance of selecting an antimicrobial which has proven efficacy against *Mycoplasma bovis* and the importance of design strong vaccination protocols and management practices to reduce the impact of viral pathogens.

Keywords: Bovine respiratory disease, prevalence, feedlot cattle.

ID-14

Prevalence of different pathogens of bovine respiratory disease (BRD) in dairy heifers in Spain

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Objectives: Bovine respiratory disease (BRD) is a common endemic disease among Spanish dairy farms, impacting mainly the young animals. BRD is the major cause of morbidity and mortality in dairy heifers, resulting in significant economic losses due to treatment costs as well as the appearance of chronic animals with a reduced productive life or with a delay in reaching the breeding time. Multiple factors such as the dairy farm environment, co-infection with viruses, and stress related to regrouping of heifers may contribute to the development of BRD. The disease complex is caused by one or more primary pathogens, including respiratory viruses and *Mycoplasma* spp., commonly complicated by a secondary bacterial infection, or by bacteria alone. Important bacteria associated with bronchopneumonia in dairy heifers include *Mannheimia haemolytica*, *Pasteurella multocida, Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis*. The studies on the prevalence of different pathogens in Spain are very few and there is no study that covers the prevalence of virus and bacteria and that focuses on the entire Spanish territory so far. The aim of the current study was to obtain real data on the prevalence of viral and bacterial pathogens in dairy heifers in Spain.

Materials and methods: The study was carried out in Spain in 2017. We collected samples from 50 dairy farms to determine the prevalence of the major viral and bacterial pathogens. Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza Virus type 3 (PI-3) and the bacterial pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Trueperella pyogenes* and *Mycoplasma bovis* are considered to be major primary and secondary pathogens in bovine respiratory disease (BRD).

Serum samples were collected from at least 10 randomly selected animals at 30-40 days after a BRD outbreak. Serum samples were collected from 50 different dairy farms with an heifer census of 33.250 dairy heifers, representing around 7 % of the total Spanish dairy heifers. The dairy farms were located in the main dairy production regions of Spain: Galicia, Asturias, Cantabria, Catalonia, Castilla León, Navarra, Valencia, Castilla la Mancha and Andalusia. A total of 540 serum samples were collected. Refrigerated serum samples were sent to a Spanish commercial laboratory (Eurofins) for assay of viral and *M. bovis* antibodies using ELISA kits.

Deep nasopharyngeal swabs or trans-traqueal aspirations samples were collected for bacteriology from 37 dairy farms from dairy calves requiring BRD treatment during the weening period. A total of 316 samples were transported to the laboratory in transport media containing activated charcoal. Upon arrival, the swabs were suspended and seeded onto a range of plates containing suitable culture media. The bacterial colonies were identified according to morphology, Gram staining, and biochemical and growth characteristics. Identification of *M. bovis*, *M. haemolytica* and *P. multocida* was confirmed by polymerase chain reaction (PCR) test.

Results: The results obtained revealed the presence of *M. bovis*, *M. haemolytica*, *P. multocida* and *T. pyogenes* in BRD cases in 75 %, 11 %, 24 % and 16 % of the dairy farms respectively. The culture individual prevalence in the positive dairy farms was 41 % for *M. bovis*, 16 % for *P. multocida*, 21 % for *M. haemolytica,* 45 % for *T. pyogenes* and 0 % for *H.somni.*

A high seroprevalence for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* was found in dairy heifers. The seroprevalence for dairy farms was 21 %, 32 %, 41 %, 44 % and 30 % for BoHV-1, BVDV, BRSV, PI-3 virus and *M. bovis* respectively. A high individual seroprevalence ranging from 45 to 88 % was also found 30-40 days after the BRD outbreaks.

Conclusions: The prevalence of the major BRD pathogens in Spanish dairy farms is high. The data indicate that contagion of the pathogen is rapid in Spanish dairy farms. The results underscore the importance of selecting an antimicrobial which has proven efficacy against *Mycoplasma bovis* and the importance of design strong vaccination protocols and management practices to reduce the impact of viral pathogens in dairy heifers in Spain.

Keywords: Bovine respiratory disease, prevalence, dairy heifers.

ID-15

Use of quantitative serology as a new practical tool for veterinarians to follow up BVD status on vaccinated farms

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Objectives: The bovine viral diarrhea (BVD) virus causes significant economic losses in cattle farms. Indeed, various EU countries have done voluntary or mandatory eradication plans. Different schemes have three central elements: biosecurity, elimination of persistently infected animals and herd monitoring. In addition, many programs include systematic vaccination against BVD as an additional biosecurity measure (EU Thematic network BVDV, 2001; Moening et al., 2007).

For monitoring, the detection of antibodies continues to be the fastest, most practical and economical method to assess exposure to BVD (González et al., 2014). Therefore, some countries and regional programs, such as the ADSG in Galicia, base their control programs on ELISA tests that are carried out periodically on youngstock serum and bulk tank.

A possible concern regarding vaccination is the interference of the vaccine response with serological monitoring of

the herd. However, previous studies in a reduced number of farms have shown how the combination of sampling age (6-18 months old) and quantitative values of BVD p80 antibody ELISA, could be a useful tool to determine the BVD status of a herd, even if vaccinated with inactivated vaccines (Carbonell et al., 2019; Eze et al.,2019).

The main objective of this study was to analyze in many farms the practical use of quantitative BVD p80 antibody ELISA to differentiate between field virus circulation and BVD vaccination.

Materials and methods: Serum samples from 50 farms included in the Voluntary ADSG Control Program of Galicia were analyzed in 2019. The farms were classified based in their BVD status into three types: RC, recent circulation of BVD (5), BOV, vaccinated with an inactivated BVD vaccine (Bovilis® BVD) (40), LV, vaccinated with a live BVD vaccine (5). In the vaccinated farms (BOV and LV) no persistently infected animals had been detected in the previous two years.

All samples were analyzed in the Animal Health and Production Laboratory of Galicia by ELISA BVD antibody (IDEXX, BVDV p80 Ab Test). For this quantitative study, the results were expressed as Inhibition Percentage of the optical density (IP-OD). The ELISA results were categorized according to the manufacturer's instructions as: negative (>50), doubtful (40-50) or positive (<40). All positive samples (OD <40) were further divided over 4 groups (<10; 10-20; 20-30 ;30-40). Furthermore, the lower the PI, the higher the titer of antibodies present in the sample. For the statistical processing a descriptive analysis was performed using Excel 2016.

Results: A total of 976 serum samples were included in the analysis (101 RC, 780 BOV and 95 LV).

21.65, 70.5 and 23.66% of the samples were seronegative while 70.1, 8.9 and 48.4% were positive to ELISA with IP-OD <10 in RC, BOV and LV group, respectively. Moreover, the average PI value of positive samples was 6.5±4.8, 21.9±14.1 and 11.3±8.2 in RC, BOV and LV group, respectively.

A more detailed analysis by segmentation into four age categories (6-12m, 13-18m, 19-24m and > 25m) reveled two clear patterns: In the BOV group, the percentage of seronegative samples was even higher in animals 6-18 months old (82.9% in both); while the percentage of samples IP-OD <10 was very low (4.9%, 2.2%, 12.4% and 16.1%, respectively in all four age groups). However, in the RC and LV group the percentage of seronegative samples was much lower (40.0 -20.0% 6-12m and 31.3-20.8% 13-18m, respectively); while the percentage of positive ELISA IP-OD <10 was very high (60.0-60.0%, 86.7-50.0%; 50.0-56.7% and 73.2-38.2% for RC and LV per group, respectively).

Moreover, if we analyze the distribution of positive results based on the IP-OD result of ELISA in a boxplot, the first, second and third quartile of the BOV group (9.5,21.0,34.0) represents a different pattern to the RC group (4.0,5.0,7.0) and LV group (6.0,8.0,12.8); showing patterns that easily allows the differentiate the positives due to vaccination with the inactivated vaccine (BOV) vs virus circulation (RC). However, this serology is not useful to monitor LV farms as their patterns are difficult to differentiate to RC ones.

Conclusions: The use of quantitative IP-OD ELISA values, using IDEXX p80 antibodies ELISA test, seems to offer a useful tool to assess BVD status in farms vaccinated with inactivated vaccines. Moreover, this interpretation adds value to the technical advisor at farm level by improving their monitoring system.

Keywords: BVD, serology, quantitative, vaccine, monitoring.

ID-16

Pasteurella multocida, Mannheimia haemolytica and Bovine Corona Virus are the most frequently detected respiratory pathogens from bronchoalveolar lavages in Dutch dairy BRD calves

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Objectives: Bovine respiratory disease (BRD) is a common cause of morbidity and mortality in dairy calves, which has long-term consequences including decreased milk production, poor reproductive performance, and poor growth and longevity. Although management practices such as commingling and group housing increase BRD risk, viral and bacterial pathogens cause the lesions characteristic of BRD. Monitoring and testing for pathogens associated with BRD may facilitate the development of appropriate, targeted vaccination pro-grams.

From November 2019 until the end of December 2021, MSD Animal Health performed several diagnoses of respiratory pathogens by PCR on bronchoalveolar lavages (BALs) on Dutch dairy farms vaccinating against *Mannheimia haemolytica*, Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza 3 virus (PI3).

This manuscript gives an overview of the first results of the BALs performed on those Dutch dairy farms.

Materials & Methods: From several calves suffering from BRD on dairy farms vaccinating against BRD pathogens in the Netherlands, a BAL sample was taken as previously described (Van Driessche et al. 2016). Samples were transported at ambient temperature and analyzed at the Veterinary Laboratory Gelderland (Epe, The Netherlands) by PCR for *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3.

Results: During the defined timeframe, 194 BALs from calves between 2 weeks and 6 months old were performed on 80 dairy farms from which 78,3%, 25,7%, 21,6% 13,4%, 12,3%, 10,3% and 1,5% of the samples were positive for respectively *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3. This is in line with similar studies from Belgium (n=3234) (Griepbarometer DGZ, https://www.dgz.be/rundvee/gezondheidszorg/bioveiligheid-en-preventie/monitoring-en-vaccinatie/griepbarometer, accessed at Jan 12th 2022) showing the following prevalences for respectively *Pasteurella multocida*, *Mannheimia haemolytica*, Bovine Corona Virus, *Mycoplasma bovis*, *Histophilus somni*, BRSV and PI3 : 74,0%,

35,8%, 19,8%, 27,7%, 25,7%,21,1% and 5,5%. Both studies show that the most prevalent bacterial pathogens are *Pasteurella multocida* and *Mannheimia haemolytica*, while the most prevalent viral pathogen is clearly Bovine Corona Virus.

Finally, we compared the results of this PCR study with the seroprevalences of respiratory samples from BRD calves on non-vaccinating dairy farms (Kuijk et al., 2022). The most remarkable finding from this comparison was on PI3: only 1,5% of the BALs in this study (BRD calves on vaccinating farms) were positive for PI3 specific RNA where 66,6% of the animals in the serology study (BRD calves on non-vaccinating farms) were positive for PI3 antibodies. This demonstrates that many animals get in contact with PI3 field virus early in life or are still seropositive by the presence of maternal antibodies. But on the other hand, it is hard to detect PI3 BALs from BRD calves.

Conclusion: From this study, it can be concluded that *Pasteurella multocida, Mannheimia haemolytica* and Bovine Corona Virus are the most frequently detected respiratory pathogens from dairy calves suffering from Bovine Respiratory Disease.

Keywords: BRD, dairy calves, Pasteurella multocida, Mannheimia haemolytica, Bovine Corona Virus.

ID-17

Prevalence of respiratory pathogens on Danish cattle farms

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Objectives: Bovine Respiratory Disease (BRD) is a costly and multifactorial disease of young and growing cattle. The factors that predispose to BRD include stress related to overstocking, moving or mixing cattle, poor ventilation or draughts, sudden climatic changes, mixing of various age groups, nutritional deficiencies, inadequate colostrum intake, and poor feed hygiene. These stress situations facilitate infection by primary viral pathogens which cause lung damage. The viral damage to the lungs may pave the way for various bacterial pathogens such as *Mannheimia haemolytica* (Mh)*, Pasteurella multocida* (PM) and *Mycoplasma bovis* (MB).

Vaccination is an important tool in managing BRD. Identification of major respiratory pathogens on farms with BRD problems can offer valuable insights helping establish an appropriate vaccination program.

In 2019 and 2020 MSD Animal Health used a testing approach BRD QuickScan to evaluate exposure to BRD pathogens present on several Danish farms. The BRD QuickScan involves detection of antibodies against Mh*,* Bovine Respiratory Syncytial Virus (BRSV), Bovine Corona Virus (BCV), Parainfluenza 3 virus (PI3V), MB and PM. The results of the BRD QuickScan and situation analysis was then used to provide the farms with a tailor-made advice including BRD vaccination and improvement of management factors predisposing to BRD.

This manuscript gives an overview of the prevalence of respiratory pathogens in Danish dairy farms and calf rearing operations with BRD problems.

Materials & Methods: The tested farms were selected based on the herd veterinarian defining them as having a BRD problem. On the selected farms, serum samples were taken from around 10 calves > 2,5 months old. The samples were subjected to the BRD QuickScan procedure in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands). An in-house ELISA test was used to measure Mh*,* PM*,* BCV and BRSV antibodies, whereas for PI3V and MB commercial ELISA kits were used (IDEXX and Bio-X respectively). The test for antibodies against BCV was only performed on samples from June 2020 onwards.

Results: In total, 51 BRD QuickScans were performed: 38 on samples from dairy farms (371 calves) and 13 from calf rearing operations (134 calves) meeting the same farm selection and calf-age criteria. At the farm level, 100% of the tested farms had samples positive for Mh and PI3V antibodies, whereas 94%, 73% and 40% were positive for PM*,* MB and BRSV respectively. All farms (100%) included in testing for BCV antibodies had positive samples.

At the calf level, no presence of antibodies against any of the pathogens tested was detected only in 1% of calves. Samples from 98% of calves were positive for antibodies against Mh*,* but only 3% of samples were positive for Mh antibodies only. Simultaneous presence of antibodies against Mh and viral BRD pathogens (BRSV and/or PI3V) was detected in 82% of samples. None of the samples had a mixed Mh - MB antibody presence.

Eighty-three percent of the samples were positive for antibodies against PI3V and/or BRSV. One percent were only positive for PI3V antibodies and none of the calves were positive for only BRSV antibodies. These findings confirm the importance of the viral pathogens in the BRD complex, mainly in combination with Mh.

Conclusion: All farms tested had calves positive for Mh and PI3V antibodies, and all farms tested for BCV antibodies had positive samples. At the calf level, the most frequently detected antibodies were those detected against Mh with a prevalence of 98% followed by PI3V with a prevalence of 82% of all calves tested with the BRD QuickScan. The results suggest that the pathogens circulating on Danish BRD problem farms were in most cases (82%) co-infections of bacteria and viruses, while Mh was involved in all cases.

The findings demonstrate that the BRD QuickScan can be a valuable tool supporting the vet and the farmer in the decision-making process around BRD control and prevention. For most pathogens circulating on farms with BRD problems, vaccines are commercially available and therefore an insight into the pathogens the animals are exposed to on a particular farm should help motivate the farmers to adopt prophylactic vaccination. Moreover, knowledge of the BRD pathogen landscape and specific farm risk factors should help the vets develop a tailor-made prophylactic vaccination programme for each farm.

Keywords: Bovine respiratory complex, pathogens, prevalence.

ID-18

Risk factors for BVDV introduction into Dutch dairy herds in a national control programme

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Objectives: After many years of voluntary control and decreasing prevalence, a national control programme for bovine viral diarrhoea virus (BVDV) is in place for Dutch dairy herds since 2018. Introduction of BVDV is minimized by regulations with respect to purchase of cattle from herds with a lower BVDV status. However, BVDV was introduced in several tens of herds in 2019-2021. The aim of the study was to determine risk factors for introduction of BVDV in the context of the national control programme.

Material & Methods: In a case-control design, herds with a confirmed BVDV introduction (cases) were compared with control herds that were located near the case herd, which remained free of BVDV. Both case and control herds were visited by a veterinarian and an extensive questionnaire was applied about the possible risk factors for BVDV introduction in the previous two years. In total, 149 cases and 148 controls were visited. Logistic regression analysis was carried out to determine the significant risk factors (P<0.05).

Results: The final multivariable model consisted of seven risk factors. Purchasing cattle from non-free herds (OR=1.25) and cattle from other herds that escaped and mingled with cattle from the own herd (OR=1.16) were risk factors related to direct animal contact. Risk factors for indirect external contact were: distance of less than 500m to beef cattle herds (OR=1.15), a permanent employee (OR=1.17) and the farmer working outside the farm in other cattle herds (OR=1.25). Risk factors that seemed more related to internal biosecurity were: housing of adult cattle and calves in the same barn (OR=1.22) and use of a group pen for calving (OR=1.16).

Conclusion: In conclusion, the risk factors for introduction of BVDV in free herds varied considerable between herds and had fairly low odds, indicating that there were many smaller biosecurity risks that need to be mitigated.

Keywords: BVDV, biosecurity, control programme.

ID-19

Voluntary control program against *Mycobacterium avium* subsp. *paratuberculosis* in cattle farms from the Livestock Health Defence Group Costa da Morte in Galicia (NW Spain)

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Objectives: Galicia is the main dairy cattle of the country, with 55% of the farms and 38% of the milk production. The mean herd size per farm is 43 cows. In 2004, a voluntary control program against *Mycobacterium avium* subsp. *paratuberculosis* (Map), causal agent of bovine paratuberculosis, was implemented in Galicia (NW Spain). 8,664 farms are currently integrated in the program, representing 26.2% of the herds (50.2% of the animals).

In the NW of Galicia, the Livestock Health Defence Group (LHDG) Costa da Morte, had 243 herds integrated in the voluntary control program (cattle and dairy farms) ranging from 539 to 5 animals (mean herd size of 68 cows).

The aim was to present the evolution of the program in LHDG Costa da Morte according to the laboratory results between 2014 and 2019.

Materials and methods: Blood samples are taken annually to animals older than 2 years for the 243 herds involved in the program, according to the following schedule:

- In Map infected herds, all cows over two years during the next years to elimination of fecal positive animals.
- 40% of animals over two years in herds not confirmed as infected.

The serums are analyzed for anti-Map antibodies with commercial ELISA and fecal samples of all ELISA-positive samples are analyzed by PCR or bacterial culture. Fecal positive animals must be sacrificed.

Additionally, all purchased animals were analyzed by ELISA. Positive ELISA or PCR animals must not be incorporated into farms.

Information of biosecurity and supervision of implementation of management measures to reduce fecal-oral contamination is also recorded in the program.

Results: The seropositive herds in LHDG studied (at least with one animal with anti-Map antibodies) varied between a maximum of 15.3% in 2015 and a minimum of 9.6% in 2018. These results are better than those obtained in all LHDG of Galicia with a maximum of 22% in 2015 and a minimum of 17.7% in 2017.

At animal level, the seropositivity varied between a maximum of 1.8% in 2014 (15.2% were positive-PCR/bacterial culture) and a minimum of 1% in 2018 (2.9% were positive-PCR/bacterial culture). In all LHDG of Galicia, it was observed a maximum of 2.8% in 2014 (20.8% were positive-PCR/bacterial culture) and minimum of 1.8% in 2017 (13.5% were positive-PCR/bacterial culture).

The percentage of seropositive in purchased animals did not exceed 2.8% any year, with a minimum of 0% in 2018 (never above 1.7% with a minimum of 0.7% in 2017 in all LHDG).

Only about 26% of the farms purchased cattle (33.7% in the whole region) and most farmers do not request information on the overall status of the origin farms.

Conclusions: Control programs are having an impact on the sanitary status. Collecting data is an important first step to identification of biosecurity shortcomings; however, apart from the control of purchased animals (mandatory), programs do not seem to have significant influence on the application of many other measures concerning biosecurity.

Acknowledgements: Animal Health and Production Lab-

oratory of Galicia, Xunta of Galicia

Keywords: Mycobacterium avium susp. paratuberculosis, cattle farms, control program, Spain.

ID-20

Comparison of different sampling sites and techniques for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in environmental fecal samples in paratuberculosis positive cattle herds

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Objectives: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is causing paratuberculosis (Johne´s disease, JD) in cattle and is known to survive for an extended period of time in the environment. The objective of this study was, to evaluate in which areas within a barn MAP can be detected in positive cattle farms and to compare different sampling sites. Thereby, potential areas for MAP transmission, as well as the most promising places for the detection of MAP in positive cattle premises, should be identified.

Material and Methods: During the study, 14 Austrian dairy and beef operations were tested for the presence of MAP twice in a six months interval. In each farm at least one confirmed clinical case of JD was detected within a year prior to the study and the herd was therefore considered to be MAP positive.

On every farm, 7-10 paired environmental fecal samples from specific sites were taken. Sampling locations depended on the type of operation and included the calving area, alleyways, equipment, milking parlor and manure storage sites. Fecal samples were tested for MAP both by bacteriological culture on Herrold´s Egg Yolk Medium and real time PCR for IS*900* (Adiagene, Saint Brieuc, France), at the Austrian National Reference Laboratory for paratuberculosis.

Results: All farms enrolled in the study had at least one positive environmental fecal sample, confirming the classification as MAP positive. Fecal samples collected from the slurry pit, the alleyways in the feeding area as well as the manure channels (tie stall barns) proved to be most likely MAP positive. Altogether, 42.3% of the samples from the slurry pit were positive by culture and 51.9% by PCR, samples from the alleyways from the feeding area showed 44.4% and 30.0% positive results, and in the manure channel 87.5% of the collected samples were MAP positive by culture an 50.0% by PCR, respectively. The sensitivity of the samples could be increased significantly by collecting two samples from each site and reached 100% at the herd level, when several sample sites were combined within a farm.

Conclusions: The results of the present study indicate, that manure storage sites, as well as the highly frequented alleyways in a barn seem to be the most promising sites for the detection of MAP by environmental fecal samples. Based on these results, the use of environmental fecal samples seems to be a useful tool to assess the MAP herd level in cattle.

Keywords: Paratuberculosis, Johne´s disease, Mycobacterium avium subsp. paratuberculosis, environmental fecal sampling.

ID-21

Examples of and lessons learned from regional control programs for the abatement of *Mycobacterium avium* subsp. *paratuberculosis* in cattle

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Objectives: Paratuberculosis (Johne´s disease, JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and may lead to substantial economic losses in infected cattle herds. Detection and control of the infection is challenging, therefore examples of regional control programs are presented and discussed.

Material, Methods and Results: In course of the program for the reduction of the MAP prevalence in Lower Saxony, Germany, dairy farms are obliged to test bulk milk samples for MAP antibodies, followed by testing of individual animals in seropositive farms. Subsequently, farmers can decide to join the accompanying MAP control program. Within the first year of the program 6,035 bulk tank samples were tested, 13% were MAP-positive and 670 farms joined the MAP control program.

Within the voluntary certification program in Hesse, Germany, the MAP herd status is evaluated using boot swab sampling (PCR and culture). In positive farms, animals are tested by individual milk or blood ELISA-serology. Until now, 100 farms participated in the program, of which 60 were MAP negative and 33 positive, respectively (no status assigned in 7 farms). The mean intra herd prevalence decreased from 7.56 % to 4.06% in participating farms.

The program for the abatement of MAP infections in cattle herds in Thuringia, Germany, is based on a yearly fecal examination of adult cattle within a herd. In 2017, fecal samples from 28,941 animals were tested of which 1.8% were MAP positive. Of the 136 participating farms, currently 64 are MAP negative and 72 positive, with 39 of the latter in the last step of the program before achieving a MAP-unsuspected status.

The biennial survey of the MAP herd status by boot swabs (PCR and culture) is the base of the MAP program in Tyrol, Austria. Positive farms may join the MAP control program to have their animals tested by individual fecal sampling. More

than 4,000 boot swab samples were tested in each run with 0.97% positive farms in 2016/17. In these farms 2,151 individual fecal samples were collected of which 2.3% were MAP positive.

Conclusions: The programs presented indicate, that a two-stage approach, with an evaluation of the MAP-herd level, followed by the testing of single animals, is generally well accepted by the stakeholders. Besides financial support, communication and cooperation of all participating parties seems to be crucial for the success of such programs. Furthermore, stigmatization has to be avoided and additional programs for positive farms should be available.

Keywords: Paratuberculosis, Johne´s disease, Mycobacterium avium subsp. paratuberculosis, regional control program.

ID-22

Wild cervids populations as Schmallenberg virus circulation sensors

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Background: The *Schmallenberg virus* (SBV) emerged in 2011 in Europe. The epicenter of this spreading was the region which straddled Germany, the Netherlands and Belgium. The dissemination of the virus is based on an arthropod/ruminant cycle. Midges belonging to the *Culicoides obsoletus complex* have been identified as the main vector population while the majority of tested ruminants supports the infection. After the remarkably fast spreading of the virus across Europe, questions raised about the virus implantation in the conquered lands.

Objectives: The objective of the study was to follow the circulation dynamic of SBV in Wallonia (Belgium) during six years after the emergence (2012-2017). We designed a seroprevalence follow-up of the wild deer populations to answer two main questions: (i) is SBV endemic in Wallonia and, if so, which kind of endemic profile characterizes its implantation? (ii) are wild deer a significant reservoir for the virus?

Material and Methods: The study is based on the annual sampling protocol of the Surveillance Network of Wildlife Diseases (SNWD) of the University of Liège (Belgium). The SNWD takes advantage of the hunting activities in Wallonia to collect, every year, during October, November and December, as many samples as possible to cover the largest number of communities. Blood is collected *post-mortem.* Sampled deer are classified as adults or juveniles (born the year of sampling) and belong to the two main deer species of Wallonia: roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*). All samples were analyzed using the commercial ELISA from IDvet: *ID Screen Schmallenberg virus Competition Multi-species*®. To assess the relative sensitivity (Se) and specificity (Sp) of the ELISA in this context, sero-neutralisation tests (SNT) were carried out on a subset of samples of years 2012, 2013 and 2014.

Results: The study led to the test of 2258 sera: 1140 from roe deer and 1138 from red deer. The geographical distribution of the samples is centered on the Belgian Ardenne. The subset of samples used for the SNT numbered 622 sera. The relative Se of the ELISA in this context was 70% and the relative Sp reached 93%. However, for the 2012 red deer cohort, an unexplained phenomenon dropped the relative Se to 30%. In consequence, the seroprevalence of this group was evaluated by SNT and not included in the statistical analysis.

The profile of the seroprevalence evolution over the six years is similar in the two species. Two years, 2012 and 2016, were characterized by a significantly higher level of circulation (roe deer: 2012 = 44±6% - 2016 = 47±7%; red deer: 2012 = 43±16% SNT-based evaluation – 2016 = 28±7%). Beside these two years, the seroprevalence was low in both species, especially in juveniles for which the seroprevalence did not exceed 6±5%, showing a very limited circulation of the SBV during 2013, 2014, 2015 and 2017. These low circulation years led to a a seroprevalence decrease in the whole population until a floor level close to 10% reached in both species in 2014.

Conclusion: Our study provides evidences that the SBV continues to circulate in Wallonia after 2011. Thus, Wallonia appears as an endemic area characterized by a global hypo-endemic state crossed by endemic pulsations. Such an endemic profile is classically explained by the Susceptible-Infectious-Recovered-Susceptible (SIRS) epidemiological model.

The intense 2016 circulation was observed and reported all across Europe. On the contrary, 2012 circulation is more specific to our study and is due to the specific topography of the Belgian Ardenne that locally slowed down the 2011 expansion of the virus.

The fact that floor level seroprevalence was already reached in 2014 for the two populations suggests that wild deer are not the main reservoir of the virus and have limited impact on the global circulation.

Keywords: Schmallenberg, Seroprevalence, Belgium, Deer, Epidemiology.

ID-23

Herd Environmental Sampling for detection of *Mycobacterium avium* subspecies *paratuberculosis* in Irish pasture-based dairy herds

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Objective: The objective of this study was to determine the herd sensitivity (HSe) and herd specificity (HSp) of Herd Environmental Sampling (HES) for the detection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Irish

pasture-based dairy herds and to compare these metrics with those of whole-herd serology and confirmatory faecal PCR.

Materials and methods: In a three-year study (2019-2021), 122 commercial dairy herds were recruited from the Irish Johne's Control Programme (IJCP) https://animalhealthireland.ie/programmes/johnes-disease/irish-johnes-control-programme-ijcp/. Herds were visited once when cows were housed for the winter. Blood samples were taken from all animals over two years old. For the HES, six composite environmental samples were collected from areas of manure concentration such as slurry storage, adult cow housing, the collecting yard for the milking parlour and calving pens. Two herd-level tests for MAP were conducted on these samples: whole-herd serum ELISA (sELISA) with confirmatory faecal PCR (fPCR) of seropositive animals (sELISA + fPCR), and herd environmental fPCR. Blood samples were tested using IDEXX MAP ELISA kit and HES samples were tested by PCR using the Indical Bactotype MAP PCR kit. Individual faecal samples were tested with various different PCR kits. A herd was considered positive on sELISA + fPCR if at least one animal was positive on fPCR; and positive on HES if at least one composite environmental sample was positive on fPCR. The HSe and HSp of HES and sELISA + fPCR were estimated using a two-test, two-population Bayesian latent class model in R.

Results: Complete test results for both test methods were available for 97 herds. Fifteen and five herds were positive on sELISA + fPCR and HES, respectively, with seventeen herds identified as infected on either test; 80 herds were negative on both tests. The median herd level sensitivity (HSe) and specificity (HSp) and 95% credibility intervals for each test were as follows: sELISA + fPCR: HSe 0.57 (0.33-0.85), HSp 0.99 (0.98-1); HES: HSe 0.31 (0.16-0.5, HSp 0.99 (0.99-1).

Conclusions: In this study, the estimated HSe of HES was lower than the current standard method of testing herds for MAP in Ireland (whole herd serology with confirmatory faecal PCR). Further research is required to determine if the test methods used can be optimised to increase the sensitivity of HES in pasture-based dairy herds.

Keywords: Paratuberculosis, diagnostic, sensitivity, environmental, pasture-based.

ID-24

Comparison of different diagnostic strategies at the herd-level for *Mycobacterium avium* subspecies *paratuberculosis* in cattle

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Objectives: Paratuberculosis (PTB) is a disease that causes significant production losses in cattle and its prevalence is expected to increase in the coming years due to the intensification of livestock systems. Knowing the status of PTB infection at the herd-level through accuracy and economical strategies would improve the control of the disease. Therefore, the objective of this work was to evaluate different diagnostic test to detect *Mycobacterium avium* subspecies *paratuberculosis* (MAP) at the herd-level from a cost-effective point of view.

Material & methods: A total of 26 dairy farms in southern Spain with an average of 107 milking cow were randomly selected for this study. The following samples were taken from each herd: blood serum samples, obtained by sterile BD Vacutainer ® from 20 randomly selected cows with at least one calf (n=520 samples). Also, a sample from the bulk milk tank (BMT, n=26) and accumulated feces (n=26) in the milking area (holding pen) from each dairy farm were obtained.

Both serum and the BTM samples were tested by enzyme-linked immunosorbent assay (ELISA) (IDEXX Laboratories, Westbrook, ME, USA), according to the manufacturer's instructions. The manure and BMT samples were analyzed by real-time polymerase chain reaction (qPCR). DNA extraction was performed following the instructions of the MagMAX™ Core nucleic acid purification kit, with a specific mechanical lysis module for MAP detection (Thermo Fisher scientific, Austin, Texas) and, in an automated manner, with the KingFisher™ mL equipment (Thermo Fisher scientific, Austin, Texas). The qPCR analysis was performed using the vetMAX™ MAP IS900-F57 kit (Thermo Fisher scientific, Austin, Texas).

The ability of each diagnostic test to detect MAP at the herd level was evaluated and compared with the other techniques. The agreement between the diagnostic tests was evaluated by Cohen's Kappa statistic (k) and interpreted as follows: k = 0.00 - 0.20, poor; k = 0.21 - 0.40, fair; k = 0.41 - 0.60, moderate; k = 0.61 - 0.80, good; and k = 0.81 - 1.00, excellent agreement (WinEpi software 2.0, Faculty of Veterinary, University of Zaragoza, Spain).

Results: In reference to individual serum samples, a total of 26 (5.0%) of the 520 serum samples showed MAP-specific antibodies. A herd was considered positive when at least one of the serum samples was seropositive, therefore, 14 herds (53.8%) were considered MAP positive by serology, most of them (64.3%) with only one animal seropositive. However, when analyzing the BMT samples by ELISA, 5 positive and 6 doubtful samples were detected, which were considered positive for herd classification, obtaining 11 seropositive herds (45.8%). When qPCR was used, the presence of MAP was detected in 11 (45.8%) fecal samples, but all BTM samples were negative with this technique.

Except for qPCR in BTM samples, the agreement between all other techniques was considered moderate (K=0.455-0.541). A total of 17 herds (65.4%) presented unanimity in the result of all the techniques studied (8 positive and 9 negative), while in 9 herds (34.6%) discrepancies were observed, standing out 3 herds (11, 5%) that were only detected by serum serology and 1 herd (3.8%) that was positive only to the BTM Elisa, which could be associated with the lack of specificity of the ELISA, described by other authors.

Conclusions: The diagnostic techniques analyzed for the classification of PTB positive and negative herds show moderate agreement. Therefore, we recommend the combination of indirect techniques (ELISA of individual animals or in BMT samples) and direct techniques (qPCR of manure) for the de-

tection of MAP at the herd-level as the most accurate strategy. However, the analysis of a sample of manure from the holding pen by qPCR turned out to be the most advantageous cost-effective strategy.

Keywords: Paratuberculosis, diagnosis, qPCR, ELISA.

ID-26

Mannheimia haemolytica serotypes detection by novel qPCR. Spanish cattle epidemiological situation

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Introduction: *Mannheimia haemolytica* is described as a primary agent of bovine respiratory disease (BRD) which remarkably impacts in cattle cost. Most of healthy animals are natural carriers; nevertheless, this agent causes acute or chronic disease under certain circumstances such as environmental stress, shipping, weaning or infections by viruses or *Mycoplasma spp*.

Twelve different serotypes of *M. haemolytica* (A1,A2, A5-A9, A12-A14, A16 and A17) have been reported so far. Other serotypes, which were formerly included, are currently classified as *Mannheimia glucosida* (A11) or *Bibersteinia trehalosi* (T3,T4,T10 and T15). Previous publications reported serotype A1 and in a less extent A6 as the most frequently found in pneumonic lesions whereas serotype A2 is found mainly in the nasopharynx of healthy animals.

Different methods have been described for serotyping *M. haemolytica* until now, however, indirect hemagglutination test (IHA) has been the most common used. The lack of commercial antisera and cross reactions avoid the laboratories from offering this kind of diagnostic.

Objectives: This work aimed to develop a novel multiplex Real Time PCR (qPCR) which detects simultaneously main serotypes described in cattle (A1, A2 and A6) not only on the isolates but also directly on the clinical samples. Furthermore, this project researched the frequency of detection of the different serotypes of the *M. haemolytica* isolates obtained from diseased cattle in Spain.

Materials and methods: A novel multiplex qPCR targeting A1(FAM), A2(HEX) and A6(CY5) was developed and tested for inclusivity and exclusivity. A complete collection of reference strains including 12 isolates belonging to the respective 12 serotypes of *M. haemolytica* and 5 isolates from the four serotypes of *B. trehalosi* and *M. glucosida* (A11) were also tested.

Then, 59 strains isolated from lungs and broncheoalveolar lavages (BAL) of diseased animals belonging to different clinical cases were analyzed by qPCR and IHA. These isolates had been confirmed as *M. haemolytica* by Maldi Tof (Bruker) and commercial qPCR (EXOone Mannheimia haemolytica, Exopol). Cohen´s Kappa value was calculated (CI 95%) considering results from both techniques. Those strains with negative result for A1, A2 and A6 were considered as concordant if IHA resulted with a different serotype different from the three above mentioned.

Finally 26 clinical specimens sampled from diseased animals which resulted positive for *M. haemolytica* were directly analyzed by qPCR. This collection included nasal swabs(n=8), BAL(n=5) and lungs(n=14).

Results and discussion: Inclusivity and exclusivity test resulted as expected and reference strains were correctly identified. Isolates which were different from A1, A2 and A6 resulted negative but A17 which resulted positive for A2 qPCR test. This misidentification might not affect the study because A17 is not expected to be present in Spain.

Once analyzed the 59 isolates, 32 isolates (54.23%) resulted positive for A1, 12 isolates (20.33%) resulted positive for A2, 6 isolates (10.17%) resulted positive for A6 and 9 isolates (15.25%) resulted negative for A1, A2 and A6. Concordance resulted very high (kappa=0.95; CI 0.95, 0.87-1.0; SD=0.03). From those 9 isolates which resulted as non-typeable (nt) by qPCR two resulted positive to A16 by IHA and the rest remained as nt. These results agree those previously reported; A1, A2 and A6 represented the 85% of the cases and A1 was found as the most frequently detected in diseased animals.

Those clinical samples (n=26) which had resulted positive to *M. haemolytica* by qPCR (Cq range 18.9-32.6) obtained positive results for A1 (n=16, 61%), A2 (n=14; 53%) and A6 (n=5, 17.24%). In everysample but one was detected at least one of the studied serotypes. Coinfection was not frequently observed; just 3 samples resulted positive for two different serotypes.

The detection rates of serotypes A1 and A6 over the isolates were not significantly different from those when clinical samples were directly analyzed. Nonetheless, important differences were found in case of A2 ($p<0.05$). Several nasal swabs were directly analyzed by qPCR and could explain this fact.

Conclusions: This novel multiplex qPCR has proven to be an accurate technique to detect the most important serotypes of *M. haemolytica* in cattle. Moreover, this molecular tool overcomes the limitation of microbiological growth and serological proceedings. Epidemiological situation in Spain is not different from those previously reported in other countries, nevertheless, serotype A2 should not be neglected as it is frequently isolated from pneumonic lungs.

Keywords: Mannheimia, serotypes, qPCR, Spain.

ID-27

Topography of the respiratory tract bacterial microbiota in cattle

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Background/Objectives: Bacterial bronchopneumonia (BP) is the leading cause of morbidity and mortality in cattle. While the bacterial composition of the bovine upper respiratory tract (URT) has not been studied in detail, the nasopharynx is generally accepted as the primary source of pathogenic bacteria that cause BP. However, it has recently been shown in humans that the oropharynx may act as the primary reservoir for pathogens that reach the lung. The objective was therefore to describe the bacterial microbiota present along the entire cattle respiratory tract to determine which URT niches may contribute the most to the composition of the lung microbiota.

Materials and Methods: Seventeen upper and lower respiratory tract locations were sampled from 15 healthy feedlot steer calves. Samples were collected using a combination of swabs, protected specimen brushes, and saline washes. DNA was extracted from each sample and the 16S rRNA gene (V3-V4) was sequenced. Community composition, alpha-diversity, and beta-diversity were compared among sampling locations.

Results: Microbiota composition differed across sampling locations, with physiologically and anatomically distinct locations showing different relative abundances of 1,137 observed sequence variants (SVs). An analysis of similarities showed that the lung was more similar to the nasopharynx (R-statistic = 0.091) than it was to the oropharynx (Rstatistic = 0.709) or any other URT sampling location. Five distinct metacommunities were identified across all samples after clustering at the genus level using Dirichlet multinomial mixtures. This included a metacommunity found primarily in the lung and nasopharynx that was dominated by *Mycoplasma*. Further clustering at the SV level showed a shared metacommunity between the lung and nasopharynx that was dominated by *Mycoplasma dispar*. Other metacommunities found in the nostrils, tonsils, and oral microbiotas were dominated by *Moraxella, Fusobacterium,* and *Streptococcus*, respectively.

Conclusions: The nasopharyngeal bacterial microbiota is most similar to the lung bacterial microbiota and therefore may serve as the primary source of bacteria to the lung. This finding confirms that the nasopharyngeal microbiota should be the focus of research as it relates to the role of the URT microbiota in BP. As well, this microbiota should be the main target for future interventions and pharmaceuticals aimed at controlling and preventing BP.

Keywords: Microbiome, bovine respiratory disease, natural cattle, lung.

ID-28

Metaphylaxis with tildipirosin did not alter the effectiveness of an experimental, monovalent vaccine of live, attenuated *Mannheimia haemolytica* administered intranasally to calves

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Objective: To determine the effect of tildipirosin administered subcutaneously (SC) on the efficacy of a live, attenuated, monovalent vaccine that contained *Mannheimia haemolytica (M. haemolytica)* administered intranasally (IN).

Materials and Methods: For this study, eighty-eight (88) healthy, single-source, Holstein or Holstein cross, male calves were approximately 14-weeks old at the time the study was initiated. Calves were seronegative for antibody to *M. haemolytica* leukotoxin; negative for persistent infection with Bovine Virus Diarrhea Virus; and, were not previously vaccinated. Calves had *ad libitum* access to fresh water and feed medicated with an ionophore. No other medications that could affect the vaccine or the virulent challenge organism were added to the water or the feed.

A completely randomized, 2 x 2 factorial, design was used; the individual calf was the experimental unit; and, 22 calves were enrolled per treatment group. Each calf was randomly assigned to one of four treatment groups:

1) an experimental intranasal (IN), monovalent, live, attenuated vaccine (VAX) that contained a proprietary seed stock of *M. haemolytica*;

2) VAX (IN) + tildipirosin (4 mg/kg; SC);

3) Placebo vaccine (PLBO) that contained the same medium and filler as in the experimental vaccine but without the bacterial antigen (IN);

4) PLBO (IN) + tildipirosin (4 mg/kg; SC).

After treatment was applied (Day 0), calves were housed in pens containing only individuals from a treatment group. The vaccine (VAX) was prepared using the same seed culture and titer of *M. haemolytica*, as commercially available in Merck Animal Health's licensed vaccines (Madison, NJ, USA).

Two calves in treatment group 2 and two calves in treatment group 4 died due to respiratory disease, unrelated to the experimental procedures, prior to the day of challenge (Day 70). The solution used for challenge was prepared with virulent *M. haemolytica* in Tryptic Soy Broth (TSB) (30 to 50 mL to deliver similar bacterial challenge); and, was administered intratracheally. After challenge on Day 70, calves remained in the same pen to the end of the study and were observed daily at approximately the same time each day. Clinical signs (respiratory scores, attitude scores, rectal temperature) of all calves were recorded. On Day 77, (7 days post-challenge) the calves were euthanized and lesions in the lungs were scored independently by two individuals. The average of those two scores was used for analyses. Samples (10 to 20 gm) of lung were submitted (within 48 hours of collection) for isolation of *M. haemolytica*. Personnel recording clinical scores, scoring lung lesions, or performing bacterial isolation procedures were blinded to the treatment group to which the animal was assigned.

The primary outcome variable was the Lung Lesion Score (LLS). Secondary outcome variables were clinical signs of respiratory disease (respiratory score, attitude score, rectal temperatures), and isolation of bacteria from samples of lung.

Results: There was no significant (P = 0.51) effect of tildipirosin on LLS whether calves were vaccinated with VAX or PLBO; however, VAX resulted in significantly (P = 0.046) lower LLS (VAX median = 1.48%) than did PLBO (median = 3.25%). Calves in all groups developed clinical signs; and, there was

no significant ($P > 0.05$) effect of any experimental treatment, on clinical signs. The percent of calves from which samples of lung yielded *M. haemolytica* was not significantly affected by VAX or PLBO.

Conclusions: Under the conditions of this study, VAX (IN) administered concurrently with tildipirosin (SC) was proven efficacious after an *M. haemolytica* challenge.

Keywords: Tildipirosin, intranasal vaccination, Mannheimia haemolytica, efficacy.

ID-29

Evaluation of risk factors of umbilical infection in newborn beef calves

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Objectives: To describe and assess the potential risk factors associated with umbilical infection in beef farms located in the center of France, focusing on the passive immune transfer (PIT).

Materials and methods: Newborn calves from 22 French beef farms were followed up during a calving season (between November 2020 to March 2021). Each farm was visited twice a week. During the first visit (calves between 1 and 5 days of age), information on zootechnical practices and calving conditions was collected. During the first and the second visits (7 days after the first visit), housing conditions (cleanliness and humidity) and omphalitis were scored. Omphalitis scoring comprised thickening of umbilical stump (0 if < 2 cm and 1 if > 2 cm), presence or absence of purulent discharge (score 0 or 1), local pain (0 if no withdrawal when slight pressure applied, 1 if any) or ultrasound of internal structure. Each calf was also sampled during the first visit to assess the quality of passive immune transfer through serum total protein and Brix value optical refractometry.

Results: Nine hundred sixty-four calves were included in the study. The prevalence of omphalitis was 34% (326/964), first and second visits coupled. Univariable and multivariable statistical analysis revealed that ($P<0.05$): (i) male calves are more at risk of developing omphalitis than females (OR=2.6); (ii) the prevalence of umbilical infection is higher for calves weighing more than 50 kg and for calves with an umbilical cord length strictly less than 3 cm; (iii) a dirty calving pen increases the risk of omphalitis (OR=1.8); (iv) calves born from primiparous dams are more likely to develop an omphalitis than multiparous (OR=1.4). Furthermore, no statistical association was found between failure of passive immunity transfer (medium or bad) and omphalitis development (χ^2 Pearson > 0.05).

Conclusions: This study is the first to report the prevalence of omphalitis in beef cattle with a prevalence of 34% (326/ 964). Development of omphalitis was not associated with a failure of passive immunity transfer in this study. Risk factors related to calves were: sex, weight and umbilical cord length. Moreover, calving hygienic conditions are of prime importance to prevent omphalitis in the field.

Keywords: Beef calves, omphalitis, risk factors, passive immune transfer.

ID-30

Identification of BRD antibodies to install a tailor-made BRD Prevention Plan on Dutch dairy farms

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Objectives: Bovine Respiratory Disease (BRD) is a multifactorial disease of young cattle. The factors that predispose to BRD include stress related to stocking, moving or mixing cattle, poor ventilation or draughts, sudden climatic changes or extreme heat or cold, mixing various age groups, nutritional deficiencies, colostrum deficiency, and poor feed hygiene. These stresses lead to infection by primary viral pathogens which cause lung damage and may pave the way for various bacterial pathogens as *Mannheimia haemolytica (Mh)* and *Mycoplasma bovis*.

Vaccination is an important tool in managing BRD. Identification of major respiratory pathogens on a BRD problem farm may be essential to establish an appropriate vaccination program.

In 2019 MSD Animal Health started a BRD Prevention Plan including a serological screening, the BRD QuickScan, to identify which BRD pathogens are circulating on a farm. The BRD Prevention Plan is a decision tree guiding veterinary practitioners to manage BRD. Depending on the results of the BRD QuickScan, a tailor-made advice including BRD vaccination and improvement of BRD management factors is given.

This manuscript gives an overview of the BRD QuickScan results in 2020 and 2021 on Dutch BRD problem farms not vaccinating against BRD.

Materials & Methods: To run the BRD QuickScan, serum samples were taken from 5 calves (3-6 months old) on a BRD problem farm or BRD suspected farm. Those samples were analysed in the Centre for Diagnostic Solutions (MSD Animal Health, Netherlands) for antibodies against *Mh,* Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza 3 (PI-3) and *Mycoplasma bovis* by ELISA. An in-house test was used to measure *Mh* and BRSV antibodies, whereas for PI3 and *Mycoplasma bovis* a commercial kit was used from respectively IDEXX and Bio-X. The results of samples collected between October 2019 to December, 2021 are presented.

Results: During the defined timeframe, 325 BRD QuickScans were performed. In 1% of the BRD QuickScans, no antibodies against the selected BRD pathogens were identified. In those farms, a tailor-made approach was provided to improve the BRD status (www.rescalf.nl).

Eighty-three percent of the QuickScans were positive for antibodies against *Mh,* 5% of the QuickScans had only *Mh* antibodies, where in 78% of the QuickScans antibodies against *Mh* and viral BRD pathogens (BRSV and/or PI3) were present. On farms positive for *Mh,* vaccination with a vaccine providing

protection against *Mh* (Bovilis® Bovipast® RSP) was advised. Additionally, if on such farms BRD was already present in very young animals, supplementary vaccination of the pregnant dams was advised to improve the maternal antibody protection in those calves.

Eighty-nine percent of the QuickScans were positive for BRSV and/or PI3. Nine percent of the QuickScans were only positive for PI3 antibodies and none were only positive for BRSV antibodies. These findings confirm the importance of the viral pathogens in the BRD complex, mainly combined with *Mh*. On farms where high antibody titers against viral pathogens were detected, and BRD problems already were apparent in very young calves, an intranasal vaccination of the calves at the age of 1 week using a live BRD vaccine (Bovilis INtranasal RSP live) was recommended.). This could be followed by vaccination with a trivalent inactivated vaccine (Bovilis® Bovipast RSP) at a later age.

Twenty-two percent of the BRD QuickScans were positive for *Mycoplasma bovis*. On *Mycoplasma bovis* farms, a specific approach was recommended as no commercial vaccines are available. It was advised to create small groups of animals, avoid mixing of calves from several groups, and to vaccinate against other major BRD pathogens.

After the implementation of the BRD prevention plan including the BRD QuickScan, over 80% of the farms started vaccinating with an inactivated BRD vaccine (Bovilis® Bovipast® RSP), 9% started with a live intranasal BRD vaccine (Bovilis® INtranasal RSP® Live) and on 3% of the farms complementary examinations were performed. About 10% of the farms started the additional pregnant dam vaccination with Bovilis® Bovipast® RSP.

Conclusion: For most pathogens circulating on BRD farms vaccines are commercially available. Implementing a tailor-made BRD management program including vaccination is important to reduce BRD related losses. Practical tools as the BRD Prevention Plan and BRD QuickScan may be useful to reduce BRD. Based on serological findings farmers can easily be convinced (in dialogue with their veterinarians) to vaccinate against BRD.

Keywords: Bovine Respiratory Disease, QuickScan, serology, dairy, Netherlands.

ID-31

Elucidation of the efficacy of fecal microbiome transplantation (FMT) in healing calves with intractable diarrhea

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Objectives: Fecal microbiome transplantation (FMT) has shown promising results that indicate the effective treatment of diarrhea in calves although the mechanisms by which FMT works has not yet been elucidated. Therefore, the aim of this study is to elucidate the efficacy of FMT in intractable diarrhea treatment and searching the potential bacterial taxa and metabolites responsible for FMT success.

Materials & Methods: Total 20 FMT trials, in which feces obtained from healthy donors were intrarectally transferred into recipient calves with diarrhea, were performed. Fecal samples were collected from donors on the day of FMT and from recipients before and on days 1 and 7 after the FMT. The samples were analyzed by high-throughput 16S rRNA gene sequencing, metabolomics via capillary electrophoresis time-of-flight mass spectrometry, and ELISA, respectively.

Results: Among the 20 FMT treatment, total 14 (70%) treatment were succeeded based on the clinical findings, diarrheal, metagenomics and metabolomics results. Considering the beta diversity, unweighted unifrac distance was found significantly different before FMT (D-success vs R0-success), but not in after FMT at day 7 (D-success vs R7-success) in successful FMT group. In unsuccessful FMT treatment, there was no significant difference observed between donor and recipient in before and after FMT. Thus, in unsuccessful FMT treatment group, calf exhibit impaired engraftment of the FMT bacterial community and failed to restore the commensal bacteria as well as metabolites, due to lack of optimal donor. On the other hand, genus *Selenomonas* confirmed donor–recipient compatibility in successful FMT treatments. A strong positive correlation between the microbiome and metabolome data, which is a prerequisite factor for FMT success, was confirmed by Procrustes analysis in successful FMT (r = 0.7439, P = 0.0001). A reduction in fecal amino acid concentration was observed in succeed treatment, which is strongly correlate with the remission of diarrhea. Additionally, weighted gene correlation network analysis confirmed the positively or negatively correlated pairs of bacterial taxa (family *Veillonellaceae*) and metabolomics features (i.e., amino acids and short-chain fatty acids) responsible for FMT success.

Conclusions: The findings obtained from the present study suggest that the FMT may directly or indirectly promote the cohabitation of certain bacterial taxa, which facilitate to recover recipient calves from intractable diarrhea in successful FMT treatment group.

Keywords: Diarrhea, microbiome, transplantation, metagenomics, metabolomics.

ID-32

Prevalence and antimicrobial susceptibility of BRD pathogens isolated from cattle with respiratory disease during over 10-years of supported testing program in Germany

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Objectives: Trans-tracheal aspiration (TTA) is a technique used for *in vivo* identification of mainly bacterial pathogens from cattle with bovine respiratory disease (BRD). MSD Animal Health in Germany has been supporting bacteriological analysis of samples from TTA for veterinarians and farmers since 2009.

The objective of this study was to investigate the dynamics in the prevalence of BRD associated bacteria (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Trueperella pyogenes*) in the lower respiratory tract of calves with respiratory disease and their antimicrobial susceptibility over the past 13 years.

Materials & Methods: During the period of 2009 to 2021 samples were collected through TTA from calves with respiratory disease after a specific request by veterinarians to identify BRD related pathogens. The samples were collected from calves located on farms in all regions of Germany, dairy farms as well as fattening units. None of the sampled animals received an antimicrobial treatment prior to sampling. If possible, samples from 3 calves were pooled (e.g. animals housed in the same pen).

Isolation and identification of BRD-associated bacteria from the TTA samples were performed using bacterial culture, MaldiTof and subsequent determination of antimicrobial susceptibility by microdilution (samples collected since 2013 only). Isolates were evaluated using Clinical and Laboratory Standard Institute (CLSI)-approved methods. For *T. pyogenes* no antimicrobial testing was done as antimicrobial therapy is not considered viable and therefore no CLSI methods are available.

Results: During the period of supported testing, 1726 calves were sampled on 361 farms. As some samples were pooled, a total of 611 samples were submitted to laboratory testing. BRD-associated bacteria were isolated from 66.3 % of all samples with a yearly range between from 55.2 – 78,8 %.

P. multocida was the most prevalent bacterium (61.1 %; 33.3 – 73.9%) followed by *M. haemolytica* (16.6 %; 5.3- 33.3%). The isolation prevalence for *H. somni* was 7.3 % (0 – 15.6 %) and 15.0 % (6.9 – 26.3 %) for *T. pyogenes.* While there was no obvious trend, the prevalence of the four pathogens showed large variation from year to year.

None or only a few isolates of *P. multocida, M. haemolytica,* and *H. somni* were resistant to amoxycillin, ampicillin, ceftiofur, florfenicol, gentamycin, and trimethoprim sulfonamide. The percentage of *M. haemolytica* isolates that were susceptible to penicillin was 75 %, to enrofloxacin 97 %, to tetracycline 85 % and to tilmicosin 60 %, with only small variation during the years. Ninety five percent of *P. multocida* isolates were found to be susceptible to penicillin, 92% to enrofloxacin, 73% to tetracycline and 66% to tilmicosin. No isolate of *H. somni* was resistant to penicillin and enrofloxacin, while 96% of isolates were susceptible to tetracycline and 75% to tilmicosin.

Conclusion: *P. multocida* was the most prevalent bacterium isolated from the lower respiratory tract of calves with respiratory disease during the period of 2009 till 2021 in Germany. However, prevalence up to 30 % were found for *M. haemolytica* in certain years.

The results of antimicrobial susceptibility testing showed that *M. haemolytica*, *P. multocida,* and *H. somni* exhibited *in vitro* resistance to some important antimicrobial products that are frequently used to treat respiratory disease. On the other hand, it was also clear that several other antimicrobial treatments are available to which the isolated bacteria showed high susceptibility.

Keywords: Respiratory disease, trans-tracheal aspiration, BRD associated bacteria, prevalence, antimicrobial susceptibility.

ID-33

Mycoplasma bovis antibody testing in purchase protocol to reduce circulation between farms

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Background & Objectives: *Mycoplasma bovis*' importance as a causal pathogen of pneumonia, arthritis, mastitis, and other diseases has been well established in the last decades. In Belgium, a steady increase in *M. bovis*' presence has been noticed in bovine herds: in 2009, only 1.5% of dairy farms tested positive on culture of bulk tank milk (BTM) (1), while in 2011, already 11% of calves sold to the veal sector (corresponding with about 11% of herds) had antibodies at arrival (2), and in 2016, 32% of all dairy farms had either PCR or antibody positive BTM samples (3). This increase is worrying, given the enormous economic impact a *M. bovis*' introduction and circulation can have on a farm. The main cause of the increase is probably the purchase of carriers, given the enormous amount of cattle trade in Belgium: between 2005-2009, 40% of the cattle born in Belgian farms changed farms at least once (4).

As detecting carriers is not evident, given the intermittent excretion and existence of asymptomatic carriers, it is currently recommended to not purchase antibody positive animals to avoid *M. bovis* introduction in negative herds. To determine the risk of purchase of *M. bovis* antibody positive animals in Belgian herds, this study aimed to determine the number of animals testing antibody-positive at purchase.

Materials & methods: Throughout 2021, *M. bovis* antibodies were determined on every purchase protocol requested at 2 reference laboratories (ARSIA & DGZ), using a *M. bovis* antibody ELISA (BIO K432, Bio-X Diagnostics S.A., Belgium).

Results: In total, blood samples of 76285 animals were analyzed, of which 14.96 % (n= 11416) tested positive on M. bovis antibodies. One thousand two hundred twenty-seven animals were retested approx. 30 days after the first sample. Of these, 87,8 % kept the same result, 5,9 % seroconverted and 6,3 % seroreverted.

Conclusions: There is a non-negligible risk in introducing possible *M. bovis* carriers in seronegative herds trough purchase. There was 6,3 % seroreversion after 30 days, which could be due to antibodies truly dropping underneath the test

limit, given the poor persistence time of *M. bovis* antibodies (5), or could indicate the presence of false positives even with a test specificity of 97 %. For the seroconversion rate, the argument is different: probably, the 5.9 % conversion is only partially due to true seroconversion, but also due to the poorer sensitivity of the test used (Se 80.95%).

Given the possible presence of false negatives and the poor persistence of antibodies, it is quite possible that a part of the animals from *M. bovis* positive farms were missed in the first analysis. As such, it is advisable for *M. bovis* negative herds to test purchased animals twice, both at the beginning and the end of the 30-day quarantine, before releasing them in the herd. If either test is positive, the animal should be considered at-risk. Another option would be to test- or interpret testing on herd-level instead of animal level.

In conclusion, further research to identify *M. bovis* carriers, or development of farm-level testing procedures is sorely needed to stop the introduction of *M. bovis* into seronegative farms.

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Keywords: Mycoplasma bovis, purchase, biosecurity, ELISA, surveillance.

ID-34

Validation and use of a new diagnostic protocol for Johne's disease control in New Zealand dairy herds

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Objectives: This study reports the diagnostic accuracy and use of an initial screening test of four serum ELISAs followed by a confirmatory test of one quantitative fecal PCR (fPCR) for the diagnosis of paratuberculosis in mixed aged milking cows in New Zealand.

Materials and methods: Data from a cross-sectional study of 20 moderate prevalence herds was combined with existing data from low and high prevalence herds to form a combined dataset of 3,845 paired serum and fecal samples. Records of incidence of clinical cases of Johne's disease (JD) were used to classify herds into three prevalence categories. High prevalence herds (> 3% clinical JD per year for the last three years), moderate prevalence herds (< 1%) and low prevalence herds (zero incidence of clinical JD for at least the last five years). Positive ELISA data were declared if > 50 ELISA units and fPCR data at two cut-points (≥1x10^4 genomes/mL or >1x10^3 genomes/mL).

Fixed and mixed Bayesian latent class models were constructed at both fPCR cut-points, accounting for conditional independence, paired conditional dependence and all possible dependencies between tests using OpenBUGS. The aim was to identify *Mycobacterium paratuberculosis avium* (MAP) infected cows that met at least one of two criteria: shedding sufficient MAP in faeces to be detected by fPCR or mounting a detectable MAP antibody response.

The effect of using this new testing method as part of Johne's control programme in a large milking herd over a four-year period is also reported. A sub-set of this data was also used to look at the effect of infection status on milk production in sub-clinically infected cows (reported at Buiatrics Dublin, 2016).

Results: ***Model validation:*** The best fit to the data was obtained by modelling either pairwise dependencies between tests in a fixed model or including all dependencies in a mixed model at a faecal cut-off of ≥1x10^4 genomes/mL. Test performance differed with prevalence: for the random model, at a prevalence of 0.38 (95% predictive interval,(PI)=0.30-0.44), sensitivity was 0.54 (95%PI=0.47-0.62) and specificity 0.98 (95%PI=0.96-1.00). At a low prevalence (0.01 (95%PI=0.00-0.03), test sensitivity was 0.60 (95%PI=0.42-0.72) and specificity 1.00 (95%PI=1.00-1.00).

Models were robust to prior assumptions and this testing protocol had a positive predictive value of 0.96 (95%PI=0.87-1.00) in high prevalence herds and 1.00 (95%PI=0.99-1.00) in low prevalence herds. Correspondingly, the negative predictive value in high prevalence herds was 0.78 (95%PI=0.71-0.84) and 0.98 (95%PI=0.84-1.00) in low prevalence herd.

Reduction in prevalence of Johne's disease: We also report the successful reduction of infectious and infected animals in an endemically infected herd, where over a 4-year period, an annual test and cull policy using this approach reduced seroprevalence of positive cows from 26% to 2% and the proportion of clinical JD culls from 5% to 0.4%. Over this period, the seroprevalence in primiparous cows fell from 15% to 2.5%.

Effect of infection status on milk production: Previously, this testing method has been used to identify under-performing MAP infected animals with clinically normal, ELISA-positive animals producing 4% fewer kg milk solids (kgMS) per lactation and faecal positive cows producing up to 12%

fewer kgMS (World Buiatrics, Dublin 2016).

Conclusions: The results presented suggest that this is a useful tool in the control of JD on dairy farms, particularly in herds with higher levels of infection, where the sampling and testing cost per animal is defrayed across more detected animals.

Keywords: Johne's disease, Mycobacterium paratuberculosis avium, sensitivity, specificity, control.

ID-35

Tuberculosis diagnosis in bovine herd and associated cross reaction in the tuberculin skin test (PPD): a case report

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Abbreviations:

PPD - Purified protein derivative ((PPD) skin test (for tuberculosis))

T3 - Officially holdings free of tuberculosis (RD 1716/2000)

PCR - Polymerase Chain Reaction

Introduction: Bovine tuberculosis is a mandatory disease under Eradication according to the Spanish Tuberculosis Eradication Program. This program is based on the detection of positive animals using both, simple and comparative Tuberculin Skin tests (PPD also called Mantoux test in human medicine) and the elimination of the reactive animals.

The characteristics of the test (sensitivity, specificity and the predictive values) as well as the characteristics of the microorganism (different etiology complexes, species and subspecies) can produce, in some cases, cross reactions or interactions with other microorganisms that cause false positivity results in the tuberculosis program.

One of those cases is presented in this communication at the same time that it reflects, based on the origin of the problem, the importance of biosecurity as a basis for prevention.

Methodology and results (the case): The case took place in a cattle herd (Blond d´Aquitaine), which began its activity with 20 heifers aged between 11 and 16 months in 2016. Animals were introduced in the farm with a semi-intensive production system that included a covered ship and a park limited by a perimeter fencing. The farm was not in contact with other livestock, but in the area there were wild boars that are under control in the Wild Tuberculosis Surveillance Program (PATUBES) on which all the results were negative (0% prevalence).

The origin of the animals was a T3 farm (Tuberculosis free qualification level in the Spanish Tuberculosis Eradication Program). Previously to the movement of the animals, all of them were tested by a simple PPD tests which resulted negative in all of them.

In the reproductive control period, one of the animals was diagnosed with a congenital disorder that excluded it for the future. Therefore, the owner and the provider agreed to change it by one another heifer that arrived pregnant and brought a negative result to the simple PPD test, which carried to the farmer to introduce it directly into the flock.

After delivery, the new animal was affected by a enteritis process that was diagnosed as Bovine Paratuberculosis by staining Ziehl-Neelsen in feces samples and serology (Elisa test). After, the animal was isolated and slaughtered in the farm. PPD test was carried out in all the animals according to the Spanish Tuberculosis Eradication Program, on which 10 of the 20 animals resulted positive (differential values between the first and the second measures of 2.5-6 mm).

Results were contrasted by Compared PPD test which resulted in five positive animals that were sacrificed. These animals were sent to the slaughterhouse where samples were taken and sent to the Reference Center for Veterinary Health Surveillance (Visavet). The microbiological study of the samples of three animals showed a positive growth in specific Mycobacterium medium that resulted negative by PCR to both: Mycobacterium tuberculosis and Mycobacterium avium subspecies suis. It was concluded that positive reaction to the PPD test was not due to bovine tuberculosis.

At the same time, four animals of the farm were seropositive to Paratuberculosis (Elisa test) and one of them evolved in a clinical way and was sacrificed. After, a Paratuberculosis control strategy was applied in the farm at the same time that PPD tests continued with negative results in all animals.

Conclusions: The final conclusion is that there was a false positive reaction to the simple and compared PPD test that was not confirmed either by clinical or microbiology, which suggests any type of interference with some another Mycobacterium spp as it has been referred in another cases. While it is not possible to confirm it, we suspected that the interference in the test was caused by Mycobacterium avium subspecies Paratuberculosis since the outbreak occurred in the exploitation in that particular period.

Keywords: Tuberculosis bovine, Tuberculin skin test, Paratuberculosis.

ID-36

A sudden outbreak of paratuberculosis in a previously test-negative dairy herd; a case report

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Objectives: Paratuberculosis (Johne's disease) in dairy cattle, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is subject of an active control policy by the Dutch dairy industry, aiming to reduce the concentration of MAP in bulk milk delivered to the milk factories. In this abstract, we describe an outbreak of MAP infections in a previously test-negative dairy herd to show the complex epidemiology of paratuberculosis in dairy farming and the necessity

to take continuous precautionary measures, even on MAP test-negative farms.

Material and Methods: The high prevalence of paratuberculosis was detected in a dairy herd of ~180 adult cattle and ~150 young stock. Cows were housed in a freestall with cubicles and grazed during the summer months. Young stock were housed in a separate barn until ~18 months of age. The herd was not closed: in 2017 a total of 79 homebred young stock were temporarily raised on pastures of two other farms and between 2013 and 2018, another 53 animals were purchased from five different herds. Young calves were fed colostrum followed by milk replacer, grass silage harvested on the farm and concentrate.

Between 2008 and 2018, milk samples of all lactating cattle were tested biannually with the IDEXX Paratuberculosis Screening Ab Test (ELISA), using an elevated cut-off S/P ratio of 1.0 in order to increase the diagnostic specificity of the test. Until 2016, negative results were obtained only. However, in July 2018, three out of 132 tested lactating cows had a positive ELISA result. MAP infection was confirmed by positive faecal qPCR in two of these three cows, aged 6 and 7 years and homebred (index cases). As a follow up, cattle ≥ 12 months of age were tested by individual faecal qPCR in April / May 2019.

Results: In April/May 2019, 253 animals were individually tested by qPCR on rectally derived faecal samples. Twenty animals tested positive. All of these animals were born on the farm between January 2010 and July 2017, except one cow that was introduced from another herd at the age of 4 years. Neither the category of animal (homebred, temporarily raised in another herd, or introduced from another herd) nor age category (1, 2, 3 or ≥4 years of age) were significantly associated with the PCR result. However, a cluster of qPCR-positive homebred heifers born in 2017 became apparent: 7 of 12 tested heifers born in April and May 2017 in the case herd were qPCR-positive. These heifers had not been temporarily raised elsewhere. The dam of the oldest of these 12 heifers tested qPCR positive as well; 4 other dams which were still present on the farm tested qPCR negative. Also a heifer born in July 2017 tested positive whilst its mother tested negative on qPCR.

After informing about the management conditions around the time of birth of these 7 heifers the farmer indicated that the calving pen hygiene had been suboptimal and pooled colostrum and bulk milk had sometimes been fed to calves.

Conclusion: In this case herd, a cluster of MAP shedding cattle could be related to birth cohort. Potential transmission routes were a contaminated calving pen, transmission through feeding of colostrum and bulk milk from multiple cows to calves and calf-to-calf transmission. Our observations stress the importance of structurally taking preventive management measures to reduce the spread of MAP in test-negative herds as well.

The aim of the Dutch milk quality assurance program is to reduce the concentration of MAP in bulk milk and to provide assurance regarding milk quality in certified test-negative herds. For this purpose, herds do not necessarily need to be free of MAP infection. However, if farmers aim to eliminate MAP infection from their herds by test-and-cull, it is important to consider that faecal qPCR has a considerably higher diagnostic sensitivity to detect MAP shedding animals compared to the ELISA test and that a considerable proportion of infected cattle start shedding MAP before adulthood - as illustrated by the observations in this case herd.

Keywords: Paratuberculosis, Johne's disease, dairy.

ID-37

Detection of *Anaplasma phagocytophilum* in bovine abortions in Flanders

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Objectives: To monitor brucellosis in Belgium, farmers are obliged by the Federal Agency for the Safety of the Food Chain (FASFC) to submit each bovine abortion for analysis. Besides brucellosis monitoring, the abortion program concurrently screens the submitted samples for multiple infectious pathogens. Despite the extensive number of analyses included in this abortion program, about 60% of cases remain undiagnosed. One of the explanations could be the presence of unidentified abortifacients like *Anaplasma phagocytophilum*. The aim of this study was to evaluate bovine abortions in Flanders for the presence of *A. phagocytophilum* during the tick season (July-September), since anaplasmosis is a tick born disease.

Materials and methods: Between July and September 2012, the placenta and fetal spleen of 150 late term (> 7 months pregnant) bovine abortions were collected and analysed using an *A. phagocytophilum* PCR. All abortion cases were also tested for brucellosis by *Brucella* spp culture on the placenta and serology on maternal serum, for *Neospora caninum* by analysis for antibodies on maternal serum and for bovine viral diarrhea virus (BVDv) by antigen ELISA. The aborted fetus was examined for bacteria by aerobic, *Listeria* spp and yeast/fungal cultures on abomasal and lung tissue.

Results: Seven of 150 abortion cases (4.7%) were BVDv Ag positive on fetal spleen. In 12 of 150 cases (9.7%) yeasts or moulds were detected. Bacteria were found in 59 of 150 (39.3%) fetal abomasal samples and in 46 of 150 (30.7%) fetal lung samples. Seroprevalence of *Neospora caninum* in the aborted dams was 15.33% (23/150).

Of the placenta samples, 2.66% (4/150) was positive for *A. phagocytophilum*, while none of the fetal spleen samples was. No positive samples were found in the 7th month of gestation, while 1 positive sample originated from an abortion case that occurred in the 8th month of gestation and 3 samples were from abortions that happened in the 9th month of gestation.

Conclusions: The detection of *A. phagocytophilum* in late term bovine abortions suggests a potential role of this pathogen in (un)diagnosed cases of bovine abortion in Flanders. Placental tissue is probably the most preferred tissue to detect this pathogen in case of an abortion. Based on these findings, it can be interesting to include an *A. phagocytophilum* PCR in

the abortion monitoring program during the tick season.

Project financially supported by the FASFC and Sanitary Fund.

Keywords: Bovine, abortion, Anaplasma phagocytophilum.

ID-38

Biosecurity and biocontainment risks of Mycobacterium avium subspecies paratuberculosis entering and spreading in UK dairy herds

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Objectives: The risks of Johnes disease entry and spread in over 3000 UK dairy herds using a risk assessment tool to measure, monitor and manage risks were studied to identify significant risks, with the objective of allowing farmers to manage those risks that predisposes their cattle to Johnes Disease.

Materials and Methods: A web based risk assessment tool was used by trained veterinary surgeons to assess, measure and monitor biosecurity and biocontainment risks on over 3000 UK dairy herds that engaged in some form of Johnes Disease management. The tool uses standard assessments and an algorithm to quantify risks and provide a summary and priority to identify and manage risks as part of the control plan.

Results: Biosecurity risks were defines as the risks of Mycobacterium Avium subspecies Paratruberculosis (MAP) entering the herd. 50% of herds were designated high risk of entry of MAP in to the herd, mostly due to purchasing cattle of unknown disease status and allowing cattle to drink from water courses that had passed through other livestock farms. Only 20% of dairy herds were designated as low biosecurity risk for the entry of Johnes disease.

Biocontainment risks were defines as those risks that predisposes to the spread of MAP within the herd. These risks are associated with prevalence of Johnes Disease within the herd and act as the multiplier of disease. 65% of herds were designated as high risk of disease spread, mostly due to the use of multiple calving areas and poor perinatal hygiene, and the use of pooled colostrum taken from high risk cows. Only 8% of dairy herds were designated as low risk of spread.

Of 2462 herds that were designated as having high risks of spread of MAP within their herds, 52% had high risks of entry of the disease, making these herds very high risk of a high prevalence of Johnes disease. 85% of these high risk herds were designated as infected by the attending veterinary surgeons.

Conclusions: Modern dairy farming systems tend to predispose to the entry and spread of MAP in dairy herds. Prevention and control of the disease will require significant changes in management and husbandry to prevent the entry and spread of the disease. The identification of specific risk factors allows rational management systems to be introduced to limit the spread of the disease in infected herds, and maintain freedom form disease in uninfected herds.

Keywords: Paratuberculosis, Johnes, biosecurity, biocontainment, risks.

ID-39

Mycobacterium Avium subspecies Paratuberculosis (MAP) Elisa tests on milk to predict future health and culling in a dairy herd

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Objectives: This study investigates the use of serial milk MAP elisa tests to predict health, productivity and emergency culling of adult dairy cows.

Material and methods: The study involved a large dairy herd of over 900 adult cows using milk MAP elisa tests on all milking cows every three months as part of a Johnes Control programme. Over 3000 adult cows that had been culled from the herd over a period of ten years, and which had milk elisa test results for their entire adult life were studied to determine if test results in the could be used to create a robust plan to manage test positive cows to predict and prevent health problems. Udder health, lifetime milk yield, fertility and emergency culling (as defined by being culled within 100 days of calving) were used to define health and productivity.

Results: Cows that had just one positive MAP elisa test in their lifetime had similar health and productivity to cows that had never had a positive test. Cows that had at least 2 consecutive positive tests had significantly higher somatic cell counts and higher emergency culling rates (34% of these cows were culled within 100 days of calving). Cows with at least four positive MAP elisa tests, with rising titres at each consecutive test had a very high emergency culling rate, such that 46% of these cows were culled within 100 days of calving for severe health issues. There was little difference in milk yields between positive and negative test cows.

Cows with repeated positive MAP test results have high risks of health problems that lead to emergency culling, with the consequent major economic losses incurred when culling lactating cattle with no salvage value.

Conclusion: Rising titres of MAP antibodies as measured by milk elisa tests indicate imminent severe health problems with poor prognosis.

Keywords: MAP, Elisa, Johnes, milk.

ID-40

Systematic review of herd-level test characteristics for *Mycobacterium avium* subspecies *paratuberculosis* in cattle

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Objective: Conduct a systematic literature review to summarise the published estimates for herd sensitivity (HSe) and herd specificity (HSp) of diagnostic tests for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in cattle.

Materials and Methods: A comprehensive literature search was performed in July 2020 to identify all published papers, including conference proceedings, published up to the date of the search, referring to herd sensitivity (HSe) and/or herd specificity (HSp) of a test method for MAP in cattle. All articles returned in the search went through a systematic four-stage screening process to identify relevant studies. Articles were included if they were written in English, available in full-text, had cattle as the species of interest (either dairy or beef) and contained an evaluation of the HSe and/or HSp of a diagnostic test method for MAP. Data extracted from each publication included sample population/s, method/s of analysis, reference tests, cut-off points, HSe and HSp. The relevant publications were classified based on the test method evaluated, and the results for each test method were summarised as a range of reported estimates.

Results: Forty-six publications with relevant results were eligible for inclusion in the final review, containing evaluations of whole-herd ELISA testing, bulk milk tank (BMT) ELISA, culture and PCR, pooled faecal testing and environmental sample testing. The ranges for HSe and HSp reported for each test method is summarised in Table 1.

Table 1. Summary of Johne's disease herd-level test characteristics.

Screening test	Herd sensitivity (%)	Herd specificity (%)
Whole-herd ELISA	40-100	21-96
Whole-herd ELISA + PCR	9-86	100[b]
BMT ELISA	8-97	53-100
BMT PCR/culture	0-85	100[b]
Pooled faecal testing	26-100	100[b]
Environmental sampling[a]	24-100	100[b]

[a] From studies evaluating a protocol using six composite samples
[b] Herd specificity can be assumed to be 100% due to direct detection of MAP bacteria.

The reported herd-level test characteristics for MAP demonstrate considerable differences in test accuracy. The wide ranges in reported estimates for each test are due to the variations in between-and within-herd prevalence, test protocols and cut-off points between studies. Whole-herd ELISA testing has potentially high HSe (40-100%) but potentially low HSp. This will result in many non-infected herds being classified as infected. The choice of seroprevalence cut-off point (ranged from >0% to 3% seroprevalence) for defining a herd as infected affects the estimates reported for whole-herd serology. The poor HSp of whole herd ELISA testing can be overcome by ancillary faecal testing of ELISA-positive animals, with an associated reduction in HSe. Bulk milk tank ELISA HSe estimates vary widely depending on the S/P ratio cut-off point used in the study. When manufacturer-recommended cut-offs are used, the HSe for BMT ELISA ranges from 8-30%. Environmental sampling and pooled faecal testing have relatively high HSe, 24-100% and 26-100%, respectively, and 100% HSp due to direct detection of MAP bacteria.

Conclusion: There are numerous options for herd-level testing for MAP in cattle. However, it is clear from this review that there are wide ranges in HSe and HSp estimates between studies. This makes it difficult to draw conclusions regarding the predicted performance of a test in a specific population with unknown disease prevalence. Decision-makers must balance the test characteristics with the resources available (funding, laboratory capacity) to identify the most suitable herd test method(s) in a population.

Keywords: Paratuberculosis, systematic, diagnostic, sensitivity, specificity.

ID-41

Control of paratuberculosis in small structured cattle farms

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Objectives: Since 2013, a voluntary survey and control program for MAP (*Mycobacterium avium subsp. paratuberculosis*), the cause of paratuberculosis (Johne´s disease) in cattle, is in place in the Austrian province of Tyrol. This alpine province is characterized by a small structured, traditional way of cattle farming. The aim of the present study was to evaluate data collected in the course of the MAP-program, related to the prevalence of MAP, as well as to the dynamics of the infection.

Material and methods: About 4,600 farms, representing approximately 70% of the Tyrolean dairy cattle, are participating in the MAP-program. In course of the program, MAP-positive farms are detected by boot swab sampling in a two years interval, followed by single animal testing and removing of positive animals. Additionally, basic hygienic measures to prevent further spread of the infection are advised in positive farms. During the program, structural date describing the farm characteristics and management practices were collected.

Results: In course of the program, the initial prevalence of 7.5% positive herds in 2013 decreased to 0.5% in 2019.

Detailed investigation of individual animal results revealed, that MAP-shedding decreased considerably after removal of single positive animals within a herd. Surprisingly, many cattle showed negative individual results and farms stayed MAP-negative in consecutive boot swab samplings thereafter, indicating possible passive shedding in some animals. Furthermore, these findings suggest, that fade out of the disease, after removal of MAP-shedding animals and the implementation of hygienic measures, may occur.

Analysis of risk factors showed, that the use of common alpine pasture seems not to be a significant contribution to the transmission of MAP. The same was found for sharing of equipment, which both seems to be in contrast to the current literature. On the other hand, rearing of calves with milk replacer, instead of whole milk, significantly decreased the chance to be MAP positive, which has been described before. Housing also seems to have an impact on the MAP-status of the farms, but the results are ambiguous and need further evaluation.

Conclusions: The data collected over a period of ten years indicate, that the use of common (alpine) pastures seems not to be a significant factor for the distribution of MAP. Furthermore, the dynamics of MAP infections in small structured cattle farms may contribute to the successful reduction of the MAP-herd prevalence by removing single positive animals and implementing basic hygienic measures.

Keywords: Paratuberculosis, Mycobacterium avium subsp. paratuberculosis, cattle, control, risk factors.

ID-42

A novel topical therapy for Foot-and-Mouth Disease improves animal welfare and reduces antimicrobial resistance risks

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Objective: Improving the therapy used by para-veterinarians and veterinarians in Foot-and-Mouth Disease (FMD) outbreaks, particularly in developing countries where FMD is endemic, is an important but largely ignored aspect of global FMD management. Whilst effective therapy is the focus of livestock owners, the priority for donors and policy makers is usually investments in vaccination programs that are frequently unsustainable. Treatment for FMD is typically use of inappropriate parenteral and/or topical antibiotics, for a viral infection, risking development of antimicrobial resistance (AMR) and food safety issues, plus increasing household and national socioeconomic losses from FMD. There is an urgent need for a therapeutic with clear animal welfare benefits, no AMR risks; one that encourages the reporting of clinical cases to authorities by farmers seeking an effective treatment for their valuable livestock.

Material and Methods: Opportunistic clinical studies were conducted during field FMD outbreaks in Laos and Cameroon in 2019, using a farmer applied 'spray-on' wound formulation, developed and registered in Australia for provision of analgesia, antisepsis and reduced healing times in animals undergoing routine husbandry procedures (Tri-Solfen®, Animal Ethics Pty Ltd, Australia). Study sites were villages with naturally occurring FMD outbreaks, occurring in April 2019 near Luang Prabang in Laos and November 2019 near Ngoundere in Cameroon. Animals treated included cattle and buffalo (n = 136) in Laos and cattle (n = 40) in Cameroon, all with clinical vesicular lesions of FMD. The therapy (Tri-Solfen®) was applied liberally (1-2mls per lesion) to all the oral and pedal vesicular lesions in the FMD-affected animals in Laos. In Cameroon, some pedal lesions were left untreated to compare healing rates and additional cohorts of FMD-affected animals were treated with a parenteral oxyteracycline antibiotic (n = 12; Moore Oxy®, Nigeria) or left untreated (n = 69), enabling comparisons of response to therapy of the pain relief (Tri-Solfen®) treated, antimicrobial treated and untreated animals. In addition to clinical observations during and following treatment, follow-up surveys involving questionnaires were conducted in both countries with the owners of treated animals approximately a week to 10 days following the clinical studies.

Results: There was a rapid response to treatment observed as a marked improvement in the demeanour of the treated animals and immediate unanimous approval of the efficacy of treatment by the livestock owners. The surveys confirmed that: oral lesions generally healed faster than feet lesions; that all topical pain-relief treated lesions generally healed within a week of treatment; in animals only treated with an antimicrobial agent (Cameroon) lesions healed after a week or more and longer for untreated animals; the appetite score was higher for pain-relief treated cases than with the antimicrobial treatment and untreated animals; pain during walking was relieved faster with topical pain relief medication compared to those treated with the antimicrobial agent and untreated counterparts; and in both countries, farmers reported a 100% appreciation for the pain relief product and were keen to have it available for general use. This product contains two topical anaesthetics (lignocaine and bupivacaine), adrenalin, and cetrimide in a gel matrix. As no antimicrobial agents are present, use of the product reduces AMR risk and with a pH of ~2.7 it is potentially viricidal and may reduce environmental transmission of FMDV if used prior to or during rupture of vesicles. Evidence from depletion studies suggests that with-holding periods of 4 days for meat and 3 days for milk will minimise any risk of tissue residues.

Conclusion: These clinical trials indicate that this novel pain relief therapeutic provided effective pain relief through blockage of nociception and ease of application and coverage encouraged the rapid repair of lesions. wounds. This may be an important intervention for improving animal welfare in FMD, potentially encouraging livestock farmers to report outbreaks as they seek supplies of a 'new medicine that works' as it provides readily visible amelioration of suffering. Sustainable FMD control requires improved surveillance and biosecurity practices, effective public awareness campaigns to encourage com-

pliance, plus in many instances, effective strategic vaccination programs. However, access to an efficacious and affordable therapy that contains no antimicrobial agents and is likely to be viricidal, is important for future FMD control programmes if it: encourages farmer reporting; decreases virus transmission; substantially improves animal welfare; and reduces AMR risk.

Keywords: FMD, therapy, welfare, AMR, cattle.

ID-43

Molecular typing of Mannheimia haemolytica isolates from UK cattle surveillance submissions

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Objectives: *Mannheimia haemolytica* is an important and commonly recognised cause of respiratory disease in cattle and sheep worldwide. *M. haemolytica* serotypes A1, A2 and A6 are considered the most prevalent in cattle worldwide (Al-Ghamdi et al 2000) and are readily isolated from the nasopharynx of healthy cattle. Serotypes A1 and A6 have been reported as common isolates from pneumonic lung tissue (Klima et al 2014). *M. haemolytica* serotype A2 is considered primarily associated with disease in sheep (Fodor et al 1984). The aim of this study was to survey *M. haemolytica* isolates derived from pneumonia cases in the UK that were submitted for diagnostic investigation. Understanding the diversity of serotypes in the sample set is of use to inform future preventative strategies.

Materials and Methods: 100 *M. haemolytica* isolates derived from bovine clinical pathology and post mortem samples from pneumonia cases were tested using a multiplex PCR assay incorporating three serotype specific primer pairs for identification of *M. haemolytica* serotypes A1, A2 and A6 (Klima at al 2017). Primary bacterial cultures were made on Columbia sheep blood agar following 18-24 hours incubation at 37°C in capnophilic conditions, identification was confirmed using routine phenotypic tests and isolates were stored at -80°C.

Isolates were selected from cases which occurred between 2016 and 2018. Isolates were from nasopharyngeal swabs (17 isolates) or broncho-alveolar lavage fluid (2 samples) submitted from cases with a recorded clinical history of respiratory disease. The remainder were from post mortem samples (81 isolates) with a clinical history of respiratory disease and consistent gross pathology. Sample selection was not random or unbiased.

Results: 45% isolates were *M. haemolytica* serotype A1, 30% serotype A2, 18% serotype A6 and 7% un-typable using the techniques employed. Isolates were recovered from animals aged between 1 day and 8 years old and an equal proportion of male and female animals. 70% of isolates were derived from animals reared for beef production and 30% from animals reared for dairy production.

Conclusions: The finding that *M. haemolytica* serotype A1 is the most common serotype in this UK sample set is consistent with other studies looking at European isolates (Andrés-Lasheras 2019). *M. haemolytica* serotype A2 has been reported in North America as a commensal organism in cattle. 97% of the *M. haemolytica* serotype A2 isolates were isolated from pneumonic lung tissue. In 60% of the cases from which *M. haemolytica* serotype A2 was isolated, no other respiratory pathogens were detected. These findings do suggest a potential, causal role for *M. haemolytica* serotype A2 in bovine respiratory disease. In the UK there is only one vaccine which has been shown to protect or cross protect against *M. haemolytica* serotypes A1 and A6, but not A2.

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Acknowledgements: This study was funded by MSD animal health. SRUC Veterinary Services disease surveillance programme is funded by the Scottish Government.

Keywords: Mannheimia haemolytica, Cattle, Serotype.

ID-44

Leptospira hardjo re-introduction in a certified-free herd in the Netherlands

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Objectives: *Leptospira* serovar hardjo (*L.* hardjo) is a zoonosis and cattle are the main carrier. The Netherlands is the only country in the world with a *L.* hardjo-free control program. In 1990 the estimated herd prevalence in dairy herds was 25%. Since 2005 only farms with a *L.* hardjo-free status are allowed to deliver milk to the dairy industry. The Dutch dairy population is free of *L.* hardjo, with very sporadic cases. The herd prevalence among non-dairy herds is 0,8%, based on national screening in 2013. Monitoring for re-introduction is necessary, as for many different reasons increasing numbers of cattle are purchased by Dutch farmers: in 2019 over 42,000 cattle were introduced from non-free herds as compared to on average around 6,000 in 2013. A considerable part of these cattle are imported.

This case report presents the results of a follow-up study after re-introduction of *L.* hardjo in a previously free herd.

Materials and methods: The *L.* hardjo-free control program is carried out by Royal GD. Herds are assigned the *L.* hardjo-free status after initial assessment consisting of testing the sera of all individual animals on the farm. Surveillance of *L.* hardjo-free herds is based on bulk milk samples every four months, serum-testing aborted cattle, monitoring cattle movement data and testing sera from cattle introduced from non-free herds. Bulk milk samples and sera are tested with an indirect *L.* hardjo antibody ELISA (Thermo Fisher Scientific).

In the event of a positive or inconclusive antibody test result, further testing is mandatory and the farm loses the *L.* hardjo-free status and receives the status 'unknown'. In case of an introduced animal with non-negative test results, the animal needs to be removed from the herd. Four weeks later, a confirmative bulk milk sample is taken, to test for spread of *L.* hardjo in the herd.

If control samples are *L.* hardjo antibody positive, individual testing of all the animals in the herd is mandatory to investigate spreading of *L.* hardjo. If spreading is confirmed, all animals in the herd are treated with an antibiotic and the herd status is changed to 'treated'.

To monitor for spread of *L.* hardjo in treated herds, a seronegative tracer group of animals > two years old are serum tested every six months.

Results: The 120 head dairy herd was *L.* hardjo-free since 1999. In the last control bulk milk sample of June 8th 2019, no antibodies for *L.* hardjo were detected.

On June 28th 2019, thirteen cows were imported from Luxembourg and introduced into the herd without a quarantine period. Mandatory testing of sera from these cows, taken on July 18th 2019, resulted in one inconclusive and twelve sero-negative test results. A second serum sample of the animal that tested inconclusive, was inconclusive as well. This animal was removed from the herd on August 15th 2019.

Four weeks later, a bulk milk sample tested positive, which was confirmed by another bulk milk sample. Subsequently, sera were taken from all cattle in the herd on October 4th 2019. Multiple animals had antibodies for *L.* hardjo, also animals that were not imported. After the confirmed spreading of *L.* hardjo, all cattle on the farm were treated with a single high dose of dihydrostreptomycin (25 mg/kg body weight, intramuscular).

The seropositive animals showed no clinical signs of *L.* hardjo infection. At the herd level, milk production was lower than expected with an increase in clinical mastitis cases.

Conclusions: In 2019, a *L.* hardjo infection was observed in a certified-free dairy herd in the Netherlands, after import of infected cattle from Luxembourg. As import of dairy cattle by Dutch farmers is increasing, import poses an increasing threat for the Dutch *L.* hardjo-free program.

Keywords: L. hardjo, control-program, bulk milk, import, re-introduction.

ID-45

Field Study of Control and Eradication of Endemic M.bovis in a Persistently Infected Dairy Herd using a Novel Testing Programme

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Objectives: A large dairy herd comprising 350 adult milking dairy cows and 700 youngstock and beef animals was enrolled in a novel management programme to attempt to eradicate M.bovis from the herd by managing biosecurity and biocontainment within the herd, concurrent with the statutory management of bovine tuberculosis by testing and culling.

The Gatcombe Herd had been persistently infected with tuberculosis for over 7 years, with the statutory single intradermal comparative cervical tuberculin test (SICCT) identifying over 100 reactors which were immediately culled in accordance with the statutory control regulations. Despite strict biosecurity during this time, the statutory controls failed to remove infection from the herd.

An assumption was made that infection was circulating in the herd, with cows shedding M.bovis without be identified by the SICCT, and thus transmitting the infection to susceptible cattle. The objective of the study was to prevent new infections from shedding cattle by management and husbandry.

Materials and methods: 192 cattle were identified and categorised in the herd using the staturoty Single Intradermal Comparative Cervical Tuberculin Test (SICCT) records to detect any animal that had ever had any form of reaction to the bovine tuberculin in its lifetime. Once categorised as high risk, these animals were repeatedly tested for the presence of M.bovis after each SICCT, which was performed every 60 days in accordance with the regulatory testing programme. 154 of these high risk animals were repeatedly tested for the presence of M.bovis in blood and faeces using viral phage testing and qPCR.

Results: 125 of the 154 high risk cows (81%) had at least one phage positive test, indicating that they were infected with M.bovis despite not being identified as reactors by the SICCT. 34 of these animals in the enhanced testing regime had at least one positive qPCR test on faeces, indicating shedding of the organism in their faeces.

Animals which were shown to be shedding were either culled or isolated to prevent transmission. High risk animals were prioritised for culling, but with economic performance

moderating culling decisions. All potential risks of transmission were identified and controlled by management and husbandry. Becasue of the unexpected high level of faecal shedding from some infected cows, risks of transmission were identified and managed to prevent further spread within the herd.

After three years of the control programme, the herd is now Officially TB Free and has had no SICCT reactors in the last 4 routine skin tests.

Conclusions: This study demonstrates the potential for controlling and eradicating M.bovis from persistently infected herds where endemic infection circulates within the herd and cannot be identified or controlled by the statutory control programmes that rely on the SICCT to identify infected animals.

The control programme allows the economic and meticulous removal of infection from the herd, and minimises the risk of re-introduction by minimising environmental contamination.

Keywords: Tuberculosis.

ID-46

Identification of potential microbial taxa involved in the efficacy of fecal microbiota transplantation (FMT) from healthy donors to recipients suffering from diarrhea in calves

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Objectives: Calf diarrhea (CD) is a common disease and has an acute negative impact in the economy of the livestock industry. Antibiotics have been consistently used to treat the disease; however, fecal microbiome transplantation (FMT) has been attracting attention as an alternative therapy for CD. The objectives of this study were to characterize the fecal microbiota profile of healthy and diarrhea calves as well as potential donor selection for FMT using a machine learning approach.

Materials and method: Bacterial genomic DNA was extracted from feces of 156 calves, which were bred in different farms and classified as healthy (n=108) and diarrhea (n=48), and they were further compared with diarrhea calves used in an FMT study in which donors (n=20) and recipients (n=20) were enrolled. High throughput 16S rRNA amplicon sequencing for metagenomics was performed using a next-generation sequencer. In addition, a random forest (RF) prediction model was established, and a variable importance analysis was conducted with the aim of identifying microbial taxa those can be considered as potential predictors.

Results: The most abundant phylum in the faces of calves were *Firmicutes* and *Bacteriodetes*, which were found in healthy (51.17 % and 38.20 %) and diarrhea (46.03% and 31.91 %) groups, respectively. A principal coordinate analysis of the unweighted UniFrac distance matrix showed a significant difference between healthy and diarrhea calves. Specifically, in the RF model *Campylobacter*, *Actinobacillus*, and *Sporobacter* were identified based on the mean decrease in accuracy. *Campylobactor*, *Sporobacter*, and *Streptococcus* were identified as the most discriminating predictors based on the mean decrease in the gini criteria. Considering microbial abundance, *Sporobacter* was abundant in the overall healthy and donor groups for successful FMT. Along with RF, LEfSe analysis was subsequently performed among these groups, in which *Sporobacter* was found to be abundant in the healthy group. Taken together, these results suggest that *Sporobacter* may be a potential biomarker for the donors associated with FMT success.

Conclusion: FMT is an effective treatment option for the prevention of diarrhea in calves, specifically by identifying a beneficial microbial cluster. These findings have enormous significance for the livestock industry because FMT could eventually address the challenge of CD treatment as well as the use of excessive antibiotics.

Keywords: Diarrhea, fecal microbiome transplantation, calves, metagenomics.

ID-47

Farmer psychosocial factors associated with bovine viral diarrhoea control behaviours

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Objectives: Psychosocial factors are important for the uptake of health protective behaviours in human health, however many have not been investigated in the context of livestock disease control where the farmer is making decisions for their livestock. There are diverse approaches farmers can take to control bovine viral diarrhoea (BVD) in their herds, from BVD-specific vaccination, testing and culling to the more general prevention of the introduction of infectious diseases from other cattle (such as by having a closed herd, isolating new cattle and preventing contact with neighbouring cattle) and these may be influenced by psychosocial factors. This study investigated psychosocial and behaviour change factors in the context of BVD control methods used by cattle farmers.

Material and methods: A survey was completed by 475 UK cattle farmers in 2020. Multiple validated measures were used to investigate trust, psychological proximity (feeling close to another person), altruism and factors from a behaviour change framework (COM-B). The survey also investigated the methods of BVD control that the farmers used. Farmers were grouped by similar BVD control practices using latent class analysis, and a multinomial logistic regression model was used to investigate associations between the psychosocial and behaviour change factors and the BVD control classes.

Results: Farmers had the highest psychological proximity with (felt the closest to) their cows, followed by their vet, neighbouring farmers, the veterinary community, dairy farmers, beef farmers, the farming community, the National Farmers Union and the Government. Similarly, the farmers had the most trust in vets, followed by the NFU, other farmers and governmental organisations. The level of altruism among the farmers was similar to that of other people in general.

Farmers split into nine classes for how they controlled BVD from the latent class analysis. Similar classes were merged for further analysis resulting in five classes: 1) Does nothing (12%), 2) Vaccinates (25%), 3) Careful introducing new cattle (sources cattle from BVD-free herds and tests/isolates on arrival) (16%), 4) Vaccinates, careful introducing new cattle and prevents contact with neighbouring cattle (31%) and 5) Closed herd and prevents contact with neighbouring cattle (15%).

There were psychosocial differences between the farmers in each class that was using some BVD controls compared with the farmers who did nothing. Farmers who were vaccinating had higher psychological proximity with dairy farmers, lower psychological proximity with beef farmers and higher motivation to control disease (due to both automatic habits and emotions and reflective plans and goals). Farmers who were careful introducing new cattle had higher trust in other farmers. Farmers who were vaccinating, careful introducing new cattle and keeping them separate from neighbouring cattle had higher psychological proximity with their vet, lower trust in other farmers and had higher motivation to control disease. Finally, farmers who had a closed herd and were preventing contact with neighbouring cattle had lower trust in other farmers, higher psychological proximity to dairy farmers and lower psychological proximity to beef farmers, as well as being more likely to feel that they had enough knowledge and understanding of how and why to control infectious diseases and enough time and money to do so.

Conclusion: Various psychosocial factors were associated with the specific behaviours that farmers used to control BVD and understanding these will help veterinarians tailor their messages to encourage clients to take up disease control measures. Of particular relevance is that farmers with high psychological proximity with their vet were more likely to be proactively controlling BVD, which was more important here than the more extensively investigated trust. Therefore, it is important that vets work to ensure a close vet-farmer relationship to encourage proactive BVD control by farmers.

Keywords: Bovine viral diarrhoea, Infectious disease control, Farmer behaviour, Psychosocial factors.

ID-50

Prevalence of *Coxiella burnetii* in Central and Eastern European dairy herds

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Objectives: The aim of the present study was to assess the prevalence of *Coxiella burnetii* in different size of dairy herds in six Central and Eastern European countries based on enzyme-linked immunosorbet assay (ELISA) and real-time polymerase chain reaction (PCR) tests.

Material and methods: Bulk tank milk samples were collected from 370 dairy herds from six Central and Eastern European countries (Croatia, *n*= 13; Czech Republic, *n*= 138; Hungary, *n*= 126; Serbia, *n*= 24; Slovakia, *n*= 53; Slovenia, *n*= 16) between March and October, 2019. Samples were taken randomly from dairy herds of different size, but with focusing on larger dairies. Forty ml samples were taken from the bulk milk tanks and tested on indirect ELISA and real-time PCR targeting the IS1111.

Results: 1. The number of examined dairy herds varied according to the country of origin, but the overall *C. burnetii* infection status (percentage of positive herds/total number of herds with ELISA and PCR tests) ranged between 62.50-100.00% in the Central and Eastern European countries. Prevalence of *C. burnetii* differed according to the country of origin with Croatia showing 100.00%, the Czech Republic 98.55%, Hungary 97.61%, Serbia 70.83%, Slovakia 90.56% and Slovenia showing 62.50% average percentages of the positive herds. The analysis of the ELISA and PCR test results in association with herd sizes revealed, that herds of ≥250 animals showed significantly higher *C. burnetii* positivity (positive test results: 100%; Spearman's rank correlation, rho = 0.716, $p < 0.001$), than herds of <250 animals (positive test results: 73.03%). On the other hand, examining only the PCR test results, similar percentages of positive milk samples (40.63-44.94%) were detected among the herds of different sizes.

Conclusions: The present research assessed the prevalence of Q fever at dairy farms in Central and Eastern European countries, revealing increased seroprevalence in bulk tank milk samples compared to other European countries. Based on the analysis of the data, it is assumed that with growing numbers of animals in dairies and farm structures moving toward concentration, the risk of *C. burnetii* prevalence is increasing, underlining the importance of monitoring the herds' infection status and implementations of control measures

Keywords: Q-Fever,Central-Eastern Europe,Dairy cattle,Milk,Coxiella Burnetii.

ID-51

An Epidemic of Salmonellosis caused by Imported Rapeseed Meal Containing Salmonella at a Dairy Farm

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Objectives: Finland has zero tolerance policy for salmonella. All Salmonella serotypes are considered to be a significant threat for public health. Zero tolerance means, that actions are always taken, if salmonella is detected from feed, animals or foodstuffs. The aim is to find the origin of infection or contamination and to prevent further spread. Within the

framework of the official Salmonella control program, lymph node and carcass swab samples are taken at slaughterhouses and raw milk is sampled at dairies. In addition, producers themselves take a large number of self-monitoring samples, e.g. in the context of animal trade. If salmonella is found on a cattle-, swine- or poultry farm, the farm is put under restrictions by the veterinary authorities and salmonella eradication must be started.

Materials and methods: A dairy farm in Western Finland was found to be positive for *Salmonella Infantis* in May 2019.

Heifers were to be sold from the farm and, hence, fecal samples were examined for Salmonella. The loose-housing dairy had 210 cows and 150 young animals. The farm was put under restrictions. This e.g. means, that animal movements to the farm an out of the farm are ceased. By special arrangements, the milk can be delivered to the dairy to be diverted to heat-treated products. The salmonella eradication was started immediately.

The principles of eradicating salmonella from the animals and the environment is to assure hygienic feed and water and to cut the contamination of feed with feces. The production facilities are cleaned and disinfected according to a separate plan. All animals were examined for salmonellosis by faecal samples. About 80 environmental swab samples were taken e.g. from feeding tables, water throughs, feeding equipment and feed storages. All samples were examined for Salmonella (ISO 6579:2017).

The animals were fed TMR (total mixed ration) with a TMR-wagon. TMR-feed itself and also each of its components were examined. The main component of the TMR-feed was pre-dried grass silage harvested from the own fields of the farm. In addition, the feed included rapeseed meal, minerals and farmed, ground barley and oats.

At first 25 % of the animals were salmonella positive. Some swab samples from feeding tables, feeding equipment and water throughs were positive but all feed was negative. The hygienic procedures were concentrated to the critical points found contaminated. Feeding tables and drinking throughs were disinfected twice a day and the wheels of the vehicles always before feeding. Feed handling and feeding equipment was cleaned and disinfected as well as possible.

Next month, however, 60 % of faecal samples were positive for salmonella. Hence, new samples from the environment and the feed were taken. Salmonella was detected in an environmental swab sample from the inner surface at the top of the rapeseed meal silo cover. This site had not been sampled before because a crane was needed for access. The rapeseed meal was imported from abroad. It was not heat-treated but tested on a sample of 1 bulk sample / 25,000 kg with negative result.

The silo was emptied, washed and disinfected. Rapeseed meal was excluded from animal nutrition and replaced by industrial heat-treated protein feed. The hygienic measures proceeded.

Results: The eradication procedure was monitored by faecal and environmental swab samples every second or fourth week. The animals got rid of salmonella in four months, as confirmed by two successive monthly faecal samples from the animals and one environmental swab sampling. Hence, the restrictions could be released.

Conclusions: The use of untreated rapeseed meal in animal feeding involves a risk of salmonella that cannot be completely eliminated by sampling. Contamination does not necessarily occur in feed samples alone but requires extensive swab and dust sampling of the feed environment throughout the whole feeding system. Feed storages and feeders may contain salmonella inoculants which, when mixed with feed, can cause infection in the herd.

Keywords: Salmonella, epidemic, rapeseed, dairy, farm.

ID-52

Facilitating the diagnosis of Q fever using FTA cards to store and ship bulk tank milk samples

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Objectives: Coxiellosis, also named Q fever, is an infectious disease caused by an intra-cellular bacterium: *Coxiella burnetii* (**Cb**). In cattle, Q fever, when symptomatic, is mainly responsible for abortions, stillbirth, birth of weak calves, retained placenta and metritis/endometritis. But it is also a zoonotic disease, ruminants being the main reservoir of Cb.

Bulk tank milk (**BTM**) is an easy, inexpensive, and representative sample to detect Cb infections in dairy herds using RT-PCR. But one major limitation under field conditions is the need to deliver the BTM samples in adequate conditions (quickly, refrigerated and safely) to a qualified laboratory. In addition, sending non-inactivated biological material via regular post may be forbidden. A new innovative, easy, and accurate diagnostic tool (**QTest**) for Q fever was developed to overcome these constraints. Farmers or veterinarians simply place some drops of BTM on a WHATMAN FTA Elute Micro Card (**FTA card**) and let it dry before posting the card to the laboratory.

The objective of this study was to validate the reliability of this innovative technique.

Material and methods: This study had two complementary objectives, carried out in two steps.

The first step aimed at assessing the preservation of Cb DNA detection from BTM spotted on a FTA card over time under two different temperatures (20-22°C and 37°C) to mimic field conditions. A milk sample was artificially contaminated with Cb to reach a load of ~5x10^6 Genome Equivalent (GE)/mL which became the master sample. The master sample was then successively diluted at different dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). Each diluted milk was then sampled several times on FTA cards and stored for up to 29 days either at 20-22°C or at 37°C. RT-PCR was performed for each dilution and each storage temperature on days 1, 4, 6, 8, 11, 15, 20 and 29.

The second step aimed at comparing, after ageing, the detection of Cb DNA by RT-PCR either when directly applied

on BTM or on BTM preserved on FTA cards. 70 BTM field samples previously tested positive for Cb by RT-PCR were stored as raw milk for 17 days before extraction or inoculated onto FTA card on day 15 and extracted on day 21. On day 21, a RT-PCR was performed for all the samples (raw milk and FTA card) and the results were compared between the two techniques for each sample.

Results: The first step showed that regardless of the duration of FTA card storage, all samples with a dilution below 10^{-3} (approximately 10^3 GE/mL) were detected to contain Cb DNA. Also, no significant loss of detectability was noted from d1 to d29, regardless of dilution or storage temperature. This means that the FTA card system ensures a stable preservation of Cb DNA in BTM samples stored at 20-22°C and 37 °C for at least 29 days.

For the second step, of the original 70 positive samples, we had 58 samples that were tested positive using one of both of the storage option. Of these 58 samples, 45 raw BTM samples tested positive and, of these, five tested negative when using FTA cards. In other words, there were 13 false negatives with older raw BTM samples while there were only 5 false negatives for older BTM on FTA cards. These five remaining were all with Ct value >35 indicating low quantities of Cb DNA. We can assume that the non-detection was likely due to the lack of reproducibility of the PCR technique for weak positive samples.

Importantly, for 13 samples, FTA cards produced positive PCR results while the equivalent raw BTM samples tested gave negative PCR results. This indicates that the detection rate was higher using FTA cards with aged BTM (91.4%) than with raw aged BTM (77.6%) samples.

Conclusion: Our study showed that the use of QTest makes BTM sampling, shipment, and storage very easy and cheap, while results do not seem to be impaired by the preservation/transportation method. Indeed, the stability of Cb DNA on an FTA card is maintained for at least 29 days at either 20-22°C or 37 °C. Therefore, this technique would facilitate an easier and more practical approach to diagnosis of Q fever at herd level and would be supportive of Q fever control strategies.

Keywords: Q fever, Coxiella burnetii, bulk tank milk, diagnosis, PCR.

ID-53

Towards the control of sheep associated malignant catarrhal fever (SA-MCF) in bison and buffalo in Wales, UK

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Introduction and background: Bison and buffalo are not commonly farmed in the UK, although they are considered an option for diversification due to the high value of the animal products. In the UK, a significant limiting factor for bison and buffalo production is the high susceptibility of these animals to sheep associated malignant catarrhal fever (SA-MCF) caused by ovine herpes virus-2 (OvHV-2) and strategies to limit this disease risk are essential to sustainable production.

Objectives: The aim of this project was to control SA-MCF in bison and buffalo in Wales, UK.

Objective 1: determine the exposure of farmed bison and buffalo to OvHV-2.

Objective 2: determine the presence/absence of OvHV-2 in species in contact with the bison/buffalo.

Objective 3: determine whether the bison/buffalo have been exposed to other infectious diseases that could potentially increase the risk of MCF following exposure to OvHV-2.

Objective 4: utilise a novel vector vaccine (Macavax) as part of a control programme.

Materials and Methods: Two farms were included in the project: 1) a mixed species farm with bison, cattle, sheep, poultry and deer; 2) a farm with buffalo and sheep.

Blood/tissue samples were obtained from a sample of the bison, cattle, sheep and deer from farm 1 and blood samples were obtained from a sample of the buffalo and sheep from farm 2.

qPCR was used to identify OvHV-2 on blood and tissue samples and commercial antibody tests were used to determine exposure to bovine viral diarrhoea virus (BVD), infectious bovine rhinotracheitis virus (IBR), *Mycobacterium avium* subspecies *paratuberculosis*, Neospora caninum and *Mycoplasma bovis*. Faeces were examined for the presence of gastrointestinal nematode eggs, *Fasciola hepatica* eggs and lungworm larvae.

Trace element analysis was carried out using commercially available tests.

Control strategies were deployed specific to the farms with a novel vector vaccine (Macavax) utilised on farm 1.

The farms were monitored for 18 months to determine the efficacy of the control strategies.

Results: Farm 1: no OvHv-2 was detected at the start of the project, however several deaths in the preceding years were confirmed as SA-MCF. The bison had evidence of previous exposure to IBR, *N. caninum* and *Mycoplasma bovis*, gastrointestinal nematodes and *Fasciola hepatica*. Copper, selenium and iodine deficiencies were also detected. The Macavax vaccine was administered twice to bison originating from the farm and once to new herd entrants that joined the herd after one year. No adverse events were observed. The health of the herd improved overall, however one death was recorded after 18 months with SA-MCF confirmed by postmortem and virus detection.

Farm 2: 4/19 (21.1%) of the buffalo had OvHV-2 detected by qPCR. 1/10 sheep also had OvHV-2 detected by qPCR. The buffalo had evidence of previous exposure to IBR and *Mycoplasma bovis*, with marginal trace element deficiencies. No deaths associated with MCF were observed prior to or during the study period.

Conclusions: Bison and buffalo can be farmed successfully in the UK, provided their health and welfare needs are met appropriately. Various commercial strategies already in place for cattle may be used in a similar way in these species with appropriate amendments where necessary. Where

possible the bison and buffalo should be kept as far away as possible from other species to reduce the risk of transmission of infectious diseases, especially OvHV-2. The novel vector vaccine (Macavax) was safe to use in bison, although further work as to the specific extent of its efficacy is needed.

Keywords: Bison, buffalo, malignant catarrhal fever, MCF, control.

ID-54

Detection rates of primary abortifacient pathogens isolated by conventional microbiology from dairy and suckler foetal submissions, 2020-2021

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Objectives: The overall study objective was to establish the national prevalence of infectious abortifacients, including *Coxiella burnettii*, *Chlamydia abortus* and *Mycoplasma bovis*, in bovine foetal material submitted to the Irish Veterinary Laboratories Service (VLS) during the main (winter-spring) calving season 2020/2021. This abstract describes the sample set, available data and the results of routine foetal and placental cultures.

Materials and Methods: Sampling of bovine foetal material (abortions and stillbirths +/- placentae) was carried out between October 2020 and May 2021 at the six Regional Veterinary Laboratories (RVLs) of the VLS. This sample collection interval was chosen to span the period over which most abortions and stillbirths occur in Irish seasonal, grass-based dairy and suckler herds.

On voluntary submission of a bovine foetus to any of the six RVLs, relevant clinical history was taken from the herd owner/keeper, including details of the herd, the foetus/es and dam. Only foetuses with uninflated lungs were enrolled in the study. Straight crown-rump length (sCRL) was measured. The foetus and placenta, if available, were examined for gross abnormalities. A sample of foetal stomach content and a swab of placenta were collected and immediately plated on blood, *Brucella* and XLD agar. Blood and *Brucella* agar plates were incubated in 8% CO_2 at 37°C. XLD agar was incubated in aerobically at 37°C. The sample was also plated on Sabouraud's agar if requested by the investigating research officer. Plates were examined daily for seven days. Up to 25ml of additional foetal stomach content, a pooled sample of lung, liver and spleen and a sample of placenta (if available) were also collected and frozen pending further processing and testing. Lung, liver, midbrain and placenta were fixed in formalin. After five days, the fixed tissues were cut and placed in cassettes. These were then stored in wax blocks until further processing.

Data were extracted from the VLS Laboratory Information Management System and processed using Microsoft Excel and R Studio.

Results: The foetal carcasses originated from 855 individual herds with number of submissions per herd over the sample period ranging from 1 to 8. Herd size ranged from 1 to 750 with a median of 110.

In total, 1181 entire foetal carcasses were examined with (305) or without (876) placenta. The median, minimum and maximum sCRL of the foetuses was 75, 22 and 130cm, respectively. This implied median, minimum and maximum gestational ages of approximately 223 days, 108 days and full term, respectively, according to the formula:

DAY=8.4+0.087CROWN-RUMP+5.46CROWN-RUMP

Of the 1061 stomach content samples cultured, primary pathogens were detected in 281 (26.4%); *Trueperella pyogenes* (124), *Salmonella* Dublin (69), other *Salmonella* species (6*), Bacillus licheniformis* (31), *Listeria monocytogenes* (42) and *Aspergillus* species (9). All *Brucella abortus* cultures were negative.

Of the 186 placentae cultured, primary pathogens were detected in 55 (29.6%); *Salmonella* Dublin (22), *Bacillus licheniformis* (21), *Trueperella pyogenes* (12), *Listeria monocytogenes* (4) and *Aspergillus* species (2). Two primary pathogens were cultured from the same placental sample in six cases.

Various other bacterial and fungal species were isolated from cultures of placenta and foetal stomach content, thought to have a secondary role (i.e. opportunistic pathogens, capable of foetal or placental infection only under certain pre-existing conditions) in the pathogenesis of bovine abortion and stillbirth.

Conclusions: These results indicate that approximately 25-30% of bovine foetal mortality in this national cattle population could be attributed to primary (mainly bacterial) abortifacients detectable using routine culture methods. This raises the question as to the causes of the remaining cases, an international diagnostic challenge. To address this challenge, the sample set collected in this study will be used to quantify the role of other, less commonly tested for, primary pathogens such as *Neospora caninum*, *Coxiella burnettii, Chlamydia abortus* and *Mycoplasma bovis*.

Keywords: Bovine abortion, abortifacient pathogens.

ID-55

Prevalence of major enteric pathogens in Turkish dairy calves

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Objective: The aim of the study was to measure the prevalence of the most important causes of diarrhea: *E. coli* K99, Rotavirus, Coronavirus, *Cryptosporidium spp*. and *Clostridium perfringens* in dairy farms located in 5 geographic regions of Turkey.

Materials and Methods: The study has been performed

between February 2015 and November 2017 in 24 dairy farms located in Aegean, Mediterranean, Southeastern Anatolia, Central Anatolia and Marmara.

280 feces samples were collected from 1 to 45 days old calves with diarrhea. Most of them had still suckling reflex and watery to creamy feces. An on-farm diagnostic test was used to identify the 5 pathogens of interest in calves with diarrhea (Rainbow Test Bio-K306, Bio-X Diagnostics, Belgium).

Results: At least one pathogen was identified in 244 calves from 280 diarrheic calves (87.15%). In 75 of the 244 positive samples, more than 1 pathogen were identified (30.74%). In total 338 pathogens were identified in positive feces samples. The most prevalent pathogen was *Cryptosporidium* with 154 cases (45.6%), then, 87 cases (25.7%) of Rotavirus, 85 (25.1%) of *C. perfringens*, 10 (3.0%) of *E. coli*, 2 (0,6%) of Coronavirus. In 94 (61%) of the 154 samples positive for *Cryptosporidium* no other pathogen was identified. A *Cryptosporidium* positive mix-infected sample was mostly positive for Rotavirus as well (n=35, 23%). *Cryptosporidium* prevalence was highest in the Aegean region (84%), followed by Mediterranean (71,8%) and Southeastern Anatolia (70%).

Conclusions: In this study *Cryptosporidium* spp. and Rotavirus are the predominant agents causing neonatal diarrhea in the respective Turkish dairy herds. Effective control and prevention of *Cryptosporidium* spp. and Rotavirus should be the major focus points in the herds to reduce the prevalence of diarrhea. Vaccination of the pregnant cows with a neonatal calf diarrhea vaccine and proper colostrum administration may help to reduce the prevalence of rotavirus.

Keywords: Calf scour, enteric pathogens, Turkey, prevalence.

ID-56

Scottish BVDV spot tests: Why do we get single animals testing positive and what is their significance?

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Objectives: Young stock spot tests are used to determine if a herd has current evidence of exposure to bovine viral diarrhoea virus (BVDV). Serum samples from a representative proportion of young stock are tested for the presence of antibodies to BVDV. Within the Scottish eradication programme, typically five animals per management group are tested. If one animal in the group tests positive for antibodies to BVDV, the herd is deemed to be "not-negative" for BVDV. Movement restrictions are placed on herds that are "not-negative" for BVDV. This study aims to determine the reasons for and significance of single antibody positive results in Scotland over a one year testing period.

Material & Methods: 4728 young stock spot tests from Scottish herds were analysed for the presence of BVDV antibodies by Scotland's Rural Colleges (SRUC) Veterinary Services between 1st October 2017 and 30th September 2018. The spot tests were divided into one of three categories: (1) all animals tested negative for antibodies to BVDV; (2) more than 20% of the animals tested positive for antibodies to BVDV or (3) 20% or less of the animals tested positive for antibodies to BVDV. The data and notes from the spot tests in the third category were analysed to determine if there was a clear reason for low numbers (usually a single animal in a group of five) testing positive for antibodies to BVDV. Retest results, herd status for that year's calf crop and the subsequent calf crop were also assessed to determine the significance of these results.

Results: 4290 of the spot tests were in category 1, 209 were in category 2 and 229 were in category 3 (20% or less of the animals tested positive for antibodies to BVDV). After further data cleansing to remove inappropriate tests (those that were originally booked in as spot tests but in fact were tested for another reason), 216 spot tests in category 3 from 192 Scottish herds were used for this study. 40% of these spot tests were identified as having avoidable reasons i.e. use of vaccination, maternally derived antibody or incorrect calf tested. Following review of herd information, and in some cases retesting, 56% of the herds were assigned a "negative" status for the year and 44% a "not-negative" status. The subsequent calf crops from these herds were also tested, where applicable, and following analysis of those results 80% had a "negative" status, 17% a "not-negative" status and 3% had been declared as non-breeding herds. Animals persistently infected with BVDV were identified in three herds, despite apparent limited evidence of exposure to BVDV in the initial spot test.

Conclusion: 5% of spot tests carried out in the study period identified 20% or less of the tested animals as positive for antibodies to BVDV. 40% of these however had a clear, avoidable explanation. The results of this study can therefore be used to give clearer, evidence based guidance to minimise mistakes and misleading results with the spot test. The significance of these results on the herd status in the current and subsequent calf crops can also inform future policy on whether the cut-point for determining herd status should be changed.

Keywords: BVDV, eradication, testing, antibody.

ID-57

Bovine abortions in southern Belgium : 10 years of results

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Since 10 years in Belgium, a national surveillance programme based on the compulsory reporting of abortions and subsequent analyses on their products reached several objectives including official surveillance of bovine brucellosis but also the monitoring of other bovine abortive diseases.

Some endemic, emerging or re-emerging pathogens could be identified, including *Brucella* spp., *Salmonella* spp., *Listeria monocytogenes, Campylobacte*r spp., *Coxiella burnetii, Leptospira* spp., Mycotic agents, Bovine viral diarrhoea virus,

Bovine herpesvirus 4, Bluetongue virus serotype 8, Schmallenberg virus, *Neospora caninum*.

In the context of the Belgian passive surveillance programme for bovine brucellosis, 46.967 bovine abortion's cases were collected from January 2010 to December 2019. They are originated from 6894 cattle farms distributed among the five Walloon provinces. In around 90% of cases, the fetus, the maternal serum and the placenta are collected and analyzed. These samples allowed a wide range of analyzes and an autopsy of the fetuses in the large majority of cases. In order to maximize the etiological diagnosis rate, a standard and systematic analysis protocol has been implemented.

To determine the cause of abortion, it is necessary to identify an abortive pathogen or congenital lesions incompatible with life. The etiologies of the abortions can be identified in approximately 30% of cases. In almost 20% of cases, an opportunistic pathogen is identified. This means that in 50% of the cases, no cause of infectious abortion could be identified. In order to improve the detection of abortive pathogens in herds with outbreaks of abortions without diagnosed etiology using conventional diagnostic methods (culture, PCR, etc.), the use of 16S RNA sequencing provides a definite advantage. Thanks to this new technology, several bacteria whose culture is complex and for which systematic PCR analysis would be too costly have been highlighted. Over 400 fetuses have been analyzed and bacteria such as *Ureaplasma diversum* have been diagnosed.

Standardizing the analysis protocol and improving it when no cause can be identified makes it possible to obtain a high rate of etiological diagnosis. The results obtained on more than forty thousand abortion's cases are an important source of information for the epidemiology and the surveillance of animal diseases in Belgium.

Keywords: Abortion, Cattle, Belgium, Diagnosis.

ID-58

Metagenomic sequencing of the respiratory virome in beef-suckler weanlings diagnosed with bovine respiratory disease

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Objectives: Bovine respiratory disease (BRD) is a global cause of morbidity and mortality of cattle placed in feedlots, despite decades of development and application of vaccines and antimicrobials. The study objective was to compare untargeted viral metagenomic sequencing (on the Oxford Nanopore Technologies MinION and Illumina NovaSeq) and targeted species specific qPCR for characterisation of the upper nasal virome in clinically-diagnosed BRD and healthy beef-suckler weanlings on the day of BRD detection (day-BRD).

Materials and Methods: One hundred and fifty-three beef-sucker weanlings (209 days old [SD: 35.8] and 306 kg [SD: 26.3]) were purchased through auction marts, transported by road, and housed indoors for the duration of the study (Cuevas-Gómez *et al.,* 2020). Weanlings were vaccinated 24 h after arrival against *Clostridia* and against three known BRD-associated viruses (BoHV1, BRSV, BPI3) and one BRD-associated bacteria *M. haemolytica*. Sterile flocked swabs were inserted approximately 12 cm into the nasopharynx of each calf and gently rotated on the day of BRD diagnosis. Thirty animals with BRD and 30 matched healthy control animals were selected for virome sequencing. Viral nucleic acid (protected by nuclease-resistant capsids) was enriched by bead-beating and nuclease treatment. DNA and RNA was extracted and purified using the Qiagen MinElute Virus Spin kit. Double-stranded cDNA was generated then aliquoted and stored at -80°C until required. One aliquot of double-stranded cDNA was sent to a sequencing provider (CD genomics) where quality of DNA was assessed by Qubit fluorometry and agarose gel electrophoresis. Sequencing libraries were prepared using VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme). Sequencing was performed on an Illumina NovaSeq PE150 (generating ~20 M read pairs or 6 Gb data). A second aliquot of ds cDNA was used to generate barcoded nanopore sequencing libraries using the Rapid PCR Barcoding Kit (SQK-RPB004; Oxford Nanopore), which were pooled and sequenced on R9.4.1 flowcells on a MinION Mk1C. A positive control library and a negative PBS extraction control library were included in all sequence runs for both sequencing platforms. Data analysis and interpretation were performed via an in-house pipeline. A One Step TaqMan™ Fast Virus 1-Step Master Mix RT-PCR Kit was used to quantify BCoV and BRAV on day-BRD in unenriched samples. qPCR data were tested for normality using PROC UNIVARIATE of SAS (9.4), and analysed using the PROC MIXED procedure.

Results: BRD naturally developed within the BRD cohort as revealed by respiratory clinical scoring and thoracic ultrasonography (Cuevas-Gómez *et al.,* 2020). The two sequencing platforms had high concordance for detection of BRD-associated virus species. The common BRD agents (BRSV,BPI3, BoHV1) were detected at low levels on both platforms and we postulate this is a result of vaccination. Using qPCR to target two viruses (Cq cut-off point of ≤ 37), nasal swabs were positive for BCoV (mean healthy Cq = 22.24 ±6.97 *vs.* mean BRD Cq = 20.07 ±7.07) in all 30 health and all 30 sick animals. Nasal swabs from 22 healthy and 23 BRD animals were positive for BRAV (healthy Cq = 14.58 ±3.29 vs. BRD Cq = 10.5 ±3.24). There was no difference (P > 0.05) in mean Cq values between healthy *vs.* BRD for BCoV.

Conclusion: A diverse and complex virome was found in BRD and healthy beef-suckler weanlings on the day of BRD clinical detection. Both Illumina and Oxford Nanopore sequencing platforms are capable of characterising the metagenomes found in BRD animals, whilst the latter offers potential application to pen-side diagnostics due to its portability and the ability to identify viruses from nasal swabs within 6-24 hours. Two viruses were selected for RT qPCR based on high read abundance (BCoV and BRAV). However, statistical differences in virus quantity or virus prevalence were not observed between nasal swabs from healthy and BRD. This suggests that healthy animals were asymptomatic carriers of BCoV and BRAV.

Keywords: Bovine respiratory disease, nanopore sequencing, Illumina NovaSeq, viral metagenomics.

ID-59

Comparison of sampling and diagnostic techniques for recovery of *Mannheimia haemolytica* from feedlot cattle

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Objective: Bovine respiratory disease (BRD) is caused by interactions among the host, environment, and pathogens. The current standard for antemortem pathogen identification in cattle with BRD is deep-guarded nasopharyngeal swabbing, which is challenging, costly, and waste generating. The study objective was to compare recovery of *Mannheimia haemolytica* via culture and real time-qPCR, and to characterize microbial community structure by 16S rRNA gene sequencing, using 75-cm deep-guarded nasopharyngeal swabs (DG), 41-cm unguarded proctology swabs (PS), or 15-cm unguarded nasal swabs (NS).

Materials and Methods: Samples were collected from beef steers and bulls (n=120, mean weight=262.2 ± 12.5 kg) 14 days after arrival at a feedlot after purchase from an auction market. One nostril was sampled with each swab type for bacterial culture, identification, and antimicrobial susceptibility testing by broth microdilution. The other nostril was sampled for DNA extraction for 16S rRNA gene sequencing and qPCR for the *M. haemolytica* leukotoxin D gene (lktD).

Results: There was high concordance among swab types for *M. haemolytica* culture and qPCR (complete concordance for 77% and 81% of animals across all 3 swab types for culture and qPCR, respectively). Microbial communities were highly similar among samples collected with different swabs types, and differences identified relative to treatment for BRD were also similar; however, sampling with NS was less effective in characterizing changes within less abundant phyla than sampling with DG or PS. Positive qPCR results for *M. haemolytica* were highly concordant across swab types (81% agreed completely), but samples collected by DG had higher C_t values (Kruskal-Wallis analysis of variance on ranks, $P < 0.05$; Dunn-test for pairwise comparison with Benjamini-Hochberg correction, $P < 0.05$) and lower frequency of positive compared to NS and PS (McNemar's Chi-square test, $P < 0.05$).

Conclusions: Though slight differences existed among results for different swab types within individual cattle, nasal swabs and proctology swabs yielded comparable results to deep-guarded nasopharyngeal swabs when identifying and characterizing *M. haemolytica* by culture and qPCR, and when characterizing the microbial community.

Keywords: Bovine respiratory disease, 16S rRNA gene sequencing, antimicrobial resistance, metagenomics, qPCR.

ID-60

Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants

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Q fever is a worldwide zoonotic disease mainly responsible for reproductive disorders such as abortion in domestic ruminants. ELISA methods are the diagnostic tools recommended for the serological diagnosis of *Coxiella burnetii* infection in ruminants but their respective diagnostic performances are difficult to assess because of the absence of a gold standard.

Objectives: This study focused on three commercial ELISA tests with the following objectives (1) assess their sensitivity and specificity in sheep, goats and cattle, (2) assess the between- and within-herd seroprevalence distribution in these species, accounting for diagnostic errors, and (3) estimate optimal sample sizes considering sensitivity and specificity at herd level.

Materials and methods: We comparatively tested 1,413 cattle, 1,474 goat and 1,432 sheep serum samples collected in France. We analyzed the cross-classified test results with a hierarchical zero-inflated beta-binomial latent class model considering each herd as a population and conditional dependence as a fixed effect. Potential biases and coverage probabilities of the model were assessed by simulation.

Results: Conditional dependence for truly seropositive animals was high in all species for two of the three ELISA methods. Specificity estimates were high, ranging from 94.8% [92.1;97.8] to 99.2% [98.5;99.7], whereas sensitivity estimates

were generally low, ranging from 39.3 [30.7;47.0] to 90.5% [83.3;93.8]. Between- and within-herd seroprevalence estimates varied greatly among geographic areas and herds. Overall, goats showed higher within-herd seroprevalence levels than sheep and cattle. The optimal sample size maximizing both herd sensitivity and herd specificity varied from 3 to at least 20 animals depending on the test and ruminant species.

Conclusion: This study provides better interpretation of three widely used commercial ELISA tests and will make it possible to optimize their implementation in future studies.

Keywords: Q fever, Bayesian, diagnostic accuracy, herd sensitivity, conditional dependence.

ID-61

Sheep associated malignant catarrhal fever (SA-MCF): a series of cases on three farms in the UK

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Introduction and background: Malignant catarrhal fever (MCF) is caused by herpes viruses of the *Macavirus* genus and disease is observed worldwide. In the UK, the sheep associated form of the disease (SA-MCF) caused by ovine herpes virus-2 (OvHV-2) appears most commonly affecting many species including cattle. Clinical signs vary but are associated with a variably distributed systemic lymphoproliferative non-suppurative vasculitis and commonly include scleral congestion, keratitis and corneal oedema (which is often considered pathognomonic) together with pyrexia, depression and anorexia resulting in a loss of condition.

The aim of this project was to study naturally occurring clinical outbreaks in the UK in order to better inform farmers and veterinarians.

Objectives:

Objective 1: document the natural history of SA-MCF in naturally occurring outbreaks.

Objective 2: where possible identify sources of infection that could be addressed through specific and tailored on-farm advice.

Materials and Methods: Three farms were identified through a single veterinary practice in Wales, UK.

Clinical examinations of clinical cases were made, together with post-mortem examinations where possible. The presence of OvHV-2 was identified by qPCR on blood and tissue samples. A diagnosis of SA-MCF was made on the basis of clinical signs together with virus detection, and, where possible, histopathology carried out by a board-certified veterinary pathologist demonstrating a lymphoproliferative vasculitis.

On two farms (1 and 2) blood samples were obtained from asymptomatic in-contact cattle and subject to qPCR for OvHV-2 to determine the presence of asymptomatic cases.

On one farm (farm 2) blood and nasal swab samples were obtained from a random sample of asymptomatic sheep and also subject to qPCR for OvHV-2 to determine the likelihood of the sheep being the source of the infection.

Treatments of live clinical cases were empirical and included antibiotics and steroids or non-steroidal anti-inflammatory drugs (NSAIDs).

Results:

Farm 1

A group of 30 non-lactating dairy cattle were relocated to a field adjacent to a group of grazing store lambs. Commencing within 24 hours, and over a 12-day period, three animals demonstrated acute clinical signs with OvHV-2 detected in two. Two of the three animals affected died with one making a gradual recovery with virus still detectable one month later. Additionally, OvHV-2 was also detected in one asymptomatic animal from the same group.

Farm 2

A group of 14 store crossbred heifers were housed in an open sided shed directly opposite a group of 50 lambing Welsh mule ewes housed in a similar open sided shed, 5m away. A single heifer became unwell. Clinical signs included: reduced appetite, pyrexia, corneal oedema, nasal discharge, a generalised fine motor tremor, hindlimb ataxia and signs of colic. Despite treatment, she deteriorated rapidly resulting in generalised seizures, whereupon she was euthanised.

An immediate on-farm postmortem was carried out with a focal, extensive, ulcerative haemorrhagic and catarrhal rhinitis observed in addition to the pathology already observed. Varying degrees of lymphoproliferative/nonsuppurative vasculitis were observed in multiple organs.

OvHV-2 was detected in the clinical case, three of the in contact asymptomatic cattle, 7/10 blood samples from the sheep and 10/10 nasal swab samples from the sheep.

Farm 3

A single bulling Stabiliser heifer was observed to be unwell with no immediate contact with sheep. She developed clinical signs including: increased lacrimation, conjunctivitis, corneal oedema, cough, nasal discharge, ulcerated muzzle, pyrexia, and a hyperkeratotic dermatitis. She gradually deteriorated despite treatment and was euthanised 50 days later. An immediate on-farm postmortem revealed multiple pathologies throughout the carcass with a lymphoplasmacytic vasculitis observed in several organs.

Conclusions: A detailed clinical history, clinical examination, together with virus detection and postmortem examination are useful for diagnosing SA-MCF on cattle farms.

A clear link to a specific reservoir host may help clinicians implement appropriate mitigation of further infections by moving the cattle as far away from the reservoir host as soon as possible.

Recovery from MCF in cattle is possible, but rare, and may be related to the amount of virus challenge.

Subclinical infection in cattle is possible and may result in latently infected cattle.

Keywords: Malignant catarrhal fever, MCF, sheep, cattle, herpes virus.

ID-63

A parainfluenza 3 virus outbreak on a Dutch Veal Farm

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Objectives: Bovine Respiratory Disease (BRD) is a multifactorial disease that can affect cattle of all ages but is predominantly seen in young cattle. Affected animals are highly infectious and shed large quantities of virus and bacteria through nasal discharge. The BRD pathogens are endemic in the Dutch cattle population. Calves may have serological antibodies in the absence of clinical disease. These antibodies may come from passive protection (e.g. colostrum) or as an active response to field infection or vaccination. A serological screening of healthy animals for several BRD antibodies, may give more clarity on the importance of these pathogens, as well as an indication to which level antibodies may have an influence on primovaccination. The latter may help to develop an adapted vaccination schedule against respiratory disease.

In this field study, antibody titers against the BRD pathogens Parainfluenza 3 virus (PI3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* and *Mycoplasma bovis* were determined in healthy calves on a Dutch rosé veal farm without major BRD problems. No vaccinations against BRD pathogens were performed on farm. The objective of the study was to determine major infectious risk factors for BRD.

Materials and Methods: Eleven randomly selected calves were blood sampled every 4 weeks from arrival (February 19th, 2019) until week 24 after arrival (August 30th 2019). The eleven sampled calves were situated in a group of 220 animals. This group changed housing once in week 9 after arrival and was always separately housed from other groups on the farm. The group received 3 times an antibiotic treatment for 5 days starting from February 21st, March 4th and March 19th with respectively doxycycline, tilmicosin and doxycycline. All animals were monitored by the farmer and veterinarian as usual on this commercial veal farm.

All the collected samples were analysed at the Centre for Diagnostic Solutions (MSD Animal Health, Boxmeer, The Netherlands) by ELISA for antibodies against *Mannheimia haemolytica*, BRSV, PI3 and *Mycoplasma bovis*. An in-house test was used to measure *Mannheimia haemolytica* and BRSV antibodies, whereas for PI3 and *Mycoplasma bovis* a commerical kit was used from respectively IDEXX and Bio-X.

Results: Antibodies against all 4 pathogens were present in the selected samples. All the samples (each calf and each time point) were positive for *Mannheimia haemolytica* antibodies. The average titers for *Mannheimia haemolytica* increased over time to very high levels at week 20. Most of the samples (91%) were positive for PI3 antibodies. The average titers decreased gradually over time which may reflect the reduction of colostral antibodies over time. The majority of samples (97%) were positive for BRSV antibodies, however most of the BRSV titers (80%) were very low. The average titers also gradually decreased which clearly reflected the reduction of colostral antibodies over time. Only 1 animal was positive for *Mycoplasma bovis* antibodies at arrival. However, at a later stage, each animal was at least once positive for *Mycoplasma bovis* antibodies with variable titers. This could mean that the passive protection at arrival was very low and that animals get infected with *Mycoplasma bovis* over time gradually.

A surprising event at this well managed farm without major BRD problems was a BRD outbreak at week 21 after arrival. The animals were treated for 5 days with chlortetracycline. This outbreak was clearly reflected in the analyzed samples, as titers for PI3 antibodies increased exponentially at week 24 compared to week 20. This confirms PI3 infection as etiology for a BRD outbreak.

Although on this farm BRD complications and potential losses due to the PI3 outbreak could be avoided by antibiotic treatment, the serological analyses convinced the veterinary practitioner and farmer about the important role of PI3 in the BRD complex.

Conclusion: Serological analyses for BRD pathogens on veal farms are essential to understand the infectious pressure and design an appropriate BRD management program including vaccination. Vaccination against PI3 virus could have reduced the complications and potential losses of a BRD outbreak and improved the wellbeing of the animals on this farm.

Keywords: Bovine Respiratory Disease, Parainfluenza 3, veal, Netherlands.

ID-64

Effects of vaccination programs on Map faecal shedding and serological response in eight French dairy herds

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Objectives: The main objective of this study was to evaluate the effects of Silirum® based vaccination programs on Map faecal shedding and serological status in French dairy herds infected with paratuberculosis. We also intended to evaluate the effect of age at vaccination on faecal shedding and serological status.

Materials and methods: The serological status (ELISA) and faecal shedding (qPCR) of 4- to 5-year-old vaccinated cows (n=237) were assessed every six months over a two-year period in 8 infected herds in the Meuse department, France. Within each herd, cows from the last non-vaccinated birth cohort (n= 249) were used as controls using the same sampling scheme. The probability of Map faecal shedding and the level of Map shed were modelled using mixed general linear regression models with herd and cow random effects, adjusting for age at sampling, days in milk within the lactation and vaccination status. The effect of age at vaccination was similarly investigated in the subset of vaccinated cows.

Results: In vaccinated cows the median age at vaccination was 5.7 months (interquartile range: 3.3-7.9 months). Only 36.3% of the vaccinated cows were positive on serum

ELISA, with cows vaccinated before the age of 6 months yielding significantly fewer positive results. Overall, 42.6% of vaccinated and 28.11% of non-vaccinated cows were positive on faecal qPCR, with strong differences between herds. However, only 5.2% of non-vaccinated and 6.7% of vaccinated cows shed more than 100 Map per gram of faeces. The probability of Map shedding (p=0.772) and the amount of Map shed (p=0.955) were not significantly different between seropositive and seronegative vaccinated cows and no effect of age at vaccination could be evidenced. Compared to non-vaccinated seropositive cows, vaccinated cows were at lower risk to shed Map in the faeces (Odd's Ratio = 0.40, 95 % confidence interval: 0.13 – 0.89, p=0.0234) and in lower amount (p<10-5). No difference was however evidenced between vaccinated cows and non-vaccinated seronegative ones, neither regarding the shedding probability (p=0.103) nor the shedding amount (p=0.410).

Conclusion: Based on these preliminary results, we conclude that the beneficial effects of vaccination on Map faecal shedding may be limited in the investigated herds. Moreover, the variability of serum ELISA response in vaccinated cows remains to be investigated.

Keywords: Paratuberculosis, vaccination, Map Shedding, serology.

ID-65

How the United Kingdom created a commercially driven National Johne's Management Plan with 95% dairy farmer participation in 10 years

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Objectives: In 2009 a National Johne's engagement plan was developed with the support of milk processors, laboratories, database providers, farming and veterinary groups. The initial objectives were to create an inclusive Johne's management plan which would be widely adopted by all dairy farmers in the UK. Farmers would have to be fully engaged for a minimum of 10 years to achieve effective control. To achieve this objective the Rules of the program would develop over time and by driven by industry and farmer demand. Creating common messages and managing peoples' beliefs, prior conceptions and increasing the importance of control were major focus points. The plan would be fully commercial with the farmer paying for advice and tests. Processors and vets would encourage participation.

By December 2020 it is anticipated that 95% of the UK Dairy Farmers will have completed a risk assessment, surveillance and have installed a JD control plan utilising an accredited vet.

Materials and Methods: The UK National Johne's Management Program evolved over a 10 year period. The initial first 5 years centred on synchronous education of farmers, vets and wider industry on the importance and benefits of control. Dairy UK (milk processor representative body) helped encourage milk processor to commit resources to help fund farmer education. A trained group of vets delivered extensive training across the UK (> 300 farmer meetings) and further farmer awareness was created through provision of training resources for private veterinarians to use in their own practices. The model was based on the RESET model (Rule, Education, Social Norms, Economics and Tools) with the greatest emphasis on Education, Economics and Social Norms in the early years.

Milk processors and retailers define the priorities for UK Dairy farmers as part of their contractual arrangements. A cohesive national plan (NJMP) for Johne's Disease (JD) control was developed in 2015 which with the objective of JD reduction in the UK. Processors encouraged their farmers to comply with the planning process.

The flexible framework for JD control was established allowing farmers to choose one of 6 potential control strategies using advice from an accredited JD vet. Less than 10% could adopt a strict biosecurity and monitor strategy. The most common strategies included improved farm management and testing. Firebreak vaccination is not commonly adopted due to complications with JD surveillance and Bovine TB testing.

Fig.1. Summary of chosen JD control strategies in 2019

Strategy	%
Biosecurity Protect and Monitor	9.3
Improved Farm Management	4.0
Improved Farm Management and Strategic Testing	50.0
Improved Farm Management with Test and Cull	29.0
Breed to Terminal Sire	7.4
Firebreak Vaccination	0.3

The success has been achieved through focusing on creating practical JD plans that match the need of the farmer and create commercial consultancy opportunities for the private vet.

The funding of the program has been minimal and has utilised stakeholder resources and commercial drivers for success. The farmer pays for all advice provided.

Results: 22 milk processors with an estimated 80% of GB milk volume engaged with NJMP. By October 2018, 6084 farmers were compliant with the NJMP. Declared strategies from 2923 farmers were improved farm management (IFM) and strategic testing (50%), IFM and test and cull (28%), biosecurity protect and monitor (10%), breed to terminal sire (7%) IFM alone (5%) and vaccination (0.3%). Data on strategies was not collated from 3161 farmers as two larger processors used their own software to collate participation.

Over 1100 vets have been accredited to deliver the JD plan through an online training portal provided by the British Cattle Veterinary Association.

In October 2019 the NJMP became a national farm assurance standard for all dairy farms supplying farm assured milk. All farms will have to undertake a risk assessment, have a compliant control plan and undertaken surveillance to track progress using the skills of an Accredited Johne's Veterinari-

an. 95% of UK dairy farmers will be compliant with the NJMP by 2020.

Conclusion: Milk processor and Retailer influence created drivers for engagement. The development of a practical, flexible and commercial approach to JD control, which appeals to all, is central to the success of the NJMP.

Keywords: Johne's, Paratuberculosis, MAP, Dairy.

ID-66

Effects of a CDK9 inhibitor RKP00156 on bovine papilloma

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Bovine papilloma virus (BPV) induces diseases of considerable veterinary importance in cattle and other ruminants. BPV infects the skin from wounds such as puncture scratches by bloodsucking insects. Papilloma grows as tumor at the site of infection, papilloma located on the teats and udder skin are often observed and it is associated with mastitis in dairy cows. Several genotypes of BPV was recently divided into three genera, Delta, Xi, and Epsilon. It is difficult to identify the virus type based on the gross characteristics of tumor located on skin. In most cases, animals are co-infected by multiple genotypes. An efficient treatment method for papilloma has not been established. Recently, a cyclin-dependent kinase 9 (CDK9) inhibitor RKP00156 that inhibit host enzymes used by the virus, was shown to be effective against a wide range of viruses in vitro and cold be used as an antiviral drug. The purpose of this study was to examine the effect of RKP00156 on papilloma and BPV expression in cattle.

Ten Holstein heifers (16 to 20 months), infected with papilloma virus were used in the present study. The left anterior teat of each animal was assigned to treatment group (T group) and the right anterior teat was used as control group (C group). RKP00156 (3%) ointment was topically applied to the teat (T group) and Vaseline ointment (vehicle) was applied to C group once a day for 2 months. Before each application, pictures of each teat was taken to evaluate the changes in the tumor size and the fate of tumors at fourth week (W4) and eight week (W8) after the start of treatment (W0), tumors samples were also collected for PCR analysis after DNA extraction. Genotype identification and virus expressions were evaluated on W4 and W8 in tissue samples. The changes in size and fate of papilloma was evaluated from the pictures, and the tumors were classified in three categories (improved; decreased in size or disappeared, no change; kept the same size, and worse; increased in size or appeared during the experiment). The differences in the size and fate of tumors between treatment and control groups were compared and analyzed by Fisher's exact test.

On W4, the improved rate of papilloma was higher and the worse rate was lower in T group than in C group ($P < 0.1$). Furthermore, on W8, the improved rate of T group further increased and the worse rate decreased. PCR analysis performed on W4 revealed that Delta and Xi types of BPV were expressed in the same number of teats in both groups, however, on W8, the number of teats expressing Delta and Xi decreased significantly compared with that of controls. These results indicate that administration of CDK 9 inhibitor RKP00156 ameliorated papilloma formation and reduced BPV expression, suggesting that RKP00156 has a good potential to be used as antiviral agent for papilloma treatment in cattle.

Keywords: Papillomatosis, bovine papilloma virus, CDK9 inhibitor.

ID-67

Seroprevalence of *mycoplasma bovis* in outbreaks of bovine respiratory disease from 2015 to 2019

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Objectives: In the UK Veterinary Investigation Diagnosis Analysis (VIDA) data there has been a steady increase in the percentage of diagnosable submissions being attributed to *Mycoplasma bovis*. In the period from January 2006 to December 2017 there were 1102 diagnoses of *M.bovis* associated with respiratory disease, mastitis and arthritis. Of these diagnoses 86.4% were associated with respiratory disease. As part of a UK wide scheme to support investigation of bovine respiratory diease outbreaks serological testing of affected groups was undertaken to gain a understanding of the role of *M.bovis* in the disease outbreaks and raise awareness of it as a respiratory pathogen.

Materials & Methods: As part of a subsidised surveillance scheme, blood samples were submitted for serological testing from outbreaks of bovine respiratory disease across the UK. The submitting veterinarian was able to choose from a screening panel of potential respiratory pathogens (Infectious Bovine Rhinotrachietis, Bovine Respiratory Syncitial Virus, Parainfluenza-3, Bovine Viral Diarrhoea Virus and *M.bovis)*. This abstract reports the findings from the samples screened for *M.bovis.* Blood samples were analysed for *M.bovis* antibodies using a commercial ELISA kit.

Results: Over the course of 5 years a total of 14451 samples were tested from across the UK. *Mycoplasma bovis* serology results are presented from samples submitted between 2015 and 2019 and summarised in table 1.

Year	Number of samples tested	Percentage of samples positive for *M.bovis*
2015	2460	50%
2016	3354	51%
2017	3351	48%
2018	2918	39%
2019	2368	41%

Table 1: Percentage of samples testing positive for *M.bovis* by year

Conclusion: The results highlight the importance of *M.bovis* as a potential respiratory pathogen on UK farms. It is important that farmers and veterinary surgeons are aware of the impact of the disease and are able to identify it's clinical presentations rapidly. The management of *M.bovis* on farm can present significant challenges. For farms that are unaffected the emphasis should be on preventing introduction through good biosecurity and stringent controls on purchasing stock. For herds where mycoplasma is already present steps should be taken to minimise spread and reduce the impact of the disease. The absence of accurate prevalence figures makes economic analysis difficult, although it is clear that the costs of mycoplasma disease include reduced production, drugs and labour for treatment, death and culling losses as well as the financial impacts of implementation of diagnostic and control measures. Because *M.bovis*-associated disease tends to be chronic, costs per case are typically high relative to other pathogens. In addition to the financial costs, *M.bovis* can contribute significantly to antimicrobial usage on farm.

Keywords: Mycoplasma, Pneumonia, BRD, Calves, Serology.

ID-68

Neurological signs in lambs as indication for BVDV circulation in a cattle young stock rearing herd

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Objectives: Twin lambs, born on a farm where young stock and sheep were raised together in the same barn, presented with neurological signs. The clinical signs resembled a border disease virus (BDV) infection without the previously described coat abnormalities. As BDV could not be ruled out, samples were sent for diagnostic testing and subsequent sequence comparison to identify potential infection with sheep or cattle pestiviruses.

Materials and methods: Both lambs were sampled and screened for bovine viral diarrhoea virus (BVDV) using an antigen ELISA. Also the young stock rearing herd, consisting of 2 adult cows, 26 heifers and 18 calves, were tested for both BVDV antibody and antigen using an ELISA. One PI calf was identified. The serum samples of both lambs and the PI calf were subsequently subjected to a 5'UTR RT-PCR. The PCR products were cloned in a pGEM-T easy vector and sequenced.

Results: The 5′UTR polymerase chain reaction (PCR) confirmed that both lambs and one calf were infected with a BVDV 1b strain. Phylogenetic analysis indicated that all BVDV (bovine and ovine derived) were type 1b. Additional analysis of the E2 and NS5b regions of the genome revealed a ~100% sequence identity for 5′UTR, E2, and NS5b.

Conclusion: Viral transmission from a PI calf to the sheep dam during pregnancy was considered the most likely route of infection. This case demonstrates that sheep can be infected with BVDV. Neurological signs and other health problems in sheep could indicate a BVDV infection in both sheep and cattle when reared in close contact.

Note: for a presentation supporting information is available:

1. A figure of the pylogenetic tree of the E1/E2 region of the type 1b BVDV viruses derived from both lambs and the calf with reference strains (GenBank reference).
2. A video of the 1-day old lambs with BVDV type 1b showing neurological signs.

Keywords: Bovine viral diarrhoea virus, BVDV, dairy cattle, sheep.

ID-69

Evaluating factors affecting recovery of *Mannheimia haemolytica* and *Pasteurella multocida*

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Microbiological diagnosis is an important step in controlling and preventing bovine respiratory disease (BRD). Moreover, adequate transport storage type, elapsed time, and storage temperature before laboratory submission are critical for optimal results. The objective was to evaluate the effect of transport storage media, time, and storage temperature on *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM) yield using an in-vitro model simulation. Semi-quantitative (quadrant method) and quantitative culture methods using colony forming units per ml (CFU/ml) were used to recover MH or PM using an in-vitro model with swabs. In both approaches, samples were grown in 5% sheep blood agar plates. In each trial, a sterile PBS solution was inoculated with MH or PM, achieving 0.5 McFarland using OD_{600} between 0.08-0.1. A total of 58 sterile cotton swabs were inoculated in a culture solution with MH or PM and placed in either: 1) sterile falcon tube (DRY); 2) Aimes culture media with charcoal (ACM); or 3) Cary-Blair transport Agar (CBA). Swabs were evaluated for recovery of MH or PM at three temperatures: 4, 23, and 36°C; and assessed at four-time points 0 (baseline), 8, 24, and 48 hrs. A multivariate mixed model was fitted to analyze the data using lme4 and lmerTest packages of R. When normality was not rejected, the dependent variable was the CFU/ml. The independent variables were storage media (DRY, ACM, and CBA), time points (8, 24, and 48 hrs), and the interaction between storage media and time points. Each swab was considered as an independent measure. When the normality was rejected, the non-parametric Dunn all pairs approach was used to compare CFU/ml between storage media, with one model created for each temperature and time point combination. The CFU/ml recovery of PM on samples stored at 4◦C was lower for ACM when compared to DRY at 8 hrs (P = 0.05) but higher

at 48 hrs ($P < 0.01$). For samples stored at 23◦C, ACM had a higher CFU/ml recovery than DRY at 24 hrs ($P < 0.01$), and at 48 hrs, ACM and CBA were higher than DRY ($P < 0.01$). At all-time points, samples stored at 36◦C had a higher CFU/ml recovery in ACM and CBA than DRY ($P = 0.02$). The CFU/ml recovery of MH on samples stored at 4◦C was higher for ACM and CBA than DRY at time points 24 ($P < 0.01$) and 48 ($P < 0.01$). Samples stored at 36◦C had a higher CFU/ml recovery for ACM and CBA than DRY at time point 24 ($P < 0.01$). These results support the value of ACM and CBA for the recovery of PM and MH isolates, especially if samples were not refrigerated properly. Also, the combination of longer elapsed time and higher temperatures can impair diagnostic accuracy.

Keywords: Mannheimia haemolytica, Pasteurella multocida, transport media, bovine respiratory disease.