



EP-P01

A Field Study to Determine Antibody Titers of BRD Pathogens on Dutch Dairy Farms without BRD Problems.

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Objectives: The major Bovine Respiratory Disease (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 young animals and lactating cows 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 vaccination. The latter may help to develop a BRD vaccination program on dairy farms.

In this field study, antibody levels against the BRD pathogens (Parainfluenza 3 virus (PI3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* and *Mycoplasma bovis*) in healthy calves and cows were determined on several Dutch dairy farms without major BRD problems and not vaccinating against BRD to have an impression on the epidemiology of some major infectious risk factors for BRD.

Materials and Methods: The study was performed on Dutch dairy farms with at least 100 lactating cows, that are not vaccinating against BRD and without major BRD problems. In 2 different farms (January 2019), all the youngstock were blood sampled as well as three groups of 10 lactating cows (respectively in first, second and third or more lactation) on the same day. On 10 other different farms (Jan-March 2019), blood sampling was performed in five calves between three and six months old, as well as in five calves between eight and twelve months.

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

Results: Although none of the farms had any obvious BRD problems, antibodies against at least one BRD pathogen could be identified on each farm, except for *Mycoplasma bovis*, that was only present in a few animals in 7 of the 12 dairy farms. Nine of the 12 farms were positive for antibodies against BRSV in several combinations with the other BRD pathogens.

Most samples were positive for *Mannheimia haemolytica* (95%), followed by PI3 (87%), BRSV (30%) and *Mycoplasma bovis* (6%). For each pathogen the level of antibodies increased with the age of the animals.

Of the 10 farms where only youngstock was sampled (n=10), 5 farms were positive for all 4 pathogens, 2 farms were only positive for *Mannheimia haemolytica*-BRSV-PI3, 2 farms only positive for *Mannheimia haemolytica* and PI3 and 1 farm only positive for BRSV-PI3. The 2 farms where all the youngstock as well as 30 adult cows were sampled, were positive

for all 4 pathogens. Especially in calves between three and six months old there are huge differences in seroprevalence between farms. All pathogens were frequently identified in animals younger than 1 year, apart from *Mycoplasma bovis* that only has been identified in 15 animals (7%) out of the 205 samples from the animals younger than 1 year.

Conclusion: Nearly all dairy farms in this study (no BRD problems and not vaccinating against BRD) had seropositive animals for *Mannheimia haemolytica* and PI3. Also, antibodies against BRSV were frequently detected, however detection of antibodies against *Mycoplasma bovis* was rather an exception. Based on these findings, vaccination to protect against *Mannheimia haemolytica*, PI3 and BRSV may be a strategy to minimize the risk of a BRD outbreak on BRD-free farms, as the presence of *Mycoplasma bovis* was rather exceptional based on the results of this study.

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

EP-P02

Prevalence of *Histophilus somni* in Spain

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Objectives: Bovine Respiratory Disease (BRD) is the most common cause of death and disease in cattle¹. The most common bacteria involved in BRD are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis*. However, *H. somni* has been underdiagnosed in the last few years since this bacterium requires specific growth conditions and is easily overgrown². Information on the prevalence of this bacterium in animals with suspected BRD using more sensitive methodologies is necessary for the evaluation of evidence-based preventive measures. The aim of this study was to evaluate the prevalence of *H. somni* as a pathogen involved within the BRD complex in the Spanish national herd, along with comparing results obtained by culture versus Taqman Real Time PCR (qPCR).

Material & Methods: In this study, the presence of *H. somni* was assessed in 1,396 samples from animals with suspected BRD from January 2017 to January 2020, representing 346 Spanish farms. Samples were passively obtained by a private diagnostic laboratory (EXOPOL) with the aim of determining the causal agents at the time of a BRD problem. The BRD panel offered by this laboratory contains the most common bacteria and viruses³, including the diagnosis of *H. somni* by both culture and qPCR. All the samples were individually tested by culture. Nevertheless, qPCR was run with a commercial kit (EXOone *Histophilus-somni* oneMIX) on pools of up to 5 samples from the same farm.

All the data were collected and analysed using Microsoft Excel. The sensitivity and specificity of both techniques were assessed.

Results: The highest percentage of farms showing BRD



problems were in Catalonia (27.7%), Aragon (13.9%) and Castile-Leon (13.6%). Out of the 1,396 samples analysed, 719 represented feedlots, 287 were veal units and 120 came from adult cattle. Samples received at the laboratory included 50.6% bronchoalveolar lavages, 33.8% organs (lungs in 89% of the cases), 12.1% respiratory tract swabs and 3.5% tracheal scrapes.

When both techniques were compared, bacterial culture detected positive results for *H. somni* in 2.7% of the samples, whereas 23.0% gave positive results by qPCR. It is worth mentioning that all the positive samples on culture were also positive by qPCR; therefore, culture failed to detect 73.0% of the positive samples identified by qPCR. Consequently, the qPCR results were selected for evaluation of the prevalence of *H. somni*.

The prevalence of *H. somni* detected in this study was 23.0%, whilst differences were observed over the 3 years (18.4%, 17.8% and 28.9% prevalence was detected in 2017, 2018 and 2019, respectively). Whereas, when the prevalence distribution is analysed by the production system, 26.4%, 20.2% and 9.3% of the samples were positive in feedlots, veal units and adult cattle, respectively. With regard to the type of sample, the highest detection rate was found in tracheal scrapes (33.3%), followed by bronchoalveolar lavages (30.2%), organs (19.3%) and finally 17.8% in the case of respiratory tract swabs.

Conclusions: In this study, samples originated from both live and dead cattle and were analysed by culture and qPCR. The difference in detection of *Histophilus somni* between culture and qPCR is likely associated with the limitation of growing these fastidious bacteria and common practices implemented in the field such as the use of therapeutic antibiotics^{2,4}. The results of this study confirm those previously published by Bell *et al.* 2014⁴ regarding the use of qPCR assays for improving the diagnosis of bacteria.

The distribution of the samples received expressed as a percentage corresponds to the distribution of the Spanish cattle census⁵. Therefore, the results obtained herein can be considered to be representative of Spanish geography, detecting a 23.0% prevalence of *H. somni* (range 17.8%-28.9%). This prevalence is in accordance with that found by PCR in other European countries such as the UK (23.3%)⁴, albeit lower than that reported in Belgium (36.4%)². These results highlight the importance of including measures for *Histophilus somni* prevention on Spanish cattle farms such as vaccination programmes, together with measures against other *Pasteurella*-*ceae* and viruses, to minimise the impact of BRD.

References:

- ¹Holman *et al.* 2015. *Vet. Microb.* 180(1-2), 90-95.
- ²Pardon *et al.* 2020. *JDS* 103 (3), 2556-2566.
- ³Available from: <https://www.exopol.com/en/diagnostic/list/bovino>
- ⁴Bell *et al.* 2014. *J. Vet. Diagn. Investig.* 26(5), 631–634.
- ⁵MAPA, 2019. "Estudio del sector español de cebo de vacuno. Datos SITRAN".

Keywords: Bovine Respiratory Disease, *H. somni*, prevalence, PCR, Spain.

EP-P03

Prevalence of bovine respiratory syncytial virus (BRSV) infection in calves on Polish dairy farms.

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Objectives: Cattle respiratory diseases are a significant cause of economic losses associated with cattle breeding. Infections are multifactorial: viruses, bacteria and the environment. Viral infections also play an important part in the pathogenesis of bacterial pneumonia in feedlot cattle. One of the viral factors responsible for this disease is Bovine Respiratory Syncytial Virus (BRSV), which is widespread throughout the world. It is classified as Bovine *Orthopneumovirus*, belongs to the genus *Orthopneumovirus* in the family *Pneumoviridae* and is a negative-sense single-stranded RNA virus. BRSV spreads very quickly by the aerogenous route and replicates in the upper and lower respiratory tract, causing mucosal inflammation. At the initial stage of infection, nasal epithelial cells are targeted, thereby facilitating subsequent adhesion and replication of pathogenic bacterial species (through up-regulation of adhesion receptors, denudation of the epithelium, etc.). The aim of this study was to determine the prevalence and seroprevalence of BRSV in calves on dairy farms located in different regions of Poland.

Material and methods: The samples were taken from calves with Bovine Respiratory Disease (BRD) symptoms or calves from herds with respiratory problems at the time of sampling.

To demonstrate the presence of BRSV, samples in the form of nasal swabs (70%) or broncho-alveolar lavages (30%) were taken from animals showing clinical signs (i.e. high body temperature, cough, dyspnoea, etc.). All the samples originated from March 2018 to December 2019 and were collected from animals younger than 3 months. Each sample analysed by PCR represented one dairy farm. These samples could be made up by pooling up to 3 individual samples on each farm. To collect material from the nasal cavity, flocked swabs (FLO-Qswabs, COPAN, Italy) were used; whereas, in the case of broncho-alveolar fluid, catheters with a 20 mL syringe containing approx. 10 mL of 0.9% NaCl (Polfa, Poland) were utilised.

For serology, blood from *v. jugularis* for serological tests was collected into serum tubes (KABE LABORTECHNIK GmbH, Germany) from 6-to-9-month-old calves. Samples were obtained from 2017 to 2019. The samples were transported, for no longer than 24 hours, at 4°C directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wrocław. Immediately after the samples were received, viral RNA was isolated using a commercial kit (RNeasy Mini Kit, QIAGEN, Germany). In the next step, another commercial kit (VetMAX Ruminant Respiratory Screening Kit, Thermo Fisher Scientific, USA) was used for detection of BRSV antigens. Real-time PCR was run using a thermocycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad, Germany).



In the laboratory, serum samples were tested for antibodies against BRSV by ELISA (test BIO K 284 - Multiscreen AbE-LISA Bovine respiratory, Bio-X Diagnostics, Belgium).

Samples for serology and PCR were obtained from different farms. The study complied with the quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

Results: Out of the 72 samples analysed for the presence of BRSV genetic material, 11.1% produced positive results. On the other hand, in 1,078 sera samples from 6-to-9-month-old calves, the seroprevalence was found to be 39.6%. From positive sera samples, slightly positive results were found in 19.3%, positive results in 35.6% and highly positive results in 45.1% samples.

Conclusions: In vivo demonstration of the presence of the BRS virus or its genetic material is possible at an early stage of infection. This test is definitely faster and more reliable than other methods, and prelaboratory activities as well as sample contamination have a negligible impact on its result. This allows you to quickly take action to reduce the negative effects of infection. Whilst the serological tests are definitely cheaper than PCR, their usefulness is limited. IgG antibodies already appear around the 13th day after infection, and in calves that received colostrum antibodies, we can detect antibodies at up to 6 months of age. In contrast, antibody detection is a good method for detecting the presence of BRSV in populations, especially calves on the farms with a potential cause of illness during the first week of life. In defence of the PCR method, authors have admitted that a low percentage of positive farms could be caused by the small number of animals sampled in most of the tested herds.

Keywords: BRSV, bovine respiratory disease, prevalence, Poland, cattle.

EP-P04

Prevalence of bacterial pathogens of Bovine Respiratory Disease in calves on Polish dairy farms.

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Objectives: The main bacterial pathogens associated with Bovine Respiratory Disease (BRD) are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis*. *M. haemolytica* is the main bacterial agent of BRD and has a considerable economic impact in cattle, especially in the feedlot industry. *P. multocida* and *H. somni* are also opportunistic BRD pathogens and are involved in the development of bronchopneumonia in cattle with clinical signs indistinguishable from pneumonia caused by *M. haemolytica*. *M. bovis* lacks a cell wall and is fastidious, requiring special media and techniques for its isolation and culture. *M. bovis* is often associated with chronic pneumonia, and its mechanism of ac-

tion remains poorly understood. The prevalence of BRD bacterial pathogens is relatively high and it has an impact on the health of calves on dairy and beef farms. The economic impact of cattle disease on calves still remains considerable, with BRD being the most significant health problem in the modern cattle industry: it is associated with pneumonia in nursing and recently weaned calves in the first weeks of life. The control of BRD in calves is aimed mainly at bacterial pathogens through antimicrobial and vaccination programmes.

The aim of this study was to determine the prevalence of bacterial pathogens involved in BRD in calves on dairy farms located in different regions of Poland.

Material and methods: The samples (n=181) came from calves with BRD symptoms or respiratory-related signs in the herd (n=90). All the samples originated from March 2018 to December 2019 and were collected from animals younger than 3 months.

In order to show the presence of bacteria, nasal swabs (70%) or broncho-alveolar lavages (BAL, 30%) were taken from live animals showing clinical signs such as high body temperature, cough and dyspnoea. To collect material from the nasal cavity, flocked swabs (FLOQswabs, COPAN, Italy) were used; whereas, in the case of broncho-alveolar fluid, catheters with a 20 mL syringe containing approx. 10 mL of 0.9% NaCl (Polfa, Poland) were utilised. The samples were transported, for no longer than 24 hours, at 4°C directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wrocław. Immediately after the samples were received, bacterial DNA was isolated using a commercial kit (DNeasy Blood & Tissue Kits, QIAGEN, Germany). In the next step, another commercial kit (VetMAX Ruminant Respiratory Screening Kit, Thermo Fisher Scientific, USA) was used for detection of *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis* antigens. Real-time PCR was run using a thermocycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad, Germany).

The study complied with the quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

Results: Out of the 181 samples analysed for the presence of *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis* genetic material, 52%, 98%, 34% and 44% of samples respectively gave positive results. When the results are shown by farm, *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis* were identified on 31%, 86%, 24% and 32% of the investigated farms, respectively.

Conclusions: Information about the prevalence of bacterial pathogens involved in the BRD complex is important in animal husbandry, as it provides evidence to effectively counteract the negative effects of infections and losses from illnesses, both in the choice of treatments and preventative measures such as vaccination. In our study, the prevalence of bacterial components within the BRD complex, especially *M. haemolytica*, *P. multocida* and *H. somni* was high. The prevalence of these pathogens is considered to be one of the most significant health problems in the cattle industry, accounting for economic losses that surpass those incurred by all other diseases of cattle combined. *M. bovis* under conditions that suppress host immunity (e.g., stress due to weaning, transportation or viral infections), can rapidly reproduce in the upper respiratory tract and gain access into the lungs through inhalation. There, they can adhere to and colonize the lung epithelial surface,



resulting in pulmonary inflammation and gross pathology.

Keywords: bovine respiratory disease, prevalence, bacterial pneumonia, Poland, cattle.

EP-P05

BVDV subtypes in cattle herds from the Czech Republic

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Objectives: *Bovine viral diarrhoea virus* (BVDV) causes significant economic losses in cattle herds worldwide. BVDV is a member of the *Pestivirus* genus of the *Flaviviridae* family. Its genome consists of positive sense single stranded RNA of about 12.3 kb with a single large open reading frame (ORF) flanked at both ends with untranslated (UTR) regions 5'UTR and 3'UTR. BVD viruses are both genetically and antigenically heterogeneous. Two BVDV species BVDV-1 and BVDV-2 are recognized and, based on the phylogenetic analysis of the 5'UTR and Npro regions, BVD viruses are further divided into several subtypes.

A previous study dealing with phylogenetic analysis of Czech isolates revealed the existence of five distinct BVDV subtypes. The aim of this study was to further evaluate the genetic heterogeneity of BVD viruses in the Czech Republic.

Material and methods: Seventy-two serum samples of BVDV infected animals collected during a 13 years long period from 67 dairy herds were used in the study. Viral RNA was extracted from 140 µl of serum using the QIAamp Viral RNA kit (Qiagen). Viral RNA was subsequently transcribed and amplified by a Transcriptor One-Step RT-PCR kit (Roche) using specific primers for 5'UTR and Npro genome sequences. Amplification primers were then used to sequence the PCR products in both directions. The obtained partial 244 bp and 384 bp long sequences of 5'UTR and Npro, respectively, were aligned using the ClustalX software and used to conduct the phylogenetic analyses in MEGA7. The phylogenetic trees were constructed using Neighbor-Joining algorithm with evolutionary distances computed using the Kimura 2-parameter method.

Results: BVD viruses clustered in the phylogenetic tree into seven different groups representing seven separate subtypes, namely BVDV-1d (n = 41), 1b (n = 19), 1f (n = 6), 1a (n = 2), 1e (n = 2), 1h (n = 1) and 1s (n = 1) out of which two, BVDV-1h and 1s, have not been detected in the Czech Republic previously. The prevalence of dominant subtypes BVDV-1d and 1b did not differ significantly during the time and the remaining subtypes occurred only sporadically.

Two BVD viruses collected from five herds four to seven years apart were sequenced. Reinfection with different BVDV-1 subtypes was detected in two herds, while in the remaining three herds, genetic analysis revealed continuous infection with the same virus.

Conclusions: Seven BVDV-1 subtypes have so far been detected in cattle from Czech herds. The subtypes BVDV-1d

and 1b predominated during the whole 13 years long period suggesting a relatively homogenous population of BVD viruses circulating in the cattle population.

Furthermore, genetic analysis is suitable to discriminate between reinfection and continuous infection with the same virus in infected herds.

The present study was supported by MZE-RO05178 of Ministry of agriculture of the Czech Republic.

Keywords: Bovine viral diarrhoea virus, phylogenetic analysis, reinfection.

EP-P06

Using a participatory approach to develop a network of sentinel veterinarians: A pilot syndromic surveillance in dairy cattle in Spain

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Objectives: As part of the of the activities carried out by the Operational Group for the improvement of dead animal collection and health alert systems (MESRASA), executed within the framework of the Spanish National Rural Development Program 2014-2020, we aim to establish a veterinary sentinel surveillance network for dairy cattle. The sentinel network will be built by enrolling private veterinarians to report routinely collected health data. The main objective of this network is to monitor selected dairy cattle health indicators, and evaluate their temporal trends within each herd. In addition, the system seeks to raise awareness among veterinarians regarding exotic diseases in order to increase the sensitivity of passive surveillance through the generation of warnings in case of an outbreak.

Materials and methods: The surveillance sentinel network is being implemented using a participatory approach in two pilot selected areas: Galicia (north-west of Spain) and Catalonia (north-east of Spain). At the time of writing, we conducted eight individual semi-structured exploratory interviews with representatives of the different profiles of private veterinarians in Catalonia. Doing this, we gained information on the main obstacles and possible incentives to the creation of the network and an overview of the existing views among private veterinarians operating in the area. We also obtained information on the files formats and management software mainly used. Moreover, a list of possible participants to the network among private veterinarians was drawn-up using non-probabilistic, snowball sampling; their final decision to participate was voluntary and free from overt. Indicators, alert levels, feedbacks and modalities of communications, which were identified through these interviews, will be further discussed in focus groups to reach a common agreement among all sentinel veterinarians. The different choices on the monitoring



system design were made by the sentinel veterinarians taking into account the herd data available in the field, the validity of indicators for herd health, and their professional interest in monitoring particular indicators (i.e. utility). In addition, the collaboration with computer engineers and software developers will provide technological support to the sentinel network and the development of ad-hoc tools for the reporting of data and the reception of automatic feedbacks.

Results: Exploratory interviews were conducted either face to face or by telephone with private veterinarians including clinical practitioners, reproduction specialists, podiatrists, and milk quality consultants. Preliminary results suggest that main factors that might inhibit veterinarians from participating to the network are linked to a low level of trust in the veterinary authorities, protection of anonymity and confidentiality and fear of the possible consequences when a warning is generated. The quality and reliability of routinely-collected data, and the effort required for data reporting were also mentioned as possible obstacles. However, the participants also showed enthusiasm for the creation of the network; they highlighted its utility in providing animal health information, contributing to create local and national references and being a useful tool for benchmarking. A set of preferred indicators were identified. These were abortions, abomasums displacements, causes of lameness, and mastitis. Moreover, the reporting of somatic cells counts and other specific laboratory-based test results was mentioned. It was proposed to report data on a monthly basis to an electronic system expressly designed for the sentinel network. Participants would like to receive a feedback report with a public section showing aggregated results and other additional health information, and a private section showing individual herd results (i.e. ranking, prevalence and trends). The definition of different warnings and response levels is currently under discussion. This study was conducted with compliance of research ethics norms and general principle established in the Code of Ethics of the American Sociological Association (ASA) and in the British Sociological Association's Statement of Ethical Practice. Before starting the interviews, participants were informed about the study objectives and formal consent was obtained.

Conclusions: The pilot sentinel network will increase data richness and facilitate the detection of changes in the occurrence of endemic diseases. Moreover, it might contribute to improve the early detection of epizootic diseases. The participatory approach used for the design of the sentinel surveillance system will increase its acceptability and sustainability.

Keywords: syndromic surveillance, dairy cattle, qualitative methodologies, benchmarking.

EP-P07

Prevalence of the main respiratory viruses in Bovine respiratory disease in Spain

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Objectives: Various viruses play a role in the onset of BRD. These include bovine adenovirus, bovine coronavirus, bovine rhinitis viruses, influenza D virus, bovine parainfluenza-3 virus, bovine viral diarrhoea virus (BVDV), bovine herpes virus type 1 (IBR), and bovine respiratory syncytial virus (BRSV). The latter three are the most important because of their clinical relevance¹.

The objective of this study was to assess the prevalence of the main respiratory viruses (BRS, IBR and BVD viruses) and their course over time to facilitate decision-making when designing evidence-based vaccination programmes.

Materials and methods: BOVIRESPCHECK is an *in vivo* diagnostic tool for identifying the main pathogens associated with BRD. Four samples from four calves are taken via nasal swabs and applied to an FTA card, thereby maximizing stability and safety when shipping the samples. Molecular diagnostic techniques (RT-PCR) are used, which have high sensitivity and specificity.

Using this kit, a total of 964 reports were created between 2016 and 2020, from a total of 661 farms distributed all over Spain (Table 1).

	2016	2017	2018	2019	2020	Total
Farms	138	134	153	154	189	661
Reports	170	169	196	212	217	964
Samples	682	684	794	842	885	3887

Table 1.

Each report corresponds to a farm that had an outbreak or respiratory problem at the time. Samples were taken from animals with early symptoms of disease at these cattle farms, which included both dairy and beef farms. If at least one sample tested positive for one of the three viruses, the report was considered positive. The number of samples refers to the number of animals sampled; 3,887 in total.

Results: The most frequently detected virus was BRSV, present in 31.5% of cases, with positivity ranging from 35.02% in 2018 to 26.42% in 2019. BVD was detected in 23.94% of all reports, varying from a peak of 31.95% in 2017 to 18.43% in 2020. IBR was detected in 17.2% of reports. Minimum positivity was detected in 2017 with 8.88%; however, the prevalence reached a peak at 28.11% in 2020 (Table 2).

55.85% of reports were positive for at least one of the three agents analysed during the study; 44.15% of reports did not provide a diagnosis, although there were variations between the years.

	2016	2017	2018	2019	2020	Total
Reports	170	169	196	212	217	964
Positive IBR (%)	17.06	8.88	19.89	10.38	28.11	17.20
Positive BRSV (%)	32.94	33.14	35.02	26.42	30.88	31.50
Positive BVD(%)	20.59	31.95	28.93	21.23	18.43	23.94
Positive Reports (%)	52.94	64.45	63.45	46.23	60.37	55.85

Table 2.



Diagram 1 shows the interactions between the different pathogens. Coinfection with the three viruses (BRS, IBR and BVD) was diagnosed in 3.34% of cases. BRSV was identified in coinfections with the IBR virus in 6.86% of cases and in 12.62% of cases with BVDV. A significant correlation (p -value = 0.03) of 0.07 was detected for an interaction between BRSV and BVDV. IBR and BVD were found together in 3.9% of cases.

Conclusions: During the five-year period presented in this study, BRSV was present in 31.5% of reports, which is equivalent to 56.4% of reports with a positive diagnosis.

BVDV was the second most commonly detected virus (23.94%). BVDV was found in combination with BRSV in 15.96% of cases, showing that there is a significant positive correlation between the two.

As for IBR, its incidence seemed to increase substantially over the course of 2020, with 28.11% of cases being detected. This marked increase could be a direct consequence of the legislative changes regarding the use of non-marker vaccines (including polyvalent vaccines), meaning that vaccination against this pathogen decreased during this period.

These results underline the significance of implementing complete vaccination programmes that cover all the most important respiratory viruses. BRSV and BVDV vaccination seems key not only because of the high incidence of both viruses, but also because of the positive correlation between them. The use of IBR monovalent marker vaccines should be added too, as their exclusion could be partly responsible for the increased incidence of IBR in 2020.

Keywords: Bovine respiratory disease, prevalence, IBR, BRSV, BVD.

EP-P08

Risk assessment as a decision support-tool to improve farm biosecurity

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Introduction and Objectives: On-farm biosecurity plays a key role in reducing the risk of introduction and spread of infection diseases. The adoption of biosecurity measures (BSMs) can be beneficial to prevent exotic diseases, but also diseases affecting production, or other endemic diseases subject to control programs. In this context, the development of tools aiming to support decision-making on which BSMs should be improved/implemented to reduce the probability of introduction of pathogens might be relevant. The aim of the present study was to develop a quantitative risk assessment model to identify farm-specific biosecurity measures that should be implemented to reduce the probability of infectious diseases into cattle herds, flexible enough to be adapted to different pathogens and production systems.

Material & Methods: The model was developed for BVDV and BHV1 for dairy cattle, and tuberculosis for beef cattle. For those pathogens and for each production system, the different pathways by which they could be introduced in a herd were identified. The probability of these pathogens introduction through the different pathways was calculated considering the characteristics of each studied farm and its region, the already implemented biosecurity measures and epidemiological data of each disease. Farm-level data were collected through face-to-face questionnaires conducted in North-Eastern Spain. Moreover, to identify BSMs that should be prioritized in each farm to reduce such risk, the model was run under several hypothetical scenarios, assuming the implementation of additional BSMs. Estimations of the impact of the additional BSMs on the farm-specific risk were obtained by calculating the risk reduction before and after their application. This allowed to identify what measures should be implemented to get a significant risk reduction. The stochastic analysis was performed and all non-fixed parameters were included as uncertain parameters.

Results: For BVDV and BHV1, a quarantine of at least 24 days, visited at the end of the workday and farms where cattle was tested on arrival, greatly reduced the probability of virus introduction. Not sharing transport with cattle from other farms had also a great influence in the probability of virus introduction through the purchase of animals. For indirect contacts, the analysis showed that providing farm specific protective clothing and boots and avoiding animal transport drivers having contact with animals present in the farm, would highly reduce the probability of infection through indirect contacts. In the case of bovine tuberculosis for most of the farms, recommended biosecurity measures to reduce the risk through the purchase of animals were a combination of quarantine together with adequate isolation and testing, especially if cattle had not been tested before transport.

Conclusions: The developed model can be a powerful tool to optimise the risk management on farms and support the development of farm-specific biosecurity plans. Moreover, it can contribute to educate and raise awareness on the benefits of BSMs by demonstrating the quantitative impact of their adoption.

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Keywords: Biosecurity, Infectious Diseases, Risk assessment, Prevention.

**EP-P09****Prevalence of selected calf scour pathogens in Slovenia**

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Objectives: Neonatal calf diarrhoea (NCD) is a highly prevalent condition. It may be caused by various pathogens of which the bovine Rotavirus and *Cryptosporidium parvum* are the most common. The objective of our study was to evaluate the prevalence of selected calf NCD pathogens in the Slovenia calf population with diarrhoea. We were particularly interested in assessing the prevalence of *Cryptosporidium parvum*.

Materials and methods: Field veterinary practitioners were asked to collect faecal samples from scouring calves, in the age group 6 to 21 days. Veterinarians were also asked to fill out the information on the calf: age, breed, days with diarrhoea, body temperature, medicines used, and on-farm type (dairy, beef, suckler), number of cattle, calf feeding routine and occurrence of other diseases. Investigators finally sent 68 faecal samples from all parts of Slovenia. All samples were screened using a rapid immunochromatographic test (Speed V-Diar™ 4, Virbac, France) for the four most common calf scour pathogens (Rotavirus, Coronavirus, *E. coli* F5 (K99) and *Cryptosporidium parvum*).

Results: The median age of sampled calves was 10 days (range 4-41) and were experiencing diarrhoea for 4 days on average. The prevalence of calf scour pathogens in Slovenian scouring calves using the rapid tests were 37%, 9%, 1% and 40% for Rotavirus, Coronavirus, *E. coli* F5 (K99) and *C. parvum*, respectively. 42 % of samples were positive for only one pathogen, while 21% were positive for two or three, 37% of calves were negative for all the most common calf scour pathogens using the rapid test. 40% of scouring calves had a history of treatment with antimicrobials.

Conclusions: The prevalence of the most common scour pathogens in Slovenia is similar to those reported for other European countries. In this survey, we observed that 37% of calves were negative for all pathogens included in rapid tests. Scours aetiology in calves is not limited to just four pathogens and can be caused by others, like other strains of *E. coli*, *Clostridium* spp., *Salmonella* spp., *Coccidia* spp., *Giardia* spp., etc. Very often, flaws in management result in NCD outbreaks. We aim to use these results to stimulate farmers to vaccinate dams against neonatal scour pathogens. The study also raised the issue of antibiotic use in non-bacterial causes of calf diarrhoea.

Keywords: Neonatal calf diarrhoea, *Cryptosporidium*, Slovenia.