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Oral Communications

BVD / Pestivirus

Voluntary BVD control - The New Zealand experience. The importance of good adult learning principles.

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Objectives: BVD is a common disease in cattle in New Zealand. Since 2005, the BVD Steering Committee in New Zealand has developed a veterinary BVD management toolkit, combining resources such as a Powerpoint presentation, breeding bull virus testing and vaccination certificates, farmer information pamphlets and an open website www.controlBVD.org.nz. In partnership with a major pharmaceutical company, these resources have been refined and presented to rural veterinarians to be used as tools to assist engagement with farmers to increase farmer participation in BVD control at individual farm level.

Methods: In 2011 the BVD Steerering Committee undertook an extensive "Road-show" programme to rural veterinarians to explain the use of the BVD management toolkit. These meetings were interactive information so that the participants could see how to control and manage BVD at individual herd level. In 2012 further developments occured with the production of a BVD power-point presentation with individual slide commentary. This has been a central tool for veterinarians to use when taking farmers through a BVD tutorial. This starts by defining and describing the disease and the various tests available and then transitions into control options and how a farmer may go about setting up a farm management plan. Veterinarians have been encouraged to use this tool as an opportunity to engage farmer attention in understanding this complex disease and the control options available. The farmer information pamphlets, bull testing certificates and website are useful aids to assist farmers reinforce the messages given in the power-point presentation.

Results: The author is a rural veterinarian and has used the power-point presentation on 62 farms in the last 2 years, initially as a pilot for the committee and more recently as a refined tool. By applying inclusive adult learning techniques with the power-point presentation the author has been able to successfully engage 100% of these farmers, whether they be farming dairy or beef herds. This success has encouraged the BVD Steering committee to further develop education of rural veterinarians by holding "Champions" meetings because it was recognised that the main limitation of farmer uptake for BVD control was how veterinarians engaged farmer attention and not disease knowledge. In short, the principles of successful adult learning are essential. Key veterinarians in a range of veterinary practices have been exposed to tutorial sessions explaining how to use the power-point presentation. This has challenged veterinarians to reconsider their approach to how they go about farmer education with BVD. During these meetings veterinarians have been challenged with such questions as 1. Who owns BVD? 2. Do you believe it is an important production limiting disease? 3. Who finds discussing BVD with farmers easy? 4. What do you think the drivers of farmer action are? Most importantly they have been encouraged to enter knowledge relationships with farmers, offer options for control and allow the farmer to "own" the decisions. When this is done well, farmer uptake in BVD control has been high.

Conclusions: Rural veterinary practice in New Zealand has been undergoing quite a transformation over recent times. There is still a call for the traditional role managing crisis events such as calving cows, lame cow examinations etc, however, in more recent times farmers have asked for more comprehensive animal health "packages" where whole flock and herd issues are addressed. To "engage" farmers in a meaningful way the veterinarian must understand principles of adult learning in order to remain relevant to the rural sector. BVD management plans allow the veterinarian to display both disease management and adult learning skills well.

Interpretation of bulk tank milk antibody responses to BVD in vaccinated herds

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Objectives: To complete a long term farm-based study of inactivated BVD vaccines and their impact on bulk tank milk antibody test.

Methods: The study was carried out in 49 farms enrolled in a voluntary BDV control program and that were going to be vaccinated for the first time. Three different commercial vaccines were used. In the studied herds, BTM samples were collected and examined at the time of the first vaccination (t1) and every six months, as mentioned, during a 3-year period (t2-t7). BTM samples were analyzed with the commercial ELISA test "BVDV p80 Ab" (Pourquier Laboratories). Data were analyzed with SPSS 11.0. A one way repeated measures analysis of variance (ANOVA) was conducted to examine changes in the % inh obtained in the serial BTM samples using the Wilk's Lambda statistic and polynomial contrast

Results: Farms vaccinates with Bovilis BDV (MSD) revealed an initial decrease in the antibody levels (comparing t2 to t1), then increased slightly to the end of the study. Globally, the polynomial contrasts indicated a significant linear increase in the antibody levels during the study. Farms vaccinated with Hiprabovis Balance (Hipra Laboratories) also showed significant variations of the antibody level during the course of the study. However, polynomial contrasts tests failed to detect any trend in the evolution. Farms vaccinated with Pregsure BVD (Zoetis) showed a wide increase in the antibody levels throughout the period. The polynomial contrasts indicated that the increase was linear.

Conclusions: The results suggested that vaccination of the farms influence the level of antibodies against the p-80 protein of the virus. The vaccine that appeared to have more influence on the serological results is Pregsure BVD followed by Bovilis BVD and, to a lesser extent, Hiprabovis Balance. Nevertheless, the use of the last two vaccines seemed to be compatible with a monitoring program provided that it is taken into account when interpreting BTM antibody test.
Oral Communications

Increased expression of ADAMTS-13, nNOS and Neurofilament correlate with severity of neuropathology in Border Disease Virus infected small ruminants

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Objectives: Border Disease (BD), caused by a Pestivirus from the family Flaviviridae, produces important reproductive losses and brain anomalies such as hydranencephaly and cerebellar hypoplasia in aborted fetuses and neonatal animals. A disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS-13), a plasma reprolysin-like metalloprotease, cleaves von Willebrand factor and expressed exclusively in hepatic stellate cells of the liver. However, it is also produced in other tissues, including brain. The aims of this study were to investigate expressions of ADAMTS-13, neuronal nitric oxide synthase (nNOS) and neurofilament (NF) in the lesions of Border Disease Virus (BDV) infected small ruminants brain tissues and to understand whether any correlation between hypomyelinogenesis and BDV intensity.

Methods: Study materials were aborted foetuses, lambs and goats which were previously confirmed as BDV positive using RT-PCR and immunohistochemical examination. Pestivirus antigens were demonstrated by immunohistochemical examinations on their formalin fixed and paraffin embedded central nervous system tissues. In this study, immunoperoxidase investigations toward ADAMTS-13, nNOS and NF were performed.

Results: Results of the study revealed that the levels of ADAMTS-13 (p<0.05), nNOS (p<0.05), NF (p<0.05) were remarkably most higher in BDV-infected brain tissues than in the uninfected control brain tissues. The most significant microscopic changes in the brainstem and cerebral hemispheres were nonsuppurative meningoencephalitis accompanied by hypomyelinogenesis. The results of the present study showed that L-arginine-N0 synthase pathway is activated after BDV infection and that is indicated NF and nNOS expressions associated with severity of BD. There is limited number of studies focusing on the ADAMTS-13 expression in the central nervous system (CNS) and its function is still unclear. The most prominent finding; ADAMTS-13, having two CUB domain, showing the highest expressions are suggested to be involved in CNS developmental processes.

Conclusions: The results clearly indicated that the interaction of ADAMTS-13 and NO may play an important role on the regulation and protection of the CNS microenvironment in neurodegenerative diseases. In addition to these comments, expression of NF might give an idea of the progress of the disease. This is the first report of ADAMTS-13 expression in cells of the BDV-infected small ruminants CNS.

A new level of standardization in real-time PCR with the IDEXX RealPCR™ BVDV RNA Test

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Objectives: Real-time PCR has revolutionized diagnostic testing over the past years. While real-time PCR is continuously developing, commercial assays often deliver a set of reagents designed for testing a precise number of samples for a specific target(s). IDEXX RealPCR real-time PCR tests aim to provide a new level of standardization to PCR diagnostics by using reagents in a modular system. The components of this modular system are shared over the entire test platform, making it possible to run any pathogen-specific detection mix with a standard master mix and a single positive control. Moreover, the IDEXX RealPCR modular system maintains a single cycling protocol for all tests and shared quality controls and guidelines across the entire platform.

Methods: The RealPCR BVDV RNA Test has been designed to detect Type I, Type II and HoBi BVDV. It has been evaluated using characterized samples and synthetic oligonucleotides. Different sample types have been tested including whole blood, serum, plasma and ear notches.

Results: The test shows an analytical sensitivity of ≤ 15 copies / reaction for Type I, Type II and HoBi BVDV with efficiencies of > 95% over at least a 7-log range. The test displays no cross-reactivity with many common bovine viral pathogens either by in silico analysis or with diagnostic specificity testing. In addition to individual whole blood, serum, plasma and ear notch claims, the test detects BVDV in sample pools of up to 50 for blood fractions or 25 for ear notches.

Conclusions: A rapid lysis protocol for ear notches has been validated, eliminating the need for full RNA extraction which greatly reduces sample handling and processing time. To ensure reliable results, the test employs a multiplexed internal sample control (ISC) to detect endogenous bovine RNA that blocks the necessity of an internal positive control (IPC) spike and controls for sample addition, sample integrity as well as proper RNA extraction and successful RT-PCR reaction.

Bulk milk samples for diagnostic, surveillance and monitoring of BVDV in milk farms

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Objectives: Surveillance and monitoring of bulk tank samples to measure antibodies against BVDV in 92 dairy farms in Costa Rica
- Evaluate the movement of BVDV antibodies during a three month period
- Run BVDV Antigen tests in the farms with high Ab levels against the disease.

Methods: Bulk tank samples were taken from 92 dairy farms once a month during 3 consecutive months, bulk tank was homogenized during 5 minutes, 200 ml of milk was transferred into a plastic bottle, kept refrigerated and transported to the laboratory, samples were frozen at -20°C, until final collection when all 3 months samples were run together. The farms in the study are located in Costa Rica, milked twice a day, vaccination programs against IBR and BVDV has a wide range of methods and application intervals,
different brands of vaccines are used with variable levels of good application and management procedures for the products, killed virus vaccines are the most used. From the 92 farms, 52 did not have a vaccination program against the disease. The ELISA test used (IDEXX® BVDV Total Ab) is an indirect enzyme immunoassay designed to detect BVDV antibodies (Ab) in serum, plasma, and milk samples from bovines. ELISA of BVDV Ag capture was performed in 100 % of the heads in one farm. Sample used was large ear notch (LEN) that measures 1 cm. The ear notcher is washed with water, then in chlorine solution, and then rinsed with water again. Ear notches are stored in plastic tubes and shipped to the laboratory for the ELISA test. The ELISA test used was the IDEXX BVDV Ag/Serum Plus which is an enzyme immunoassay designed to detect BVDV antigens (Ag) in serum, plasma, whole blood and ear notch tissue in cattle.

Results: The prevalence by farm for BVDV Ab was 79.35 % (73 of 92), measured by the average of the S/P relation of the monthly samples on March, April and May 2013. (Table No.1) Graphic No.1 shows the Ab levels for BVDV on all 92 studied farms arranged from low to high Ab levels, there are 7 farms completely negative, this suggests that the animals in that farm has not been in contact with the virus; there are 10 farms over de Standard Deviation which suggest contact with the virus (PI’s). Negative farms with some Ab levels suggest that some animals have been in contact with the virus in low levels or a long time ago. A high Coefficient of Variation (CV %) (58.81 %), Ab levels have a high variation between farms because of different health programs against the disease, different vaccines used, vaccination interval, immune status of the herd, and management. We can determine in which farms we should do an Antigen test to detect the PI’s, this farms would be the ones with high Ab levels, because the PI’s are infecting other cows that will produce Ab against BVDV. In the vaccinated farms group there are 4 negative farms (1-4) this tells that vaccination programs against BVDV is not working properly on those farms, vaccination program must be evaluated to correct any error in order to have antibodies against BVDV produced by the vaccine, that guarantee protection against the disease.

Conclusions: With bulk tank testing, surveillance and monitoring of antibody levels is possible. Bulk tank testing in a scheduled and continuous way is a helpful tool to measure Ab levels against BVDV in dairy herds. After the Ab tests in bulk tank, farms with high probability to have a PI in their herd can be identified by high and continuous Ab levels, followed by Ag tests. Zone segregation can be done and used to determine different sanitary programs for each zone depending on Ab levels and movement.

Reproductive performance in pregnant ewes experimentally infected with BVDV and transmission rates in sheep co-mingled with BVDV PI calves
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Objectives: The aims of this study were to investigate the potential for natural transmission of BVDV from cattle to sheep and determine the impact an experimental infection, with a local Australian strain of BVDV, would have on the reproductive performance of pregnant ewes. In order for these outcomes to be able to be monitored, a commercially available enzyme-linked immunosorbent assay (ELISA) was validated for the use on sheep serum.

Methods: Serum samples (n=99) were tested by ELISA and results compared to the agarose gel immunodiffusion (AGID) assay which was used as the reference standard. Nine BVDV naïve wethers were co-mingled with three BVDV persistently infected (PI) calves in a small paddock for four weeks and observed for seroconversion. Twenty-three BVDV naïve, pregnant ewes were experimentally infected with BVDV and their subsequent reproductive outcomes monitored.

Results: A cut-off threshold of ≥35 percent inhibition was established in the ELISA to maximise diagnostic sensitivity for the detection of BVDV antibodies in sheep serum. Cross-species transmission from PI calves to naïve sheep occurred in 4 out of 9 wethers after four weeks of co-mingling, while severe reproductive losses, including a high percentage of abortions and early lamb deaths, were seen in the BVDV infected ewes. One persistently BVDV infected lamb was also produced in the trial; it however died at 15 days of age.

Conclusions: Cross-species transmission of BVDV into sheep populations occurred with severe reproductive losses being observed, as was the possibility for PI lambs to be produced. These results suggest that sheep populations may have the ability to act as a reservoir of BVDV infection in cattle and should be considered in the establishment of future monitoring and/or control programmes.

Etiologic diagnosis of bronchopneumonia in calves associating transtracheal lavage and molecular biology techniques
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Objectives: The "Bovine Respiratory Disease Complex" has a high degree of complexity due to multifactorial and synergism between viral and bacterial agents. The main established measure in the raising calves is treatment with antibiotics, however, the identification of risk factors, including identification of viral agents involved in this process, it is essential to control the disease. The use of serologic tests traditionally used for diagnosis of viruses in the raising of calves is limited by the presence of antibodies (Abs) maternal in the calves blood circulation up to 6 months old, pointing to direct tests for agent detection. The installation of pathogens in the lungs points to the transtracheal lavage association with microbiological and molecular biology techniques, as polymerase chain reaction (PCR). The objective of this research was to propose flow chart for diagnosis the situation in the raising of calves focus on bronchopneumonia.

Methods: The study was conducted in a farm high-producing dairy where the calves were kept in individual cages suspended approximately 1m², distributed in barn covered area with 112.5 m² and a height of 4.5m, where they are installed 95 calves between 1-60 days of life (1.18 unit animal/m²). The monthly rate of respiratory problems reported in the raising of females calves was 5.5%, showing especially in animals between 40 to 60 days of age. The expansion of the herd from 900 to 1680 lactating cows resulted in higher birth rate and increased occurrence of...
Methods: For etiologic diagnosis was made transtracheal lavage (TL) in ten calves with clinical pneumonia for identification of bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) by polymerase chain reaction (PCR) and determination of bacterial species involved by cultivation. Blood samples were also collected for determination of Abs titers for BVDV, BoHV-1 and bovine respiratory syncytial virus (BRSV) by serum neutralization, traditionally used in adult cattle.

Results: Among viral agents investigated, it was possible to prove the involvement of BVDV by positivity in 2/10 (20%) calves to the PCR. At this step, was not possible to detect for PCR positive BoHV-1. Antibody titers obtained was 4-512 BoHV-1; 40-5120 BVDV, and 32-256 for BRSV. Importantly, the two PCR positive calves in the TL for BVDV presented titers of 40-640 for BVDV. Regarding the bacterial isolation, was observed growth of Pasteurella multocida in 5/10 (50%) of TL obtained. The remaining samples showed no bacterial growth, probably because some animals were treated with antibiotics. Pasteurella multocida strains were resistant in vitro to 6/14 antibiotics tested: amikacin, ampicillin, amoxicillin associated with clavulanic acid, gentamicin, neomycin and trimethoprim sulfas associated. Observed sensitivity to cephalinex, cephalexin, cefoxitin, cefetin, ciprofloxacin, enrofloxacin, norfloxacin and tetracycline.

Conclusions: The detection of BVDV by PCR from transtracheal lavage had not yet been reported in the literature, highlighting the importance of this method for the diagnosis of BVDV in calves, especially in the moment where maternal Abs are present and make it impossible to use the serum neutralization technique. The BVDV infects leukocytes involved in innate and specific immune response in the respiratory tract mucosa, allowing the installation of Pasteurella multocida. Immunization programs of the raising calves through colostrum and early vaccination of calves are strategies to be inserted to prevent the BVDV in raising calves.

BVDV control in New Zealand: progress in a non-regulated environment
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Objectives: Efforts to coordinate BVDV control in New Zealand got underway in 2005 when a steering committee composed of interested veterinarians formed with the support of the Sheep and Beef and Dairy veterinarian's branches of the New Zealand Veterinary Association. Initial activities focused on trying to quantify the costs of BVDV through research efforts while increasing awareness of the disease among veterinarians and farmers.

Methods: Efforts to promote virus testing of breeding bulls led to significant increases in testing by bull suppliers. Simultaneous encouragement of industry groups led to advances in test development, and the best use of available vaccines. A web site to act as a repository of information was set up, and an online discussion group formed. The steering committee partnered with industry to produce a veterinary BVDV disease control manual tailored for dairy and beef farms. This increased veterinarians' understanding of the disease and increased their confidence in promoting on-farm control. Once the understanding of disease among the veterinary profession had been raised, the next step was to select BVDV champions from rural clinics throughout the country. Targeted teaching of these champions about adult education and the intricacies of BVDV testing and control promoted a ripple effect as they returned to their clinics. More and more trained and BVDV interested veterinarians spread the message through their clinics and farming communities.

Results: As a voluntary control scheme, with no regulatory backing, it is the enthusiasm and passion of the bovine veterinary community contributing to significant BVDV control efforts in New Zealand. PCR testing of milk for BVDV was introduced to New Zealand in 2007, and in the first year of testing, 600 herds (about 200,000 cows, mean herd size 333) were tested. Virus was detected in 14% of the herds. By 2012, about 5235 herds (about 2,057,355 cows mean herd size = 393) were tested. Virus was detected in 11%. During this period pooled serum antibody testing was developed for non-milking animals and large numbers of cattle were screened individually for the presence of virus.

Conclusions: This talk will outline the history and goals of BVDV control in New Zealand with a special emphasis on the development and use of diagnostic tests.

Performance characteristics of an ELISA for the detection of Bovine Viral Diarrhoea Virus (BVDV) antibodies when using bovine colostrum compared to serum samples
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Objectives: There are a number of livestock diseases which are characterised by an extended subclinical disease phase. With diseases like this, such as Johne's disease, diagnostic tests often display low sensitivity, causing difficulty in detection of infected animals. Due to the relatively high concentrations of immunoglobulins present in the colostrum following parturition, it is likely that testing of colostrum, rather than serum or milk, will improve test sensitivity. Bovine Viral Diarrhoea Virus (BVDV) is a pestivirus in the family Flaviviridae and is considered to be one of the most economically important diseases of cattle worldwide. Ab-ELISA is the test of choice for the routine diagnosis of exposure to BVDV as it is a rapid and relatively inexpensive test to perform. If the sensitivity of the Ab-ELISA can be increased when using colostrum, it will increase the assurance of absence of disease, or improve identification of exposure at the herd level. The collection and testing of serum and colostrum samples from a BVD vaccinated South Australian dairy herd and a bulk-milk negative New Zealand dairy herd was a ‘proof-of-concept’ study to determine the performance characteristics of colostrum, when compared to serum, using a commercially available ELISA for detection of BVDV antibodies.

Methods: Serum and colostrum samples were collected from 30 Holstein-Friesian dairy cows from South Australia that vaccinates against BVD, following a previous outbreak (Pestigard®, Zoetis - an inactivated vaccine) and from 20 Jersey dairy cows from a dairy in New Zealand that was identified as bulk-milk negative (S/P ratio ≤ 0.2) following 3 consecutive bulk-milk antibody tests over a 10 month period. The colostrum samples were collected within 24
hours of calving, and the blood samples were collected 30-60 days following calving. The samples collected from the South Australian dairy herd (antibody positive herd) were tested in-house and in triplicate, as per the manufacturer’s instructions. The samples collected from the New Zealand dairy (antibody negative herd) were tested by a commercial laboratory (Gribbles Pathology, Palmerston North, NZ) using the recommended dilution for serum (1:5). Frequency histograms and Q-Q plots determined that the data was non-normally distributed. Minimum, median and maximum S/P ratios for all dilutions for serum and colostrum were used to create box-and-whisker plots using Microsoft Excel 2010. A Wilcoxon signed rank test was used to identify any significant differences using the statistical program R (version 3.0.2).

Diagnostic sensitivity (DSe) was calculated using results from the antibody positive herd and diagnostic specificity (DSp) was calculated using results from the antibody negative herd.

**Results:** The median S/P ratio for colostrum collected from the positive herd was significantly higher compared to the corresponding positive serum samples, at all dilutions (P<0.001). The median S/P ratio for colostrum collected from the negative herd was significantly higher compared to the negative serum (P<0.05). The S/P ratios for positive serum and colostrum were significantly higher compared to negative serum and colostrum (P<0.001) at the 1:5 dilution. All other comparisons were not significant. Using the recommended serum sample dilution and suspect cut-off S/P of 0.2, both DSp and DSe for serum was 100%. Using the recommended undiluted colostrum and the same suspect cut-off threshold, DSp and DSe for colostrum was 70% and 100%, respectively. An increase in cut-off S/P to 0.5 increased colostrum DSp to 100%, while DSe of colostrum also remained at 100%. From a cut-off S/P of 0.5 upwards, colostrum showed an increasingly and significantly higher DSe compared to serum, whilst maintaining high DSp comparable to that of serum.

**Conclusions:** Colostrum may be used as an alternative sample to serum for the detection of BVDV. Colostrum can achieve 100% DSe and DSp when the cut-off is increased from the recommended S/P of 0.2 to 0.5. Further research has been conducted to investigate these same parameters on colostrum collected from BVDV infected animals.

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**Diagnostic opportunities in the ‘Trojan cow’ and her persistently bovine viral diarrhoea virus (BVDV) infected calf**

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**Objectives:** Infection with bovine viral diarrhoea virus (BVDV) during early gestation can result in a variety of gestational outcomes including abortion, stillbirth, the birth of calves with neurological deficits or the production of immunotolerant, persistently infected (PI) calves. The diagnosis of young calves persistently infected (PI) with bovine viral diarrhoea virus (BVDV) by antigen enzyme-linked immunosorbent assay (Ag ELISA) is complicated by interference from colostrum-derived specific antibodies, while pre-natal diagnosis would have to rely on amniocentesis - an invasive, expensive and potentially risky procedure. Therefore, this study aimed to explore opportunities for the simple, rapid and inexpensive diagnosis of BVDV PI status in the “Trojan” dam and fetal or neonatal calf.

**Methods:** Seventeen seronegative pregnant heifers were infected with BVDV via exposure to a PI cow from days 69-90 of gestation. Serum, nasal, saliva and vaginal swabs were collected from each heifer weekly from 7 days prior to exposure until six weeks post-parturition. Ear notch samples were collected every four weeks. Serum, nasal swab, saliva swab and ear notch samples were also collected from each calf, prior to colostrum ingestion, and weekly until three to four months of age. Samples were analysed by antibody and antigen enzyme-linked immunosorbent assay (ELISA), agarose gel immunodiffusion (AGID) and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Of the seventeen heifers, four experienced pre-term abortions, one delivered a calf that died soon after birth, three delivered non-PI calves that exhibited clinical neurological deficits (one of which was euthanized at birth), three delivered clinically healthy PI calves and six delivered apparently healthy calves. A novel pre-treatment method was applied to calf samples to improve sensitivity of detection of PI calves in the presence of colostrum-derived specific antibodies.

**Results:** All heifers seroconverted within 28 days post-exposure. Low levels of BVDV antigen were detected in five heifers on days seven, nine and/or fourteen post-exposure. No BVD virus or viral antigen was detected in any samples after seroconversion. AGID results of 3+ were achieved by all but one heifer, with 3+ results persisting until calving in some heifers. Heifers carrying PI fetuses (n=3) were observed to have significantly higher antibody ELISA results than heifers carrying non-PI calves. Heifers carrying calves with neurological deficits tended to exhibit lower antibody ELISA results than other heifers, but this difference was not statistically significant.

Following colostrum ingestion, antibody levels in non-PI calves rose by a mean S/P ratio of 0.95 (95% CI: 0.64 - 1.25) and a mean 1.72 (95% CI: 1.55 - 1.89) in PI calves, before declining to approximately 1.2 S/P ratio in non-PI calves and 0.5 S/P ratio in PI calves at 60 to 80 days of age. Negative antibody results were observed in PI calves at approximately 6 months of age. In PI calves, testing for antigen in serum, nasal and saliva swabs was subject to interference by colostrum-derived antibodies up to three weeks of age. Ear notches maintained positive antigen results at all timepoints, despite a substantial drop in the signal following the ingestion of colostrum. When a novel pre-treatment method was applied to samples from the three PI calves, diagnosis was improved such that the ‘diagnostic gap’ was eliminated in all calves.

**Conclusions:** Antibody difference between heifers carrying PI fetuses and those carrying non-PI fetuses by antibody ELISA could be used as a method of pre-natal diagnosis, generally with diagnostic sensitivity of 100% and specificity of ≥70%. After birth, pre-treatment methods may represent a substantial opportunity to improve diagnosis of young, colostrum-fed PI calves.
Establishment of presumptive diagnostic cut-off for persistently infected cattle and pooling of ear punch samples for the detection of BVDV PI animals with VetMAX®-Gold BVDV detection kit
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Objectives: Bovine Viral Diarrhea Virus causes infection in cattle that has led to major economic losses in both the beef and dairy industries. During pregnancy infected cows between the 30th and 125th day of gestation non-cytopathic BVDV can induce immunotolerance, causing newborns to be persistently infected (PI) for life. PI animals continuously shed the virus and are the main source of BVDV infection in herds. Animals that are acutely or transiently infected will pass the disease and will show negative BVDV results if re-tested 2 weeks after an initial positive test. In this study, we show a method for presumptive PI determination based on real-time RT-PCR results.

Methods: Life Technologies's Bovine VetMAX®-Gold BVDV Detection Kit has been shown to be a rapid method for BVDV detection. Ear punch samples were used since ear punches will show high viral titers if an animal is PI, but not if the animal is acutely infected. Purified RNA from 63 BVDV PI positive samples of various subtypes and 53 BVDV negative samples co-mingled with PI animals to create transiently-infected (TI) cattle that were tested with the Bovine Virus Diarrhea RNA Test Kit. A 3mm ear punch re-suspended in 200µl PBS, using 50µl of supernatant for with PI animals to create transiently-infected (TI) cattle that were tested with the Bovine Virus Diarrhea RNA Test Kit. A 3mm ear punch re-suspended in 200µl PBS, using 50µl of supernatant for

Results: In the context of high prevalence and monitoring strategies, pooling individual samples together is desired as a way to reduce the cost of testing entire herds for BVDV PI animals. In a pooling workflow, ear punches from individual animals are collected and re-suspended in PBS. The individual PBS supernatants are then combined into a pool which is then treated as a single sample used for RNA isolation followed by real-time RT-PCR. If the pooled sample tests positive for BVDV, the individual samples are then re-tested to determine which animal or animals caused the positive result. The sensitive nature of real-time RT-PCR allows samples to be pooled without losing the ability to detect a single PI animal from the pool. The VetMAX®-Gold BVDV Detection Kit was evaluated to determine the kit’s performance when pooling up to 24 bovine ear punch samples into a single pool. Ear punches from confirmed PI or TI animals were pooled with 23 negative BVDV ear punches to determine the effect on assay sensitivity and specificity due to pooling. A total of 63 PI pools, 12 TI pools, and 51 negative pools were used for this study.

Conclusions: We showed that the sensitivity and specificity was 100% for the detection of a single PI animal in a 24 sample pool while the TI pools were all negative. This indicates that pooling up to 24 samples is a viable method for the screening of PI animals in herds with the VetMAX®-Gold BVDV Detection Kit.

Bovine viral diarrhoea virus infection in beef heifers in commercial herds in Australia; a risk assessment approach is more appropriate than a partial budgeting approach when considering control strategies
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Objectives: To describe mob-level seroprevalences and incidences of seroconversion to bovine viral diarrhoea virus (BVDV) in beef heifers in commercial herds in Australia.

Methods: A prospective longitudinal study was conducted using thirty eight mobs (management groups) from Queensland, New South Wales, Victoria and Western Australia consisting of approximately 4,600 non-pregnant beef heifers (i.e. female cattle that had never calved) not vaccinated against BVDV selected by eleven collaborating veterinary practitioners. Sero prevalences were assessed in each mob between 2 and 7 months before mating start date using an agar gel immunodiffusion (AGID) test for BVDV antibodies. Fifteen mobs considered to be at higher risk of BVDV transmission during the upcoming mating period (those with lowest seroprevalences at this initial serological assessment and greatest risks of BVDV introduction through anticipated contact with other cattle) underwent further serological monitoring using the AGID test, with sera collected from between 17 and 26 randomly selected unvaccinated heifers in each mob a) shortly before mating start date and b) at pregnancy testing (between 99 and 218 days after mating start date). (All heifers in these mobs were also included in a double blind controlled trial to assess vaccine efficacy; approximately half of the heifers in each mob were vaccinated. Results of this trial are reported in Morton et al, Australian Veterinary Journal 2013:91:517-524.)

Results: Distributions of seroprevalences were both bimodal, with seroprevalences commonly being either 10% or less, or greater than 80%. These results indicate that widespread infection had occurred in a substantial proportion of beef heifer mobs before mating start date, so most animals in these mobs are likely to have acquired natural immunity to BVDV before mating. These results also suggest that a substantial proportion of beef heifer mobs have probably not been exposed to BVDV before mating start date, and so are probably susceptible to an epidemic of BVDV infection. Proportions of animals that seroconverted during the mating period were assessed in 9 mobs where at least 9 heifers were seronegative shortly before mating start date. Proportions of animals that seroconverted were 0% (4 mobs), and 6%, 7%, 9%, 15% and 89% (1 mob each). In the latter 3 mobs, final pregnancy rates (proportion of heifers that were diagnosed pregnant) were 87 to 90%. All calves in these 3 mobs (i.e. progeny of study heifers) were tested for BVDV infection using an antigen-capture ELISA test applied to ear notch samples. Respectively, 1/29, 1/25 and 14/55 of calves born to unvaccinated heifers were antigen-capture ELISA test positive and thus likely to be persistently infected with BVDV. Thus, widespread seroconversion was uncommon and only one mob experienced a serious impact attributable to BVDV infection. However, the single infected calves were a potential cause of loss if grazed with non-immune cattle during subsequent mating periods.

Conclusions: Results from this relatively small study are consistent with epidemics of infection with BVDV during the mating period being relatively uncommon in mobs of beef heifers.
Efficacy of Pestigard® vaccine against foetal infection in heifers exposed to persistently infected cattle (PI) with BVDV Type 1a.

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Objectives: To demonstrate the efficacy of Pestigard® vaccine against foetal infection in pregnant heifers exposed to cattle persistently infected with Bovine Viral Diarrhoea Virus (BVDV) subtype 1a in New Zealand.

Methods: Fifty five (55) BVDV antigen and antibody free crossbred Friesian heifers were randomly allocated into three treatment groups. One group of twenty five (25) heifers received 2 mLs of Pestigard® Vaccine (Zoetis Australia) twice at an interval of 8 weeks (Days 0 and 56 of the study), and another 20 heifers remained unvaccinated as the negative control group. A third group of ten (10) heifers received 2 mLs of Bovilis® BVD vaccine (MSD Animal Health) twice at an interval of 4 weeks (Days 28 and 56 of the study). Blood samples were collected at regular intervals for measurement of Virus Neutralising Titres (VNT). The 55 heifers were synchronised and bred. For the challenge phase, only 38 pregnant heifers were selected with fifteen (15) each from both the Pestigard and negative control groups and eight (8) from the Bovilis BVD group. All heifers were not greater than 74 days of gestation at the beginning of the challenge phase. Six months after the second vaccination, all 38 pregnant heifers were exposed to four calves that were persistently infected (PI) with BVDV Type 1a for 4 weeks. Approximately 3 weeks after the PI removal, heifers from each treatment group were euthanised separately on Days 288 (Pestigard group), 289 (Bovilis group) and 290 (negative controls) for foetal harvest and sample collections. Various tissues were collected from each foetus under sterile conditions for the detection of BVDV by ELISA (in the foetal heart blood) and PCR (in the foetal tissues).

Results: Two weeks after the second vaccination, heifers in both the Pestigard and Bovilis BVD groups demonstrated at least a 10-fold increase in VNT compared to Day 0, with titres ranging from 256 to ≥2048 for the Pestigard group and 24 to 1024 for the Bovilis BVD group. Prior to challenge, the VNT declined to lower levels (≤256 in the Pestigard group and ≤24 in the Bovilis BVD group). All the heifers in the negative control group were seronegative (VNT <1:2) immediately prior to challenge. Following the challenge, all the vaccinated heifers showed an anamnestic rise in antibody titres. Negative controls also demonstrated a rise in titres. BVDV was detected in all foetuses in the negative control group by both ELISA antigen test (14/14) and by PCR (15/15), compared to 3/8 foetuses in the Bovilis BVD group and 3/14 foetuses in the Pestigard group. An additional foetus in the Pestigard group tested negative on ELISA but positive on PCR for BVDV.

Conclusions: The results of the study indicate that the efficacy of Pestigard in prevention of foetal infection was 71%, whilst the efficacy of Bovilis BVD in the prevention of foetal infection was 63%. Both vaccines demonstrated a significant reduction in the proportion of foetuses testing positive compared to the negative control group (p<0.002).
Objectives: To develop a stochastic simulation model to simulate herd employing either seasonal, split or year-round calving. Innate milk production, mortality, group membership etc.) were simulated increments. Events (oestrus, insemination, conception, calving, herd was developed using R (The R Foundation for Statistical computer simulation model of a pasture-based Australian dairy stochastic, dynamic, discrete time, individual-animal based behaviour of contagious disease in populations such as herds. A Computer simulation modelling can assist understanding of difficult to forecast behaviour of an infectious agent in a population. unpredictably; not all contacts result in transmission. This makes it transmitted following contact. Interactions leading to contact characteristics of the infectious agent determine whether disease is determine probabilities of contact between individuals and replacement, group segmentation, etc.). Population dynamics with an unrelated protein. A booster was applied after 30 dpi and serum samples from all animals were taken up to 360 dpi. Virus neutralization was used to measure humoral immune response of guinea pig and cattle against BVDV after vaccination.

Results: The Master Bank and the Working Bank of SF9 Cells and APCH-tE2 recombinant baculovirus were generated in compliance with the international requirements of identity, purity, stability and suitability for manufacturing procedures (ICH Q5, EMEA). The concentration of the Recombinant APCH-tE2 protein in SF9 supernatants was 5 μg/ml and it was recognized by several E2 specific Mabs and by bovine anti-BVDV sera. No protein degradation was observed throughout the stability test, when the protein was stored from 2 to 8°C. Immunized guinea pigs showed a strong NA response after vaccination with the three doses tested. Also, the subunit vaccine induced high titers of NA in calves after immunization up to 360 dpi, while animals of the control group remained seronegative throughout the experience.

Conclusions: Results showed that titres of BVDV NA elicited by the subunit APCH-tE2 vaccine were comparable to those required for a satisfactory BVDV killed vaccine according to CFR 113.215. All the information about protocols, controls and experiments were registered following the National Argentine Regulation and constituted the core of the APCH-tE2 vaccine dossier.

Computer modelling of bovine viral diarrhoea virus transmission, impacts on productivity, and the effectiveness of control measures within pasture-based Australian dairy herds

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Objectives: To develop a stochastic simulation model to simulate bovine viral diarrhoea virus (BVDV) transmission and impacts on productivity within pasture-based dairy herds in Australia.

Methods: The behaviour of contagious organisms in a population is determined by: innate characteristics of the infectious agent and the host-agent relationship (infectivity, duration of infection, immunity etc.) and dynamics of the population (animal survival, replacement, group segmentation, etc.). Population dynamics determine probabilities of contact between individuals and characteristics of the infectious agent determine whether disease is transmitted following contact. Interactions leading to contact between individuals are complex and transmission of infection is unpredictable; not all contacts result in transmission. This makes it difficult to forecast behaviour of an infectious agent in a population. Computer simulation modelling can assist understanding of behaviour of contagious disease in populations such as herds. A stochastic, dynamic, discrete time, individual-animal based computer simulation model of a pasture-based Australian dairy herd was developed using R (The R Foundation for Statistical Computing). Individuals moved discretely through time in daily increments. Events (oestrus, insemination, conception, calving, milk production, mortality, group membership etc.) were simulated to reflect the management of an Australian pasture-based dairy herd employing either seasonal, split or year-round calving. Innate characteristics of BVDV and the host-agent relationship (infectiousness, impact of infection, immune response and duration of infection and immunity, etc) were obtained from literature and data. A BVDV transmission module was developed using Reed-Frost principles that simulate movement of animals between susceptible, infected and immune statuses in and between management groups. The model will be used to examine the range of outcomes and the effectiveness and cost-benefit of controls following entry of BVDV to a herd.

Results: The model's simulated reproduction, milk production, mortality and culling were recently validated using industry data. BVDV modelling revealed the cyclical nature of infection (and re-infection) within a herd and showed a demonstrable reduction in individual cow and herd reproductive performance. More detailed studies of the transmission of virus, animal infection statuses and productivity within Australian pasture-based dairy herds over time will now be undertaken to determine the range and likelihood of impacts of disease within Australian dairy herds. Control strategies such as vaccination, biosecurity (testing of introductions, control of over-the-fence contact with neighbouring stock), and removal of persistently infected animals will be examined for different calving systems, herd sizes and under different challenge scenarios to identify key drivers of disease and to determine the economics of various control strategies. Interim results will be presented at the conference.

Conclusions: Effects of a contagious organism in a herd will vary over time. BVDV infection can negatively impact the reproductive performance of individuals. In seasonal and split calving dairy herds, the conception pattern is a major determinant of calving pattern which, in turn, strongly influences the subsequent conception pattern. Determining the impact of BVDV in dairy herds requires consideration of this complex sequence of events. Computer simulation modelling over multiple consecutive years provides an effective way of studying these impacts.

Humoral and cellular immune response in vaccinated calves against Bovine Viral Diarrhea Virus (BVDV)

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Objectives: The goal of this research was to evaluate the humoral and cellular immune response to BVDV in young calves, by vaccination stimulus after the end of the colostral immunity.

Methods: Ten Holstein calves born to vaccinated mothers to BVDV in the peripartum period were selected. Newborns received 6L of colostrum of their mothers in the first 12 hours of life, and were kept isolated during the trial period. When they reached 6 months of age, were randomly divided into two experimental groups consisting of 5 animals: non-vaccinated (VAC-) and vaccinated (VAC+). The vaccination protocol adopted included the use of two doses of vaccine, at 180 days after birth (a.b.) and 210 days of life, by subcutaneous applying of commercial polyvalent vaccine (5mL), containing the BVDV type 01 (5960) e BVDV-2 (53637) inactivated strains, diluted in the adjuvant containing "Quil A, cholesterol and amphigen". Whole blood and serum samples
were collected at the following times: before applying the 1st dose - 180 days (d) (T0), before applying the 2nd dose - 210d (T1) and 30 days after application 2nd dose - 240d (T2) of life. The blood serum of calves was tested using a serum neutralization test (SN) to BVDV; the whole blood samples were used to perform flow cytometry to determination of lymphocyte subpopulations and activation of these cells by the expression of the CD25+ receptor.

Results: The BVDV Abs were not detected in T0 to T2 of VAC (-), except for 1/5 (20%) calves in T1 with a 1/10 liter. The VAC (+) group were seronegative at T0 time, and had seroconversion rates of 40% (2/5) at T1 and 60% (3/5) at T2. These animals showed neutralizing antibodies titres corresponding to 1/20 and 1/40 at T1 and 1/10, 1/320 and 1/1280 at T2. The ratios obtained for B lymphocytes (CD21+), T (CD3+), T helper (CD3+CD4+), cytotoxic T (CD3+CD8+), and gamma-delta T lymphocytes (CD3+WC1+) in VAC (-) and VAC (+), respectively, were 24 and 28%, 53 and 53%, 27% and 22, 20 and 11%; 30 and 38% at T0; 23 and 23%; 47 and 55%, 31 and 29%, 19 and 15%; 35 and 39 % at T1; and 23 and 35%, 44 and 48%, 32 and 24%, 12 and 17%, 39 and 20% at T2. It was possible to verify that the VAC (+) calves had higher proportions of activated T helper cells (CD3+CD4+CD25+) and gamma-delta (CD3+WC1+CD25+) at T1.

Conclusions: The humoral response stimulated by inactivated BVDV vaccine showed a partial response, considering that some animals did not respond to vaccination. The cellular immune response was higher in the group of vaccinated animals as indicated by the higher proportion of TH helper and gamma-delta lymphocytes, however, the cell assessment tests used were not specific to vaccine antigens. Natural exposure to pathogens may have influenced in this response.

A study of Bovine Viral Diarrhoea Virus (BVDV/Pestivirus) in eastern Australia, Part 1: farmers’ understanding of the disease – preliminary findings.

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Objectives: Previously a Meat and Livestock Australia report (Sackett, Holmes, Abbott, Jephcott & Barber, 2006) identified that there is little information on the economic impact of disease caused by the Bovine Viral Diarrhoea Virus (BVDV) in southern Australian cattle herds. Before investigating the economic consequences of this disease, and assessing the welfare and financial advantages possible through applying control measures, it is important to gain an understanding of farmers’ current knowledge and attitudes in relation to BVDV infection. The current study aims to better understand the level of knowledge relating to BVDV disease among cattle breeders in eastern Australia. Reference: Sackett, D., Holmes, P., Abbott, K., Jephcott, S. & Barber, M. (2006) Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. MLA Report AHW.087, Sydney, Australia: Meat & Livestock Australia Limited.

Methods: A cross-sectional study was conducted among eastern Australian cattle breeders from southern Queensland to Victoria using a commercial agricultural marketing company database (producers’ details were blinded to the researchers). A sample-size of 400 was required to estimate the proportion of producers conducting a specific practice, with a 95% confidence level, and assuming 50% would conduct the practice. A total of 1500 candidates were selected with an expectation of a 25-30% response rate. Candidates were randomly selected and the number in each state was proportional to the total number held in the database for each state. Consequently a total of 76, 951 and 473 questionnaires were posted to cattle producers in Queensland, NSW and Victoria, respectively. Two mail-outs were used. The study was approved by the Charles Sturt University School of Animal and Veterinary Sciences Human Ethics committee (Number 416/2013/20). Information was sought about general property information (e.g. postcode, size, herd-type) and farmers’ understanding of BVDV disease. The latter asked respondents to identify factors from a list which they believed were a) outcomes associated with BVDV infection, b) factors involved in BVDV spread, c) how the timing of infection may result in a persistently-infected (PI) calf, d) whether veterinary advice was sought in relation to this disease, and e) whether, and how, testing for BVDV occurred. Data were analysed by the Chi-square test.

Results: The first mail-out resulted in a total of 108 responses (Victoria 36; NSW 67; Queensland 5). Herds were described as being: ‘closed’ (38.0%), ‘occasional introductions’ (50.0%), ‘regular introductions’ (12.0%). Ninety-two producers responded when asked to identify outcomes that they recognised to be consequences of BVDV infection. Reponses were: abortion (80.4%); birth of a Persistently Infected (PI) calf (78.3%); poor fertility (77.2%); immunosupression (66.3%); weakly neutanes (66.3%); calf deaths (53.8%) and deformities (46.7%). A proportion of producers identified all these outcomes as possible; NSW 16/57; Victoria 5/31; P<0.05. When asked to identify the routes by which BVDV could spread the following responses were made relating to virus shedding by: a PI calf (84/93, 90.3%), salvia and nasal secretions (57/92, 62.0%), abortion material (51/92, 55.4%), transient infection (47/93, 50.5%), faeces (37/92, 40.2%), semen (31/91, 34.1%). Regionally, 17.5% and 12.9% of producers from NSW and Victoria, respectively, identified all routes as potential risks. Eighty-one producers answered the question relating to the timing of infection from which a PI calf may result, with 86.4%, 22.2% and 21.0% replying foetal infection, suckling-calf infection and post-weaning infection, respectively. Respondents utilised veterinary advice regarding BVDV as follows: general advice (72/94, 76.6%), BVDV testing (56/83, 67.5%) and vaccination (56/85, 65.9%). The proportion of producers seeking veterinary advice was higher in NSW than Victoria (P<0.05). Overall, antibody testing and antigen testing, respectively, was conducted by 27/75 (36.0%) and 26/75 (34.7%) of respondents, with 13 undertaking both. Regionally, NSW respondents performed more (P<0.05) testing than Victorian participants (31/49 v 8/24).

Conclusions: Preliminary results suggest that participant producers have varying levels of understanding in relation to the pathogenesis of disease caused by BVDV. The role of the PI calf, and how it arises, seems to be well-understood, but further education is required to achieve a wider appreciation of the many routes by which the virus can be transmitted, and also the potential outcomes post-infection. Currently the veterinary profession appear to be under-utilised as a source of advice and education for general advice and specific advice on testing and vaccination for BVDV, and there are potential opportunities to remedy that deficit.