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# **Abstracts**

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# Paratuberculosis Control in Cattle in Europe

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## Introduction

Paratuberculosis, or Johne's disease (JD), is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and is found in ruminants worldwide. The disease is untreatable and can cause considerable economic losses in affected herds, both dairy and beef. Clinical JD represents only a small proportion of cattle actually infected with MAP within a herd. Economic losses include culling of cows with clinical JD as well as reduced performance (milk yield, fertility...) and an increased susceptibility to other disease of animals subclinically infected with MAP. As JD is difficult to diagnose, shows a long incubation period, is untreatable and poses a herd problem, control programs including the whole herd are most important.

## Paratuberculosis control in Europe

Europe consists of many different countries with a very diverse cattle population, leading to heterogeneous frame conditions for dairy and beef production. The prevalence of MAP infections in The European cattle population also varies in a wide range, including countries almost MAP-free and regions with a seroprevalence of above 50 % infected dairy herds in many states.

Control efforts and programs for JD in cattle are very diverse among European states. Some countries, such as Sweden, which are almost free of the disease, perform a rigorous mandatory control program with a stamping out policy. In most countries voluntary control programs for MAP are in action. The majority of these voluntary programs are aligned for dairy farms. The programs show different aims, levels of measurements and acceptance by the producers.

In Austria clinical JD is rated as a notifiable disease since 2006. Ruminants with suspected clinical JD have to be tested by ELISA (enzyme linked immunosorbent assay) and PCR (polymerase chain reaction). Confirmed clinical cases have to be culled, hygienic precautions are set by the state veterinarian and the farmer gets compensation for the culled animal. The aim of this compulsory program is to reduce clinical JD, decrease the MAP shedding into the environment and thereby protect uninfected herd mates and farms. Additionally, the intake of MAP into the food chain shall be reduced by elimination of cattle with an advanced MAP-infection.

Most control programs for JD are based on a test and cull strategy, combined with hygienic precautions, which are mainly focused to prevent new MAP-infections in calves and young livestock. Unfortunately, the acceptance of such programs by farmers and veterinarians is limited due to high costs, high workload, long duration and the lack in sensitivity and specificity of laboratory tests, leading to limited success of the programs.

## Suggestions for a "minimum control program for JD in cattle"

To overcome this lack and to harmonize the control of MAP in Europe a basic "minimal control program" with defined minimum standards should be established. This suggested "minimal program" to control JD in cattle consists of 3 steps. Step 1 includes diagnostic evaluation of every case of diarrhea in adult cattle and culling of all animals with clinical JD. Step 2 is the implementation of

basic management measures adapted to the situation and potentials of the individual farm to reduce new *MAP*-infections. Step 3 consists of regularly evaluation of the *MAP*-herd status. The focus of these herd evaluations should be on *MAP*-shedding animals, for example by the use of environmental fecal samples.

This “minimal control program” can be performed with reasonable costs and work load in most cattle herds and might serve as an international minimum standard for *MAP*-control in cattle. The aim of such a program might be the reduction of clinical JD as well as to reduce the shedding of *MAP* into the environment. Thereby new infections within the herd and between herds might be reduced and the introduction of the bacterium to the food chain can be decreased. As an additional side effect, these simple measures might also help to reduce other diseases and to increase production efficiency and animal welfare.

### **Conclusion**

Infections of cattle with *MAP* are difficult to control, have a great economic impact and will be an important topic in cattle medicine in the future. As trading of subclinically infected cattle is the most common route of disease transmission, efforts to control *MAP* in Europe should be coordinated on an international level. Although it might be very difficult or even impossible to eradicate *MAP* in infected farms, the reduction of new infections within infected cattle herds and the protection of uninfected farms can be achieved. To reach these goals, a pan European “minimal control program for JD in cattle” is suggested. Such a cheap and easy to perform program might help to establish minimal standards for *MAP*-control in Europe and serve as an incentive to decrease *MAP*-infections for those not willing to participate in more sophisticated control programs.

## Prevalence and Prevention of Paratuberculosis in North America

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The United States of America (U.S.) has made several attempts over the years to develop a voluntary program that was acceptable to producers. The focus of the U.S. Voluntary Bovine Johne's Disease Control Program (VBJDCP) is to provide producers with the tools to control Johne's disease on their farms and identify herds with a low risk for the presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection, instead of certifying herds as free. Producers' perception of Johne's disease has attributed to the current U.S. situation. Without the immediate connection to clinical disease, producers tend not to understand the effect of their management practices. Also the lack of immediate economic loss contributes to slow corrections to risky management. Past restriction on known positive animals has caused producers not to test for Johne's disease for fear of having the animals restricted to their premises. This has led to slow adoption of control practices.

Most work in the U.S. regarding prevalence rates has been done within the dairy industry. Work done by the APHIS's National Animal Health Monitoring System (NAHMS) has created the best national estimates available. In 2007, MAP was isolated from at least one environmental sample from 68.1 percent of U.S. dairy operations. Few studies have been carried out looking at beef populations. Only one national level attempt has been made for U.S. beef operations back in 1997 which showed only 7.9 percent of the studies beef herds tested positive for MAP. However the beef estimate was not weighed and cannot be considered a national estimate. Given the sensitivities of the tests used both prevalences are likely less than the true prevalences.

The education component of the VBJDCP is intended to inform livestock producers about the costs of Johne's disease and to provide information about management strategies to prevent, control, and eliminate the disease. The impact of these activities is hard to gauge since changes in producers management practice cannot be easily measured. The NAHMS 2007-2008 beef cow-calf study looked at producer knowledge of Johne's disease. By 2008 31.3 percent of beef producers felt that they were fairly knowledgeable or knew the basics about Johne's disease. In contrast to the beef industry, 94 percent of dairy producers considered themselves fairly knowledgeable or knew the basics about Johne's disease. Dairy producers also responded to the education programs by increasing testing and in 2007, 31 percent of dairy producers participated in a Johne's disease control or certification program.

The management component of the VBJDCP includes an evaluation of producers' operations to identify practices that could potentially allow the transmission of MAP among animals or between premises. Once risky practices have been identified, a herd management plan is developed to assist the producer in correcting risky practices. The National Johne's Disease Demonstration Herd Project (NJDDHP) was initiated to evaluate the long-term feasibility and effectiveness of management. For dairy herds, addition/replacement management and preweaned heifer areas appears to be most important with regard to risk of dairy cattle being fecal-culture positive for MAP. Specific factors associated with the greatest risk for cattle to be MAP-positive included feeding pooled colostrum to

calves, possible manure contamination of milk or colostrum, and additions. These results suggest that placing more emphasis on preweaned calf management and increased caution in obtaining herd additions can aid in management of MAP on dairy operations. Results from the beef herds enrolled in the NJDDHP suggests that keeping cow-calf pairs separate from Johne's disease clinical or suspect animals, limiting manure contamination of water for preweaned heifers, and limiting cow access to accumulated or stored manure are the most important control points to reducing MAP prevalence on beef operations.

In addition to management changes, vaccination is a control tool allowed in the U.S. because it reduces the clinical signs of Johne's disease and there is evidence that it can reduce the shedding of MAP. While it is allowed in the program, widespread use is not encouraged due to MAP's ability to cause cross-reactions on tests for other mycobacterium. Testing is a second tool widely used in addition to management changes. While the classification component of the VBJDCP dictates the amount and type of testing herd owners are required to conduct, the education and management component of the VBJDCP does not specify testing protocols. The testing for control is intended to fit the needs and resources of producers and can be quite flexible.

The amount of testing has varied in the U.S. over the past decade primarily due to the levels of federal funding that supported individual animal testing. A downward trend in the number of tests being conducted reflects the decrease in federal support. The only variance to this trend is the milk ELISA testing through the Dairy Herd Improvement Association laboratories which has continued to increase through 2010. This shows that although producers may not have an interest in enrolling in the national program, at least dairy producers continue to monitor the disease situation on their own.

Without a strong regulatory component of a program, industry adoption of a voluntary certification program has been limited and has shown there are few economic drivers for a national program. Over the past decade, the cattle industry, State animal health agencies and APHIS has continued to change and modify its program to best meet the needs of the industry. Although producer participation is a common measure of success, changes that producers make in their management scheme continue beyond formal enrollment and this impact is easily overlooked. To this end, federal resources continue to be used for education and outreach to producers. Management changes on the farm remain the key to control and programs cannot replace well thought out plans by producers that are specific to their resources, facilities, and operation.

# Epidemic situation and control of paratuberculosis in Japan

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In this talk, I'd like to outline the history of the epidemic situation and national practical control strategies against paratuberculosis in Japan.

**Low incidence in Japan:** Paratuberculosis (Ptb), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic and progressive granulomatous enteritis that affects many livestock and wild animals worldwide. The clinical disease is called Johne's disease (JD). In Japan, all dairy cattle (one million head) are examined for Ptb every five years. About 1,000 of 500,000 officially examined cattle annually are diagnosed as having Ptb, but most of them exhibited only minor or no clinical signs and typical lesions. In contrast to the situation in Japan, the disease prevalence in western countries is very high. In the United States, for example, over 70% of dairy herds are contaminated, and Ptb causes an estimated annual loss of \$220 million to the agricultural economy. The prevalence of Ptb in cattle in Australia, New Zealand, and Europe range from 10 to 60%. I will discuss why the prevalence is low in Japan.

**Powerful control strategy backed by Japanese law:** The current status (i.e., low prevalence) of Ptb in Japan suggests that continuous diagnosis with proactive culling of positive animals (test-and-cull strategy) could be an effective way to eradicate Ptb. Japanese law requires that cattle officially diagnosed with paratuberculosis be killed. The government will compensate farmers for about 80% of the value of the cattle according to the Act on Domestic Animal Infectious Diseases Control. Combining the law and test-and-cull strategies has been successful.

**Practical control methods:** We have mainly used ELISA and bacterial detection (culture or fecal PCR test) to diagnose paratuberculosis. Until 2007, about 50% of the positive cases diagnosed annually were detected by ELISA, and the remaining positive cases were detected by bacterial culture. A few percent were detected by clinical findings. However, the Ministry of Health, Labour and Welfare of Japan instructed that the use of bacterial culture be terminated after October 2007. The ministry recognized Ptb as a suspected zoonotic infection agent according to previous papers. Therefore, they provided guidance that the milk and meat of cattle diagnosed as having paratuberculosis should not be used for human consumption, and that companies must recall all products retroactive to the day of sampling. Since it takes more than three months to get the results of a MAP culture, a gold standard of Ptb diagnosis could not be applied. Many bottles of milk and huge number of dairy products thus went to market and were consumed during the culture period.

**Application of real-time PCR:** In September 2008, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan introduced a quantitative PCR method to detect IS900, a MAP-specific DNA sequence, as a semi-official diagnostic method. Nonetheless, we are still concerned about an increased incidence of Ptb. Although the PCR method is known to detect Ptb earlier than the current ELISA method, we should not forget that PCR-positive cattle must have already shed Map organisms in the herd in the past.

**Guidelines for controlling bovine paratuberculosis:** On Nov. 1, 2006, MAFF released guidelines for controlling bovine paratuberculosis. I was involved as a member of the committee. We divided

farms into two categories. Category 1 was a clean farm and category 2 was a farm under control. Prefectural governments direct adequate hygienic management, regularly check herd condition, and issue a certificate of the category and test history of individual cattle. The administrative direction on hygiene control includes cleaning cow houses and the environment, disinfection at entrances, disinfecting boots, carefully observing animals, maintaining good communication with veterinarians, keeping the delivery room clean, using a replacement for colostrum, quickly separating calves from mothers after birth, and carefully aging manure. Every Japanese cattle farm receives an official diagnostic test for Ptb every five years or at the onset of the clinical Ptb by a clinical veterinarian. ELISA and/or PCR testing will then be repeated at three-month intervals until no positive animals are detected. The farm will then be classified as category 1.

**Control at the emergence:** Prefectural governments take preventive measures such as isolating infected animals, ordering culling within two weeks, disinfecting the farm environment, and performing quality checks of manure compost.

**Recommendation of autonomous culling:** In addition to killing animals diagnosed as positive in official testing, prefectural governments can recommend autonomous culling for animals with close epidemiological relations to positive cattle and cattle in which real-time PCR detects higher levels of MAP DNA. The government will compensate farmers for this culling to some extent.

**Problem of non-specific reaction in ELISA diagnosis:** We have experienced nonspecific positives in ELISA testing of cattle sensitized by mycobacteria other than Ptb. The ELISA test is very easy and practical, but should be used as screening to find animals sensitized by mycobacteria, and specific diagnosis with the isolation or PCR detection of specific DNA should be used for confirmation.

**Need for eradication according to many public-health studies:** There is ongoing concern that MAP may be an etiological factor of human Crohn's disease. Our recent study suggests the risk of MAP-contaminated milk and dairy products in the onset of Crohn's disease. Therefore, we need to eradicate Ptb by accumulating careful control trials and checking dairy foods for Map contamination.

# BVDV control and eradication programmes in Europe—an overview

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Infections with bovine viral diarrhoea virus (BVDV) are endemic in cattle populations worldwide and result in major economic losses. For long, attempts to control BVD were limited to prophylactic vaccination practices, implemented primarily to reduce or prevent clinical disease on a herd basis. However, the benefit of preventing clinical disease in transiently infected animals is negligible when considering the overall prevention and control of the disease [1]. Instead, because the persistently infected (PI) animal is the key to the evolutionary success of BVDV, prevention of foetal infection is the key to BVDV control [2]. Consequently, modern vaccination programmes are designed not only to prevent clinical disease, but also to protect against viremia and to prevent foetal infection. Several challenge studies indicate that inactivated as well as live vaccines may prevent foetal infection under controlled experimental conditions [3–5]. However, the efficacy of these vaccines to protect fetuses against infection under field conditions have been questioned [6], and field observations, where PI calves have been born in vaccinated herds, support this concern [7–9], and because 100% efficacy and coverage is needed to prevent the infection from being established, if it is introduced, vaccination has, despite the widespread use, failed to reduce the incidence and prevalence of BVDV [10].

Another more systematic strategy to control BVDV evolved during the 1990s within eradication programmes in the Scandinavian countries. This was based on an initial determination of herd BVDV status, followed by implementation of systematic zoo-sanitary measures at a regional or national scale (without the use of vaccines) to prevent introduction of BVDV in non-infected herds, and to reduce the prevalence of infected herds by identification and elimination of PI animals [2]. These programmes have been very successful, and all of the Scandinavian countries are currently either free, or almost free from BVDV [11–13].

Today control programmes are underway in several European countries [14–18]. In all, the necessary elements biosecurity, virus elimination and monitoring constitute the basis. The role of vaccination, on the other hand, is still controversial but could, in addition to the three necessary elements, constitute an optional element in areas where the risk of re-introduction into free herds is perceived as very high [18].

This paper will discuss the general model of BVDV control, and give an overview of different strategies used within, and the current status of, the ongoing control programmes in Europe.

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## **Preventive strategy for BVDV infection in North America**

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Despite 60 years of vaccination, bovine viral diarrhoea virus (BVDV) infections remain a source of significant economic loss for producers in the United States. The success of control efforts in Scandinavia has demonstrated that BVDV eradication is possible. It is difficult to design “one size fits all” control program for the United States. Program design in the United States varies by region within the country based on the incidence of BVDV, density of animal populations, animal movement, contact with wildlife populations, level of producer compliance, variation among circulating BVDV strains, prevalent type of production unit or industry and support offered by state institutions. The Upper Peninsula BVDV Eradication Program in Michigan, the Montana BVD-PI Herd Biosecurity Project and the Alabama Voluntary BVD Control Program illustrates three different regional approaches. A large component of all three programs is the identification and removal of animals persistently infected with BVDV. All three programs are voluntary rather than government mandated. The Montana and Alabama programs focus on herd screening to eliminate PIs but do not have eradication as a goal. The participating production units are mainly beef cow/calf or feedlot operations and represent a fraction of the herds in the region. These two programs differ in regards to the practice of pooling of samples before testing. The Michigan program is unique in the United States in that its goal is to eradicate BVDV from a defined geographic region. The Upper Peninsula (UP) of Michigan is ideal for this project because it is an isolated geographic region, has a variety of large and small herds, and most cattle movement is out of the UP. While the Alabama and Montana programs are beneficial to individual producers they did not have a significant impact on the prevalence of BVDV. In contrast, the Michigan program has reduced incidence of herds harboring PI animals in the region.

While the details of control programs may differ based regional differences listed above, the most efficacious programs are built around a three-pronged attack consisting of biosecurity (aimed at the development of management practices that prevent BVDV from being introduced into a herd), surveillance (to detect and remove PI animals) and control (use of means, such as vaccination, to limit BVDV spread if it is introduced into a herd).

Selection of the tools used in BVDV control programs will impact on success. The design of BVDV diagnostics and vaccines should take into account variability among BVDV strains and regional differences in subgenotype prevalence. Designers of diagnostics and testing protocols need to understand that PI animals differ in their BVDV load. Thus, determination of sensitivity and specificity of diagnostics should be done using a number of different strains and samples from a number of different PI animals. Further, design must reflect the production setting in which tests will be used. There is typically a trade-off between cost, sensitivity, and speed of the test; the weighted importance of these 3 factors will vary depending on production unit. The speed of test is less important in smaller production units with ready access to animals and limited likelihood of transport of animals between sample collection and determination of testing results. In contrast, obtaining the results of testing quickly is important to production units with large populations of animals, units in

which access to animals is limited, or units in which animals will be sold or transported soon after testing. Similarly, the importance of cost of testing versus the cost of missing a PI animal will vary by production unit. The cost to risk ratio for cheaper, less sensitive test may be acceptable in a feedlot situation in which there are no future generations that will be impacted by overlooking a PI animal. The same test may not be acceptable from a cost-risk standpoint for a breeding herd operation in which the failure to eliminate a PI may result in reproductive losses for future calving seasons.

Design of vaccination programs also needs to also take into account stressors that reduce an animal's ability to respond to vaccination, differences in immune response related to age and pregnancy status, periods of greatest vulnerability to infection and negative outcomes of infection. Thus for optimum efficacy different vaccination strategies may need to be devised for production units which focus on neonates, breeding herds, stocker calves, replacement heifers, dairy or feedlot production.

Finally, program design must take into account the human element. It has been demonstrated that compliance with control programs is directly linked to education and the presence of a support network composed of fellow producers, engaged veterinarians and knowledgeable diagnosticians.

# **Risk Analysis and Management of BVDV**

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Experts have claimed that bovine viral diarrhoea (BVD) is the most important infectious disease of cattle worldwide. Despite this the consequences of BVD virus (BVDV) infection are not well recognised or well understood by many cattle farmers. Where they are aware of the threat of BVDV induced disease farmers are, however, fortunate in that they have a range of response options: do nothing; vaccinate; eradication or combinations of these. At the national/regional level some countries such as Sweden and Scotland have already recognised the threat and government/industry have taken varying degrees of collective action to minimise BVDV associated risks. Other nations such as The Netherlands understand the problems BVDV presents but stakeholders so far have chosen to use limited collective resources to combat other disease threats such as paratuberculosis. Elsewhere, such as the USA, an intermediate option that relies heavily on vaccination to dampen down, but not eliminate, the threat appears to have been preferred overall. These variations in approach to BVD control suggest that where risk analyses have been carried out different conclusions have been reached. It is not really surprising that working through cycles of risk identification and assessment, risk communication and risk management for different systems in different environments the conclusions differ. Although, almost certainly, such assessments take place intuitively at a local level between farmers and their veterinary advisors, if this process has occurred at regional or national level the evidence is not readily available through the academic literature.

Recently the case for systematic BVDV control in Scotland has been agreed by stakeholders at a national level although more local eradication and vaccination programmes have been successfully implemented for many years. In this presentation the authors will describe risk analysis and management for BVDV with some, but not exclusive, reference to the evolving European, UK and Scottish situations. Our evidence base will be derived from results previously presented in a series of papers that will form the basis of this review. These results are the products of a series of policy linked research projects funded by Scottish Government (RESAS), UK Government (Defra), EC, Animal Health Ireland and the Scottish red-meat industry (Quality Meat Scotland). Such research is aimed at answering questions such as: how much BVDV exposure do we have (prevalence studies); what are stakeholder priorities; how much does it cost; what are the implications of BVD for whole farm management; what are the best tests and what are the optimal testing strategies; what are the net benefits of control at farm level and the wider consequences for trade and the environment; which farmer attitudes and other barriers to action make the “do nothing” control option so prevalent and what may be done to overcome such barriers? The approach includes exploration of risks in a context beyond the farm level. It starts with consultation between researchers, farmers and their veterinary advisors and radiates out to quantify the scale of the risk and explore aspects of economic risk such as stochastic cost benefit analysis and variable, competing, demands for expenditure on animal health. We will review approaches derived to manage BVDV exposure in Scotland.

Neither improved recognition of the disease complex attributable to cattle exposure to BVDV nor the usually compelling cost benefit arguments in favour of control seem to be sufficient to convince the majority of farmers. This leads us to explore behavioural aspects of disease control and biosecurity and try to understand the risk that negative attitudes to systematic BVD control present to proposed

control schemes and how these might be overcome through enhanced communications and knowledge exchange. The authors recognise that circumstances differ between farming systems, regions and countries as do the risks and that the results of risk analyses for BVDV management will vary. Our aim is some standardisation of approach that embraces greater exchange of relevant information now available internationally.

# Prevalence of leptospirosis in farm animals

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Leptospirosis is a worldwide zoonosis caused by infection with pathogenic spirochetes that belong to the genus *Leptospira*. *Leptospira* spp. colonize the proximal renal tubules of various mammals and are excreted in the urine of carrier animals. Transmission of leptospirosis in humans and animals occurs by exposure to water or soil contaminated by the urine of infected animals or by direct contact with infected animals. Farm animals such as cattle and swine are not only infected as incidental hosts but also are maintenance hosts of specific *Leptospira* serovar strains and serve as reservoir animals for the same animal species and/or humans. Incidental host infections of cattle with serovars such as Grippotyphosa, Icterohaemorrhagiae and Pomona cause acute, severe clinical disease. Clinical signs of acute bovine leptospirosis include high fever, haemolytic anaemia, haemoglobiuria and jaundice. Infection in pregnant cattle can result in abortion. In lactating cows, infections are often associated with agalactia. Cattle also serve as a maintenance host of serovar Hardjo, which consists of two different genotypes, Hardjobovis and Hardjo-prajitno. Antibodies against this serovar were detected in 25 to 65% of cattle in the US, Europe, South America and Australia and *L. borgpeterseni* serovar Hardjo type Hardjobovis (Lb Hardjobovis) is the most common in cattle worldwide. Acute infection with Hardjobovis results in asymptomatic or mild cases, but chronic infection is associated with infertility and reproductive failures such as abortion, stillbirth and weak calves. *L. interrogans* serovar Hardjo type Hardjo-prajitno is often associated with acute infection in dairy cows leading to milk drop syndrome.

Leptospirosis in cattle is a notifiable disease in Japan but there are almost no reports on the disease in recent years and the actual prevalence of bovine leptospirosis remains unknown. Therefore, in order to reveal the current situation of leptospiral infection and the relationship between abortion and leptospirosis in cattle, we carried out testing for anti-leptospiral antibody among 303 healthy dairy cattle from 18 farms in Hokkaido by microscopic agglutination test. Anti-leptospiral antibody (reciprocal MAT titer  $\geq 100$ ) was detected from 43 cattle on 9 farms (14.2%). Predominant reacting serogroups were Sejroe to which serovar Hardjo belongs (41/43, 95.3%), followed by Hebdomadis (10/43, 23.3%) and Autumnalis (1/43, 2.3%). Leptospiral DNA was detected in 2 of the 39 urine samples from 3 seropositive farms. Both PCR-positive cattle had histories of abortion. The nucleotide sequences of the two amplicons were identical and the leptospiral species was deduced to be *Leptospira borgpeterseni*. These serological and DNA analyses strongly suggest wide spread of Lb Hardjobovis among cattle in Hokkaido, although attempts to isolate leptospirae failed. Next, we examined the relationship between history of abortion and presence of anti-leptospiral antibody on 5 farms where the history of abortion for each cow was recorded. The seroprevalence was high on 2 of the 5 farms (30.7% and 38.9%) but there was no difference between cattle with and cattle without histories of abortion. On 3 other farms, low seroprevalence was detected in both abortion-experienced and abortion-naive cattle. On the other hand, comparison of the embryonic age at the time of abortion between seropositive and seronegative cattle revealed that abortion occurred earlier in seropositive cattle than in seronegative cattle (positive; 51.5 days (median, N = 8), negative; 82.5 days (N = 76),  $p = 0.037$ ). The presence of anti-leptospiral antibody in abortion-experienced cattle and the differences in the fetal age at the time of abortion

between seropositive and seronegative cattle suggested positive correlation between abortion and leptospiral infection in Hokkaido, although further verification with a larger sample size is needed.

In this study, of two highly seroprevalent farms, one bought cattle from the other, and the other entrusted their cattle during the summer season to another operation, suggesting that contact with carrier cows may have caused infection. Thus, it is important to identify and eradicate carrier animals introduced from outside. Carrier animals can be identified using urine samples by isolation, fluorescent antibody staining or detection of leptospiral DNA using polymerase chain reaction. However, it is difficult to carry out these methods in the field. We have developed a loop-mediated isothermal amplification method for the detection of leptospiral DNA in urine, which is applicable to point-of-care testing. We will report results of evaluation of this LAMP method using field animal urine samples.

## **Weaning weights in calves from cows with positive ELISA for *Mycobacterium avium* subspecies *paratuberculosis* antibody in US beef cow-calf herds**

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The US National Johne's Disease Demonstration Herd Project (JDDHP) was launched to evaluate management related control measures for cattle and to educate veterinarians and producers about the importance of diagnostic testing, management and control strategies for Johne's disease. JDDHP data were obtained to evaluate losses associated with Johne's disease in beef cow-calf herds based on serum ELISA (IDEXX) for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) antibody. Data for 205-day adjusted weaning weights (AWW) of calves and serum ELISA (n = 3,482) results from their dams were analyzed. ELISA results were classified into four categories based on manufacturer recommendations: negative, suspect, positive, and strong positive. To account for clustering of observations, multilevel mixed models were developed including random effects to account for repeated tests within cow, and cow nested within herd. Potential confounding of the associations between test status and AWW associated with herd and animal-level covariates was evaluated on the basis of change in regression coefficient after inclusion of the covariate in the model. Multivariable model adjusted for the effects of cow age and years since the inception of a control program in the herd was used to estimate the differences in AWW. Compared to the AWW of calves from test negative dams, calves from suspect cows were estimated to have 5.9 lbs (95% CI: -2.38 to 14.16) lower AWW, calves from positive cows were estimated to have 5.8 lbs (95% CI: -5.37 to 17.03) lower AWW, and calves from strong positive cows were estimated to have a significantly lower AWW by 45.2 lbs (95% CI: 23.25 to 67.26). Results from this study indicate that there is a lower AWW in the offspring of cows with positive result for MAP antibody with serum ELISA. These findings will be important for producers and other stakeholders in the industry to better understand the importance of control of Johne's disease and use of serum ELISA in beef herds.

# **Uptake and use of cattle vaccines in the UK**

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## **Objective**

Farmers in the UK have expressed concern with regards to the 'best practice' to vaccinate their cattle. Currently there is limited data present that describes current uptake and use of cattle vaccines. The existing evidence indicates a lack of compliance from farmers with regards to correct application of a vaccine. The aim of this study was to investigate current uptake and use of vaccines available to the dairy and beef industry in the UK.

## **Materials and Methods**

An online and paper based questionnaire was distributed to dairy and beef farmers throughout the UK between September and October 2011. The questionnaire collected information on which vaccines were used, how and why farmers were using these vaccines and requested descriptive information about the respondent.

## **Results**

Preliminary results indicate a vast variety of vaccine uptake and use amongst farmers. In addition, the farmer's assumptions with regards to the effect of vaccination are frequently inaccurate. Compliance of respondents with regards to the correct execution of the vaccination appeared to be adequate. The veterinarian is seen as an influential adviser on vaccination. The reason to choose a particular type of vaccine and vaccination protocol against a disease syndrome (e.g. respiratory disease) is often unclear to the farmer. A variety of advice is given by different sources available to the farmer; there is the opportunity to improve evidence based advice from an independent adviser, such as the veterinarian. This study is still ongoing and will be completed in December 2011. The research was confined to the UK, however conclusions from this study are expected to be relevant in countries where vaccination of cattle is common.

## **Conclusion**

Vaccination is one of the cornerstones in controlling infectious disease worldwide in the cattle industry. Although excellent research continues to develop novel methods to control disease, results of this study support that successful disease control by vaccination is dependent on more than the quality aspects of the vaccine alone.

## **Molecular characterization of immunoinhibitory factors PD-1/PD-L1 in bovine leukemia virus infection**

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The immunoinhibitory receptor programmed death-1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1) are involved in the immune evasion mechanisms of chronic infections and tumors. Blockade of the PD-1/PD-L1 pathway by antibodies specific to either PD-1 or PD-L1 restores anti-pathogen immune responses, and is expected to be applied to new therapies for the chronic infectious diseases and tumors. However, few functional analyses of these molecules have been reported for domestic animals. Thus, in this study, cDNAs encoding for bovine PD-1 and PD-L1 were cloned and their expression and roles were analyzed in the bovine leukemia virus (BLV)-infected cattle.

A deduced amino acid sequence of bovine PD-1 and PD-L1 showed high homologies with those of human and mouse PD-1 and PD-L1. The proportion of PD-L1<sup>+</sup> cells in peripheral blood mononuclear cells, especially B cells was upregulated in cattle at the late stage of the disease compared to cattle at the aleukemic infection stage or uninfected cattle. The proportion of PD-L1<sup>+</sup> cells were correlated positively with prediction markers of the disease progression such as leukocyte number, virus load and virus titer, and inversely correlated with the degree of the interferon-gamma expression. Blockade of the PD-1/PD-L1 pathway *in vitro* by PD-L1-specific antibody upregulated the production of interleukin-2 and interferon-gamma via T cell dependent mechanism, and downregulated BLV provirus load.

These data suggest that PD-L1 induces immunoinhibition in disease progressed cattle during chronic BLV infection. We are now generating recombinant PD-1 protein for the blockade *in vivo*.

## **Bovine viral diarrhoea virus infection in a dairy herd with high prevalence of persistently infected calves**

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A dairy herd that was detected as a high prevalence of animals persistently infected (PI) with bovine viral diarrhoea virus (BVDV) infection was investigated. At first in this dairy herd, a newborn PI calf was detected as a clinical case suffering from nervous manifestations. Then all cattle in the herd were examined and 2 PIs (a milking cow and a calf) were also detected. In order to eradicate BVDV from the herd, monitoring of all newborn calves was continued for PI detection. During 15 months of surveillance, 36 newborn calves were produced. 6 of them were identified as PI within the first 4 months of surveillance period. All detected PI viruses were identified as BVDV1b. In the comparison of the virus genes of the detected PI animals based on the PI milking cow virus, a very high homology was recognized among nucleotide and amino acid sequences. 100% nucleotide homology in the 5'UTR and 96.43-100% amino acid sequences in the E2 region were recognized. The PI milking cow was introduced to the herd 5.5 months before the birth of the first PI calf. The PI milking cow was strongly suspected to be the source of BVDV infection into the herd because of the sequence identity of the detected virus genes and the epidemiological information of the herd. Prevalence of PI animals in this herd estimated 7 % during 15 months of surveillance which is very high compared to that estimated in previous reports. In the present study, all PI animals had a single origin of infection and the high prevalence of PI was recognized in a short period.

## Subcellular formation and localization of *Bovine viral diarrhea virus* replication complex

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**[Introduction]** *Bovine viral diarrhea virus* (BVDV) has two biotypes, cytopathogenic (cp) and noncytopathogenic (ncp). As the onset of mucosal disease is related with both biotypes, their replication mechanisms *in vivo* and *in vitro* are very much concerned. We previously reported that apoptosis caused by the viral double-stranded RNA (dsRNA) accumulation in cultured cells is important for the cytopathogenicity. However, detailed mechanisms of this procedure have yet to be elucidated. In this study, we tried to detect the viral replication complexes to clarify their formation and localization in virus-infected cells.

**[Materials and Methods]** Bovine kidney cell line, MDBK cells and LB9.K cells, and primary bovine fetal muscle (BFM) cells were infected with cp BVDV (Nose and KS86-1cp) or ncp BVDV (KS86-1ncp), stained with the specific antibodies against a nonstructural protein 3 (NS3), NS4B and NS5A, and analyzed by fluorescence microscopy. In addition, the specific antibody against dsRNA was used for staining of the infected MDBK cells and LB9.K cells.

**[Result and discussion]** Each NS was colocalized in endoplasmic reticulum (ER) of either cp or ncp BVDV-infected cells. The localization of dsRNA in the cp BVDV-infected cells was also shown in ER, while dsRNA was not detectable in the ncp BVDV-infected cells. This result supported our previous finding, showing that the amount of dsRNA in the cp BVDV-infected cells was 100 times more than that in the ncp BVDV-infected cells, and may show the amount of dsRNA in the ncp BVDV-infected cells was not enough to be detected. This study revealed that there was no difference of the subcellular formation and localization of the BVDV replication complex between the both biotypes. Thus, to explain the reason why the yield of dsRNA in the cp BVDV-infected cells is much higher than that in the ncp BVDV-infected cells, further investigation will be required.

# Innate immune response elicited by host pathogen interactions of bovine caruncular cells with abortifacient bacteria *Leptospira* and *Listeria*

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## Objective

Infections, caused by the abortifacient zoonotic pathogens *Leptospira* and *Listeria*, are responsible for economic losses to the beef and dairy industry and are widely known to cause significant public health concerns. Limited research has been performed on the mechanism by which *Leptospira* and *Listeria* can cause abortion when interacting with the bovine reproductive tract. The aim of this study was to investigate the host response of bovine caruncular epithelial cells to *Leptospira* and *Listeria* infection.

## Material and methods

Using a bovine caruncular epithelial cell line, mRNA expression of Toll-like receptors (TLRs)1-4, 6, 9, and oestrogen and progesterone receptors was demonstrated. The study subsequently investigated the modulation of TLR 2 and 4 expression and the production of inflammatory mediators Tumour Necrosis Factor  $\alpha$  and prostaglandin E2 after exposing the cell line to lipopolysaccharide, *Leptospira borgpetersenii* serotype Hardjo Bovis and *Listeria monocytogenes*. In these experiments the effect of pregnancy on TLR expression and inflammatory mediators was simulated by the addition of progesterone.

## Results

While TLR2 mRNA expression was increased in response to lipopolysaccharide, neither TLR2 nor TLR4 mRNA expression was modulated by *Leptospira* or *Listeria* infection. *Leptospira* increased prostaglandin E2 release in a dose-dependent manner. However, in contrast to uterine epithelial cells, neither TLR expression nor prostaglandin production was effected by pre-stimulation with progesterone.

## Conclusion

Caruncular epithelial cells recognize and respond to *Leptospira* infection, although their response is relatively modest when compared with the response to lipopolysaccharide stimulation. *Listeria* can infect and survive in caruncular cells without eliciting PGE2 release. This difference in the inflammatory response to these abortifacient bacteria compared to lipopolysaccharide may explain the pathogens' ability to sustain subclinical infections.

## Detection of *Mycoplasma* species in the uterus of postpartum dairy cows and its relation to endometritis

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This study was carried out to investigate the incidence of mycoplasma infection in the uterus of postpartum Holstein dairy cows and its relation to the occurrence of endometritis. The genital tracts of 104 cows distributed in three dairy farms in Iwate Prefecture, Japan, were examined at week 5 (W5) and week 7 (W7) postpartum. The condition of the cervico-vaginal mucus was assessed by Metricheck device (Five scales from score 0, clear mucus, to score 4, purulent material with fetid odor). Moreover, intrauterine samples were collected using cytobrush. Following its withdrawal, swab samples were taken and placed in mycoplasma culture broth at 37°C for 72 h. A novel and rapid PCR was used to detect seven mycoplasma species (*Mycoplasma bovis*, *M. arginini*, *M. bovigenitalium*, *M. californicum*, *M. bovirhinis*, *M. alkalescens*, and *M. canadense*). The cytobrush was also rolled gently along the length of glass slide for polymorphonuclear neutrophil (PMN) count. At W5 and W7, the diagnostic criteria for cytological endometritis were  $\geq 6\%$  and  $\geq 4\%$  PMN, respectively. The incidence of dystocia at the last calving was compared in mycoplasma positive and negative cows by Chi-square test.

*M. bovigenitalium* was detected in seven out of the 104 cows (6.7%). Three cows were diagnosed positive at W5, three were positive at W7 and the other cow was positive at W5 and W7 and had a Metricheck score of 4 and high PMNs (15%) at W7. Interestingly, the incidence of dystocia was significantly higher ( $P = 0.002$ ) in mycoplasma positive (71.4%) compared to mycoplasma negative (3.7%) cows. Moreover, the incidence of cytological endometritis was higher in mycoplasma positive (57.1%) compared to mycoplasma negative (34.4%) cows, although it didn't reach a significant difference.

These results indicate that *M. bovigenitalium* might be associated with dystocia at the last calving and with endometritis in postpartum dairy cows.

# Opinions and practices of vets and dairy farmers towards herd health management in the UK

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## Objective

The objective of this study was to compare farm veterinary surgeons' and dairy farmers' opinions on Herd Health Plans and Herd Health & Production Management with the aim to discover and better understand differences in opinions of vets and farmers.

## Materials and methods

For the purpose of this study 'Herd Health Plans' were defined as 'the current paper document issued by the British Cattle Veterinary Association or other organisations'. 'Herd Health & Production Management' was defined as 'regular scheduled farm visits that go beyond the 'one-off' tasks such as pregnancy diagnosis, castrations and dehorning; the purpose being to prevent disease and/or improve animal health and production by introducing long term strategies focusing on the herd as a whole.'

Two comparable questionnaires, one for farm vets and one for dairy farmers, were distributed to 436 dairy farmers and 160 farm animal practices throughout the UK between June and September 2008.

## Results

Vet and farmer respondents differed when listing the 'major roles' of the vet on the farm; although vets see 'Optimising milk production' and 'Being an independent advisor' as important roles this does not seem to be perceived as such by the farmer. Furthermore, when presenting themselves to clients, vets seemed to favour the 'friend of the farmer' style approach; a much smaller proportion of farmers seemed to prefer this approach. The majority of farm respondents (81%, n = 98/121) valued the discussions with their vet and it was apparent from the relatively small proportion of vets instigating a discussion on farm (26%, n = 33/125) that there is the opportunity for a more proactive approach from vets. The study underlined that 'demonstrating cost effectiveness' is still a main concern for vets and farmers.

## Conclusion

The vet is an important stakeholder to motivate change on dairy farms and the results of this study identified areas that can be improved by more training and effective communication.