Second European Symposium on: BVDV Control

Session 1: Virus properties and diagnostic assays relevant for control of BVDV

Selection of diagnostic assays for BVDV control programmes

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A mandatory objective of any control programme aiming at eradicating BVD from an infected bovine population is the removal of animals persistently infected (PI) with BVDV. In practice this will require a selection of reliable diagnostic assays. Approaches on how to identify PI animals can be indirect, by first identifying herds with a high probability of containing PI animals through diagnostic surveillance. Subsequently individual testing all animals in suspicious herds may identify PI animals.

Several factors influence what diagnostic tests should be chosen for a given BVD control programme. Currently two species of bovine viral diarrhoea virus (BVDV) have been recognised, and also within each of these species a certain degree of viral diversity can be seen. To some extent viral diversity may affect the ability to detect BVDV, or indirectly to diagnose infection with them. Furthermore, the choice of diagnostic tests to use will depend on the epidemiological status of the population. In high cattle density areas BVDV spreads more easily than elsewhere, and the initial value of serological surveillance is less. Nevertheless, once a control programme has been running successfully for some time, monitoring the rate of reinfection may be very important in high cattle density areas. To some extent serological surveillance may be difficult for populations vaccinated against BVD, since distinguishing between immunity derived from vaccination and natural infection cannot easily be done. Dependent on the production system, the nature of sample material easily available may differ considerably. Bulk tank milk samples have proven to be a very cost-effective and reliable way to monitor the BVD prevalence in non-vaccinated dairy herds. Similar surveillance of beef cattle herds is much less convenient since it requires sampling of individual animals. Finally, cost, investigation time and ease of use are parameters that influence whether an otherwise technically reliable test is suitable for mass testing during control programmes.

Historically, most laboratory investigations for bovine viral diarrhoea (BVD) diagnosis have been performed in virological laboratories equipped with cell culture facilities. During the last 15 years, the development of enzyme-linked immuno-sorbent assays (ELISA) has revolutionised the capacity of large-scale diagnostic investigations, allowing the bulk of laboratory investigations for BVD to be performed away from the scrutiny of pestivirologists. Similarly, during the last decade many assays based on in-vitro amplification of nucleic acids (RT-PCRs) have been refined for diagnostic applications. Thus, the laboratory investigative capacity may today easily outperform the ability to analyse and describe the dynamics of infection on the individual farm, or in the cattle population under investigation.

Both for testing of individual animals and serological surveillance, antibody ELISAs are the principal choice of assay today. One shortcoming over the reference test for serology, the virus neutralisation test, is that quantification of the antibody level can only be approximate with ELISAs, especially for individual sera. Antigen ELISAs are similarly convenient to identify PI animals, although RT-PCRs may soon become realistic alternatives. A principal advantage over reference assays based on isolation of BVDV in cell cultures is a much shorter turnaround time. Most importantly, in well organised BVD control programmes the performance properties of all diagnostic assays should be known, and used to design a diagnostic network that is able to operate at diagnostic sensitivity and specificity levels as close to 100% as possible.

Impact of BVDV Type 2 on American BVD control approaches

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In the early 1990’s research groups in North America reported that a newly recognized severe acute form of bovine viral diarrhea virus infection, termed hemorrhagic syndrome, was associated with a distinct subgroup of BVD strains (Pellerin et al., 1994; Ridpath et al., 1994). This new subgroup was named BVDV genotype 2 or BVDV2. All BVDV strains previously characterized in the literature belonged to a separate genotype, BVDV genotype 1 or BVDV1. However, not all BVD strains identified as BVDV2 were associated with severe acute infections. One of the earliest studies cha-
racterizing BVDV2 strains found that 11 out of 76 had been isolated from persistently infected animals born to dams previously vaccinated against BVDV (Ridpath et al., 1994). Characterization of BVDV strains used in the vaccines revealed these strains belonged to the BVDV type 1 genotype. Subsequent surveys of BVDV strains isolated from clinical submissions to diagnostic laboratories and contaminated fetal calf serum suggested that the ratio of BVDV2 to BVDV1 strains in the U.S. approached 50% (Bolin and Ridpath, 1998; Evermann and Ridpath, 2002; Fulton et al., 2000; Ridpath and Bolin, 1998). Further, while antigenic cross reactivity has been noted between BVDV1 and BVDV2 strains, cross neutralizing titers are typically a log or more higher between BVD viruses within the same genotype compared to viruses from different genotypes (Fulton et al., 2003a). These observations prompted vaccine manufacturers in North America to produce vaccines against BVDV that contained antigens from both BVDV1 and BVDV2 strains. As of January 2004, at least five major biologics companies were marketing modified live and/or killed vaccines containing both a BVDV1 and a BVDV2 strain. Under experimental conditions these new vaccines offered improved protection against type 2 strains, however field data is still insufficient to assess their efficacy in practice.

Recently questions have been raised regarding the possibility of recombination between BVDV vaccines strains contained in the same modified live vaccine. The concern raised was whether such a recombination would give rise to a noncytopathic virus that could pose a danger for fetal infection. These concerns arose from a reported observation of recombination between a type 1 and a type 2 BVDV (Ridpath and Bolin, 1995). However, this was not between two cytopathic vaccine strains, but between a cytopathic type 1 vaccine strain and a noncytopathic type 2 strain. The recombinant virus was isolated from a persistently infected animal that succumbed to mucosal disease (MD) several months after vaccination. It was theorized that a recombination between the cytopathic virus in the vaccine and the noncytopathic virus present in the persistently infected animal gave rise to a third virus that was cytopathic. This “new” cytopathic virus was identical to the persistent virus with the exception of an insertion in the NS2/3 coding region. This sequence of this insertion was identical to sequences found in the vaccine virus. To date, there have been no observations, either in vitro or in vivo, of recombination between cytopathic viruses giving rise to noncytopathic viruses. In addition there have been no reports of increased reproductive disease within herds under vaccines programs using combination type 1 and type 2 vaccines.

Subgenotypes and BVDV1 and BVDV2 have been recognized. Recently, surveys of prevalence of BVDV1 subgenotypes in North America have led to suggestions that protection may be improved by inclusion of strains from BVDV subgenotype 1a and BVDV subgenotype 1b in vaccines in addition to BVDV2 strains (Fulton et al., 2003a; Fulton et al., 2002; Fulton et al., 2003b). The cost to benefit ratio of this proposal is currently a matter of debate.

References

Genetic diversity of BVDV: consequences for classification and molecular epidemiology

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At present genetic diversity of BVDV isolates is intensively studied using rapid sequencing of RT-PCR products coupled with computer-assisted phylogenetic analysis. The most often used parts of the pestivirus
genotype for this purpose are the 5'-untranslated region (5'-UTR), Npro and E2 coding regions. In our laboratory we type pestiviruses several years selecting the 5'-UTR (using panpestivirus 324/326 PCR primers which amplify a 288 bp DNA fragment) and Npro (using BD1/BD2 PCR primers which amplify a 428 bp DNA fragment) regions. Here we present selected results which according to our opinion significantly contribute to the understanding of genetic diversity of BVDV and usefulness of this approach to resolve practical epidemiological problems.

ii/ Recently we performed a very comprehensive genetic typing of BVDV-1 isolates originating from various countries around world and revealed that this pestivirus is very variable at the genetic level: Two BVDV-1a and BVDV-1b subgenotypes were extended to 11 (BVDV-1a – BVDV-11) genetic groups. Additional experiments on genetic typing of BVDV-1 isolates led to the identification of the 12th subgenotype (BVDV-1k).

iii/ We also used genetic typing for BVDV isolates originating from two non-European countries, India and Australia, showing that there are predominant BVDV-1b and BVDV-1c subgenotypes, respectively.

iv/ The above-mentioned approach was used to resolve the problem of exact typing of BVDV isolate causing no typical BVD/MD symptoms with suspicion for a BVDV-2 infection. However, genetic experiments identified that the infectious agent belonged to the BVDV-1b subgenotype.

v/ In other study we have shown that BVDV-1 vaccine strain used for the vaccination of cattle can be spread into cattle population.

vi/ BVDV-2 isolates where detected not only in USA, Canada, South America but also in Europe, namely in Germany, Belgium, Italy and UK. Our laboratory contributed to these studies by the first identification of BVDV-2 isolates in cattle farms in Austria, France and Slovakia. These results demonstrated that BVDV-2 spreads to European countries.

No doubt that cumulating data on genetic typing of BVDV isolates will contribute not only to the taxonomy of these viruses but for the development of molecular epidemiology of BVDV infections as well.

Genetic heterogeneity of pestiviruses of Ruminants in Switzerland

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Vaccination against BVD is uncommon in Switzerland, and a similar prevalence of both antibody-positive (appr. 60%–70%) and persistently infected animals (appr. 0.7-1%) was found in several studies conducted over a time period of approx. 10 years. Moreover, very few animals are imported from other countries. This offers interesting possibilities to study the genetic heterogeneity and dynamics of the viral strains circulating in the cattle population under equilibrium conditions.

To determine the genetic heterogeneity of BVD viral strains, we sequenced the 5' UTR and in selected cases also the Npro region of some 150 strains isolated in routine diagnostic work from 1990 to 2004. To exclude BVDV contaminating the cells, the uninfected control cells were routinely stained for viral antigen. In these control cells, we detected several contaminants derived from fetal calf serum. Sequence data indicate that these are novel pestiviruses that do not cluster with the known pestiviral species and genotypes.

Representatives of BVD genotype 2 were not observed in the strains isolated from cattle. According to the classification proposed by Vilček et al. (Arch. Virol. 146, 99, 2001) the viruses isolated from cattle were assigned to phylogenetic groups 1e, h/k, and b, in decreasing order of frequency. Although detailed information on the virulence of the strains is lacking, several cases were observed of acute BVD with severe clinical outcome, including hemorrhages in internal organs and muscle. Preliminary sequence data indicate that the strains associated with severe clinical signs can be assigned to different phylogenetic groups of BVDV genotype 1. Only one attenuated and one inactivated BVDV vaccine are licensed in Switzerland. The attenuated strain has been shown to induce mucosal disease in persistently infected animals (Becher et al., J. Virol. 75, 6256, 2001) and the strain contained in the inactivated vaccine belongs to BVDV genotype 1a, which to date has not been documented in Switzerland.

BVDV is genetically conserved in persistently infected animals as well as during chains of transient infection. Moreover, the virus is also conserved when transmitted from persistently infected cows to their offspring, even when more than one calf is born to one persistently infected cow. The existence of persistently infected animals may be viewed as “genetic repository”, suitable for the reconstruction of chains of infection. Using this approach, we have detected chains of infection involving several farms that were associated over several years by sending their animals in early pregnancy to the same Alp for grazing during the summer months. The results of molecular epidemiology confirm earlier epidemiologic studies (Braun et al., Zb. Veterinaermedizin 45, 445, 1998).

With some 20%, the prevalence of sheep seropositive to Border Disease virus (BD virus) is well below that of cattle seropositive to BVDV. To date, BD virus virus was isolated only on two occasions in Switzerland. All strains analysed of these outbreaks belonged to the BD virus group 3, originally proposed by Becher et al. (Virology 311, 96, 2003). No BVDV were isolated from sheep but we detected a group 1e BVDV in a persistently infected goat.

Animals seropositive to BVDV were occasionally
found in red deer, ibex and chamois. However, no viruses were isolated from animals of these species, and the genetic properties of the pestiviruses responsible for the antibodies to BVD remain unknown.

**Diagnostic assays applied in BVDV control in the Netherlands**

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In the Netherlands a voluntary BVD-virus-free certification programme was started in 1998. After an intake procedure in which all cattle are tested for the presence of BVD-virus, the herd obtains the status “BVD-virus-free”. To maintain this status, a monitoring procedure is executed twice a year to verify absence of BVDV circulation in the herd.

Several diagnostic tests are used: PCR in bulk milk and pooled blood samples, antigen-ELISA (based on Erns) and antibody ELISA (based on p80) in individual blood samples. Sensitivity and specificity of these tests and consequences for the certification programme will be discussed.

In addition, a diagnostic “quick scan” has been introduced, consisting of a combination of bulk milk testing for antibodies and virus, and antibody testing in a sample of 5 calves. Preliminary results will be presented.

**Detection of Bovine Viral Diarrhea Virus (DVDV) harboring seropositive cattle**

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Detection of virus harboring cattle is vital for the prevention of BVDV. In a previous paper a very sensitive cELISA, able to detect BVDV in 10 out of 107 seropositive cattle with or without previous virus amplification on polycation treated MDBK cells (cELISA-FCV) was presented. In order to avoid the use of cell lines, peripheral blood leukocytes (PBL) from these virus positive animals were directly treated with polycations, giving negative results. Mitogens may enhance virus expression in leukocytes. PBL were stimulated with different mitogens, and Phytohemmaglutinin (PHA) showed to be the mitogen giving best results. Using PHA stimulation with polycation treatment of the same PBL, 229 seropositive cattle were studied and could be classified in 4 different states of BVDV infection. Lysed PBL from 4 animals were directly positive in c-ELISA (Category I), 17 animals were positive after the PHA stimulation (Category II), 15 animals were positive only after PHA stimulation plus PBL treatment with polycations (Category III), while virus could not be detected in 193 seropositive cattle (Category IV). Wild type BVDV strains were isolated by co culture on polycation treated MDBK cells from 11 of these virus harboring animals. The circulating antibodies of these same animals were able to neutralize their autologous virus strain, indicating that virus may persist in the spite of the presence of strain specific antibodies. No gross immune malfunction could be detected in any category of virus positive animals. BVDV virus may be persistently harbored in cattle, albeit with different capabilities, in spite of a specific active immune response.

**Testing of cattle ear notch samples using a Bovine Virus Diarrhea antigen ELISA kit**

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The samples used in this study were from veterinary diagnostic labs at Cornell university, University of Nebraska, and Oklahoma State University. Reference method BVDV testing (virus isolation, PCR, or IHC) was performed by each of these labs on their own samples. 100 samples from calves ranged in age from 1 day to adult (more than 3 months) were tested by reference technique. In the following proportions: 61 by IHC, 23 by PCR, 10 by virus isolation on buffy coat, 5 by virus isolation on sera and 1 by ELISA on serum. The kit tested in this assay (SERELISA™ BVD E0 Ag Mono Indirect, Synbiotics Europe) was evaluated on ear notch samples. This is an indirect mono well antigen ELISA. Ear notch approximately 1cm x 1cm in size was cut from the edge of the ear of each subject animal. Each of the samples were mixed to 2mL of phosphate buffered saline pH 7.4 then allowed to soak in PBS for a minimum of 10 minutes at room temperature. The ELISA was carried out exactly as described in the kit enclosure, except that 100 µL of the ear notch/ PBS supernatant was pipetted into each reaction well in place of serum. The data reduction and the cut-off’s for the ear notch samples were the same as detailed in the kit enclosure for serum samples. Complementary studies on the ELISA kit using ear notch samples were performed: relationship between size of ear notch and assay results for three representative BVDV positive samples, as well as effect of soak time of this three ear notch samples in PBS.

The cattle included in this study ranged in age from 1 day to adult (more than 3 months). This included 60 young animals (less than 3 months), 47 of which were from 1 day old pre colostral calves. All results obtained with ELISA on ear notch samples correlate perfectly with results obtained with reference technique. Ob-
erved correlation is 1 (100/100 animals). Sensitivity index of the ear notch/ELISA was 100% (39/39) relative to the reference methods, and specificity index of the ear notch/ELISA was 100% (61/61) relative to the reference methods. At a risk = 5%, the minimum guaranteed indexes are 91% for sensitivity and 94.1% for specificity. Data obtained from relationship study between ear notch size and assay result are positive. No significant decrease was observed on BVDV-positive ear notches down to 0.25cm x 0.25cm in size. Data obtained from relationship study between soak time in PBS (from no soak time to 10 days of soaking) and assay results are all positive. Even with no soak time the results obtained are clearly positive.

The excellent agreement between ear notch/Ag ELISA results and the reference methods on these samples indicates that no age limitations are necessary when assaying ear notch samples by antigen capture ELISA. This is not the case when this ELISA is performed on serum samples: this is due to the potential interference of anti-BVDV maternal antibodies (Palffı et al., 1993). These circulating maternal antibodies would not be expected to be present at appreciable levels in the case of these ear notch samples. This is supported by the results obtained with sample from a 1.5 week old persistently infected animal. This animal was BVDV positive by PCR and by ELISA performed on ear notch but negative by ELISA performed on serum. The recommended size of 1cm x 1cm for ear notch represents an adequate margin of safety to insure that BVDV-positive samples are correctly identified. The results obtained with different soak times give significant positive results even with no soak time protocol.

The testing of ear notch samples with the antigen capture ELISA tested here represents a very effective means of assessing the BVDV-status of cattle of all ages, especially young ones under colostral protection. This test is an attractive second choice to expensive means of assessing the BVDV-status of cattle of all ages, especially young ones under colostral protection. However, the high correlation between assay results are all positive. Even with no soak time the results obtained are clearly positive.

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The testing of ear notch samples with the antigen capture ELISA tested here represents a very effective means of assessing the BVDV-status of cattle of all ages, especially young ones under colostral protection. This test is an attractive second choice to expensive and difficult techniques that were the only available for testing young animals less than 3 months of age with suspected presence of maternal antibodies. In this context, control refers to a systematic reduction of the incidence of infection with bovine viral diarrhea virus (BVDV), and the prevalence of infected herds, with or without the use of vaccines. A systematic control approach for BVDV can be described as consisting of three different activities; 1) initial determination of the infective status of herds, 2) interventions aimed at eliminating the virus from infected herds, including monitoring of the effectiveness of such interventions and 3) preventive measures aimed at reducing the risk of introducing the virus into non-infected herds.

BVDV has developed strategies for persistence that are very efficient, but there are also “weaknesses” that work in favor of control. For within-herd transmission of BVDV, one such feature is its strong immunity after natural infection, which gives long-lasting protection against new fetal infections, and consequently the birth of new persistently infected (PI) individuals. If the infection is to be sustained, herd demographics/management need to allow for contacts between animals in early pregnancy and PI animals. It also needs to allow PI animals to remain in the population long enough to propagate the infection. If these conditions cannot be met, the infection will be eliminated from the herd. This is why it is possible to clear infected herds from the virus simply by removing PI animals, without taking special measures to prevent horizontal spread of infection. Elimination of the infection without intervention is referred to as self clearance. This phenomenon is most common in small herds where animals are kept in the same compartment, but can occasionally appear also in larger herds.

For large-scale control of BVDV, the key factor to manipulate is the between-herd contact pattern. This does not necessarily mean that the frequency of contacts has to be reduced, but rather the risk associated with having such contacts. For example, many control schemes use knowledge about herd status as the basis for how livestock can be moved/traded, thereby changing the way in which animal movements occur and providing safer trading patterns.

Another key element of systematic control is to allocate resources to where they are needed, i.e. to take appropriate measures depending on the status of each herd. Features in BVDV epidemiology that facilitates such activities are, once again, the efficient transmission of BVDV in the presence of PI animals, and the strong immune response in surrounding cattle. This allows the infection to be detected indirectly through serology and by the use of herd level tests on specimens such as bulk milk. The high correlation between the serological status in individuals and the infectious status of the herd also allows the use of spot samples on a small number of individuals as an alternative diagnostic pathway. Thus, every control program need to take the special features of BVDV epidemiology into consideration both in the intervention and in the surveillance part.

Session 2: Characteristics in BVDV epidemiology of relevance to control

Characteristics in BVDV epidemiology of relevance to control

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Successful control of infectious diseases requires a good understanding of the epidemiology of the agent.
Epidemiological investigations in populations where BVDV is endemic have shown that demographic factors such as, herd size and herd density are significant predictors for the prevalence of infection. However, experiences from controlling BVDV in high-density areas like Denmark and South East Sweden show that it is not a predictor for the prospects of successful control. Rather, it is the way in which control activities are organized and implemented that will determine the progress.

To this day, non-vaccination control approaches have been more successful in reducing the level of infection than approaches using vaccination. One major difference is that with the former, animal movements require reconfirmation of the herd’s free status before cattle can leave their herd of origin. Thereby, PI animals and PI carriers are efficiently prevented from being put on the market. In other words, the risk of acquiring BVDV infection through trade is being managed at the source. Vaccination in itself does not prevent introduction of the infection – it has to be combined with biosecurity to prevent PI animals or PI carriers from being purchased. However, once a vaccination scheme has been put in place, such a message can be difficult to convey to farmers as they may have the false perception of already having taken sufficient action to protect the herd from BVDV infection. It should also be noted that non-vaccination approaches more often have been implemented at a level where they are likely to have a significant impact (region/nation), whereas decisions on vaccination have been left to the farmer.

An understanding not only of the biology, but also of the social factors - human behaviour, the motives that make stakeholders follow advice and the cultural differences in this respect - will be important factors in forming recommendations on alternative European strategies for BVDV control.

Epidemiological associations in BVD controlled Greek dairy herds: results of an ongoing study

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BVD infection is endemic in most cattle raising countries where between 1 and 2% of the animals are persistently infected (PI). PI animals are the main reservoir of BVDV within herds and play the most important role in spreading of the disease. Economic losses are due to reduced milk yield, death by either classical mucosal disease or severe secondary infections and a range of reproductive problems including abortions and congenital defects. Control and eradication of BVDV in cattle herds depends on the identification and subsequent elimination of PI animals. The objective of this work was to identify epidemiological associations in Greek dairy herds where an ongoing eradication program based on the identification and removal of PI animals is applied.

Thirty-two dairy herds enrolled in this voluntary BVDV eradication program. In these herds blood samples were collected from all their cattle (total 5097 animals) and tested for BVDV antigen, using an antigen capture ELISA (BVD-VIRUS II, Bomelmi, Switzerland). After a period of three weeks, a second sample was collected only from the positive animals; those re-testing positive were classified as PI. For analysis of the data the herds were classified into two groups, one with and another without clinical manifestations at the time of initial testing. Clinical manifestations included various combinations of the following: deaths in adult and young cattle, increased embryonic losses and abortion rates, abnormal estrous cycles, reduced milk production, high incidence of retained membranes, metritis, lameness and mastitis. The percentage of PI were compared between the two groups by the exact Pearson's chi square test in StatXact 4.0. (1).

RT-PCR was performed in 10 blood samples from cattle that were ELISA-positive. The RNA extractions were carried from serum or plasma using the TRIzolTM LS reagent. The sequence of the primers and the reverse transcription and PCR conditions were the same as described previously (2). Sequence analysis of the isolates was commercially performed, by MWG Biotech (Germany). Nucleotide sequences from the other BVDV isolates were retrieved from the EMBL database. They were aligned and compared using Clustal X and the tree was drawn using TreeView.

At first sampling, 20.2% (744/2930) and 17.92% (255/1423) of the animals in the clinical and non-clinical group respectively, were positive (P>0.05). The respective percentages for PI were 2.7% (101/3573) and 2.4% (34/1389) (P>0.05). Phylogenetic analysis showed than only one isolate was identified as type II while the remaining were BVDV type I.

The percentage of the positive and PI animals did not differ between the herds with or without clinical manifestations. Hence, clinical manifestations may not be associated with either the presence or the spread of BVDV infection in these herds. The ongoing detailed analyses of the accumulating data will reveal further epidemiological associations. To the best of our knowledge, this is the first time a BVD type II isolation was identified in Greece.

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References
Prevalence of Bovine Viral Diarrhoea Virus in the Entre Douro e Minho region of Portugal


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Bovine Virus Diarrhoea is a serious cattle disease (Dubovi, 1994, Houe, 1999, Vale 2001) and its control can be carried out using different strategies (Brownlie, 2002). Having reported the presence of BVDV cattle carriers in this region (Niza-Ribeiro et al., 2004) it was important to quantify the presence of BVDV in the Entre Douro e Minho Region (EDM). Decisions about the adoption of control measures at regional level and the best strategy to be followed can only be taken when information is available. The authors carried out a study to estimate the proportion of dairy herds infected with BVDV and their annual rate of contact with the virus. The results of the study are presented with information about the vaccination policies adopted at the farm level that was considered relevant to help their interpretation.

One hundred and twenty four herds were randomly selected from a sampling frame containing all 1208 herds in the Dairy Herd Improvement Scheme of the region. The dimension of the sample allows estimating, with 95% confidence, the proportion of infected farms and was calculated for an expected prevalence of infection of 10%. Farms from all the relevant municipalities were stratified to ensure a representative sample. The results can therefore be used to estimate the occurrence of BVDV infection at the farm level, for the region.

The number of sera samples to be taken from each herd was calculated to detect presence of disease, assuming a prevalence of sero-positive BVDV animals of 20% at a confidence interval of 95% (Noordhuizen et al., 1997). The following protocol for individual sample collection was adopted, whenever possible: collection of sera from not less than 5 animals aged between 6 and 12 month. Additionally, 80% of the total sera from each herd should belong to animals with less than 24 month and the other 20% from older cows. The sera and the milk samples were analysed using a blocking ELISA test (LSI) directed at antibodies against the NS2-3 protein of the BVDV. Usually tests with these monoclonal antibodies only show positive titres on animals or farms (in the case of BMT) which had previous contact with live BVDV, either from field strains or from attenuated vaccines.

In each herd a structured inquiry was conducted by a trained veterinarian, at the time of the visit. This inquiry was designed to characterize the management of the herd. The questions covered aspects like animal density, opportunity for contact with cattle from other farms, observation of the clinical disease and treatment records of the farm, breeding, replacement and purchasing policies and vaccination practices adopted.

Results from the analysis of 117 BMT samples showed that 36% of the farms had positive results, with blocking levels equal or greater than 60%. The rest of the farms, 64% had less than 60% blocking percentage, exhibiting doubtful (39%) or negative (25%) results. Herds with positive BMT results have 20% chance of having persistently infected animals, as shown by Niza-Ribeiro et al. (2004) for the EDM region conditions, whereas for the herds having less than 60% blocking in BMT results, the probability of having PI animals is only of 4%. Interpreting BMT results using these criteria allow us to propose an overall estimate for the region of less than 10% of herds harbouring PI animals. The BMT results among vaccinated and non-vaccinated herds showed roughly the same proportion of positive results, respectively 23/64 (35.9%) and 15/45 (33.3%). The probability of the differences is p>0.1, suggesting that vaccination does not interfere significantly with the blocking response in BMT results measured by the test.

The global prevalence for the 1268 individual samples was 27%, 23% among the non vaccinated animals (n = 878) and 36% among the vaccinated ones (n = 390). These results are compatible with the postulated low prevalence of PI animals at the farms.

Combining the BMT results with individual results, especially tanking in account the proportion of positive individuals among the non-vaccinated population and, in particular the proportion of positives among young stock we classified the herds according to the probability of recent exposure to the BVDV. The criteria to look at the herd as positive according to the young stock were adopted. We have estimated that in the EDM region 35% of the herds had contact with BVDV each year or harbour PI animals, whereas 40% of the herds do not show signs of exposure to the virus. The rest of the herds (25%) are probably at risk of exposure although no conclusive evidence of it could be drawn from our results.

Among the 124 farms, 53% were vaccinating against BVDV using monovalent vaccines (12%) or polyvalent vaccines under a wide range of possible combinations and schedules. Only the inactivated vaccines were used at the farms. Many farms were not using veterinary advice or supervision for vaccination. For the rest of the farms, 39% had never used the vaccine whereas 8% have vaccinated at some point in time but stopped. A question emerging from the analysis of our results relates with the intensity of the risk of exposure of BVDV at the farms and the low level of herds containing PI animals. One hypothesis to explain this situation is the expanded use of different types of vaccines. Further analysis is needed to clarify the true infection status among the suspected herds in order to evaluate the effect of being vaccinated on the existence of PI animals.

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In conclusion, the results of our study permit to estimate the proportion of herds having PI animals in the Entre Douro e Minho region among 10% which is relatively low, when compared with estimates from many other European countries currently infected. The results from this study are in agreement with those from a previous study carried on the region Niza-Ribeiro et al. (2004). On the other side, the annual risk of exposure of the farms to BVDV is relatively high. Only 25% of the farms showed no evidence of BVDV contact. This is probably due to the high rate of purchase of replacement stock (heifers and cows) of these farms. Vaccination is widely used in the region, most of it without veterinary supervision and following many different schemes of application. Based on the present results, it is not possible to have an evidence based opinion about the real benefits from the use of vaccines, although it is also not possible to advise the farmers to stop vaccinating.

Control activities that might be taken in the future in the region should take in account the specificity of the production system of the region and its vulnerabilities, namely the high risk of exposure to the BVDV, the socio-economical context of the farms as well of the expected costs and benefits from the control.

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Persistent BVDV infection in Mousedeer infects calves. Do we know the reservoirs for BVDV?

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The isolation of pestivirus from a perfectly healthy Lesser Malayan mousedeer in Copenhagen Zoo as part of a routine health investigation initiated an epidemiological follow up investigation. Based on cell susceptibility and nucleotide sequence information, the virus was characterized to be a BVDV 1f, (Grondahl et al., 2003). The mousedeer (Tragulus javanicus) is the smallest ungulate known, adults weighing about 2 kgs; it inhabits the rainforests in Asia. The males have large teeth but no horns and this nocturnal animal eats fruit and leaves. The animals have their own family Tragulidae. The BVDV 1f subgroup have never been detected in Denmark. In order to determine the time of infection, serum samples from the parents and a sibling in Artis Zoo of Amsterdam were analysed. Surprisingly, virus could be isolated from serum of the mother and the sister, whereas the father was virus negative but had antibodies to BVDV. This picture resembles a typical BVDV persistent infection (PI) in the mother and her offspring and seroconversion after acute infection in the father.

These findings led us to analyse more animals related to the male mousedeer in Copenhagen. A total of 15 animals (7 females and 8 males) could be analysed. A family tree revealed that all descendants from the mother in Amsterdam were virus positive; whereas animals kept in contact to PI animals had antibodies to BVDV but were virus negative. It could be speculated that if the mousedeer population was infected by a cytopathogenic BVD, the entire family could die from mucosal disease. Recently we have found a sibling to the PI mother in Amsterdam, and this animal is reported to be virus negative. If this information can be confirmed we can determine the time of introduction – presumably from cattle - into the captive mousedeer population to the summer 1998 shortly before the birth of the mother of the Copenhagen mousedeer.

To analyze the horizontal spread of virus to cattle, two calves were placed in a pen adjacent to the PI mousedeer. The animals had no direct contact but after 2 weeks an indirect contact was established. The calves were bled daily to detect infection by BVDV. Eight days after the contact, virus could be detected by PCR in the blood from one calf. Virus-RNA was detected on 4 consecutive days but from one calf only. The level of virus was too low to be isolated in cell cultures. The virus positive calf seroconverted 11 days later, whereas the other calf remained seronegative till the termination of the infection study. Nucleotide sequence studies on the BVDV isolated from Copenhagen mousedeer and the calf showed 100% homology.

The detection of persistent BVDVirus in exotic animals is worrying; controlling BVDV is only possible
if all animals, which may be infected, are known. Our finding of PI mousedeer underlines that the host spectrum of pestiviruses in general urgently needs to be determined.

Bibliography

Sero-conversion in the absence of a PI animal


A closed dairy herd was serologically tested for BVD antibodies twice a year during a six-year period. Seroconversion was found at every herd sampling. Each year all animals were also tested for BVDV. During the entire monitoring period BVDV was only isolated twice, with an interval of three weeks, in one newborn calf of which the mother had sero-converted during pregnancy. Although the introduction of BVDV from outside the herd can never be excluded it seems highly unlikely on this closed farm. The fact that transmission of BVDV can take place in the absence of a PI animal can have serious consequences for control programmes. Consequences will be discussed.

Assessment by simulation of strategies to control BVDV spread in dairy herds

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Different strategies to control infection by the bovine viral-diarrhoea virus (BVDV) within a herd are available to farmers: either protection by vaccination, or strategies combining monitoring, screening and elimination of Persistently Infected (PI) animals (test-and-cull programmes) with biosecurity. Strategies without vaccination are generally preferred in areas where the risk of new introduction of the virus in a herd is lowered by collective programmes. Modelling can allow to assess ex-ante the efficiency of possible strategies. The objective of this presentation was to study, by simulation, the expected efficiency of strategies to control BVDV spread within a dairy herd without vaccination in a context of low risk of new infection.

A stochastic simulation model was developed to represent the BVDV spread in a dairy herd under different conditions. It represented horizontal and vertical transmission of the virus (by PI and transiently infected animals) in a structured population with controlled size (demography and separation into subgroups). In the modelled herd, actions were assumed to be done to avoid virus transmission from herd’s neighbourhood. PI animals were assumed to be detected before any movement between herds and not to be sold. In such a context, the most probable remaining origin of virus introduction is the purchase of an immune dam carrying a PI foetus which cannot be detected by available tests. The virus introduction was simulated as the purchase of an immune heifer carrying a PI foetus. No reintroduction of virus over time was simulated. Four scenarios representing four strategies were studied: (1) no other action, (2) prevention of contacts between animals of different subgroups of age, (3) test and cull of PI animals and (4) combination of (2) and (3). Test and cull programme was assumed to be used after a positive result from periodic initial screening for antibodies in bulk-milk. In scenarios (2) and (4), two levels of prevention were studied. Simulation experiments were done for a typical western-France dairy herd (38 cows, 34 youngstock, all of which were susceptible). For each scenario, 600 replications were run. The BVDV spread was described by the simulated duration of infection in the herd (clearance was obtained when no shedding or PI-carrier animal was present anymore) and the simulated extent of infection (number of new infections, of PI animals and of present animals with post-infectious antibodies). The duration and extent of infection were compared for the different scenarios.

After 4 years, probabilities of persistence of the virus in the herd decreased from 0.34 to below 0.10 with improved biosecurity or test-and-cull programme. Duration and extent of infection showed a large heterogeneity in each scenario. In 75% of the replications, clearance occurred before 1171 days for scenario (1) 296 to 482 days for scenario (2), 684 days for scenario (3) and 252 to 390 days for scenario (4), respectively. Total number of infected animals in the herd reached 90, >22 to >38, >70, and >22 to >32 in 25% of the replications. The duration and extent of infection showed a large heterogeneity in each scenario. In 75% of the replications, clearance occurred before 1171 days for scenario (1) 296 to 482 days for scenario (2), 684 days for scenario (3) and 252 to 390 days for scenario (4), respectively. Total number of infected animals in the herd reached 90, >22 to >38, >70, and >22 to >32 in 25% of the replications. The duration and extent of infection showed a large heterogeneity in each scenario. In 75% of the replications, clearance occurred before 1171 days for scenario (1) 296 to 482 days for scenario (2), 684 days for scenario (3) and 252 to 390 days for scenario (4), respectively. Total number of infected animals in the herd reached 90, >22 to >38, >70, and >22 to >32 in 25% of the replications. The duration and extent of infection showed a large heterogeneity in each scenario. In 75% of the replications, clearance occurred before 1171 days for scenario (1) 296 to 482 days for scenario (2), 684 days for scenario (3) and 252 to 390 days for scenario (4), respectively. Total number of infected animals in the herd reached 90, >22 to >38, >70, and >22 to >32 in 25% of the replications.

Molecular epidemiology and surveillance of BVD during the final phase of the Swedish BVD-programme.

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The Swedish BVD eradication programme has been successfully running since 1993 and is now in its last phase. The incidence of BVDV infection have continuously diminished and by March this year, 96 % of
all herds had been officially declared free from BVDV. Nevertheless, new infections are being detected despite the strict biosecurity rules within the programme. The importance of indirect routes of transmission of BVDV between herds is relatively higher in this phase as the biosecurity measures undertaken efficiently reduce the risk of transmission via the conventional routes. Since 2002 there are established routines within the programme to trace routes of transmission and to identify risky behaviour. Still, in 40-50% of the cases where new infections are detected in previously free herds, the route of transmission remains unidentified. Previous studies have shown that BVDV strains found in Sweden belong to either subgroup 1a, 1b or 1d of BVDV type 1 and that they are herd specific (1). This unique situation allows new infections to be traced to their origin and the routes of virus spread to be identified and cut.

During a three-year period (2003-2005) a BVDV-bank consisting of isolates from all BVDV infected farms detected within the programme will be built up. Isolates obtained from these farms will be molecularly characterised. Three specific regions of the BVDV genome, the 5'NCR, the Npro gene and parts of the E2 gene will be analysed from the isolated viruses by means of RT-PCR and sequencing. The sequences will be used for phylogenetic analysis to seek epidemiological relationships between occurrences. If a relation is found between isolates from different herds, information will be retrieved from the data bank of the Swedish Dairy Association to trace the origin of mothers of persistently infected (PI) animals and to identify potential risk factors. This information will be used to identify relationships in time or space between the herds, and to trace routes of transmission. In case of isolation of BVDV-strains previously not described in the country, phylogenetic comparison will be performed using sequences of known strains within the GenBank database. Introduction of foreign BVDV-strains by e.g. imported semen or embryos will thus be detected at an early stage.

Since the start of the project, isolates from around 200 herds have been collected and so far 54 out of these have been sequenced. Alignment of the 5’NCR of the isolates has resulted in a total of 44 different sequences and 7 groups of between 1 and 5 identical sequences. So far we have identified relationships between isolates with identical sequences in two of the 7 groups. Phylogenetic analysis have shown that all isolates belong to genotype 1; subtype 1a (n=3), 1b (n=15) or 1d (n=36).

The characterisation and mapping of all Swedish BVDV isolates will give us a unique possibility to trace routes of infection and to survey the national BVDV situation. The molecular epidemiological approach will speed up the last phase of the BVD-programme and help to reach total eradication within stipulated time.

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**Session 3: Strategies for BVDV control**

Strategies for BVDV control & the relevant use of vaccines

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Bovine Viral Diarrhoea Virus (BVDV) is a widespread, important and, until recent years, underrated infection of ruminants. Its worldwide distribution and its high prevalence in many national herds have enticed many veterinarians and farmers into the false security of accepting that BVDV control was unnecessary. Furthermore, its complex pathogenesis has deterred many from undertaking strategies for controlling, eliminating and thereafter preventing new BVDV infections. For some, the sole use of vaccination for control has been simpler both to recommend and maintain on an annual basis; this has been most evident in North America where some 70-80% of livestock owners use a BVDV vaccine. Whereas for others, in particular the Scandinavian countries, control has been on the basis of elimination of persistently-infected (PI) animals, excellent biosecurity and no vaccination. These are stark differences in these strategies and their relevant merits will be discussed in this lecture.

There are several countries (the 3 Scandinavian countries and Finland) that have made good progress to national eradication whereas others have committed themselves to national programmes of control (e.g. Austria) or regional eradication schemes (e.g. Shetlands and Orkneys within the UK & the region of Italy). These have all been undertaken through elimination of PI animals, excellent biosecurity and without vaccination. If this is possible, then the central question must be – why vaccinate and, if agreed, what vaccines should one use?

The use of vaccines to protect against infectious disease and/or to reduce the level of infection within the population has been the cornerstone of preventive medicine for over a century. It is not surprising that there are a number of BVDV vaccines licensed for use in Europe (in the USA, it has been estimated to be greater than 100 BVDV combination vaccines available) but there is some controversy for their appropriate role in control. It would appear from the national schemes already mentioned, where there is full commitment of all stakeholders to disease control and where there is government support, eradication can proceed without undue risk for re-infection.

However, there are cattle-dense regions with high
BVDV prevalence and countries within Europe where there is only partial compliance to control and where the risk of clearing the circulating virus, thereby creating groups of immunologically naïve animals, creates vulnerable herds and a substantial disease risk following re-infection. On these occasions, there is advantage—in conjunction with the systematic test and removal of persistently infected animals—to use vaccines. Some vaccines have published data of good efficacy both experimentally and in the field. The selection and proper use of such BVDV vaccines should ensure (fetal) protection against circulating viruses, although there is still concern that vaccine-induced antibodies may interfere with diagnostic serology. For this reason, there is a future need for efficacious, broad spectrum and safe ‘marker’ vaccines.

We will discuss the value of the different control schemes and the role of present and future BVDV vaccines.

### BVDV-infection-risk in the course of the voluntary BVDV-eradication program in Styria/Austria

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In 2001 a voluntary BVDV-eradication program in breeding herds started in Styria/Austria. Since then all animals to be sent to common pastures or markets have been tested for BVDV to ensure that BVDV can not spread from herd to herd during the grazing period or by the purchase of persistently BVDV infected (PI) animals. Like in Scandinavian countries herd level testing on the basis of bulk milk samples and of blood samples of young stock has been performed since autumn 2001.

During the first 30 months of the BVDV-eradication program 859 PI-cattle in 439 different breeding herds were identified. This is equivalent to a prevalence of 4.58 % PI-cattle in infected herds and 0.58 % PI-cattle in all breeding herds. Within the same period of time the number of infected herds, which had to carry out follow-up tests in all calves born during one year, decreased from 312 (7.3 %) of initially 4,295 herds to 108 (2.4 %) of 4,409 herds covered by the program in 2004.

In most cases new infections and the spread of BVD between herds were caused by PI-animals coming in contact with seronegative dams in early pregnancy on common pastures or by the purchase of dams carrying PI-foetuses. Movement data of all Styrian cattle, including information about purchases and the time animals spent on common pastures are stored in a database, developed for the administration of BVD-test results and for the purpose of organizing the eradication program.

By using the information stored in the BVD-database, it is possible to calculate the risk of BVDV-infections at any stage of the eradication program for any kind of exposure. The risk of new BVDV-infections of herds sharing common pastures with other herds and/or with regular purchase of cattle, appears to be higher than in herds with no animal movement. Compared to the time before the beginning of the eradication program, the risk of BVDV-infections in herds using common pastures, was at least 4 times lower (Odds Ratio OR = 5.77; 95 % Confidence Interval CI = 4.19 – 7.97; p < 0.05) during the first 30 months of the eradication program. The decrease of the risk of BVDV-infections in herds with purchased cattle was even more significant. Generally it is considered to be at least 8 times lower (OR = 11.45; 95 % CI = 7.89 – 16.62; p < 0.05) after eradication program had been established. 2001 the overall risk of BVDV-infections in Styria appeared to be 5-9 times higher (OR = 6.81; 95 % CI = 5.16 – 8.97; p < 0.05) than 30 months later.

After the implementation of the eradication program new BVDV-infections can be explained in almost all cases by incompliance with the conditions of the program. A further decrease of the risk of BVDV-infections requires the introduction of a compulsory BVDV-eradication program for all herds.

### Detection of PI animals: evaluation of the strategy implemented in Belgium

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Limited studies on the prevalence of BVDV infection have shown high animal seroprevalence (65.5%) and a prevalence of pi animals of 0.75%.

No official control program has been implemented in Belgium and the only legislation concerns the purchase of animals.

A voluntary program, based on the vaccination of cows before insemination and the identification and elimination of pi animals has been started both in the Northern and the Southern part of the country.

The strategy of pi detection relies on PCR testing of pools of blood or milk samples. The individual bloods of each positive pool are then tested by antigen ELISA.

Data obtained in 2003 are presented and discussed regarding the validation of the laboratory strategy, the genotype of circulating viruses, the prevalence of positive herds, the outcome of positive animals and the need for legislation improvement.
BVD control in Finland 1998-2003 based on annual screening

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The majority of the Finnish cattle resides in dairy farms. A voluntary control programme for BDV was launched in 1994 but very few farms joined it. Therefore, the control in Finland has relied on thorough annual screening of dairy herds and further testing of the herds with suspected BVDV infection. The prevalence and geographical distribution of BVD in Finnish cattle herds has been reported up to 1997 (Nuotio et al. 1999). This report summarizes the progress in BVDV eradication and the sources of infections in 1998-2003.

The material consisted of bulk-milk samples of annual screenings and individual blood samples from animals in bulk-milk antibody positive but previously untested herds. Indirect antibody ELISA (SVANOVA, Sweden) was used for testing bulk milk and blood samples. For virus isolation serum samples were cultured with bovine turbinate cells. The sources of infection were traced by epidemiological investigations.

The results of the annual screenings show a moderately decreasing trend in prevalence of bulk-milk antibody positive herds from 0.37% in 1998 to 0.15% in 2003. A similar trend is seen in the new bulk-milk antibody positive herds. Intensified testing of herds with BVDV antibodies in bulk-milk since 1998 led to discovery of 22 persistently infected (PI) dairy herds during 1998-2002. No PI herds were found in 2003. PI animals were found in 50% of newly infected herds, but only from 17% of the herds with old infection. In most cases it was not possible to trace the source of infection, but where worked out it was most often purchase of a PI animal or a dam carrying PI fetus, direct or indirect contact with a PI neighbour and, in one case, insemination with semen collected during the acute infection of the donor bull. Indirect transmission via animal transport personnel or vehicle was occasionally suspected.

In addition to the control measures the progress in BVD eradication probably was also due to the structure of the husbandry with small fairly isolated herds, only a modest level of animal trade between herds, BVD control of the AI bulls and voluntary control of imports, and because no viral vaccines for cattle were in use. In voluntary eradication it was not possible to restrict animal trade from PI herds, this has been amended by the new legislation effective as of 1st of May 2004.

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Immune responses to non-structural protein 3 (NS3) of BVDV in NS3 DNA vaccinated and naturally infected cattle

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Immune responses to non-structural protein 3 (NS3) of bovine viral diarrhea virus (BVDV) were investigated. cDNA encoding NS3 from type 1a BVDV was cloned into a mammalian expression vector. Five calves were then vaccinated intradermally with 500 micrograms of the NS3 plasmid DNA on 3 occasions, 21 days apart. Another 5 calves remained unvaccinated. Three weeks after final vaccination, animals were challenged intranasally with heterologous type 1a BVDV (5e6 TCID50/dose). Elevated rectal temperatures were evident in both groups 7 days post-challenge. The mean increase in the controls was twice that observed in the vaccinees. Anti-NS3 antibodies, determined using a novel indirect ELISA, were detected in one animal post-vaccination. An increase in anti body titre of 37% 14 days post-challenge was also seen. All other animals seroconverted to NS3 21 days post-challenge. Virus was not isolated from nasal mucosa in 2 vaccinees, and virus clearance from nasal mucosa was faster in the vaccinees compared to the controls. In conclusion, NS3 DNA vaccination induced humoral immunity in one calf, prevented fever and virus establishment in the nasal mucosa in 2/5 calves, demonstrating the efficacy of NS3 vaccination, which may benefit future development of pestivirus and flavivirus vaccines.

Implementation of two-step vaccination in the control of BVD

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Identification and removal of persistently infected (pi) animals is the method of choice to control and ultimately eradicate BVD infections from cattle populations. In general vaccination is not allowed. Herds that have been freed from pi cattle are getting seronegative over time and will become BVD-free, i.e. virus- and seronegative. However, this straightforward strategy is
BVD-eradication in Sweden – the goal in sight

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BVD-eradication in Sweden – the goal in sight
High cattle densities and high BVD seroprevalence, because seronegative herds are facing a very high risk of reinfection followed by high economic losses. In this situation herds at risk could be protected by a high level of immunity against BVDV. Systematic vaccination of all female cattle in pi animal negative herds is a way to prevent accidental reinfection and the generation of new pi animals. When considering vaccination strategies the antigenic variation among BVD virus isolates and between BVDV-1 and BVDV-2, respectively, are complicating factors. Therefore the choice of vaccine and vaccination method is crucial. We have decided to use a two-step vaccination, combining the advantages of both inactivated (i.e. safety) and modified live vaccines (i.e. efficacy and foetal protection). Cattle are first immunised using an inactivated BVD vaccine and four weeks later the animals are boostered with a modified live vaccine. In experimental vaccination trials we have followed the development of immunity over periods of up to 3 years. In addition challenge experiments with pregnant cattle were performed using heterologous BVDV-1 and BVDV-2 strains, respectively. These experiments showed a high level of foetal protection in vaccinated cattle. Modified live vaccine virus was not shed by the vaccinees. Ideally young heifers are vaccinated at the age of 7 months or later, in any case well before first pregnancy. However, at later ages this vaccination schedule might be applied as well.

Two-step vaccination has become an integral part of the voluntary BVD control scheme of the federal state of Lower Saxony. After removal of pi animals from herds farmers in cattle dense areas have the choice of systematically vaccinating their female cattle. In addition strict biosecurity measures are recommended and animal trade is monitored. The aim of this policy is to gradually reduce the incidence of pi cattle with the final aim of BVD eradication and ban of vaccination.

BVD-eradication in Sweden – the goal in sight

The Swedish control scheme on BVD was initiated in 1993 at the request of the farmers’ organisations. The objective was to halt the spread of infection among herds, to clear infected herds and thus eradicate bovine viral diarrhoea virus (BVDV) from the country in order to prevent the big financial losses caused by the disease. The target group has been all dairy and beef herds, fattening units excluded. The Swedish Dairy Association runs the scheme, authorised by the Swedish Board of Agriculture. From the beginning the scheme was voluntary and fully paid by the farmers.

Basic design of the scheme: For reaching and maintaining free status repeated serological tests on either individual blood samples on 5 to 10 animals above 12 months of age or bulk milk are used. If any sample is seropositive further actions has to be taken. For clearing an infected herd, all animals over an age of 12 weeks (to avoid interference of maternal antibodies) has to be individually tested for antibodies and if negative also for virus. Follow up tests has to be made on all calves born in one year after the last persistently infected animal is removed at an age of at least 12 weeks.

From being a very common disease in Sweden 1993 with 65% of the dairy herds in the south having very high levels of antibodies against BVDV in the bulk milk the situation has changed and today, 10 years later BVD is a rare disease. In May 2004 96% of the herds are certified free and 1.2% (272 herds) are still undergoing clearance. The missing 2.8% are recently cleared and are now taking herd samples to reach certified free status. The goal for July 2005 is that 99.6% of the herds are free which means that less than 100 herds are still left to certify.

From a situation during the first years of the scheme were quick results could be reached by voluntary affiliation by the majority of the farmers and making the most important livestock traders sell BVD-free animals it is now important to find and convince the few farmers and livestock traders who doesn’t care or know about BVD to follow the rules in order to get the country totally free from BVDV. As the disease becomes more rare the awareness among farmers also tend to decrease and therefore more efforts are needed to prevent new infections. To meet this problem BVD-control is now made compulsory for the farmers and partly paid by the government, the scheme rules have stepwise been made stricter, mainly those regarding contacts between herds, and trade. Rules that were “political impossible” when a lot of herds were infected and the main goal was to convince as many as possible to join the scheme is today seen as absolutely necessary to fix the holes in the bucket. In the paper this journey will be described, problems turning up along the road discussed as well as our choice of solutions.

Control of BVDV-infection on common grassland - the key for successful BVDV-eradication

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A bovine viral diarrhoea/mucosal disease (BVD/MD) control and eradication program was introduced in Lower Austria in 1996, according to the Swedish model. In principle, cattle herds are identified as infected or not by using herd-level antibody tests on bulk milk or on groups of young animals. To achieve virus clearance
from infected herds, persistently infected (PI) animals have to be identified and slaughtered. At present 9800 out of 17000 cattle herds take part in this voluntary BVDV-eradication program without vaccination. An important risk factor for BVD transmission under local conditions is communal grazing, approximately 3-4% of livestock shares common pastures, where susceptible pregnant cattle from several herds may be mixed with unrecognized PI animals.

Following rules and regulations for communal grazing were defined: to bring animals of a herd as free from BVDV-infection to common grassland, the latest herd-level test with appropriate result must have been done within the last three months. Calves younger than five months are tested compulsory, even if originating from BVDV-free herds. Animals from herds with unknown BVDV-status must be tested individually before communal grazing. Additionally at the end of the common grazing season, at least 10% of the cattle stock of each common grassland has to be tested for BVDV-specific antibodies. The animals with an antibody negative result at the beginning of the communal grazing season have to be also negative at the end of the season. This result confirms the stock of common grassland to be free of a PI animal.

In 1999 out of 4630 animals from 732 herds 24, 4% were seropositive, 33 PI animals were detected. In 2002 the prevalence of seropositive animals was reduced to 12, 96%, only 2 PI animals were found on the occasion of the spring tests. The last seroconversions on a single common grassland were detected 1999 caused by a PI animal admitted to communal grazing without testing for BVDV.

With a reliable system for identification of PI animals and a high certainty of prevention of PI animals on common grassland, the mode of BVDV-infection can be stopped, even if the animals are derived from infected herds and transiently infected animals cannot be excluded. That’s why since beginning of the eradication program, the number of BVDV-free certified herds have increased continuously. At the moment a number of 5067 herds are certified as free from BVDV. A number of 3386 herds are preliminarily free of BVDV-infection.

Due to the successful BVDV-eradication in Lower Austria and in other regions of Austria a draft law was developed to commit all herd owners to eradicate BVDV.

Eradication of BVDV-infection in norwegian cattle 1992-2003 – a success story

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The Norwegian national control and eradication program on BVDV started in the autumn 1992, as a joint venture between the national authorities and the cattle industry. The current program was set up after using an economic simulation model to identify the most cost-effective way of controlling the disease in relation to the Norwegian cattle population at that time. The program included yearly sampling of bulk tank milk from all dairy herds. In seropositive herds, pooled samples from primiparous cows were tested. In herds positive at this stage, also young stock was sampled. Positive analyses at this level were followed by official restrictions. A similar program was adapted to the beef herds. In the beginning, the simulation program pointed out the importance of using bulk milk testing as the most effective screening method. Later in the program, with few positive herds left, the use of blood sampling was increased.

In 1993, in the first bulk tank screening test of 26,424 milk producing herds, 63.0% of these herds were classified with a sample to positive (S/P) ratio<0.05, 14.1% 0.05< S/P ratio <0.25, 15.9% 0.25< S/P ratio <0.55 and 7.1% with an S/P ratio>0.55. Ten years later, 96.6% of the dairy herds are classified with an S/P ratio>0.05 and 0.02 % with an S/P ratio>0.55 and only three herds are still under official restrictions.

Positive herds identified after examination of pooled blood samples from young animals (7 to 12 months), were put under official restrictions as to animal trade and admission to common pasture. Restricted herds were not declared free from infection until all persistent infected (PI) animals were identified and slaughtered and two consecutive pooled blood samples from young stock, at least four months apart, had been serologically negative. The farmers had to choose either actively to get rid of PI animals in order to lift the restrictions or passively to wait until the infection eventually faded out.

The progression of the program was evident with a continuous increase in the number of negative herds recorded by milk sampling and further, a reduction of the number of restricted herds through the first six to seven years of systematic control. However, in 1998-2000, the progression seemed to slow down, and several herds were left that took no action as to get rid of PI animals. At that stage, the cattle industry, that had not been an active part in the program administration over the previous three years, suggested an “End phase project BVDV” – a classical mopping up strategy as also was indicated by the initial simulation model.

The purpose of the end phase project was to intensify the control and following up procedures in remaining affected herds. By mopping up in these “risk management” herds, mainly from areas of the country with rather many restricted herds left, it was possible to avoid any severe backlashes in the program.

In close cooperation between the national authorities and cattle industry, such a mopping up strategy was successfully completed in July 2003 with 13 restricted
herds, and only three herds left in the end of the year. In the remaining three herds, the restrictions will hopefully be lifted during the second part of 2004. The overall economic gain during these years is estimated to 130 mill NOK, corresponding to 16 mill €.

Session 4: Economic and social pressure for BVDV control

Assessing economic and social pressure for the control of bovine viral diarrhoea virus

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Within the BVD V Thematic Network, the team has been exploring the socio-economic issues relating to herd infection with bovine viral diarrhoea virus (BVD V). We have carried out surveys and have adopted a farm level modeling approach to try and provide answers to the most important questions. For those working with BVD V infections in European livestock, it comes as a shock when we describe this disease to farmers, to find most do not know what we are talking about. We believe BVD to be such a fascinating disease and such an important virus causing so much serious damage in host animals with potentially such serious income loss to farm businesses. We are preoccupied by the different possibilities for control: vaccines, marker-vaccines, control programmes, eradication, improved tests, virus isolation and so on. So why do farmers and veterinary surgeons not know about BVD? Why will producers and their veterinary advisers not listen to us? What could be more important? Where farmers are aware of this disease, why do the majority choose to do nothing about it? Conversely, why is it that in certain countries and some regions every farmer understands about BVD V and that the disease has almost been eradicated? What then does the future hold for BVD V control at a European level? How can we help shape that future? The economics team has started to evaluate these issues and this presentation will present preliminary results and explore possible answers to these questions.

So the reality is that for much of Europe there is little interest in controlling BVD V at farm level. Yet we know that BVD is very important from several perspectives: production (farmer and government concern), welfare (farmer, government and society concern) and increased commodity price (society concern). So there are real objective reasons to give BVD a higher priority than it is awarded at the moment. If such arguments are correct then we must question why the perception of both producers and the general public are not in agreement. Our survey indicates a large variation between different countries, production types, etc. Our team believes that we should seek to convince producers and the general public that BVDV deserves a greater attention. To assess economic pressure our focus is on producers: estimating the economic impact at farm level. We have customised existing farm BVD V models to help (Groenendaal, H. (1998) and Gunn G.J. et al (2004)).

The reality is that endemic disease frequently, but not always, comes quite low on the overall list of priorities for farm business resource allocation. Labour costs and food costs are usually of more immediate importance. The less predictable nature of disease often means that disease issues are postponed. Diseases such as acute mastitis and lameness are more obvious and their link to impaired welfare and production is equally obvious so such diseases grasp the farmer’s attention. BVD probably comes quite low on most lists for disease expenditure prioritisation because the producer does not make such strong production/welfare associations for BVD. We can further explore this issue of rank using other pestivirus infections of livestock as examples. With Classical Swine Fever/Hog Cholera (CSF) you have much higher farmer awareness. That is because CSF results in highly evident reproductive losses in a species with a considerably shorter reproductive cycle resulting in OIE list A epizootic disease status; confirmed infection results in herd slaughter. Because of the OIE disease status the consequences of CSF infection are severe at herd, regional and national level and everyone pays attention. BVD occupies an intermediate position in this series, it is accepted as a significant endemic disease, mucosal disease cases attract attention but it is not OIE listed and the major effects of herd infection are frequently not obvious. At the other end of this spectrum border disease in sheep receives scant recognition by any farmer and often fails to register as a potential cause of infertility or abortion with veterinary practitioners. This is because the effects are so occult and the diagnosis so difficult to make.

The general public in different EC countries hold different views on animal welfare but generally are increasingly concerned about cattle welfare. Endemic BVD V certainly constitutes a welfare problem. However there is low public awareness of BVD. This disease does not usually generate the horror images now associated with CSF and FMD. Nor does BVD generate the public health concerns that exist about zoonotic diseases such as BSE, toxic E. coli and salmonellosis. So at society level it is unlikely that much impetus will be generated for BVD control.

Our survey raised interesting issues and obviously the perspective differs depending upon the level of focus. There are issues relating to the variable central/regional government support for eradication of endemic diseases. National, regional and farm level biosecurity...
and the source of replacement cattle are big issues in many countries. We have explored the effect of farm herd size and national herd size. The farmer’s perspective is framed by what is happening at a regional and national level. Beyond that the European perspective and even world trade influence must be taken into account. This disease is certainly important but we have yet to properly quantify the economic losses for different farming systems in different countries. Further we do not appear to agree among ourselves what the correct approach should be for control. For some veterinary practitioners the answer will always be to do nothing – many veterinarians still believe this. The fact that the Nordic countries have largely eliminated BVD V is a wonderful example to everyone. However in countries with much greater cattle populations many veterinary college students are not taught how to eradicate BVD V. Farmers are advised to vaccinate or to eradicate and many farmers are instructed to do both with ensuing confusion. Clearly there is a need for us to establish many more facts and then pass this information on through a range of educational media, so that better, informed decisions can be made. The only route to greater control of BVD V must be through improved information transfer to local government, the public and particularly the farmers.

References

Effect of Bovine Viral Diarrhoea Virus (BVDV) infection on milk yield and somatic cell counts in 7,252 dairy herds
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In order to assess ex-ante the profitability of control schemes for BVDV-infection, quantitative information on its production effects are needed on a large scale. Production effects in case herds with acute infection were described, but very few studies investigated the possible difference in milk yield and somatic cell counts associated with BVDV-infection in large samples of commercial dairy herds with known BVDV status. This study aimed at quantifying the variation (i) in cow test-day milk yield (TD-MY), and (ii) in test-day bulk milk somatic cell count (TD-BMSCC) according to BVDV-infection status of the herd.

Five herd BVDV-infection statuses were defined, based on levels of BVDV-antibodies measured in bulk tank milk four months apart (BVDV-status definition-period): presumed (1) not-infected for a long time (NI), (2) not recently-infected (NRI), (3) past-infected recently-recovered (PIRR), (4) past-but-still-infected (PSI), (5) recently-infected (RI) (Robert et al., 2004). On each test day, TD-BMSCC was calculated as the weighted mean of the individual cow-level somatic cell counts. A total of 982,745 individual test-days in 7,252 herds located in Bretagne (western France) were considered in analyses. The effects of the BVDV-infection herd-status on TD-MY and TD-BMSCC (after logarithmic transformation) were assessed using mixed linear models, controlling for herd (random), lactation number and days in milk (for TD-MY) and for herd (random), proportion of primiparous cows and average days in milk on test-day (for TD-BMSCC). Both immediate and carry-over effects were investigated.

Among the 7,252 herds under study, the proportions of NI, NRI, PIRR, PSI and RI herds were 7.92, 37.60, 49.75, 3.39 and 1.34 % respectively. The BVDV-status was significantly associated with both TD-MY and TD-BMSCC. Considering test-days within the BVDV-status definition-period, the reduction in milk yield was 0.41 (P<0.001), 0.58 (P<0.001) and 0.02 (NS) kg/day for cows in PIRR, PSI and RI herds respectively, compared with cows in NRI herds. Over the same period, PIRR, PSI and RI herds had respectively a TD-BMSCC that was on average 12 000 (P<0.001), 27 000 (P<0.001) and 6 000 (NS) cells/ml higher than NRI herds (having a mean TD-BMSCC of 217 000 cells/ml). A carry-over effect (at least 1 year) of BVDV-infection on TD-MY and TD-BMSCC was also evidenced in PIRR, PSI and RI herds.

The present results were consistent with first estimates by Lindberg and Emanuelson (1997) and Valle (2000) who reported a decrease in average annual milk yield per cow in the current year or in the one next to the detection of BVDV-infection. A slight increase in BMSCC in presumed infected herds was also reported by Lindberg and Emanuelson (1997). From the present study, BVDV-infection had not only a short- but also a long-term impact on parameters. This suggests that the effect of BVDV-infection on milk yield could be mostly indirect, probably in relation to an increased susceptibility to infectious health disorders (e.g. intramammary infections), due to the known BVDV-immuno- depressive effect.

References

How best to model uncertainty in BVD and its control

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Modelling is frequently used to provide, in the absence of sufficient empirical data, best estimates for the outcome of an event such as an outbreak of BVD. In addition, models ought to estimate the uncertainty associated with such predictions and in this presentation we compare two models: a herd-based model that was designed to predict expected outcomes compared with an individual-based model that uses the same parameter values but which should provide better estimates of uncertainty.

A brief description of our primary epidemiological model and its results for BVD in the beef herd is presented. This original model is a herd-based state-transition model. Stochasticity is included primarily because a random process was the most appropriate way to ensure that periodicity (for which there is no empirical evidence) was not predicted (Gunn, Stott & Humphry, 2004). Stochasticity, however, now appears important in its own right, as greater recognition is given to farmers’ concerns regarding financial variation (risk). The response from farmers when presented with the results, has been that they are very keen to have a good estimate for ‘worst case scenarios’. This supports the generally held belief that farmers are risk averse (Oglethorpe 1995).

The estimates of financial variation that are provided by this model have been used in combination with empirical estimates of variation from other parts of the farm business to demonstrate methods by which farmers can minimise their risk at the whole farm level (Stott et al. 2003). Furthermore financial variation or risk can be equated with a loss in expected income depending on the level of risk aversion and this will be presented by Stott in this same symposium. From a modelling perspective this means that we have to model uncertainty accurately. Expected losses, for example, are often not of as much interest as the predicted distribution around the mean.

To get better estimates of uncertainty we have translated our herd-based model into an individual-based code-driven model in which each animal is tracked separately. We present a comparison of expected results, variation and utility between the two models.

Interestingly, whilst in the first few years of an outbreak the results of the individual-based model give higher estimates of uncertainty than the herd-based model, the differences between the two are not as large as might be expected. A priori, it is assumed that the individual-based model should provide better estimates of uncertainty whilst the herd-based model provides a useful presentational tool because of its simplicity.

References

Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus

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Assessing economic consequences of bovine viral diarrhoea virus (BVDV) infection at the farm level contributes to support decision-making by the farmer for control of the disease. Total disease costs include extra expenditures for treatment of sick animals and losses resulting from production effects of the virus. Although several studies aimed at assessing production effects, few data were published on the economic consequences of BVDV infection in a farm. The objective of this paper was to quantify economic losses consecutive to on-going BVDV infection in a dairy herd, considering all documented production effects.

Production effects were calculated for a 100-cow herd, based on the available estimates of the effects of BVDV infection on reproduction, milk yield, calf mortality, and occurrence of diseases in calves and cows. Economic consequences of the calculated production effects were then estimated using a partial budget model developed by us. This model calculates losses consecutive to diseases and reproductive disorders in a farm with management, production costs, and prices representative of western France situation. Two cases were considered: an average case farm where production effects were calculated from point estimates available in the literature for farms with on-going infection and a severely affected farm where production effects were issued from higher limit of confidence intervals of the effects. Transformation of production effects into economic effect on the gross margin were considered under two different assumptions. Under the first
one, it was assumed that reduced milk yield resulted in reduced milk sales from the herd, whereas under the second one, it was assumed that the farmer had increased the size of the herd to reach a milk delivery equal to the quota. This second assumption was based on the observation that in most dairy herds, despite disease occurrence, the herd milk yield is sufficient to produce the quota and therefore, no extra milk sale is possible under the French quota regulation. In the partial budgeting calculation, the reference situation for milk yield, reproductive performance and disease incidence was set at average values observed in a sample of 205 dairy farms in western France.

When the quota was produced despite effect of BVDV, losses consecutive to BVDV infection were estimated at 48 € per cow-year in the average case with on-going infection and 85 € per cow-year in the severe case. This resulted in losses equal to 6.8 € and 12.2 € per 1000 litres of milk, respectively.

When milk delivery was below the quota, value of losses (with the same production effects) was increased to 67 and 121 € per cow-year, equivalent to 9.6 and 17.3 € per 1000 litres of milk for the average and severe cases, respectively.

Losses resulted mainly from direct effect of BVDV infection on milk yield, indirect effect on milk yield (consecutive to increased disease incidence and increased calving interval), effect on calf mortality and reduced milk price consecutive to increased somatic cells.

To assess total economic consequences for the farm, extra-expenditures for extra diseased animals can be added. They varied from 8 to 12 € per cow-year in average versus severe case.

Economic consequences of BVDV infection in a dairy farm with on-going infection can be compared to economic consequences of major production diseases. With a comparable method applied to the 205-farm sample used here to define the reference situation, total costs of production disease were estimated at 32 € per 1000 litres of milk (21 € losses + 11 € expenditures). Among those, on average, mastitis resulted in total costs of 11 € per 1000 litres of milk (78 € per cow-year).

Assessing BVD control options by their relative contribution to risk management

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Stott et al. (2003) found that cow-calf herds in Scotland that were free of BVD could justify significantly more expenditure on biosecurity measures in order to reduce the risk of re-infection than if the BVD status of the herd was unknown. As farmers are generally considered to be risk averse, the authors concluded that the least-cost disease-control option might not always be the preferred option. It follows that any assessment of the relative value of alternative BVD prevention measures must take into account the reduction in risk as well as changes in expected returns. It must also be recognised that the attitude of decision-makers towards risk will vary and that this too must be accounted for.

Data for this analysis were generated using the model of Gunn et al. (2004). It simulates the transmission of BVD in a typical Scottish cow-calf herd over a 10-year period. The disease status of the herd at the start of the epidemic and at annual intervals thereafter can be adjusted to reflect the expected risk of BVD infection/re-infection in the region. This will also depend on the precautions taken and hence expenses incurred to improve biosecurity at farm level as functionalised by Stott et al. (2003). Vaccination may also be represented in the model at alternative levels of effectiveness. Output from the model is expressed as the annualised expected net present value of prevention costs and output losses due to BVD. By using Monte Carlo simulation a range of possible outputs can be generated, representing the risks associated with a particular control option.

This paper addresses the problem of devising ways of quantifying the risks associated with the various factors of production so as to rank alternative options open to decision makers. In this study, risk measurement techniques proved very useful tools in identifying the strategy that best accounts for the specific risks presented by an outbreak of BVD. The approach is one of computing expected values of returns based on amounts of losses conditional on a range of outbreak probabilities. Net expected returns are then further corrected to account for whether or not “insurance” (vaccination or biosecurity) is purchased and to what level of coverage (income loss protected). In effect, a particular increase in risk can be accepted, from a human’s point of view, so long as it is offset by a certain increase in the expected return. This equivalence relationship depends on the level of risk aversion of the human (i.e. the farmer). We can then apply this equivalence relationship using a range of likely risk aversion coefficients (Pratt, 1964) and compare the relative value of vaccination against BVD over this range of risk aversion.

Results showed that, given the losses that can be incurred, there exists a strong case supporting the purchase of insurance from which optimum returns can be realised. Unsurprisingly, which of the two control options maximise the income depends on the assumptions made. As the level of risk aversion decreases the biosecurity option is more likely to be preferred and vice versa for vaccination. This is because of the two control options biosecurity is thought to provide greatest expected income but with greater financial variation. It is probable that the financial variation associated with biosecurity is lower when biosecurity decisions are im-
implemented at a regional level because in such cases the probability of reinfection on a particular farm is likely to be lower. Future work should allow us to examine such regional level decisions and to compare more control options - in particular, using a mixture of biosecurity and vaccination.

References


Financial-economic considerations on decision making on control and prevention of Bovine Virus Diarrhea in the European Union

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Bovine Virus Diarrhea (BVD) is a viral disease of cattle that can cause severe clinical disease in a cattle herd with consequential severe economic impact. Prevalence of BVDV varies enormously amongst countries within the European Union (EU): Scandinavian countries such as Denmark and Sweden are almost free of the disease, whereas in The Netherlands and the United Kingdom sero-prevalence exceeds 50%. Current approaches with regard to control and prevention of BDVD also differ between countries. These vary from more or less mandatory country-wide eradication programs (e.g. in Sweden, Norway and Denmark) to regional eradication programs (Shetland and Orkney Islands and Lower Saxony) and voluntary programs, in which the farmer is free to participate or not, to no control.

At present, BVD has no ‘official’ EU status, i.e. occurrence of the disease does not have any adverse consequences with regard to movement of animals, sales of products, etc., neither for the farm nor the region nor the country. Nevertheless, in order to prevent introduction of BVDV, farmers are free to refuse trade with infected farms on a voluntary basis. Hence, the financial-economic impact of BVDV is comparable with other endemic production diseases such as mastitis, lameness, etc.

Currently, discussions at various levels are taking place on how to proceed with BVDV control and prevention in the future. E.g., Scandinavian countries would prefer BVDV to be regarded as a OIE List-B disease, thereby ‘institutionalizing’ their BVDV-free situation.

Changes in the ‘official’ BVD status, with corresponding restrictions for those who still harbor BVDV (i.e. farmers, regions or countries), will have consequences for the financial-economic importance of BVD. Hence, careful consideration of these consequences would help support decision making. The aim of this paper is to reflect qualitatively the possible financial-economic impact of a change of the official BVD status of a region or country.

A qualitative inventory will be presented on the financial-economic impact of BVD, given a particular BVDV status, on various stakeholder levels, including e.g. affected and non-affected farmers, the agri-industry, consumers and the national economy. Starting point is the current situation, i.e. no official status, hence BVD is merely a production disease. It will be shown, that, changing this current status to a status that involves some level of trade restrictions (on regional or national scale), will have serious financial-economic consequences. Depending on the economic level of concern, these consequences can be favorable or unfavorable. Nevertheless, in case of occurrence of BVDV, not only affected but also non-affected farmers can face adverse financial-economic effects.

The paper concludes with a review of data, methods and information, required for a sound science-based decision to change the status of BVDV within the EU. Some examples of existing research and knowledge will be presented, as well as an identification of gaps of knowledge still to be acquired.

Session 5: Experiences with BVDV eradication and/or control in Europe

Ten years of Bovine Viral Diarrhoea Virus (BVDV) control in Norway: a beneficial joint effort in three parts

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The Bovine Virus Diarrhea Virus (BVDV) eradication strategy chosen by the Nordic countries and a few others was initially met with skepticism and a “wait and see” attitude. It was therefore of interest to perform a retrospective cost-benefit evaluation of the BVDV control program in Norway starting in 1993. An analysis of the first five years has been presented earlier and here the analysis is expanded to the first ten years.

The Norwegian BVDV control program can be divided into three parts: (a) It started as a joint effort between the National Animal Health Authorities (NAHA) and the cattle industry (Norwegian Dairies, TINE, The Cattle Breeding Association, GENO, and Norwegian Meat) lasting for five years up to 1998, (b) the NAHA took over the control program incorporating it into the
national surveillance scheme and, finally, (c) in 2001, the last phase of control program started with additional support from The industry in order to intensify the screening for the last infective herds and to reduce the length of the ‘epidemic tail’.

Based on information regarding the program cost parameters gathered from the participating parties, the cattle industry, the NAHA, the National Veterinary Institute and cattle farmers themselves, cost related to the BVDV control was gathered. Only variable costs directly associated with the control program, and costs carried by the farmers as a consequence of the program, were accounted for in the present calculations.

The benefit of the BVDV control program was estimated as the difference between the assumed losses without control, represented by a static 1993 BVDV infection level through out the ten years period, and the observed losses during the same period. The financial losses associated with BVDV infection were estimated from studies of the herd level effects of BVDV infections on health, reproduction, and production in BVDV sero-converted herds (indication of infection of earlier free herds) and in herds with a BVDV antibody positive young stock (indication of persistent infection).

The cost-benefit calculations were performed in Microsoft Excel, and the add-in program @RISK was used to account for the uncertainty in the program cost and financial loss input estimates. The annual net benefits over the tens years were discounted to a 1993 net present value (NPV) for the BVDV control program using a 6% discount rate.

Prior to the start of the control program the national losses in Norway were estimated to about 40-50 million Nkr per year. In this calculation an annual loss of 34 million Nkr per year without a control program in place, was used and the observed losses were estimated to 26 million Nkr in 1993 decreasing down to 1 million in 2002 due to a successful control decreasing both sero-conversions and presence of herds with persistent infections down towards zero.

The total cost of the BVDV control program is estimated to 52 million Nkr of which the cattle industry (farmers included) covered about 62% of the costs. The cost-benefit analysis yields on this background an estimated to 52 million Nkr of which the cattle industry (farmers included) covered about 62% of the costs. The cost-benefit analysis yields on this background an estimated to 52 million Nkr of which the cattle industry (farmers included) covered about 62% of the costs.

The epidemiology including mode of disease transmission must be sufficiently clarified to allow for effective risk-reducing and transmission-preventing measures to be introduced.

This presentation will describe the essential knowledge, tools and steps needed for official authorities to participate in infectious animal disease control programs, such as the Danish BVDV control program.

Risk assessment and risk management

Government involvement in disease control should follow the principles of risk assessment and risk management as separate and independent activities, in the interest of transparency and trust in the decision process.

Even for animal diseases with no human health implications and without serious consequences for international trade should these basic principles be guiding the establishment of a control program. It is not only a question of whether government funds are being utilized or not – it is a question of trust, openness and fairness towards all interested parties.

Core issues

All disease control programs should be scientifically sound as well as efficiently managed. The following knowledge and tools are needed to establish a national disease control program with a properly designed basis and a well-founded chance of success:

- The aim of the control programmes must be clearly formulated and generally accepted by both farmers and by the general public.
- Advantages and disadvantages of the program must be clearly identified and described to ensure that also the political system can accept the goals and the tools used in the control program.
- The official veterinary authorities must have the power to establish the legal framework for the control program.
- There must be an agreement of how to finance the plan. In case of co-operation with stakeholders associations their contribution must be agreed on beforehand.
- The costs and benefits of a potential control program should be estimated, including an assessment of the influence of the disease on animal welfare and any direct or indirect impact on human health and welfare.
- The epidemiology including mode of disease transmission must be sufficiently clarified to allow for effective risk-reducing and transmission-preventing measures to be introduced.
- There must be efficient laboratory tests available for screening. These tests should be sensitive, cheap and easily applicable. In addition, a confirmatory test with high specificity to differentiate between true positive and false positive screening test results must be
available. Cross-reaction to other diseases must be known.

- A register of all holdings with susceptible species in the country must be available. The location of the holding including the coordinates should be stored in a database that can be used for mapping of holdings and disease outbreaks using a GIS system.

- Susceptible animals must be individually tagged and their identification numbers stored in a database with the ID of the holdings. The database must be constructed in such a way that it gives easy access to information on animal movements to be used for tracing.

- In case of a disease mainly spreading by trade of live animals a system must be in place to ensure that no animal movement can take place from a holding which is infected or has an unknown disease status.

- If vaccination is a tool for control an approved and efficient vaccine must be available.

The Danish BVDV control program

On that basis a voluntary control program for Bovine Virus Diarrhoea (BVDV) was implemented in Denmark in 1994 (Bitsch et al., 2000). The voluntary program was replaced in 1996 by a compulsory program, which is a co-operation between the Danish Veterinary and Food Administration and the Danish Cattle Federation. The legislation has been continuously adjusted according to the evolution in the BVD program.

Status of Danish herds

To be able to trade live animals the herd owner is obliged to have the holding’s status determined. If a holding with free status exceeds the deadlines for testing, the holding’s status will change so that trading and movement of live animals is prohibited until clarification has been obtained.

Bulk milk samples from all dairy herds must be tested 4 times per year.

Blood samples from beef holdings are collected at an abattoir or in the holding. At least 3 samples per holding must be tested every year if the holding has less than 25 animals. If the holding has 25 or more animals 3 samples must be tested every 4 months. In co-operation with the Danish Cattle Federation a database containing information on herd status for use in abattoirs has been developed. The database is connected to the Central Husbandry Register.

A holding is considered as ‘suspect of infection with BVDV’ if laboratory tests show unexpected antibody changes in blood or in bulk milk samples.

Herds remain registered as infected until laboratory tests from all epidemiological units have documented no BVDV and no animals are suspected of giving birth to new PI animals.

Status of the control program

In 2003, 28,270 cattle herds were registered in Denmark of which 6,000 were milking herds. Only 72 herds are currently under restrictions due to BVDV infection, and 4 herds have so far become infected in 2004.

Reference


Description and first results of a BVDV control scheme in Bretagne (Western France)

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BVDV control in France is either defined at the regional level or decided at the farm level. Bretagne is a region grouping 4 “départements” from western France with 19,700 dairy herds (780,000 cows) and 5,300 beef herds (142,000 cows) for a total of 2,200 000 cattle on a total area of 27,208 km².

In 1986, animal health organisations in Bretagne (Union Bretonne des Groupements de Défense Sanitaire (UBGDS)) started a control scheme for BVDV, focusing on herds experiencing clinical signs due to BVDV: mucosal disease, runting disease, abortions. This scheme aimed mainly at detecting and slaughtering PI animals in such herds. Until 1996, 500 to 600 herds with clinical signs and 1000 to 1500 PI animals were detected every year (annual herd incidence rate of clinical disease of 3%). During the same period, many recontaminations were observed; for instance, in 1997, about 60 herds (10% of the annual incidence rate) were reinfectcd. Therefore, farmers asked to consider a collective BVDV control scheme extended to infection with or without clinical signs.

The first step was to assess the prevalence and the dynamics of the BVDV infection in dairy herds. A preliminary study in 134 herds aimed at evaluating a blocking NS2-3 ELISA for the detection of BVDV antibodies in serum and milk and its possible use in bulk tank milk to assess within-herd prevalence of cows with antibodies. Bulk-milk results expressed in percentage inhibition can be split into three classes: 0, 1 or 2 corresponding to an estimated within-herd prevalence of antibody-positive cows of [0-10], [10-30], [30-100] %, respectively [1]. Then, 2,135 randomly selected herds were followed during 24 months: bulk-milk BVDV antibodies were measured every four months from February 1998 to February 2000. The herd status was based on results from three consecutive tests. The 27 possible combinations were gathered into 5 different statuses: status A for herds presumed non infected i.e. with three consecutive
results with a low to moderate level of BVDV antibodies (e.g. 1,1,1), status D for herds presumed infected i.e. with high levels of antibodies (e.g. 2,2,2), status C for seroconverting herds (0,0,2), status E for herds requiring further results (e.g. 1,2,0). In the sample, the initial proportion (October 1998) of A, B, C, D, E herds were 39.4, 19.6, 1.9, 33.7 and 5.3% respectively. These percentages were almost the same 16 months later. During this time-frame, transition rates from status A to statuses A, B, C, D, E were 84, 11, 1, 3 and 1%, respectively, whereas those from status D were 2, 22, 1, 74, and 2%, respectively [2]. A Markov Chain simulation over a 15-year horizon with these transition rates confirmed that it was illusory to expect any decrease in the incidence rate of BVDV-infection without further control measures.

UBGDS then decided to implement a collective control scheme for BVDV-infection, aiming at controlling the risk of new infection in all herds. Detection and slaughter of all PI animals were chosen. Target periods and animals were: before going to pasture (to limit the risk of transmission by contacts), and before trading (to limit the risk associated with introduction in a herd).

Since February 2001, a BVDV-infection status has been established for each herd. In non presumed free herds, detection of PI animals is done as follows: (i) a bulk milk sample of the first lactation cows is tested by blocking NS2-3 ELISA test; in the case of a positive result, whole bulk tank milk is tested by RT-PCR; in the case of a positive PCR test, all dairy cows are serologically tested then virologically for the negative ones; (ii) spot tests by antibody ELISA are carried out on sera from five pregnant heifers and five young heifers (older than 6 months). When more than 2 heifers are found positive, the whole group comprising the positive-tested heifers is serologically tested, then seronegative animals are virologically tested. PI are slaughtered within one month following detection. This procedure is implemented six and twelve months later when young calves are older than 6 months. Investigations are stopped when three consecutive groups of heifers are seronegative. Costs of the scheme are shared between farmers and animal health organisations.

The first results are encouraging: 80% of non presumed free herds started the test-and-cull programme in 3 out of the 4 departments. The percentages of B, C, D, E herds with PI animals are respectively of 7.3, 20, and 2%. Bulk milk testing of first lactation cows enabled to identify the majority of herds with a PI animal. Overall, it was estimated that less than 10% of the herds in the region had a PI animal. Proportion of seroconverting herds in the total population was lower in February 2004 (0.5%) than in February 2001 (1.2%). Transition probabilities between statuses from February 2001 to February 2004 showed that the survival rate in A-status were higher for herds located in the 3 departments with a collective scheme (83%) than for herds in the department with a volunteer scheme (69%) whereas the transition rates from A to D over the period differed accordingly (5% vs 10%).

Animals purchased are either tested or their BVDV-status has to be previously known to prevent trade of PI animals. All available information (on both herds and animals) are used to qualify individual animals. Each animal with a favorable result is included in a “guaranteed non-PI animal” database and is exempted from individual testing when sold to another farmer. The non-PI status is given to an animal from different possible results (i) ELISA-antigen negative animal older than 6 months, (ii) PCR-negative animal, (iii) antibody-positive animal older than 6 months, (iv) antibody-negative animal belonging to a group of at least 10 animals where at least 90% are antibody-negative, (v) dairy cow from a A-status herd, (vi) multiparous cow from a B-status herd, (vi) dairy cow from a bulk-milk PCR-negative herd. All together, 40% of total present cattle are now qualified as non-PI; other possible criteria will be evaluated (e.g heifers in a A-status herd or calves younger than 6 months in a BVDV-free herd). The objective is to reach 80% cattle with a known non-PI status.

Beef herds are submitted to a similar programme where herd status is determined using serological spot tests on 2 bulk sera samples taken in 24 to 48 month-old cows.

Further steps now considered to improve the control programme are (i) a re-definition of herd-statuses, not only based on serological results but on viral presence, presumption or absence, ii) new modalities of serological survey, especially for herds with high levels of antibodies in bulk milk but known as virus free and (iii) legal means to constrain farmers not complying to the programme.

In France, this BVDV-infection control scheme is specific to the Bretagne region. Results from the scheme could be used to define control programmes in other regions.

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Experiences with the use of vaccines in BVDV-control

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Succes full longterm BVDV-control on a farm basis can only be achieved if vaccination is applied next to eradication.
Veterinary Services “Midden Salland” is a mixed private practice in the eastern part of Holland (Oversijssel), where 5 specialised Bovine Practitioners take care of 31,000 head of dairy cattle (spread over more than 300 dairy farms) in an area with a high density of livestock.

In this private practice, without the existence of an official BVDV eradication program in Holland, BVDV related problems are encountered on a regular basis. In this presentation an overview will be given of the frequency of BVDV diagnosis, used diagnostic tests and protocols.

After confirmation of the presence of BVDV in a herd, farmers are advised to run a voluntary eradication program of the Dutch Animal Health Service (Certified BVDV eradication program) which consists of a whole herd screening on Persistent Infected (PI) animals, a twelve month’s screening of all calves born after the last PI animal is removed and a continuous monitoring program after this screening. With some years of experience with this eradication program, reintroduction of BVDV was encountered on 10-20 % of the certified free farms each year. The possible reasons for reintroduction on these farms will be shown.

Since 2001, as a result of the disappointing monitoring outcomes on BVDV certified farms, BVD vaccination was added to the BVDV control program of this practice, before 2001 BVD vaccination was only applied on a small scale for preventive measures.

Several aspects considering the choice of vaccin and the vaccination scheme will be shown. Vaccination scheme is according to producers advice, where 30% of the vaccinated farms are on a twice a year scheme and 70% on a prebreeding program. Farmers which are on the BVDV-control program can be divided in 3 categories: 1. eradication, 2. eradication and vaccination and 3. only vaccination.

Monitoring of the BVDV-control program is done on a regular basis, results and protocols will be shown.

Currently a (simple) stochastic infection model of BVDV is developed in our practice, wherein diagnostic data can be fitted and will give the farmer an overview of his herd serological (protection) status and from which future BVDV spread can be predicted with a certain probability. With this tool improved consultation on BVDV control will be possible.

Experience with clearing herds for BVDV infections

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Bovine Virus Diarre has been the greatest challenge in my years as a veterinary practitioner. Eradicating the disease has been the greatest step towards better animal welfare that I have witness. All the cattle herds I visit today is BVD free and I can look back at a period with a lot of frustrations of lack of cure, animals with pain and dead cattle. Also periods with great frustrations to the cattle owner until they understood, that the loss, were not bad management and veterinary assistance, but a complex infectious disease. Today I rely can say we found the light in the tunnel.

I graduated in 1980 and in the first 10 years, to me BVD was just a disease related to a single or group of young cattle dying with Mucosal Disease. The cattle owner was satisfied if we diagnosed BVD virus as he got insurance money for the dead calves.

After our IBR eradication in the early 80-ies, and practising alone in a new practice area with close contact with fewer herds I began to relate BVD outbreaks with Diarrhea among cows, abortions, lack of fertility, birth difficulties, birth of PI calves, calve diarrhea and death, outbreaks of lung diseases and Mucosal Disease in young stock. Placing the events at a timescale according to the birth date of the PI calves and with good record keeping I was able to convince farmers about the ½ to 2 years of problems after BVD infection in a cattle herd. I also saw great problems with a lot of different diseases in cattle 1-2 month after seroconversion, this also includes calves seroconverting in uterus. And I still postulate that this immune suppression were of high importance.

In the late 80-ies I started to certify herds as infected or free of BVD by milk antibody titter and testing for antibodies among calves of 1 year as Houe had documented.

This convinced me that cattle herds with reputation of well management and low calve deaths rates were BVD free and managed to stay free in my practice area despite visits from veterinarian, insimminator and other persons, indicating possibilities of controlling the spread of infection. So the step to try convince cattlemen to eradication of BVD were taken.

The eradication plan were bleeding of all herd, removing PI animals and controlling eventual inlet of new cattle.

The first eradication of a herd was made in 1990-91 with success. A few others were made in the next years and when the Danish National control program started in 1994 the eradication took high speed.

In 1994, 54 of 94 milking herds were infected and in 1997 also Beef herds were tested and 7 of 141 beef herds were infected

20 herds were cleaned of the infection in 1994, 12 in 95 and 4 in 1996. In 2000 solely 1 herd were infected and this last herd were cleaned in 2002. This herd with 105 cows had been infected since 1994. Declared free in 1999 and the infection reoccurred in 2000.

Bigger herds with highly separated young stuck, young stuck on pasture or selling of bull calves have given some prolonged eradication of the infection in herds all over Denmark
Essential for the eradication program were the cattlemen understanding that they rely on themselves could see the advantage of a free herd. So a lot of work was made in educating farmers all over Denmark. As an example in, 1992 I arranged a meeting for veterinarians with J. Brownline, S. Alenius, K. Kalis, V. Bitsch and H. Houe and in 1994 I held 19 meetings for farmers and their veterinarians on BVD.

Essential for the program were reliable tests and I must say the tests we have in Denmark are highly reliable. I have in the early days 1992 found one seronegative cow that were virus negative in the culture test at our National Laboratory but 2 years later virus positive. This is the only mistake I have found. Since 1994 both virus and antibody testing were by ELISA testing in the laboratory Ladelund.

The oldest PI animals I have found were a 4 and a 5 years old cow.

The highest number of PI animals born in a short period in a herd, were 22. Herd size 110 cows.

I have found a normal cow giving birth to a PI calve in two different years. As the one calve were death it was not possible to confirm the identity of the parents so it might have been wrong ear tag and the farmer must have been a very certain.

I have found seroconversion in 5 of 7 calves being de horned the day after the same veterinarian had de horned in a PI infected herd. As the saw wire were changed the most possible way of spread of infection were in the medicine used lidokain or xylazin containing in the PI herd.

At last I would say if you haven’t seen a BVD free herd: Try it.

**Poster Session 1: Virus properties and diagnostic assays relevant for BVDV control**

**Use of site directed mutagenesis to identify pestivirus E2 amino acid residues involved in virus cellular interactions**

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In order for pestiviruses to be propagated it is essential that the virus interacts with the hosts cellular machinery. The first step of this interaction is the attachment of virus to cell surface receptor(s), and this interaction usually determines the host range of the virus.

Alignment between different species of the pestivirus genus was performed and we consistently found two cysteine residues conserved in all strains of ruminant pestivirus (BVDV and BDV) tested but replaced by asparagine and phenylalanine in CSFV, specifically at amino acid positions 751 and 798 of BVDV strain NADL. We hypothesised that these sequence differences between BVDV and CSFV, might lead to differences in E2 conformation and affect the viral host range.

To test this hypothesis, a NADL infectious clone was propagated and a portion of the clone including 9 amino acids of E1, the whole of E2, P7 and 60 amino acids of NS2 was subcloned into the pGEM-T Easy vector. Site directed mutagenesis was used to introduce two restriction sites into the E2 region to facilitate exchange E2 from different species into NADL infectious clone. In addition the two cysteine residues identified (Cys751 and Cys798) were mutated to asparagine and phenylalanine respectively using an inverse polymerase chain reaction. The effect of these changes on viral viability was assessed in PT and MDBK cells. We found that the transfected cells with in-vitro transcribed RNA from the double cysteine mutant cDNA clone was negative for E2 using an immunoperoxidase test whereas cells transfected with the wild type RNA under the same experimental condition were positive. After partial sequencing of the NADL clone we identified a further amino acid substitution in the NS2 region at position 1163 leading to the substitution of methionine for isoleucine in addition to the cysteine substitutions.

We hope to be able to present further work on the effect of the site directed mutagenesis of cysteines 751 and/or 798 on the host range of BVDV in vitro.

**Experiences with the control of BVDV excretion with semen of transiently infected bulls according to Council Directive 2003/43/EC**

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**Introduction**

Persistently with BVDV infected bulls commonly excrete the virus in semen. Consequently bulls in semen collection centres must have been tested for PI with negative results. Extending these requirements the Council Directive 2003/43/EC (“amending Directive 88/407/EEC laying down the animal health requirements applicable to intra-Community trade in and imports of semen of domestic animals of the bovine species”) recognises the potential risk of BVDV shedding with sperm of transiently infected bulls by introducing a two-step-procedure outlined as follows:

1. Serology: During quarantine bulls entering appro-
2. Virological examination of semen: In quarantine semen has to be examined virologically for absence of BVDV following sero-positive results. If sero-conversion is observed in the annual test of former seronegative bulls, all semen batches taken since the last serology are tested for BVDV. For these virological examinations virus isolation or (actually inappropriate) BVD-antigen-ELISA have to be applied.

Observations
Serology: In our laboratory EDTA-plasma-samples are routinely tested using two commercially available antibody ELISAs. Following the manufacturers’ instructions poor results with insufficient reproducibility, sensitivity and specificity became evident. Improvements were obtained after heat-inactivation of the plasma. VNT was done for confirmation. Comparing CPE-reading and immunostaining of the VNT more reliable results were observed with immunostaining.

BVDV detection: In a field study semen of 109 seropositive bulls were examined for BVDV by Real Time RT-PCR. Only one 15 months old bull was found positive in RT-PCR for several months but remained negative in virus isolation over the whole period.

Animal test: In an additional animal test this bull was held together with 5 sero-negative heifers for 9 weeks. Sero-conversion of these heifers was not observed and all became pregnant in due time. Obviously at least in this case the risk of BVDV infections resulting from artificial insemination with semen of a transiently infected bull seems to be overestimated. Also in literature only few reports are present. In principle virus concentration in semen was much lower compared to PI-bulls. After insemination clinically manifest infections of the female reproductive tract were obtained once. Generation of PI animals was not reported. Occasionally seroconversion occurred in sero-negative recipients.

Conclusion
An additional intention of the directive 2003/43/EC is to improve BVD-control by the exclusion of virus containing semen of transiently infected bulls from artificial insemination. This aim should necessarily be accomplished by appropriate diagnostic procedures, i.e. largely standardised protocols for antibody tests with ELISA and VNT as well as semen examination using RT-PCR for screening additional to virus isolation.

The Npro region as a genetic marker for tracing closely related isolates of bovine viral diarrhoea virus (BVDV)

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Molecular epidemiology of bovine viral diarrhoea virus (BVDV) isolates, circulating between years 1998 and 2003 in the breeding cattle herds in Slovenia was studied. A selection of 128 isolates, collected on 48 farms from persistently infected cattle, aborted foetuses or died animals were subjected to genetic typing.

The viruses were propagated on cell culture. Most of them were of non-cytopathogenic biotype, except one strain, which was of cytopathogenic biotype. Viral RNA was extracted from infected cell cultures, reverse transcribed and amplified by one-step access RT-PCR with primers targeting the 5’-non translated region (5’NTR) and Npro region of viral genome, followed by direct sequencing of purified PCR products obtained for both genomic regions. Sequences of 5’NTR (238-243 nt) were assembled for all 128 BVDV strains, whereas the sequences of Npro region (392 nt) were determined for 80 BVDV isolates.

Phylogenetic analysis of each data-set revealed identical grouping of viruses into four genetic subgroups within BVDV 1. BVDV type 2 was not found among collection of 128 BVDV. Most viruses were found in two subgroups 1f (59) and 1d (52), while viruses of subgroups 1b (14) and 1g (3) were less common. Subgroup 1f, the most common BVDV genetic group, was only found in the two Western and Nord-western regions Gorenjska and Primorska. The subgroup 1d isolates were found mainly in the Gorenjska and Štajerska region and in one herd from Primorska region which was concurrently infected with 1f virus. From 25 persistently infected animals several virus isolates were collected during the period of one to three years after first sampling. Only one to two nucleotides differences were found in both regions, meaning that viral genome is relatively stable in persistently infected animals. Similar small differences of a few nucleotides among viruses isolated from the same herd were found in both sequenced regions. However in several herds the strains with identical sequences in 5’NCR could be determined. They could only be distinguished when Npro region was sequenced. For closely related viruses, identical in 5’NCR, the more variable and larger data sets of Npro gene sequences provided better genetic resolution, and gave also higher statistical support for phylogenetic classification of the viruses and can be used as genetic marker for tracing viruses from different herds.

The use of different diagnostic tests in a herd with an unexpected case of a BVD virus positive calf

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BVD virus has been under eradication in Denmark for many years and the herd prevalence is presently below 1%. At present the majority of farms do not have PI calves. After removal of PI animals the bulk milk will contain antibodies for many years.

The detection of BVD virus in a blood sample of a 6 month old clinical healthy calf routinely tested before entrance to an AI centre was therefore unexpected. The calf was virus negative and antibody positive when tested eleven days later indicating that it was acutely infected at the time of the first sampling. The herd was a Danish dairy herd that had been declared free of the infection for the last year (the last PI calf was removed in 2001). In subsequent follow-up investigations blood samples from the herd were analysed by ELISA for antibodies and antigen ELISA, PCR and isolation in cell cultures were used for detection of BVDV. By cell culturing BVD virus was detected in two calves confirming that the herd was in the course of an acute infection. Test of the samples by PCR revealed six positives, because PCR is more sensitive and independent of maternal antibodies. Still it was necessary to retest virus positive animals after 2-3 weeks to determine if they were acutely or persistently infected. One animal, born in May 2004, was identified as persistently infected.

Routinely all Danish dairy herds are surveyed by bulk-milk BVDV antibody analysis every 3 months. The bulk-milk is analysed by ELISA and data from this herd showed a slow but steady decrease in bulk-milk blocking OD value since the removal of the last known PI-calf in October 2001. The birth of the PI calf in May 2004 caused acute infection in several young calves, and the bulk milk titre increased significantly in subsequent samplings. The epidemiological follow up of the herd will be presented including the future advises to the farmer. The strengths and weaknesses of the methods used will be discussed.

**IFNα-induced Mx protein confers resistance to cytopathic BVDV**

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Mx proteins are type 1 IFNs-induced GTPases of which some isofoms (human, mouse, rat) restrict the replication of an array of RNA viruses. Initially discovered in mice, where they confer resistance to influenza viruses, their known antiviral spectrum now includes members of the Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae and Bunyaviridae families, thus typically negative-stranded RNA viruses. In addition, positive-stranded Semliki Forest and Coxackie B4 viruses are also inhibited, but are considered to be rare exceptions. In this context, this study aimed at establishing whether the known inhibitory effect of IFNα against bovine viral diarrhea virus (BVDV) could be assigned to the implementation of such an “Mx pathway”. The porcine kidney cell line PK15 was transfected either with the vector pTracer-huMxA or with the empty parental vector (control) and clonal lines constitutively expressing human MxA (huMxA) were obtained. Control and huMxA-expressing cell monolayers were then infected with the cytopathic Osloss strain at successive dilutions ranging from 10-1 to 10-5. Seven days after infection, a dramatic contrast was observed between the cultures, with only minor alterations, if any, in huMxA-expressing cells whereas a clear cytopathic effect was observed up to dilution 10-3 in control cells. Afterwards, virus yields were titrated within the supernatants obtained 6, 12, 24, 48 and 96 hr after infection at a multiplicity of infection of 0.1. On and after 48 h, control cell monolayers systematically produced ~100 times more virus particles than huMxA-expressing cells.

It is concluded that the inhibitory effect of IFNα on BVDV replication is, at least partially, mediated by the Mx pathway. This is the third positive-stranded virus ever shown to be repressed by Mx proteins, the first among Flaviviridae.

**Frequent recombination between a non cytopathogenic BVD virus and a vaccine strain causes Mucosal Disease in immunotolerant cattle.**

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Cytopathogenic bovine viral diarrhea viruses were isolated in Belgium during the 1999-2001 period from suspected cases of mucosal disease. Amongst them, a clinical mucosal disease was diagnosed in an animal that was previously vaccinated with the RIT4350 strain. This live attenuated cytopathogenic virus vaccine is widely used in Belgium and is characterized by duplication of regions coding for NS3, NS4A and part of NS4B, and insertion of part of the cellular sequences derived from the ribosomal S27a and ubiquitin proteins. A cellular insertion, identical to that of RIT4350 strain was detected in 8 of 14 cytopathogenic field isolates, demonstrating that the recombination between a non cytopathogenic strain harbored by a persistently infected and a vaccine strain is a common phenomenon. Furthermore, one cytopathogenic virus consisted in a chimeric virus. Sequences located upstream the 5’ recombination site were similar to those of the non cytopathogenic counterpart, while the sequences located from this recombination site to the 3’UTR were similar to the RIT4350 strain.
Clinical, pathological and genetic relationships of bovine viral diarrhea virus isolates in Argentina

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The goal of this work is the description outbreaks caused by Bovine Viral Diarrhea Virus (BVDV) in commercial beef cattle ranches in Argentina. Genetic affiliation and their association with the clinical manifestation were carried out with 5 BVDV isolates from an outbreak of mucosal disease (MD) (Outbreak #1), acute enteritis (Outbreak #2 and #3) and generalized dermatitis (Outbreak #4 and #5). Upon genetic analysis CP BVDV isolate of Outbreak #1 clustered to closely to BVDV Oregon (Genotype 1). BVDV isolates from the outbreaks of generalized dermatitis (Outbreak #4 and #5) were located close to BVDV Osloss within Genotype 1. The identification by immunohistochemistry of BVDV in exudative dermatitis indicates the epithelial cell tropism of the virus. Phylogenic characterization of BVDV from Outbreak #2 and #3 locate them as BVDV-2. 5’UTR sequence of these viruses revealed a homology of 88% and 90% to BVDV-890 (genotype 2) and a 77% and 75% to BVDV-SD1 (genotype 1), respectively. The association of BVDV-2 with severe disease indicates the presence of highly virulent strains. Data from natural outbreaks where BVDV-1 and BVDV-2 were isolated revealed that pathology overlaps and not clearly allows the differentiation between genotypes based on gross or microscopic lesions. Thus, for a definitive diagnosis, further virology and molecular studies are necessary. Additionally, the results of this work focused on the origin and consequences of genetic variations of BVDV with regard to pathogenesis and suggest the association between genotype and a defined clinical syndrome.

Phylogenetic analysis of bovine viral diarrhea virus isolated in Portugal between 2001 and 2004

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The 5’-UTR region of 43 isolates of bovine viral diarrhea virus (BVDV) was partially amplified by RT-PCR using total RNA extracted directly from spleen, lymph nodes and blood of suspected animals. The resulting 236 bp amplicons were cloned, sequenced and compared with published sequences of BVDV, classical swine fever virus (CSFV) and border disease virus (BD). The phylogenetic analysis segregated the Portuguese viruses into two genotypes, BVDV-1 (n = 39) and BVDV-2 (n = 4). BVDV-1 viruses were further grouped into subtypes –1a (n = 7), -1b (n = 22), -1d (n = 7) and –1e (n = 3). In one occasion, BVDV-1a and –1b was detected in the same animal and BVDV -1b and –1d was detected in different animals of the same herd. Despite the small number of viruses analyzed, this study showed a higher level of incidence and genetic diversity of BVDV-1 in Portuguese herds than that of BVDV-2, which occurred only in the south of the country.

Testing tissue samples derived from ear tagging for detection of bovine viral diarrhea virus antigens in persistently BVDV infected cattle


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Reliable detection of cattle persistently infected (PI) with Bovine Viral Diarrhea Virus (BVDV) is important for efficient BVDV control and eradication. Diagnosis of PI animals is based on the detection of BVDV from several tissues or body fluids. The detection of BVDV from ear notch tissue samples by immuno-histochemistry or antigen capture ELISA is commonly practiced in the USA. The tissue samples, usually are collected using ear notchers, a tool that cuts about a 1 cm2 wedge of tissue from the edge of the ear. This collection method carries a risk of contaminating the ear notcher when sampling multiple animals without proper disinfections between samplings. The large tissue sample cut by ear notchers may be unacceptable in Europe for animal welfare reasons.

A protocol has been developed which combines ear tagging, which is mandatory in many European countries, with the testing for BVDV. The testing for BVDV utilizes a 2-3 mm plug of tissue that is punched during the ear tag process. Some commercially available ear tag systems allow the collection of such tissue samples without contamination by collecting directly into a labeled sampling device. Ear tag systems are not a requirement for this new test. Any ear tissue sample or plug 2-3 mm in diameter can be used as a testing specimen.

Mini ear notches were tested in a new version of a commercially available BVDV antigen capture ELISA. The ear notch tissue samples were soaked overnight in...
a special soaking buffer, and this buffer was then tested in the ELISA.

Testing ear notches from 68 characterized positive and 318 negative cattle indicated a sensitivity and specificity of 100% . Positive results from up to 8 ear notch punches taken from the same animal, showed a high degree of reproducibility. Multiple samples from PI calves were stored for up to two weeks at room temperature and 37°C, and were still detected as positives. In order to test for detectability of PI calves under influence of colostral antibodies, 11 calves which had been infected intrauterine by a PI heifer introduced into a BVDV negative herd, were tested before and up to 70 days after colostrum intake. These calves were detected positive by testing ear notch tissue samples during the entire period without showing a diagnostic gap, while other specimens from those calves did not continuously test positive.

Testing mini ear notch tissue samples using BVDV antigen capture ELISA might be a new, reliable and economic approach for BVDV eradication and control. There is an advantage to testing calves as early in life as possible and testing ear notch tissue samples could potentially allow a valuable means to assess the BVDV status even under the influence of colostral antibodies.

Presentation of a new Test using Taqman probes for BVDV detection by real-time PCR

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Introduction

In France, some regions as Brittany started voluntary programs of BVDV controls. Additionally to classical ELISA diagnostic tools of control (NS2-3 BVDV Serology, NS2-3 or E0 Virology), these regions started to use since about 1 year real-time PCR for BVDV detection in different types of samples. Test Kit (Taqvet BVDV) has been developed for this purpose. In 2004, about 20 French veterinary laboratories use Real-Time PCR for BVDV diagnosis.

Description

This Kit is designed to run BVDV detection in One-step RT-PCR by using MultiScribe TM Reverse Transcriptase, and AmpliTag Gold DNA polymerase, dNTP, passive Reference (ROX) and optimised buffer from Taqvet One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Les Ulis, France).

The Kit contains Taqman One-Step PCR-Master Mix (2 Vials), Positive BVDV Controls (2 Vials), Pools of Primers and Probes (1 vial), Internal Positive Control (IPC) (1 Vial) and instruction manual.

Principles Of The Kit

The samples tested are: Serum, Plasma or whole blood (EDTA Tube), Milk, Organs (Spleen, lymph nodes, pieces of intestine), semen, nasal swabs.

After shipment of the samples to the laboratory, RNA is extracted from samples with Qiagen reagents:
- Serum, plasma and whole blood are treated with Qiamp Viral RNA mini Kit.
- Milk, PBL (Peripheral blood leukocytes), organs, semen and nasal swabs are treated with RNeasy mini kit.

RNA could tested immediately in RT-PCR or stored at -20°C for 8 days maximum.

RT-PCR used a Reagent Mix prepared just before run. Reagent Mix is prepared by adding adequate volumes of RT-PCR Master-Mix, Pools of primers and probes and IPC.

Pools of Primers and Probes is mix of both specific BVDV and IPC primers (Forward and reverse) and probe and probes.

Primers and probes for BVDV detection has been designed in 5’UTR region of BVDV genome. BVDV probe is MgB (minor groove binding) probe labelled with FAM.

IPC is an inactivated virus, never described in bovine sample and added in the samples before extraction. Primers and

The conditions of the RT-PCR are: 48°C - 30 minutes (RT), 95°C - 10 minutes, and 45 Cycles of 95°C - 15 seconds and 60°C - 1 minute.

The RNA volume per sample is 5 µl. The volume of the reagent mix is 20 µl. So the final volume per well is 25 µl and PCR plates of 96 wells are used. 2 BVDV Positives Controls (Inactivated BVDV, one BVDV1 and one BVDV2) are included in each run. Negative Control has to be provided by the user. It’s recommended to use 2 Negative Control (NC):
- PCR-NC: The water used by the user for sample extraction and Mix preparation is tested as a RNA sample.
- PCR-NCS: The water used by the user is tested as a serum sample.

The run is validated if both Positive Controls are scored as positive and if both Negative Controls are scored as negative.

The results are provided in Ct (Cycle Threshold). In individual sample the Ct Value could be a goon evaluation of the “Charge Virale”.

Validations and results

The Kit has been validated on different individual and pooled bovine samples:
- Individual serum, plasma and whole blood on adults animals and calves with high titres of maternal antibodies.(22 observations of PI with High colostral antibodies and kinetic survey)
- Individual and tank of milks: maximum of 400 milking cows by tank is recommended (More than 200 observations in Tank milk with BVDV status of all in-
individual milking cows).

- Pools of sera, plasma and whole blood: maximum of 20 animals by pools is recommended (Fig 1)

Concerning Pestivirus and Pestivirus genotypes detected by the kit, the following genotypes has been tested and scored as positive: BVD1a, BVD1b, BVD1d, BVD1e, BVD1f, BVD2, BD, HCV.

In individuals samples, some preliminary results show that the kit could be used as an interesting tool to help the distinction between transient viremics and PI animals (Fig 2)

In the validation procedure, we sequence and genotype of about 30 BVDV strains isolated in 2003 and 2004 coming from different areas of France. We confirm, as VILCEK in 2001, that BVDV1e is frequent in France. We confirm also the diversity of BVDV1b.

Antibodies to bovine viral diarrhoea virus in persistently infected calves: a source of misclassification?

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In large-scale control schemes using a “test and cull”-strategy, the detection of serum antibodies to bovine viral diarrhoea virus (BVDV) is a central element. Based on the assumption that persistently infected (PI) calves do not produce antibodies to BVDV, antibody positive samples are usually not tested for the presence of virus. Thus, PI cases with antibodies may be misclassified as non-PI.

In the present study, six calves experimentally persistently infected with BVDV were included. The calves were fed colostrum without BVDV antibodies, kept strictly isolated, and superinfected with a cytopathogenic strain of BVDV at five months of age. The calves were screened for antibodies against four different strains of BVDV using a serum neutralisation assay. Antibodies against at least one of the strains were found in all calves at least at one time point. The highest recorded titre was 1:512. However, when the same samples were tested in the antibody enzyme-linked immunosorbent assay (ELISA) used in the Norwegian surveillance and control programme, they yielded negative results. Even though significant levels of antibodies were detected in the neutralisation assay, these calves would have been regarded antibody negative if tested in the scheme.

Antibody positive PI calves have been described previously, but the prevalence of such animals is unknown. The possibility of the existence of such partially tolerant animals should be taken into account when deciding which tests and what cut-off values to use in a control programme. A highly sensitive antibody test increases the possibility of excluding PI animals from virus detection. If misclassified, such animals could be a source of unexplained re-infections and unsolved BVDV-mysteries.

Development of a real time heminested single tube RT-PCR TaqMan assay for the simultaneous detection and genotyping of BVDV1 and 2.

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Conclusions

Taqvet BVDV is a very accurate tool for BVDV diagnosis. The efficacy of the kit on pools of sample reduces highly the cost of the BVDV analysis and is very helpful for plans of BVDV eradication or control
In the UK, BVDV type 1 is considered to be endemic with subgenotype 1a predominating. Using a single tube RT-PCR TaqMan assay designed to detect the 5’UTR of the genome of type 1 BVDV (McGoldrick et al., 1999) an exotic BVDV was found circulating in cattle in the UK in 2002. Phylogenetic analysis revealed that the virus was a BVDV type 2a. Further analysis of the herd from which this positive individual was identified revealed a further BVDV type 2 virus positive animal. In the light of this finding we have modified our existing single tube RT-PCR TaqMan assay such that only a single probe labelled with FAM is required to detect both genotypes 1 and 2. By using a second probe labelled with a different fluorophore (Yakima Yellow) we can discriminate between genotypes 1 and 2 in the same tube. By combining this single tube heminested RT-PCR with standard Q-PCR we have developed a novel approach, with general application, to increase the sensitivity of diagnostic PCR assays. An increase of 1000 times in the sensitivity of the heminested assay over the non-nested TaqMan assay has been demonstrated on the Stratagene MX3000P PCR machine.

A major limitation of molecular diagnostics generally is the time consuming extraction of nucleic acids. We have addressed this part of our assay by the application of the MagNA Pure extraction robot to the extraction of pestiviral RNA from both anticoagulated blood and abortive foetal tissues. The robot was used not only for extraction purposes but also for dispensing template to 96 well plates prior to RT-PCR, effectively eliminating cross-contamination between wells. Comparison of results obtained from archival blood samples tested by BVDV antigen ELISA has shown that the TaqMan assay is more sensitive than the ELISA. Analysis of 93 samples previously tested by antigen ELISA identified yet another BVDV type 2, the ampiclon hybridising not only with the generic BVDV but also the BVDV type 2 specific probe. This result was confirmed by sequencing and phylogenetic analysis of the 5’ UTR which demonstrated that the virus was very similar (99.6% identity) but distinct from the second BVDV type 2 identified in the UK in 2002. The sequence was that of the UK isolate from which this positive individual was identified revealed a further BVDV type 2 virus positive animal. In the light of this finding we have modified our existing single tube RT-PCR TaqMan assay such that only a single probe labelled with FAM is required to detect both genotypes 1 and 2. By using a second probe labelled with a different fluorophore (Yakima Yellow) we can discriminate between genotypes 1 and 2 in the same tube. By combining this single tube heminested RT-PCR with standard Q-PCR we have developed a novel approach, with general application, to increase the sensitivity of diagnostic PCR assays. An increase of 1000 times in the sensitivity of the heminested assay over the non-nested TaqMan assay has been demonstrated on the Stratagene MX3000P PCR machine.

A new sequence database designed to simplify genotyping of bovine viral diarrhea virus isolates

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In the last decade, a great number of studies were published where phylogenetic analyses of bovine viral diarrhea virus (BVDV) isolates were performed, with the aim of defining genetic groups and subgroups which would allow to differentiate individual or groups of isolates from each other. In most cases, genetic typing was performed based on the nucleotide sequences of the 5’ untranslated region (5’-UTR), the Npro- and the E2-coding genes. Due to the high number of sequences that were generated (and often not published), the nomenclature of genetic groups and subgroups became greatly inconsistent. To define unambiguous genetic groups and subgroups and to unravel nomenclature, it seemed appropriate to generate a new database with the nucleotide sequences that have been used for genotyping of BVDV strains and isolates, accessible by the World Wide Web. The sequences should be in a format that allows download and direct use for genotyping of new isolates. The inclusion of additional data such as year of isolation and geographic localisation should also allow to determine whether the genotype has any relevance for the epidemiology of BVDV.

The program consists of the virus database and the web frontend running directly on the server. The server runs under LINUX (Debian 3.0) on a 1700 MHz AMD-Athlon computer using the Apache web server (v. 1.3.26). Virus and sequence entries are in an SQL database (MySQL v. 3.23.49). The web frontend consists of dynamic CGI scripts programmed in PERL (v. 5.6.1). New entries can be added directly to the SQL database, thus making it possible to keep it permanently updated.

The database consists of two modules, which can be accessed by individual passwords:
1. Administrator module: here, entries in the SQL database can be edited, added or deleted, new sequences can be added, and accession to individual entries by the users can be determined.
2. User module: full listing can be accessed, fields can be searched and printed, and sequences can be searched and downloaded.

The database was designed to be searchable, the sequences can be downloaded. They are in FASTA format and in the right length so that they can be directly


used for alignment and phylogenetic analysis. In addition, it will allow automated typing of new isolates by performing the phylogenetic calculations, albeit these modules are currently being prepared.

**Serological and virological investigation of bovine viral diarrhea virus (BVDV) infection in bulls examined to be used in artificial insemination centers by enzyme linked immunosorbent assay (ELISA) methods.**

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Bovine Viral Diarrhea (BVD) is a common infection all over the world. It causes important economical losses in cattle breeding.

In this study, blood samples were examined taken from bulls into tubes with and without EDTA brought to use in Artificial Insemination Centers.

Blood sera samples were tested to detect by the presence for being antibodies against Bovine Viral Diarrhea Virus (BVDV) and leukocytes samples were tested for BVDV antigens by ELISA methods.

8 out of 46 sera samples were found as positive by the means of antibodies against BVDV while 3 out of 46 leukocytes samples were detected as positive for BVD antigens. One of the seropositives bulls was detected as positive for BVD antigen. Two of the bulls which were detected as seronegative were detected as positive for BVD antigens.

**Poster Session 2: Characteristics in BVDV epidemiology of relevance to control**

**Bovine viral diarrhea virus: a Pestivirus shed via the skin?**

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Of the many aspects of virus-host interaction the excretion of virus has been the least studied. Yet, viral shedding is a critical component of viral persistence in the host population.

Nonlycopathic BVD viruses have the ability to cause persistent infection which is associated with immunotolerance to the persisting strain. This unique feature of virus-host interaction is due to viral entry into the host very early in its intrauterine development, i.e. at a time before the establishment of immunocompetence.

The aims of our work were (i), to determine the routes of viral excretion and (ii), to determine if the same viral population is excreted via the different routes. For our experiments we used three persistently infected calves. The experiments confirmed that BVD virus is shed via saliva, nasal secretions, tears and preputial secretions whereas no virus was detected in swabs taken from feces and the auricles. Since previous histological investigations had shown that BVD viral antigen is present in the skin of persistently infected animals, we investigated if virus might be shed via the skin. To differentiate between contamination of skin by saliva or other secretions and shedding via the skin, part of areas investigated were initially disinfected and subsequently kept covered. Viral RNA was detected on the surface of the skin both in covered and uncovered areas. However, infectious virus was isolated from unprotected areas only. By contrast, hair taken from unprotected as well as covered areas contained infectious virus. Comparison of the nucleotide sequence of the 5’ untranslated region by direct analysis of amplified DNA showed that the virus was identical in all samples analysed which argues against the possibility that evolution within a chain of infection might be influenced by the source of virus transmitted by an infected animal.

Spiking the skin surface with BVD virus grown in tissue culture revealed rapid inactivation of infectivity which explains why very little, if any, virus may be shed through the intact skin. However, our experiments do not rule out the possibility that virus may be transmitted by mutual grooming, as this involves intensive licking. Similarly, infection might also be transmitted by epilated hair when brushing animals with the same curry comb.

**Risk factors associated with bovine viral-diarrhoea infections in dairy farms from the Galicia region of Spain**

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A survey of bovine viral-diarrhoea virus infection was carried out in 92 farms from Galicia (northwest of Spain) during 2004. All the farms had more than 25 cows and voluntary programs were developed for BVDV prevention and control.

The potential risk factors were gathered in a questionnaire through a personal interview with the farmer.

During the same period all the cows, older than one year, were bled and the samples were analysed with a commercial ELISA based on the detection of antibodies against p-80 BVDV antigen, in a cross sectional study.

All data were processed by logistic regression. This method seems to indicate the importance of the purcha-
se of animals and the vaccination patterns.

In general, we consider as a priority the redefinition of the use of vaccines, taking into account the epidemiology and risk situation of the farm, as well as, to check new animals.

In dairy farms, we recommended the use of inactivated vaccines in order to reduce the incidence of new PI foetus.

The study also let us establish two types of farmers:
- Producers who show interest in the sanitary improvement, in which farms could think about sanitary qualification of BVDV.
- Producers who were not interested in control and biosecurity measures.

Spatial distribution and risk factors for spread of BVDV between cattle herds in Denmark

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In 1994, the Danish cattle farmers’ organisations initiated a voluntary control and eradication program of bovine virus diarrhoea virus (BVDV) infection. The program was supported by national legislation introducing the first BVDV order in March 1996.

The objective of the present study was to identify municipalities with increased risk of BVDV infection in Denmark on January 1, 1995. Further, to evaluate spatial risk factors for spread of BVDV during 18 months in the period January 1995 to July 1996.

Data were extracted from a BVD database. The BVD database was made from existing databases (Danish Cattle Database and Danish Husbandry Register) including information on herd (e.g. size, type) and animal level (e.g. BVD test results, transfer, birth, calving). The BVD database included information regarding: herd identification number, herd size, type of possible PI transfer, geographical coordinates and estimated date of occurrence of first PI-animal in the herd. The date of occurrence of the first PI-animal in the herd was retrospectively estimated based on information on the official BVDV-test results of individual animals 1995-1999, date of movement, slaughter and deaths of animals and delivery of calves. A herd was classified as a PI-herd at occurrence of the first PI-animal in the herd.

All cattle herds (N=37,041) registered in the period January 1, 1995 to June 30, 1996 were extracted from the BVD database.

The relative risk of BVDV in municipalities on January 1, 1995 was estimated relative to the risk of the overall risk of BVDV in Denmark. A beta-binomial model was used in hierarchical Bayesian modelling. Further, spatial scan statistics was used to identify clusters.

The analysis of risk factors for becoming a PI-herd was performed for dairy herds participating in the monthly milk-recording scheme (N=11,382), as data were nearly complete for these herds. Spatial risk factors were derived such as mean distance to neighbours in the neighbourhood, mean distance to PI-herds in the neighbourhood, number of infected neighbours in the neighbourhood and herd size. A generalized linear mixed model was used to evaluate the significance of the spatial and non-spatial risk factors.

Regional differences in occurrence of BVDV infections at study onset January 1, 1995, were identified. Three clusters were identified, one in the Northern part of Jutland and two in the Southern part of Jutland.

The analysis showed that risk factors for spread of BVDV in the 18 months period were mean distance to neighbours, number of infected herds in the neighbourhood and herd size (number of cows). The risk for becoming a PI-herd was increased as the number of infected herds in the neighbourhood increased and as the herd size increased. The risk for becoming a PI-herd decreased as the mean distance to the neighbours increased.

Prevalence and risk factors of BVDV in 3 Italian regions.

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A preparatory step towards the design of an eradication program consists in investigating the prevalence and the identification of factors related to BVDV infection. The classification of areas characterized by different epidemiological status is critical for the virus eradication strategies. In Italy the BVDV national prevalence is unknown, however several studies have reported high prevalence of BVDV virus in some area of the country. To study characteristics of BVDV epidemiology and prevalence, a cross-sectional, serological study was carried out during 2003-2004 in 3 Italian regions: Piedmont, Sicily (only Ragusa district) and Campania. Dairy, beef and mixed cattle herds were included in a two-stage cluster sampling (herd and animal). Blood samples were collected during mandatory tests against bovine brucellosis and Blue Tongue surveillance from 5088 animals belonging to 280 herds. A questionnaire was administered to the farmer by public veterinarians at the time the blood collection, to gather information relative to herd size, introduction of cattle, breeding method, occurrence of immunization. The serum samples were tested for antibody against BVDV by ELISA (Bommeli® CheKit - Se 98.6, Sp 100). Doubtful test results were classified negative in the data analysis.
Into unvaccinated herd prevalence corrected for test Se and Sp was 78% (CI 73.8-92.3) in Piedmont, 79% (CI 75.4-82.7) in Campania and 98% (CI 97.5-100) in Sicily. Sero-prevalence within herds corrected for Se and Sp was 31% (CI 29.8-32.2) in Campania, 36.1% (CI 34.8-37.3) in Piedmont, 76.1% (CI 74.9-77.2) in Sicily. Vaccination interested 35% of sampled herds in Piedmont, 27% of herds in Sicily and 5% in Campania. Vaccination was administered with inactivated (52%) and modified live vaccines (48%). Reproductive and respiratory disorders related to BVDV were reported in Sicily (47% of sampling), in Piedmont (10%) and in Campania (14%).

To identify predictors of seropositivity against BVDV at herds level, a logistic regression model was performed. To take into account correlation between serological status of heads that were in the same herd a subset of eleven herds was selected for individual testing. All samples were tested for specific BVDV antibody using an indirect ELISA. The results demonstrated a moderate level of exposure (73%) but low proportion of herd with high antibody-level (13%). The bulk milk results and the low individual seroprevalence among the young stock suggested a low prevalence of active infection. A progressive self-clearance was also indicated by the results from the individual testing. We found evidence that at least 22 out of 35 BVDV seropositive cows in the eleven herds had been imported and introduced to the region. Moreover, a surprisingly low seroprevalence of BVDV among the dairy herds at one of the milk centres was found. This centre was established 5-10 years before the others. We believe that replacement of imported seropositive animals has occurred over the years resulting in a nearly total self-clearance of BVDV.

Furthermore a low incidence of BVDV infections in 209 herds was determined by analyzing two samples of bulk tank milk taken one year apart (2002 and 2003). Only 1 out of 103 low antibody level herds had a high antibody level in the subsequent test.

Based on our experiences and on these results we are convinced that the self-clearance process of BVDV will continue as long as there is awareness of herd biosecurity. This is especially important in the context of a future intensification of the dairy production.

### Present status of BVDV infection in Lithuania

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The bovine viral diarrhoea virus (BVDV) infection to continue to be a major problem worldwide affecting a large scale of ruminants. The initial studies in Lithuania from 1994 to 1995 showed a widespread distribution of BVDV infection and evidence of other respiratory, enteric and reproductive disorders that could be associated with BVDV were frequent. On the background of this and because of lack of information on the possible influence of different risk factors the further detailed studies of BVDV infection were established in 1997-2001. The objectives of the present work were to estimate level of the immune response into bovine viral diarrhoea virus (BVDV) in cattle herds at the different Lithuanian districts and to determine the possible influence of such risk factors like as animal density in herds, animal age and sex of the animals. Further on, it was the objective to estimate incidence risk of infection in different age groups. Lithuania is divided in 10 counties which are divided in 44 regions. The studies were explored in 147 intensive dairy cattle breeding herds from 27 different Lithuanian regions. Naturally, more samples were investigated from regions in which

### Epidemiology of BVDV infection in dairy herds in Thailand

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Bulk milk samples from 220 dairy herds were collected during year 2000 at nine public milk collecting centres in the north-eastern and northern Thailand, and a subset of eleven herds was selected for individual testing. All samples were tested for specific BVDV an-
cattle husbandry is more developed. In addition, we investigated cattle blood samples from Lithuanian Artificial Insemination (AI) Centres among the breeding bulls sperm donors. A total of 4098 blood sera samples of different age and sex were investigated. The commercial ELISA kits was used for serological tests. Our studies demonstrated that BVDV infection is a widespread cattle disease in Lithuania. The number of seropositive animals ranged from 11.9 to 100%. It must be pointed out, that 29.9% of the herds were not infected with BVDV and one-third of the herds (32.7%) had between 70 and 100% antibody carriers. This also indicates that BVDV is highly prevalent in Lithuania. A positive correlation between the number of seropositive cattle, and the size of herds and age of animals was determined. An infected herd in an area with large herds has double as high impact on the overall prevalence of infection compared with infection of a herd in an area with small herd sizes. It was estimated that with age of animal the number of seropositive animals have tendency to increase reaching its maximum in the age groups 5-7 and >7 years. The seroprevalence of BVDV could be affected by various risk factors. Therefore, was important to calculate the annual incidence risk of this infection on the basis of relevant dataset. The values obtained according to specific prevalence of seropositive animals among the animals of the different age groups were comparable. Hence, was determined that the incidence of infection showed a tendency to lower risk among older animals compared to younger animals in Lithuania. The same general trend was determined among cattle from dairy herds and among bulls from AI Centers. The influence of animal sex on BVDV distribution was analysed in our studies as a complementary factor. It was estimated that percentage of seropositive animals in groups of cows and bulls was comparable and not differ statistically.

Key words: Bovine viral diarrhoea virus; Cattle; Epidemiology

Some examinations of prevalence of BVD infection in Serbia and Montenegro

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On the bases of clinical signs and pathomorphologic findings, in 1966, in the area of former Yugoslavia, BVDV infection was, for the first time, described by Durićković et al. and it was serologically approved by Cvetnić et al. in 1968.
During the last several years a control on presence of BVDV infection has been carried out in Serbia and Montenegro. In the northern parts of the country antibody ELISA test was applied in examining 94 (4.34%) samples of sera from 7 big herds with a total of 2164 animals and 94 sera samples (12.13%) from small herds with a total of 775 animals. Small herds are located in 17 settlements in 5 municipalities. It was discovered that the prevalence of seropositive animals in big herds ranged between 0% (1 herd) to 71.43%, with an average of 22.34%. In one big herd there were no seropositive animals. The percent of antibody positive sera samples from small herds was 59.57%. Seropositive animals were located in all 5 examined municipalities.
In the area of Južnobачka and Srem district presence of VN antibodies against NADL strain BVDV was the following: out of 2076 samples from 13 big herds it was detected in 1088 (52.41%) and out of 581 samples from small herds from 7 area it was detected in 266 (45.78%). In all of 7 investigated areas serologically positive animals were detected.
In the Belgrade surrounding 440 blood sera samples from big herds and 2024 blood sera from animals of small herds were examined for the presence of antibody. The average prevalence of seropositive animals in big herds was 66.80%, and in small herds it was 4.24%.
In the area around Valjevo two big herds were examined. In the herd of fattening cattle out of 86 examined animals there were 48 (55.81%) seropositive animals, while on the farm with dairy cows out of 178 cows, percent of seropositive animals varied depending on age and it ranged between 30.55% and 52.24%.
In 9 municipalities from the southern part of Serbia, antibody ELISA test was applied on 188 sera samples of dairy cows from small herds. Prevalence of seropositive animals was between 0% and 45%, with an average of 10.64%. In the samples from 4 municipalities we did not find specific antibodies against BVDV.
ELISA test was applied for 217 single and 135 bulk milk samples in the area of Montenegro. In 217 milk samples that came from small herds located in 6 municipalities there were 85 or 39.17% seropositive samples. Examining bulk milk samples from 135 big herds from 5 municipalities, prevalence of over 30% seropositive animals was detected in 34 or 25.18% herds, while prevalence under 10% was detected in 85 or 62.96% herds.
On the bases of these investigations we can concluded that BVDV infection is present in the whole country.

Epidemiology and clinical features of an outbreak of mucosal disease in the south of Portugal

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The epidemiology of an outbreak of Mucosal Disease (MD) was investigated after the death of 14 young (6 to 9 moths) beef cattle.
The herd was never vaccinated until July 2003 when
an adult cow, with diarrhoea and fever, was suspected to be infected with BVD. Blood samples at that time showed a high prevalence of antibodies to BVDV. All the animals were then vaccinated with inactivated multivalent vaccine (BVDV, IBR, BRSV, PI3) in July and August 2003.

Between 10-09-2003 and 15-10-2003, 14 young animals died showing signs of MD. The non-cytopathic virus was isolated and classified as Type 1, sub-type b1. The cytopathic virus belonged to the same Type and sub-type.

The herd anamnesis confirmed that 8 new-born calves (1 to 34 days old), born more or less at the same time as the 14 that died with MD, died with diarrhea and pneumonia even after several days of treatment. Three calves showed congenital defects.

The first cases of MD showed severe clinical signs of the disease – fever, anorexia, oral and nasal erosions and ulcers, conjunctivitis, diarrhea, tenesmus and death after a few days. The following cases showed less severe signs and death only occurred after 5 to 7 days. The hemogram of these animals, compared with healthy herd mates, showed high MCV, neutrophilia (with left shift) and thrombocytosis: 1.074; 3.173; 2.575; 1.112 (x103). Necropsy findings: oral, abomasal and intestinal erosions and ulcers, conjunctival erosions and splenomegaly. Histopathology: increased size of the Lieberkuhn crypts with herniation into the Peyer patches lymph nODULEs.

Also interesting was predominance of mortality in males, although because of the small number it can not be considered statistically significant. During the period epidemiologically important (October 2002 and March 2003), 21 females and 36 males were born. First phase (new-born calves) mortality: 0 females and 7 males. Second phase mortality (MD outbreak): 2 females and 11 males. There were no differences in the management, vaccination or contact with other animals.

Some questions arise from this case: was the vaccination related to the MD outbreak? Can a non-cytopathic virus recombine with an inactivated cytopathic virus of a vaccine? What were the predisposing factors involved in the MD outbreak? Why were the males more affected by the disease? Is the unusual thrombocytosis a relevant sign?

**Epidemiology of bovine viral diarrhea virus (BVDV) in Turkey: a description in the light of antigenic characterization data**

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Bovine viral diarrhea virus (BVDV) is thought to be distributed world-wide. Studies have been performed in Turkey show that the disease is prevalent in cattle population. Prevalence of persistently infected (PI) animals in governmental intensive dairy farms was reported to be 0.25% (Burgu et al., 1999) and overall prevalence of BVDV antibody positive animals was 24.8% (ranged between 0.6% and 70%). The prevalence of herds, which include PI carriers, was 11.5% (Burgu et al., 2003). Although studies are continuing to determine prevalence of the infection in small capacity public establishments, it is thought to be harmonious to the results described above.

In this study, a cluster of 60 BVDV isolates previously isolated by field screening studies between 1997 and 2000 were subjected to antigenic characterisation. These viruses had been originated from 12 intensively managed dairy farms around Turkey, of which 1 was privately owned and 11 were governmental. BVD viruses were received from virus collection of Virology Department at Ankara University Faculty of Veterinary Medicine. Isolates characterised for their biotype, mixed biotypes were cloned and antigenically characterised by the means of 16 monoclonal antibodies (mAbs). Virology Institute of Hannover Veterinary School-Germany kindly provided Mabs, specific to NS2-3 and E2 proteins. MAb binding results were statistically analysed by Chi-square and Fisher’s exact tests in order to determine herd specificity, intraherd and interherd relatedness of viruses isolated from PI.

Our preliminary results conducted on limited number of isolates from 5 farms indicated a possible transmission of BVDV from one farm to others (Yesilbag and Burgu, 2003). This farm had a central role because having a frozen sperm production unit, which make service to others. In following stages, viruses isolated from the other farms were included in the characterisation works.

Viruses produced too different reaction patterns indicating high level of antigenic diversity. Some viruses were reacted with only 1 mAb, contrary to some others, which reacted with 13 mAbs. This situation was a case for viruses from different herds as well as viruses from the same herd. Viral isolates were divided into 3 groups in phylogeny tree. It is thought that animal importation from European and other countries may be one of the reasons of great antigenic differences. Two viral subpopulations could be generated among persistent viruses originated from the same herd. MAb binding patterns of those subpopulations were significantly different (p=0.001). If viral subpopulations ignored, there was not intraherd and interherd antigenic difference statistically significant (p>0.05).

In conclusion it is thought that there are many groups of BVDV strains, antigenically too different, circulated in the country. If a program will be developed to control the infection by the means of vaccination policies, vaccine viruses should be well selected. Transfer of biological products and infected animals among herds (belongs to government, boards etc) without control
measures may be the reason for generating persistent viral subpopulations circulating in same herd.

References

A description In the light of antigenic characterisation data.

Bovine Viral Diarrhoea (BVD) Infection in Galicia: Bases For The Development Of A Control Program.

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To establish a control and prevention program of BVD it is necessary to know the real situation of the disease. With this objective we realized a seroprevalence study on the basis of the cattle census of Galicia (North-west of Spain) in 2000 (36,481 dairy and 38,241 beef cattle herds). After establishing the global percentage of each productive aptitude in the total census of each province (A Coruña, Lugo, Ourense, Pontevedra), animals older than one year of age were sampled at random for each aptitude in every council.

Other seroprevalence studies on BVD in Spain reported 80-100% of dairy cattle herds positive. From these results, we expected a prevalence of 80% for dairy and 50% for beef. The final size of the sampling was of 624 herds (6,854 animals).

A commercial ELISA (Institute Pourquier) based on the detection of antibodies against the p80 antigen was used for the analysis of the samples. According to the manufacture’s data, the sensibility and specificity of this test are 97.3% and 97.6%, respectively.

The results of real prevalence were of 79.2% (dairy) and 65.2% (beef) for herds, and 30.5% (dairy) and 30.7% (beef) for animals. So, the animal prevalence obtained was similar for both aptitudes. However, the prevalence was higher for dairy than for beef herds. This result might be due to the fact that a herd was considered positive with at least one positive animal. As the medium number of animals per dairy herd (16.6) is bigger than per beef herd (7.2), the possibility of a positive result for dairy herd is higher.

The herds were classified into 4 groups depending on the infection rate. A herd was considered not infected with a seroprevalence rate of less than 5% (30.8% of herds). With seroprevalence rates of 5-25% the probability of infection was considered low (26% of herds) unless the infection was in the first stage. Herds with a seroprevalence rate of 25-65% could develop persistently infected (PI) newborns (26.8% of herds), and a seroprevalence rate higher than 65% would mean high probability of presence of PI animals or high risk of developing them (16.5% of herds).

When we analysed seronegative animals from 35 herds with a serum-prevalence higher than 65%, we detected three PI animals from three different herds (8.6% of event probability). This percentage would be higher if we have analysed also animals younger than one year of age.

So, to prevent the development of infection or its diffusion in those high risk groups, it would be necessary the establishment of a control program based on bio-safety measures and periodic sampling among the different groups of age.

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Poster Session 3: Strategies for BVDV control

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Bovine virus diarrhoea (BVD) prevalence in cattle dense areas in Northwestern Germany is very high (90%). A recurrent infection with BVD virus in a herd within the endemic region was successfully eliminated by removal of persistently infected (pi) animals, intensive surveillance including serological and virological investigations and –at a later stage – systematic vaccination of female offspring. All measures taken were recorded from the beginning. The process was performed in two different phases: From 1989 - 1996 a virological examination of the complete offspring was performed in order to identify and eliminate pi animals, followed by virological and serological monitoring of the herd. In addition, during the second phase from 1997 – 2002 all female cattle were systematically vaccinated using...
a two-step vaccination program in order to avoid reinfections and the generation of new pi animals. A total of 32 pi calves was identified from 1989 to 2002, the majority (26) of them in 1989. Between 1994 and 1999 five more pi animals were identified and their origin was traced. In the last years of the ongoing control process no more problems attributable to BVD viral infections were noted. The overall results of the control attempts demonstrate that the strategy of “test and removal” of pi animals in conjunction with systematic two-step vaccination is suitable for the protection of herds in cattle-dense areas with a high BVD prevalence.

Birth of persistently infected calves in two herds using inactivated BVDV vaccines

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Efforts to control the detrimental economic and welfare implications of bovine viral diarrhoea virus (BVDV) in cattle herds focus on vaccination and/or eradication, in conjunction with appropriate levels of biosecurity. There are currently two inactivated BVDV vaccines designed to provide foetal protection licenced for use in the UK (Bovidec, Novartis Animal Health UK Ltd and Bovilis BVD, Intervet). During 2003, BVDV was isolated from two calves born into two different dairy herds which routinely practiced BVDV vaccination, one using Bovidec, the other Bovilis BVD. Resampling of these calves confirmed that they were persistently infected and serological testing revealed widespread exposure among cohorts in both herds. Both calves were born to first calving heifers. Neither dam was persistently infected. Analysis of partial sequence data of the 5’-NCR of the isolated viruses indicated that they were both BVDV subtype 1a, in common with the majority of local isolates and the vaccinal strains of virus. Investigation of vaccination protocols in the two herds showed that following primary immunisation, boosters were given annually. This deviates from the datasheet for Bovilis BVD, which recommends re-vaccination at 6-monthly intervals to maintain herd immunity. These findings highlight the need to maintain vigilance for BVDV in vaccinated herds, and to incorporate vaccination into overall herd strategies for BVDV control that also address issues of surveillance and biosecurity.

Using a commercial indirect antibody detection ELISA to identify dams PI-carrying PI foetus, a complementary measure in BVDV control/eradication program

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Bovine viral diarrhoea virus is the causative agent of bovine viral diarrhoea and mucosal disease. Infection with non-cytopathicogenic strains of the virus in early gestation may lead to abortion, teratogenic defects, or to birth of calves persistently infected with the virus. Persistently infected (PI) animals are carriers of BVDV virus for life, and therefore the main source of virus in infected herds.

When attempting to control or eradicate BVDV, between-herd transmission is a primary target for measures. In most countries, the routes with the highest impact for spread are trade with PI animals and with non-PI dams pregnant with PI foetuses. The latter would be even more important if trade with PIs is controlled. These dams are indistinguishable from other seropositive cattle since they are virus negative and seropositive. However, they have a significantly higher level of antibodies, most likely due to a continuous immunological challenge by to the PI foetus.

To minimize the risk of introducing dams carrying PI foetus into naive herds, monitoring maternal response to foetal BVDV infection could be one way of identifying these animals.

This method was in fact applied in the livestock trade in Sweden at the start of the voluntary national control scheme on BVDV in 1993 to facilitate low-risk trade of pregnant dams from non-certified herds. Today, the same principle is used for detecting dams at risk of carrying BVDV infected calves in herds undergoing clearance from the virus. The main tool used for antibody detection in the Swedish BVDV program is an indirect ELISA, for which it has been shown that the optical density (OD) has a good correlation with the actual antibody titre. All assays are performed by the National Veterinary Institute of Sweden (SVA). The indirect ELISA is performed on serum samples diluted 1:100. An optical density of 1.0 has been used as cut-off for identifying potential PI-carriers.

The aim of this study was to examine whether the commercially available version of this indirect ELISA (SVANOVIR® BVDV-Ab, Svanova Biotech AB, Sweden), where samples are run at a 1:25 dilution, can be
used to identify dams carrying PI foetuses. The performance of a p80 blocking ELISA (Institute Pourquier, France) in this respect was also investigated.

A total of 11 BVDV negative and clinically healthy cows were experimentally infected with BVDV type 1. Nine of these animals were in the first trimester of gestation. Blood samples were collected before inoculation and then regularly for eight months.

Three weeks post infection all of the animals scored positive for BVDV antibodies in the indirect ELISA while the blocking ELISA required another two weeks to detect the seroconversion in all animals.

A continuous rise in optical densities could be observed in all animals with the indirect ELISA; however those pregnant with PI foetus showed markedly higher OD’s which continued to increase up to the time they gave birth. With the blocking ELISA no discrimination between the PI-carriers and the control animals could be observed.

As a conclusion, the commercially available indirect ELISA kit can be used to identify potential PI carriers in an experimental setting. A clear advantage with the indirect ELISA is with this ELISA a single dilution of serum is a good estimation of the BVDV neutralisation titre compared to a blocking ELISA.

Thus it could be used for this purpose in large scale BVDV control/eradication programs. The sensitivity and specificity of the assay under specific field conditions needs to be further evaluated.

The comparative efficacy of commercially available BVDV vaccines in cattle challenged with European BVDV Type 1

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Bovine viral diarrhoea virus [BVDV] is an important cattle pathogen with a global distribution. Two genotypes of BVDV are recognised, types 1 and 2, both of which can cause severe, if rarely fatal, infection and devastating economic losses. The pathogenesis of BVDV-related disease is complex and most commonly includes the intestinal mucosa, the immune system and the reproductive system. The clinical impact of BVDV infections depends upon the virulence and biotype of the strain involved. As a result of the tropism of the virus for the reproductive system it is not surprising that BVDV is thought to affect fertility in cattle.

In this study a novel inactivated BVDV vaccine [PregSure BVD, Pfizer Animal Health S.A.], Bovilis BVD [Intervet Nederland B.V.], Bovidec [M.F.M Laboratories/Virbac Laboratories] and Mucobovin & Vacoviron [Merial SAS/Merial B.V.] were administered to three to seven month-old calves according to the manufacturer instructions. A similar number of calves were given saline as a control. All animals were negative for BVDV serum antibody and virus before enrolment. All animals were challenged intranasally 21 days after completion of the recommended two dose immunisation course with 105-106 TCID50 of a heterologous noncytopathic strain of a European BVDV type 1.

PregSure BVD stimulated a significantly [P<0.05] higher geometric mean neutralising antibody titre against a BVDV-1a strain (heterologous to the inactivated vaccines but homologous to Vacoviron) than Bovilis and Bovidec at seven and 21 days after completion of the basic vaccination, and higher than Mucobovin/Vacoviron at seven days after vaccination. All saline control animals remained seronegative until challenge. A higher percentage [44.4%] of animals was protected from post-challenge leucopaenia following vaccination with PregSure BVD than with any of the other three vaccines [0-30%].

In conclusion, PregSure BVD was shown to be superior to competitor BVDV vaccines following challenge with a heterologous noncytopathic European BVDV type 1 strain under controlled laboratory conditions.

BVDV - control in Germany

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Until now BVDV-Control in Germany is more or less a private initiative of farmers to reduce individual economic losses in cattle herds. The measures taken are based on the “German Guide Line on Protection of Cattle Herds against BVDV-Infestations and on Sanitation of Cattle Herds Suffering from BVDV-Infestations”.

Since the economic importance of BVDV-infestations is increasing the decision was actually made that an official BVDV-control and eradication program should be established as soon as possible. This program should come into force by a national regulation. Additionally, BVD will become a notifiable disease in Germany within 2004. Therefore, German government is establishing a national reference laboratory for diagnostic of BVD at the Friedrich-Löfler-Institute (former Federal Research Centre for Virus Diseases of Animals) to optimise and harmonise serological and virological diagnostic. Actually prepared national BVDV-Control and Eradication Regulation will define the health status of herds and individual animals, the conditions for vaccination measures, the examination schedule and testing procedures, the conditions for trade, and the safety measures taken on farms. Each individual animal detected as persistently BVDV infected has to be slaughtered as soon as possible.
In summation the planned measures hopefully will lead to an effective BVD eradication within an acceptable time frame without stressing German cattle business too hardly in the meantime. Motivation for the planned program is to increase the competitive capacity of farm business and to optimise the animal health status in German farm business.

**BVDV control program in one dairy herd**

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Bovine viral diarrhea virus (BVDV) is one the most economically significant pathogens of cattle worldwide. The unique feature of the virus is the ability to evade the host immune response by induction of persistent infection. The persistently infected (PI) animals are a major source of virus spread within the herd and therefore the BVDV control programs are mainly based on their detection and eradication. In our study, we describe the strategy of elimination of PI animals in one dairy herd.

The BVDV control program in the herd (600 dairy cows, 150 heifers and 300 calves) started in winter 2002. To screen the BVDV status, somatic cells isolated from the bulk milk sample were examined for detection of the viral genome by a nested RT-PCR test. Subsequently, individual serum samples were analyzed in two steps. First, indirect Ab ELISA was used to detect anti-BVDV antibodies. Animals 6 months of age and older were examined to avoid interference with maternal derived Abs. In the second step, single PI animals were identified from serum samples without detectable anti-BVDV antibodies using Ag capture ELISA. The BVDV status was then monitored in six months intervals by detection of BVDV genome in the bulk milk samples and by examination of the newborn animals after they reached 6 months of age by Ab and Ag ELISAs. The Ab value in the milk samples was also determined.

In the herd, eight PI animals were detected during the winter 2002 and spring 2004 (1 dairy cow in December 2002, 4 heifers in January 2003, and 3 calves in June 2003, January 2004 and March 2004). All PI animals were discarded from the herd immediately. BVDV viral RNA was detected only in bulk milk samples from 2002, and after discarding of one PI dairy cow all subsequent samples were negative for the presence of viral genome. The decrease of anti-BVDV Abs in the milk samples was also recorded. No new PI calves were identified in June 2004, although of 40 animals tested, 55% had antibodies against BVDV in serum samples.

Our experiences confirmed that RT-PCR detection of viral RNA from bulk milk samples is appropriate for herd screening. Our results showed that this method is sensitive enough to detect 1 PI animal of 600 cows. After testing of all individuals in the herd for the presence of PI animals, only the newborn calves can be tested provided, no animals enter the herd. We tested all animals aged 6 months before they came to the contact with pregnant heifers to prevent intrauterine infection and birth of PI calves. Although 55% calves serum samples were positive for anti-BVDV Abs during the last testing in June 2004, we suppose that virus infection will be eliminated soon because the birth of PI calves is avoided. Our assumption is also supported by the decrease of anti-BVDV Abs in bulk milk samples suggesting that virus circulation in the herd is broken.

**Vaccination of cattle against bovine viral diarrhoea virus**

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BVDV has a worldwide distribution and readily establishes endemic infections in cattle populations. The disease has a major impact on the productivity of affected cattle populations. After maternal infection, non-cytopathic BVD viruses crosses the bovine placenta and infects the conceptus. This leads to transient reproductive problems, abortion, generating persistent infected animals, and stillbirths. In contrast, most postnatal infections run only a very mild course. Therefore the primary aim of vaccination is to prevent congenital infections. Vaccination to prevent severe postnatal infections may be indicated only when virulent BVDV strains are prevalent.

In this study we compared the efficacy and safety in calves of four BVDV vaccines that are currently on the market. Post-vaccination the effects of these vaccines were compared with respect to the development of immune responses and to the local injection site reactions. Vaccinated animals and controls were challenged. Post-challenge, clinical symptoms, immune responses, the degree of immune depression, the degree of viraemia and nasal excretion were determined.

We demonstrated that all four vaccines primed for humoral and cellular immune responses and that calves with lymphocytes specific for BVDV in this study were protected from a virulent BVDV challenge.

The results of this study are of strategic value for BVDV control, but also to contribute to better understanding the consequences of BVDV vaccination and its effects on modulation of immune responsiveness.
Poster Session 4: Economic and social pressure for BVDV control

Estimating the economic impact of bovine viral diarrhoea virus (BVDV) infection during pregnancy in commercial dairy cattle

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During the past 30 years BVDV has been shown to be associated with significant early reproductive loss including ovulation and fertilisation failure, embryonic mortality and abortion (McGowan and Kirkland, 1995). The objective of the present study was to estimate the economic impact of BVDV infection in commercial dairy herds located in south-east Queensland, Australia. The Expected Money Value utilised by Houe et al (1993) was further modified and used in the present study. To obtain the most accurate estimate of reproductive losses (from 14 days prior to mating through is 6 months of gestation) due to BVDV infection, data from various published experiments and field studies were used. The following model was constructed:

\[ E.L = (N_{suscept} \times P_{infection} \times P_{pregnant} \times P_{preprod} \times losses) \]

Where EL is economic losses; Nsuscept is number of susceptible animals; Pinfection is probability of infection; Ppregnant is probability of pregnancy in a defined risk period; Ppreprod. loss is probability of reproductive loss (includes delayed expression of loss, i.e. birth of P1 calves, non-viable calves); F is financial cost of reproductive losses. In the model herd, the numbers of susceptible cows were assumed to be 100. The herd was assumed to be a moderately-high producing herd (approximately 7,000 L per 300 day lactation) managed for year-round calving. A PI cow was introduced into the herd and an outbreak of BVDV infection initiated. The data were manipulated and analysed by a computer spreadsheet programme (Microsoft Excel® version 7.0). Statistical analysis was performed on EPI INFO (version 6.04a). The model predicted that 6 months after the introduction of a PI animal, 80 out of the 100 susceptible animals would be infected, with 42 suffering losses related to in-utero BVDV infection. Thirteen PI calves were expected to be born. The average days open for the infected cows was 60 days longer than the uninfected cows. Losses were predicted to occur over a 21 month period (represents the midpoint of duration of infection, 3 months; the gestation length, 9 months; and the mean life of PI animals, 9.3 months). The estimated economic loss during this epizootic phase of infection was A$6,124 per 100 cows. Within 6 months of introduction of the PI animal, the pattern of infection would change to an endemic state. Assuming an annual incidence of infection of 0.25, a seroprevalence of 90%, and no introduction of susceptible replacement females, the annual loss was estimated to be A$191 per 100 cows, with no PI animals born.

References


Impact of bovine viral diarrhoea virus (BVDV) on the economics of fertility of beef suckler herds in marginal farming areas of Scotland.

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Fertility is the major determinant of production efficiency for a cow-calving system (Morrison et al., 1999) and is affected by both infectious agents (e.g. bovine viral diarrhoea virus (BVDV) and Campylobacter spp.) and managerial factors (e.g. length of breeding season, feeding and bull performance). These factors produce financial losses due to failure to conceive, abortions, increase in culling rate for infertility, decrease in live-weight of calves and heterogeneity at sales. BVDV is a major reproductive pathogen, which can lead to a fall in semen quality in bulls, and poor conceptions rates and abortions in cows (Fray et al., 2000). This paper examines the impact that BVDV, acting in isolation from the other factors, has on fertility in beef suckler herds in marginal farming areas of Scotland.

A Markov chain model of a suckler herd, based on the cow’s reproductive cycle, was used to estimate the financial results of alternative BVDV scenarios for infected beef suckler herds. This model provides stochastic simulations of reproductive performance for a breeding herd over a number of years. Secondary data combined with data derived from a survey (n=106) of farm activity and performance in marginal rural areas were used to parameterise the above model. It provides technical performance indicators and cost-revenue streams. Comparisons of the associated financial results demonstrates the often hidden impact that BVDV can have via infertility on the viability of suckler herds in marginal rural areas of Scotland and hence the importance of controlling the disease. Survey results (Figure 1) highlight the importance of veterinary input...
as a means to enhance fertility and hence economic performance. The effects that BVDV has on fertility in beef suckler herds are explored in order to improve understanding of the role for BVDV control in development of sustainable farming systems.

Poster Session 5: Experiences with BVDV eradication and/or control in Europe

Eradication program for BVDV in Saxony-Anhalt (Germany)

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Dimension of BVD in Saxony-Anhalt and control strategy

The history of BVDV in Saxony-Anhalt is rather short but much the worse. Along with large scale import of breeding cattle mainly during 1993/94 the virus was introduced into a partly seronegative population. Approximately 60% of herds were affected. In 265 herds (200 cows per herd on average) BVDV outbreaks were confirmed with severe clinical manifestations as abortion (52%), malformation (58%), embryonic death (32%), infertility (45%), mastitis (42%) and calf weakness (94%). Consequently prophylactic vaccination was started and still is widely used.

In 2002 a voluntary BVDV control program was initiated by an administrative regulation based on federal German Guidelines. In 2004 the voluntary program has been modified and was transformed into obligatory. The immediate object of both programs is the establishment of BVDV unsuspicious herds (virus negative irrespective the serological status) by elimination of persistently infected cattle. The long term perspective is the eradication of BVDV in Saxony-Anhalt.

Diagnostic procedure

The diagnostic program and particularly the choice of diagnostic tools were based on local laboratory experiences as well as economic and logistic aspects. Routinely pools of 50 (48) samples are tested in a highly sensitive Real Time RT-PCR. Positive pools are split into pools of ten. Individual samples of positive 10-sample-pools are submitted to commercially available Erns-ELISA testing. Both the high sensitivity of PCR and a low prevalence of PI-animals contribute to low examination fees per sample. Blood samples are preferred to milk to ensure simultaneous examination of a whole herd.

Obstacles for eradication

Diagnostic gap: The diagnostic gap caused by the uptake of high concentrations of maternal anti-BVDV antibodies with colostrum can lead to false negative virological results (ELISA, virus isolation) in calf for a so far not exactly defined period. Consequently no animals under 3 months of age are allowed to testing which results in the risk of virus spread and in additional logistical efforts when calves are on pasture. (In a recent study with 11 PI we detected BVDV in blood procedure at any day after birth using Real Time RT-PCR.)

Vaccination: Despite application of inactivated BVDV-vaccines accredited for fetal protection 12 respectively 14 PI-animals have been generated in two herds.

Sensitivity of ELISA: ELISAs are known to provide sensitivity less than 100%. If only ELISA testing is used for detection of PI-animals some may remain unrecognised.

Limiting the number of new BVDV infections in Pays de Loire area (France)

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In France, Pays de la Loire is an area where both dairy and beef productions are developed. A third of
the farms manage beef and dairy productions together. It is also an area of fattening with animals coming from beef areas of the center of France who register a higher incidence of BVDV.

This means difficulties in testing beef herds, various herd managements, and thus variability of the risk of transmission by contact between and within herds. Geographic distribution of seropositivities confirms the importance of contact transmission.

Considering these difficulties sanitary organisations of cattle owners decided to implement actions for a best knowledge of the infection among farmers. Testing of milk or young animals is a way to make cattle owners aware of their status and propose adapted recommendations. The aim is to reduce new infections, thus limiting economic consequences of the disease. It was decided to avoid actions implementing a free status while risk of infection was not under a better control.

It is expected to slowly lower the incidence of BVD in an economic way.

BVD control program in Lecco and Como provinces (Italy): herd risk categories to modulate interventions

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A BVD voluntary control program for dairy herds has been started in two provinces of Northern Italy since 2002. Among the 425 dairy herds located in the area, 86 (20%) asked to be included in the control program (May 2004). The results from 58 herds, monitored for at least 6 months and where BVD vaccine was not applied, are reported. The basic intervention was based on serological screening on individual blood samples and a questionnaire to collect information on BVD risk factors (livestock trade, exhibitions, pasture, natural insemination, embryo transfer, other susceptible species). Questions were in closed format and farmers were directly interviewed by a trained practitioner.

Blood samples (n=1448) were stratified by age (0-6 months, 6-15 months, pregnant heifers, uniparous and pluriparous cows) and in a number sufficient to detect presence of seropositive animals at a prevalence of 10%. On the basis of screening results and on the presence of risk factors, the herds were classified in four categories: negative at low and high risk of BVD introduction, positive at low and high risk of infection. This classification modulates follow up strategy: all initially seronegative animals and 3-5 calves of 0-6 months of age were tested at interval of 6-12 months, respectively in high and low risk herds. Moreover, in the herds with 6-15 months seropositive animals, persistently infected (PI) animals were identified. Prevalence of seropositive herds was 43/58 (74.1%), among these, 36 were classified as positive at high risk. The follow up showed 22 herds (37.9%) with ongoing infection (seroconversion and/or PI animals), 20 of them were observed in the high risk category. A single new infection was observed in a seronegative herd in low risk category. Questionnaires allowed to qualify risk factors, and livestock trade showed to be the most widespread (60,3%).

Considering the BVD high prevalence and frequency of risk exposure, a monthly serological test on bulk milk and yearly spot test on 0-6 months calves were introduced in seronegative herds. Moreover an improved biosecurity was applied and a killed vaccine in herd with ongoing infection was suggested. The results of these program showed that predictive serological and risk profiles could be useful to modulate diagnostic and control measures in high prevalence area, even in absence of a systematic control program.

BVDV control: the use of a sustainable control programme

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Once a control programme (BVDV) is put in place the necessary tools should be available to run such a programme in a cost effective and efficient way. To that extent the Animal Health Service developed the Certification Operating System (COS). COS is a tool that automatically manages the monitoring and certification rules and regulations of a number of health schemes. A software programme based on the functional description and translation of the health scheme rules and regulations is the core of COS.

In a nutshell the following steps are supported by COS: it combines and integrates information from the national Identification and Registration system on animal movement, stock keeping and mutations, it does keep track of the registration of health scheme participants, it produces timely and proper listings for inspection and sampling, it provides a selection of animals to be sampled (barcode), mailing of sampling instructions to vet and farmer, it provides proper lab flow: available lab capacities are matched and/or compared with required capacities. Lab results are interpreted based on predefined criteria, and based on these results the current herd status is determined. Examples will be presented.

There are several benefits in organising health schemes this way: one system fits all, COS has a modular architecture and therefore other health schemes can be incorporated using most of the existing modules. A reduction in cost of ownership and maintenance is the result. The payback period is approx. 3 years. There is a
transparency of procedures and traceability of samples at all times. The process is running as designed. Disease-specific data become available for various analytical and modelling purposes. COS can also be of use in case of a List A disease outbreak. In such an event a high number of actions have to be undertaken within a distinct time period and COS can provide the necessary workload or input data for GIS mapping. Examples will be given.

Other cattle health schemes included in COS so far are: L. hardjo, IBR, paratuberculosis, Salmonella dublin, and recently Neospora. By mid 2005 a total of 42 schemes will run under COS.

Data on COS performance, costs of development, costs of maintenance and updating, and labour input will be presented.

The BVD situation in Denmark, development and surveillance program

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In the early nineties, Danish veterinarians began more systematic testing of herds for BVD virus (BVDV) and antibodies. The testing was performed by means of ELISA-techniques applied on milk or blood samples. All tests were performed at the Danish Institute for Food and Veterinary Research.

Based on these results, the herds were categorised as free or not free of BVDV. Following these initial investigations Danish Cattle Federation was involved, and a few years later, the Danish Veterinary and Food Administration introduced a law, containing the principles for control of BVDV infections.

The first law came into force March 21.1996, and during the following years, the law was updated nearly every year, concurrently with better knowledge about the disease.

From the early beginning the infection was surveyed through either bulk milk or blood samples, and from the start the cattle had to be followed by a health document, stating that they were free of BVDV. All farms were tested on yearly blood tests, - or bulk milk four times a year.

Furthermore, it was forbidden to put PI animals out on pasture, and vaccination was not allowed either.

In year 2000, we had almost 1000 infected farms, but this number has been reduced every year.

Today the BVDV infections in Denmark is controlled to such an extent, that we have a surveillance programme instead of eradication programme.

As of February 8, 2004, farms are considered free of BVD, unless test results shows differently. There is no longer a demand for yearly tests. Only when selling or showing cattle, there is a demand for a clarified status.

Today, cattle are surveyed through bulk milk or blood from slaughtered animals.

As seen on the figure we have about 80 farms, which are classified as infected with BVD. We find about 10 PI calves each year. Total numbers of farms and cattle in Denmark are shown below.

Figures on Danish cattle in 2003

| Cattle in total | 1,700,000 |
| Dairy cattle    | 1,500,000 |
| Beef cattle     | 200,000   |
| Farms with dairy cattle | 7,400 |
| Farms with beef cattle | 19,300 |
| Average herd size dairy cattle | 90 |
| Average herd size beef cattle | 11 |

Voluntary local BVDV eradication programmes in Austria leading to a national compulsory regulation

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Up to the 1st of August 2004, BVDV eradication programmes in Austria had been only established on a voluntary basis at a provincial level. Within the last few years eight of the nine Austrian provinces decided to implement such programmes especially for cattle breeding farms. In principle two different approaches were chosen. Whereas the two western provinces of Vorarlberg and the Tyrol were trying to detect persistently BVDV-infected (PI) animals by regular blood screenings using a BVDV antigen ELISA, the provinces of Salisbury, Styria, Carinthia, Upper Austria, Lower Austria and Burgenland established programmes similar to those in Scandinavian countries. In the latter provinces a serological screening of bulk milk samples and of blood samples from young stock was used to detect herds suspected of containing PI-animals. Consequently such suspect herds were examined thoroughly by using BVDV antigen ELISA or PCR in all samples showing no antibodies against BVDV.

The common principles of the two different eradication programmes were the prohibition of BVDV vaccination, the slaughter of identified PI animals and rules for defining the status of a herd. Although these voluntary eradication programmes had proven to be rather successful, experiences showed, that further improvements could only be reached by extending the programme to all Austrian cattle farms. Especially the problem arose, that contacts between animals from cer-
Identified BVDV-free herds and unknown PI-animals from animals originating from herds not participating in the programme could not be ruled out by 100%. Due to this fact some of the farmers were reluctant to comply with the rules of the programme and faced BVDV reinfections as a consequence. Therefore it was decided to create a draft for a national compulsory BVDV eradication programme. After several attempts in the last few years had failed because of various reasons a new draft was broadly accepted by all stakeholders. This new compulsory BVD-regulation has been published now, and was set into force on 1st of August 2004. One of the main challenges is the way how herds certified as BVDV-free by the former voluntary programmes can be certified by the new regulation. Another important topic is the necessity of strict rules for animal movements. These regulations provide that every contact of animals with different BVDV health status is prohibited. In order to keep an overview over the various test results and the status of the herds it is of vital importance to have in place a comprehensive database, which also allows to deliver health certificates and forms for veterinarians taking samples. At the moment all provincial veterinary services try to establish such database like those already used in Styria or Lower Austria.

The costs of an eradication programme are always a major concern of farmers. The national BVD-order only compriases a public compensation for the slaughter of PI-animals, whereas all other costs are to be beared by the farmers. Nevertheless at the moment at least the costs of the laboratory diagnosis are funded by means of the provinces.

Targeted sampling of beef animals at slaughter

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Annual surveys based on testing of bulk-milk samples meet the needs for screening for BVD in dairy cattle. However, these surveys do not cover beef suckler herds. The latter were supposed to be dealt with phenotype-based sampling at slaughter. Each abattoir collected a specified number of serum samples, adding up to an annual total of 3000. Nevertheless, analysis of the origin of samples taken at slaughter in 1999-2001 revealed that only a third originated from the target population. A higher precision was considered necessary.

A new way of directing the sampling at slaughter was launched in 2003. It is based on the central bovine register, which includes also all the animals in the beef suckler herds (n = 1275) that do not have registered dairy cows. The abattoirs get the sampling requests from the Ministry of Agriculture and Forestry (MAF), when they check up the information of the animals intended to be slaughtered. The meat inspecting veterinarians take the requested blood samples and send them to the National Veterinary and Food Research Institute (EELA), with the identification codes of the individual animals (ID). EELA queries the farm identification numbers corresponding to the animal IDs from the central register. All the information of the sampling is compiled to one data base in EELA. When a minimum of 10 samples per herd is reached, the sampling request from MAF is lifted. Sera of 10 animals are pooled and tested for antibodies by indirect antibody ELISA (SVANOVA, Sweden). If the pooled sample tests positive, the individual samples of the pool are retested. The results of the testing are sent to the farmer and to the communal veterinarian.

A total of 6 811 blood samples from 892 herds were obtained during 2003. Of these 99% originated from beef suckler herds. The sampling covered 70% of the herds (at least 1 sample/herd) and the request to take samples was lifted from 34% of herds during 2003. Only two seropositive animals from two herds were detected, which agrees with the earlier inference (Nuotio et al. 1999) that BVDV infection is rare or non-existing in the beef suckler herds. A sample size of 10 per herd was chosen, because BVDV status of the herd would be easier to evaluate, and samples could be used as a part of the voluntary BVD control programme. In conclusion, the new sampling scheme for beef suckler herds relies heavily on serviceable information technology but does give a highly targeted result.