THREE YEARS OF MANDATORY BVD CONTROL IN GERMANY – LESSONS TO BE LEARNED

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Introduction
Contact of naïve, non-pregnant cattle with bovine viral diarrhea virus (BVDV) results in most cases in an unapparent infection or in a mild or moderate clinical disease with fever, respiratory or enteric symptoms, reproductive disorders or milk drop. A subsequent immunosuppression can promote other secondary infections leading to an impairment of the general health conditions on the herd level. Even more importantly, infections of pregnant animals in the first trimester with a non-cytopathogenic (ncp) biotype of BVDV can result in the birth of so-called persistently infected (PI) calves. Those animals are immunotolerant against "their" BVDV, and are therefore unable to develop specific antibodies and shed lifelong enormous amounts of BVDV. PI calves are the major source for both the spread and the perpetuation of BVDV within and between herds, and PI animals are in consequence the major target of all attempts to reduce the clinical diseases and economical losses of one of the most significant disease in bovines. After years of a worldwide practice of more or less "half-hearted" vaccination programs the Scandinavian countries started a systematic approach in the early 90s. The "Scandinavian model" is based on large scale bulk milk serology pre-selecting farms with the risk of PI animals, followed by individual testing of all animals from those herd and removing of the detected PI animals. A strict non-vaccination policy as well as biosecurity measures to prevent infections of free herds and an ongoing monitoring on the herd level proved as very efficient to achieve the freedom of BVDV within a timeline of about 10 years under the Scandinavian conditions (Lindberg and Alenius, 1999, Stahl and Alenius, 2012). Other countries like Austria (especially Lower Austria) followed that approach with a voluntary program in 1997 which became compulsory in 2004, and they are now in the final stage of eradication (Roßmanith et al., 2014).

An alternative way of BVD control proved as beneficial especially for countries with a high initial BVDV prevalence, a high level of cattle trading and transport partly together with ongoing vaccination programs, is to detect and to eliminate PI animals as early as possible by direct antigen or genome testing without the serological pre-screening. Switzerland started a very consistent three-step-program following that approach without vaccination in 2008, and the PI prevalence dropped to 0.02% until the end of 2012 (Bachofen et al., 2013). The successful measures in detection and removing of animals are supported by strict interdictions of trade by farms with positive animals. Ireland implemented a test run on a voluntary basis in 2012 (Graham et al., 2014), progressing to an obligatory program in 2013 which is also focused on the testing of ear notches of all newborn calves.

Approach and results of the German BVD control strategy
Bovine viral diarrhea/Mucosal Disease (BVD/MD) is a notifiable disease in Germany since 2004. At the same time, a National Reference Laboratory was established at the Friedrich-Loeffler-Institut (FLI), Insel Riems. Germany started a voluntary program in 1998 in the responsibility of the Federal States ("Laender"), and after 10 years of no or only moderate progress, a consistent, obligatory nation-wide BVD control program was finally implemented. Since the 1st of January 2011, an official “BVD Regulation" is in force. The major goal of the program is a fast and efficient reduction of the prevalence of PI animals, and the establishment of so-called "unsuspicious (virus free)" animals and farms with a certified status. Interestingly, in contrast to other countries the complete eradication leading to a BVDV-free (antibody negative) status is yet not claimed explicitly. A major reason is the failure of a common European BVD control strategy like it is existent for other diseases, e.g. BHV-1 (directive 64/432/EG). Therefore, transnational rules and measures are difficult to accomplish, and for the German authorities a complete eradication process requires an Europe-wide concerted action.

Four basic rules were defined:

1. Obligatory investigation of all newborn calves for BVDV (antigen/genome) before the age of 6 months
2. Immediate elimination of all PI animals
3. Trade with certified “unsuspicious animals” (non-PI) only
4. Prevention of reinfections by qualified measures (e.g. biosecurity, voluntary vaccination)

A PI animal is defined according to the German legislation as an animal which is tested antigen/genome-positive two times with methods described in the official collection of methods within an interval of 60 days as a maximum or animals tested positive without confirmation. Furthermore, the offspring of a PI is declared as PI as well as animals with Mucosal disease.

In Germany, about 12.535 million cattle are kept in 163110 holdings (status at the 31.12.2012). About 43% of the total number of animals are kept in farms with more than 200 animals by only 8.8% of the holders. Otherwise, there are still more than 90 000 farms with less than 50 cattle. Thus, the diagnostic and control strategy has to consider that there is a big number of small farms but that otherwise the majority of animals are kept in large holdings.

Furthermore, the traditional differences regarding agricultural, commercial and trading structures between the German Laender influence the efficacy of the control measures.

The number of births per year is about 4.8 million over the years without seasonal peaks. Together with the necessary follow up tests and the investigation of older animals, nearly 5 million diagnostic tests for BVDV are performed every year since 2011. More than 43000 PI animals have been detected and removed within the last three years in Germany, and the prevalence was reduced from 0.50% in 2011 to 0.24% in 2012, and
0.14% in 2013 (see Figure 1). In addition, outbreaks with more than 100 PI animals within a short period were observed in several cases after reintroduction of the virus into free or unsuspicious farms showing the necessity and impact of biosecurity measures, and demonstrate that the observed decline of vaccination densities increases the economic losses after reinfection.

Disease control in Germany as a federally structured state is the responsibility of the Laender. Therefore, it is not surprising that differences in implementation, speed and flanking measures are observed. However, it also turned out that there are some common risk factors which can influence the progress markedly:

- Animals detected as PI often remain in the farms for weeks/months although an immediate elimination is prescribed
- Trading with non-tested (male) calves, i.e. for export
- Contact between animals with a different status during exhibitions, livestock markets, fair and animal trading points
- Existence of older cattle without certified status; e.g. 1265 PI animals older than 12 month were detected in 2012
- Insufficient control of success by inadequate serological monitoring measures
- Deficits in management, biosecurity and awareness for actual risks of the introduction of BVDV

In contrast to the most European countries with a BVD control program, a voluntary vaccination is being used in Germany primarily as a two-step vaccination procedure consisting of a primary vaccination with an inactivated vaccine followed by a second shot with an attenuated live vaccine. This strategy combines the advantages and minimizes the disadvantages of both vaccines. It is targeted to achieve an efficient fetal protection.

**Diagnostic experiences**

The majority (about 95%) of tests are done with ear biopsies (ear notch sampling) taken during the tagging procedure what has to be done for every calf in the European Union within the first seven days of life. Both the antigen ELISA (~80%) and the real-time RT-PCR (~20%) are applied by the local veterinary laboratories of the Laender which are in charge of BVDV diagnostics. In addition to about 25 veterinary state laboratories distributed over Germany, about 10 private laboratories are involved in some of the “Laender”. Blood samples are investigated too, but due to the strong inhibitory effects of maternal antibodies during the first 30 days for the E<sub>RNS</sub>-ELISA, respectively 40 days for a pool-PCR (diagnostic gap) its use is very limited. Therefore, less than 5% of the specimens derive from blood samples at the moment. In contrast, according to our experiences, there is no crucial diagnostic gap for ear notches using these test procedures. However, in a few cases we have observed reduced OD values in the E<sub>RNS</sub> ELISA around the cut off most likely due to maternal antibodies in the related tissues (e.g. lymph) or due to accidental blood in the specimens. Nevertheless, after improvement of the analytical sensitivity by the test producer of the E<sub>RNS</sub> ELISA, no further problems have been observed. In the German program, antigen capture ELISAs based on the detection of the p80 (NS3) protein of BVDV are not allowed to be used prior the age of 90 days both for serum and ear notches. Reasons are on one hand the instability of the p80/NS3 protein and the stronger inhibitory effect of colostral antibodies compared with E<sub>RNS</sub> on the other hand. All methods are prescribed in detail in an official “Collection of Methods for the Diagnosis of Notifiable Diseases”.

In Germany only tests and methods are permitted to apply which are officially approved by the respective authority at the FLI. Licencing, batch releasing and organization of proficiency tests are part of a system of quality assurance and contributes to the acknowledged high standard of BVD diagnostics in Germany.

Collectively, seven real time PCR kits and three ELISA are licensed in Germany. But, only two to three real-time PCRs and only one ELISA are really used on a large scale. The type of specimen and the age of the animal determine the method employed. Thereby, the diagnostic gap proved as the limiting factor for the selection of and decisions for methods (table 1).
as BVDV of subtype 2c. In all cases the affected animals were characterized by pneumonia, bloody diarrhea, hemorrhages and extensive lesions in the oral cavity and the oesophagus. A pestivirus was detected in the first samples, and further characterized by sequencing and phenotyping with monoclonal antibodies as BVDV of subtype 2c. In all cases the affected animals had an unsuspected BVDV-status with a negative ear notch result for BVDV after birth. BVDV-2c could be also found in the past in Germany in a few cases, but never connected with dramatic clinical signs on the herd level. The current BVDV-2c strain, which has spread later also into farms of Lower Saxony and the Netherlands, formed a distinct cluster within the BVDV-2c subgroup. Deep sequencing analysis revealed the mutual existence of three genome variants with or without a nucleotide duplication and adaptive mutations in the highly conserved p7/NS2 region (Jenckel, et al., 2014). It is hypothesized that these alterations contribute to the highly virulent phenotype. Furthermore, the very high virulence was confirmed in an animal experiment. Eight calves which were infected with a BVDV-2c outbreak isolate showed fever, severe leukopenia and thrombocytopenia, and died or had to be euthanized until day 11 post infection. Severe purulent-fibrinous pneumonia comprising more than half of the lung tissue could be observed as one of the prominent findings at necropsy. The viral load both during the viremia and in the post-mortem specimens was extraordinary high and comparable to a persistent infection. It becomes evident that acute BVDV infection should be also considered when Mucosal disease like lesions are observed.

### Conclusions

The history of BVDV control over the last 25 years in different Europe has clearly shown that a voluntary based strategy is not adequate to achieve a freedom from the disease and could even enhancing the damages induced by BVDV. All countries which started with that approach have changed their programs sooner or later to a consistent and compulsory strategy. Several countries like Norway, Sweden, Finland and Denmark have succeeded in eradication or are almost free after ten years of a governmental program. Some other countries like Switzerland, Austria and Germany are in an advanced or final stage, and still others implemented a program recently or decided to start in the near future (Ireland, Belgium). The experiences reveal that both fundamental approaches, the “Scandinavian model” as well as the “PI detection and eradication approach” are suitable to achieve substantial successes. The implementation of the ear notch testing enables a PI-detection at a very early stage, and is a fundamental step towards an efficient and reliable diagnostic strategy. Both the E\textsuperscript{RNS} antigen capture ELISA and the real-time RT-PCR have proved as efficient and suitable tools for molecular epidemiology. Especially in the final stage of eradication programs, it is of interest to identify relationships between infections, to compare current virus isolates with those from the past or to find new types or those which might be circulating for a longer time than others. We have therefore characterized 592 BVDV isolates sampled between 2008 and 2014 by sequencing and phenotyping with monoclonal antibodies

<table>
<thead>
<tr>
<th>method</th>
<th>sample</th>
<th>diagnostic gap</th>
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<tr>
<td>E\textsuperscript{RNS} Ag ELISA</td>
<td>serum, plasma, EDTA blood organs, ear notches</td>
<td>&lt; 30 day of age no diagnostic gap</td>
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<tr>
<td>NS3 Ag ELISA (p80)</td>
<td>blood leukocytes</td>
<td>&lt; 90 day of age</td>
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<tr>
<td>real time RT-PCR</td>
<td>serum, plasma, EDTA blood, leukocytes, organs, milk, ear notches</td>
<td>pooled: &lt; 40. day of age individual: no diagnostic gap</td>
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<tr>
<td>virus isolation</td>
<td>blood, leukocytes, organs, (serum)</td>
<td>7 – 40. day of age</td>
</tr>
<tr>
<td>FACS analysis</td>
<td>blood leukocytes</td>
<td>&lt; 90 day of age</td>
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Pen-side applications could be useful e.g. for the self-monitoring to allow a quick detection of BVDV in blood or ear notches under special conditions. However, the risk of false negative results due to a lower diagnostic sensitivity has to be considered, and therefore such tests are not used within the official German control program currently. Moreover, the potential possibilities of manipulation minimize the acceptance of such tests by the veterinary authorities. However, with a special permit the tests can be applied also in Germany, e.g. for the immediate testing for incoming animals in veterinary clinics.

### Molecular analysis of circulating virus strains in Germany

Phylogenetic analyses are widely used in studies of molecular epidemiology. Especially in the final stage of eradication programs, it is of interest to identify the relationship between infections, to compare current virus isolates with those from the past or to find new types or those which might be circulating for a longer time than others. We have therefore characterized 592 BVDV isolates sampled between 2008 and 2014 by sequencing within the 5’ non-translated region (5’-NTR). The analyses clearly showed that in Germany two BVDV subtypes (BVDV-1b and BVDV-1d) are dominating, and both types represent 69% of all analyzed strains. Apart from this, strains of subtype BVDV-1f (10.5%), BVDV-1e (4.1%), BVDV-1h (2.9%), BVDV-1g (0.5%), and BVDV-1k (0.2%) could be observed. However, also regional differences were seen, and in general, the Southern part of Germany (especially Bavaria) displayed a higher diversity of the analyzed BVDV isolates than the Northern part. The percentage of BVDV-2 was 9.5%, and while the subtype 2a Germany was dominating within the genotype 2 strains formerly, the newly emerged subtype BVDV-2c is currently found more frequently.
the reliable detection of PI animals. The diagnostic standards for BVDV detection are at a very high level what is also reflected by the results of several proficiency tests which are organized by the German National Reference Laboratory on a regular basis. Hence, limitations and relapses in the BVD control cannot be attributed to the diagnostic methods and standards. Other factors and influences outside the diagnostic systems, like national and transnational trade, or inadequate management practices, proved to be more crucial for the efficacy and success of BVDV eradication programs.

References


