Screening for persistently infected (PI) animals among newborn calves in Belgian cattle

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Introduction

Bovine viral diarrhoea or BVD is a viral disease affecting cattle that is caused by the Bovine Viral Diarrhoea Virus (BVDV), which, together with Border Disease Virus (BDV) and Classical Swine Fever Virus (CSFV), belongs to the genus *pestivirus* in the *Flaviviridae* family. The virus is endemic in most cattle-producing countries and causes significant economic losses worldwide. BVDV can manifest itself in several ways on cattle farms, which often impede a fast diagnosis. Despite its name Bovine Viral Diarrhoea Virus, the absence of diarrhoea does not rule out circulation of the disease on a farm. BVD can, for instance, be present on a farm subclinically as well as clinically, in which case it causes mortality, mainly of calves. In addition, it can give rise to reproductive impairments and production losses, ranging from a decrease in milk production to runts and stunted growth. The virus also gives rise to a diminished immunity and a general immunosuppression, causing the infected bovine animal to have an increased sensitivity to a number of secondary infections such as diarrhoea, problems with udder health, scab and respiratory disorders. A “typical” clinical picture is difficult to describe for BVD. Often, BVD has to be considered after ruling out the obvious causative agents for a certain clinical picture.

Transient infection versus persistent infection

When a cow becomes infected for the first time with the BVD virus after birth, an acute transient infection will manifest itself, independent of the age of the animal. This infection may pass unnoticed, or may present clinical symptoms. In case no complications occur, the animal recovers within 2 weeks and develops an immunological response against the BVD virus, causing it to be protected against re-infection for a long time. In most cases, a transiently infected animal will only be able to infect other animals to a limited extent, since only small amounts of the virus are excreted and this only during a period of a few days.

When a pregnant female cow that has never been infected with BVDV, and consequently hasn’t developed antibodies against the BVD virus (naive animal), goes through an acute infection during gestation, the foetus will also be infected by the virus. This foetal infection can lead to a strongly diminished fertility (embryonic mortality), abortions, premature birth or the birth of weak calves and calves with congenital defects. Moreover, if the infection of the mother takes place between the 30th and 125th day of gestation, the foetus will recognize the virus as a part of itself, which leads to the birth of the persistently infected calves, the so-called BVD carriers or IPIs (immunotolerant & persistently infected) bovines. Generally speaking, these BVD carriers lag behind in growth and die
of “mucosal disease” (or MD) sooner or later. Yet, it is estimated that about 10% of the BVD carriers become older than 2 years, and these animals often appear to be healthy, whereas they spread the virus continuously without being suspected to be BVD carriers, with enormous damage being caused to the cattle farm as a consequence. Unlike transiently infected animals, BVD carriers do not develop immunity to the virus they are infected with and are consequently seronegative. On the other hand, these animals excrete enormous amounts of BVD virus during their entire life cycle, which makes BVD carriers the most important source of new infections for naive animals on cattle farms. The early detection and immediate removal of these animals is therefore an essential step in any BVD control program.

Table: Overview of possible serological and virological statuses of cattle with respect to BVDV

<table>
<thead>
<tr>
<th>Serological status</th>
<th>Virological status</th>
<th>Virus transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive animal</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transiently infected animal</td>
<td>During infection</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After infection</td>
<td>+</td>
</tr>
<tr>
<td>IPI</td>
<td>Lifelong</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: -, negative; +, positive; +++: strongly positive

Screening for BVD carriers in newborn calves

Because of the large impact of BVD on the cattle sector, successful BVD eradication programs have already been started in several European countries. The Belgian cattle sector has also been interested for some time in starting a Belgian eradication program. This has led to the drawing up of a national control plan by different stakeholders in the cattle sector, which is currently being finalized. The basis of the control program is the detection and elimination of BVD carriers (=IPI) and the granting of an IPI-free status to all bovine animals that aren’t carriers, to eventually get a livestock that is disease-free. In the first stage of this control program, all newly-born calves will be tested for BVD within 7 days after birth. As of January 2015, all newborn calves will be systematically tested, which comes down to about 900,000 calves that will be tested on a yearly basis.

In order to have this screening taking place as efficiently and cheap as possible, the choice is made to test these calves by means of ear notch samples collected at the moment of the identification of the newborn calves. Indeed, each calf that is born has to be registered within 7 days after birth and upon this birth notification it receives a unique identification number that corresponds to the number mentioned on the earmark. The ear notches can be sampled by the cattle farmers themselves, simultaneously with the earmarking to identify each calf. For this purpose new earmarks are already being used, which will become mandatory as of January 2015. In addition, serum and blood samples of individual animals can also be used as a test matrix. Yet, these samples have to be taken by the company veterinarian.
BVD antigen-ELISA versus RT-qPCR

In order to be able to eradicate BVD, there is a need for accurate diagnostic tests which can be used efficiently and effectively. In the first stage of the control plan, there’s a need for tests aimed at detecting the BVD virus, where a high specificity is required (detecting all infected animals, avoiding false negative results), in combination with a strong sensitivity (avoiding false positive results, every animal that tests positive is actually infected with BVDV). In addition, these tests should be used routinely and in a fast, cost efficient and user-friendly manner. Both the BVDV antigen-ELISA and the real-time reverse transcriptase (RT RT-PCR or RT-qPCR) may be used for this purpose.

In the first stage of the eradication program, the intention is to detect all IPIs and subsequently remove them from the herd. Since the “viral load” of IPIs is generally much higher than in transiently infected (TI) bovines, the detection of these animals can be done with the antigen-ELISA as well as with the RT-qPCR with a high probability of detecting positive animals. Since the antigen-ELISA is, however, less sensitive than the RT-qPCR, transiently infected animals will often not be detected by this test. These animals are most often detected with the RT-qPCR, since this test can detect relatively low “viral loads”. The obtained Ct-value will give an indication of the “viral load” of the sample and will point out whether an animal may be IPI or TI. In order to be certain that there is no circulation of BVDV on a farm, the preferred method is the RT-qPCR method. On the other hand, an ELISA-antigen is sufficient if the only intention is to identify BVD carriers. Moreover, antigen-ELISA tests are cheaper and easier to conduct, which is the main reason why these tests are preferred for systematically testing newborn calves within the Belgian eradication plan.

In certain cases, as for instance export, participation to animal gatherings or markets and the introduction in AI-centers, testing of animals is demanded so as to prove that these animals are BVDV free, regardless of the type of infection (IPI or TI). For such a certification, an RT-qPCR-test is strongly recommended, since this is the only test that is sensitive enough to identify each TI-animal.

In addition to detecting TI-animals, the higher sensitivity of the RT-qPCR also allows for the testing of pools of serum or blood samples, so as to reduce the screening costs associated with the testing of larger numbers of individual samples. This method is often used in combination with the antigen-ELISA, by first analysing pools of sera or blood samples using RT-qPCR and subsequently identifying IPI animals based on antigen-ELISA tests on the individual samples from the positive pools. This combination of RT-qPCR and antigen-ELISA is often used for screening older animals in the framework of the (voluntary) screening of farms. In this respect, it should be noted that when antigen-ELISA tests are carried out on blood or serum of calves younger than 3 months, there is a chance of false-negative results due to interference with maternal antibodies, as opposed to when the RT-qPCR is carried out.

Conclusion

Eradication of BVDV from Belgian bovine herds is only possible by means of a combination of effectively testing all animals and the use of appropriate diagnostic tests for a specific predetermined objective. Testing and eliminating IPI-animals is essential at the start of an eradication program in order to remove the biggest source of the infection from the herd and to reduce the current high BVDV prevalence in Belgium. From an epidemiological point of view slaughtering TI-animals has little use. Besides, this would be a very expensive affair for the sector, due to the high number of BVDV infected farms in Belgium. Both the antigen-ELISA and the RT-qPCR can play an important role in this respect. In a later stage of the control program, when the BVDV prevalence has dropped sufficiently,
the detection of transient infections will begin to play a more important role, when companies want to prove that they’re actually BVD virus free. In this case, the RT-qPCR is the only method that is sensitive enough to detect transient infections. Choosing an appropriate test method, depending on the objective of the test, will thus always have to be put first.

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