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Full Article

Prevalence of Bovine Viral Diarrhoea Virus antibodies and antigen among the aborted cows in industrial dairy cattle herds in Mashhad area of Iran

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ABSTRACT

The measurement of antibody responses of animals exposed to BVDV either through a natural exposure or an immunization protocol is still a standard procedure. For BVDV, the test formats have been largely limited to ELISA which is a valuable diagnostic test to measure the level of BVDV specific antibodies as well as antigen in blood samples. In the present study, 120 blood samples were collected from the cows with the history of abortion in different period of pregnancy from different industrial dairy cattle herds of Mashhad area of Iran. Also 30 samples were collected from the cows with no history of abortion as control. The presence of antibody against BVDV from the 120 serum samples was investigated by indirect ELISA. From 120 serum samples which were collected from aborted cows, 89 samples were positive (%74.16). From these positive samples, 12(13.48%), 54 (60.68%) and 23 (25.84%) samples belong to the first, second and third trimester of pregnancy, respectively. From 89 positive samples, 12 (13.48%) samples were related to stillbirth and 8 (8.99%) samples were belongs to the mummified fetus. From 89 positive samples, 71 (79.78%) were related to cattle between 2-5 years old and 18 (20.22%) were associated to cattle more than 5 years old. In control group, 20 samples (66.66%) were antibody positive. Also the presence of BVDV antigen in serum samples was investigated by Ag-capture ELISA. From 120 serum samples, 2 samples were positive (1.67%), which belongs to the second period of pregnancy. In control group, none of the samples were antigen positive. The results of this study showed that the prevalence of BVDV infection is high among the aborted cows of Mashhad area. Although this prevalence is higher than the control group, the observed difference is not significant.

Keywords: Bovine virus diarrhoea virus, ELISA, antibody, antigen, abortion

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) infection is a worldwide distributed animal disease characterized by bovine reproductive disorders that can severely affect the developing embryo and fetus (Talebkhan Garoussi 2007). BVDV belongs to the *Pestivirus* genus in the *Flaviviridae* family (Pringle 1999). BVDV infection is listed as a group-B disease (milder infectious disease) on the list of noticeable animal diseases (Valle *et al*

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2001). The disease was first described by Olafson et al (1946) and diagnostic tests for individual animals and test strategies for cattle populations have evolved considerably. Transiently infected animals with high, long-lasting antibody levels and persistently infected (PI) animals with high levels of viremia have provided important targets for diagnostic efforts (Houe *et al* 2006). The accuracy of available diagnostic tests is crucial for the success of a control program. For BVDV infections, several diagnostic tests, aiming either to detect the virus itself or to detect viral-specific antibodies, are available. In general, the analytical sensitivity and specificity of these tests are high (Goyal 2005). The measurement of antibody responses of animals exposed to BVDV either through a natural exposure or an immunization protocol is still a standard procedure. Among different serological assays that have been used for BVD over the years, the most commonly used antibody detection techniques are the virus neutralization test (VNT) and ELISAs. VNT is a labor-intensive and also expensive test (Sandvik 2005). As an alternative to the VNT, indirect and blocking ELISAs are commonly used (Schrijver and Kramps.1998). ELIS As have many advantages over the VNT and for BVDV, the test formats have been largely limited to ELISA which is a valuable diagnostic test to measure the level of BVDV specific antibodies as well as antigen in blood samples. In general, ELISAs have proven to perform well in practical use, with sensitivity and specificity values between 95 and 100% (Sandvik & Krogsrud 1995, Brinkhof *et al* 1996).

Bovine viral-diarrhoea virus (BVDV), endemic in most cattle-raising countries, also causes reproductive failure. Infections during pregnancy can result in embryonic death, abortions, birth of stillborn or weak calves, or can lead to birth of persistently infected (PI) calves that will shed virus throughout their lifetime (Grooms 2004). Infection with BVDV is generally subclinical, however, when a dam is infected with BVDV during pregnancy, transplacental infection may occur, and as a result, fetal abortion, mummification or congenital defects may occur depending on the gestation stage (Kozasa *et al* 2005). More importantly, infection in the first trimester of pregnancy can result into the birth of immunotolerant calves that are persistently infected (PI) with BVDV. The PI animals are a major source of virus spread and thus, it is very important to identify and remove them from the cattle herd (Lindberg 2003). In general, PI cattle show varied clinical manifestations such as diarrhea, pneumonia (as a result of immunosuppression), poor growth, some succumb to mucosal disease, and some PI cattle indicate no clinical manifestations. The PI cattle on dairy farms are suspected as the cause of milk production loss and/or increase in occurrence of secondary or opportunistic infections (Baker 1995, Chi *et al* 2002, Kelling *et al* 2002). Although detecting animals carrying virus is essential for identification and removal of PI animals from an infected herd, screening herds for antibody carriers is also important to identify PI animals (usually seronegative) and to determine the herd's infection status and susceptibility (Mainar-Jaime *et al* 2001)**.** Seroprevalence in non-vaccinated herds differs among areas or countries, ranging between 20 and 90% (Alenius *et al* 1986, Loken *et al* 1991). Area differences could in part be explained by factors such as cattle density, herd size or livestock trade (Houe *et al* 1995).

Mashhad is the capital of north-eastern province of Iran with high agricultural economic values. Previous studies in this region have shown a high prevalence of BVDV infection among industrial dairy cattle herds (Talebkhan Garoussi *et al* 2008 & 2009). However, none of the previous studies have addressed the potential risk factors contributing to BVDV infection in this region. Abortion is a major problem in herds of Mashhad area and so far, no study has been undertaken to show any relation between BVDV infections in aborted cows in Iran. Therefore, the purpose of the present study was to evaluate the prevalence rate of BVDV infection among cows with the history of abortion in Mashhad area of Iran using ELISA technique and to determine any association between the rate of BVDV infection and abortion.

MATERIALS AND METHODS

Collecting sera. In total, 120 Holstein aborted cows blood samples plus 30 blood samples from cows with no abortion history as control were obtained from different industrial dairy cattle herds in Mashhad area of Iran. The samples were centrifuged at 2000 × g at room temperature for five min to separate sera. Sera were stored at -20ºC until used.

Indirect ELISA. The assay was performed by Bovine Viral Diarrhoea Virus (BVDV) Antibody Test kit manufactured by IDEXX (HerdChek, IDEXX Laboratories, Westbrook, ME, USA), in a 96-well micro titration plates which were coated with BVDV antigen. The sensitivity and specificity of the test as manufacture instruction were mentioned 96.3% and 99.5%, respectively. The serum samples were diluted (1:1) by wash solution. One-hundred µl of sera was loaded into wells and incubated for 90 minutes at room temperature. Positive and negative control sera were used as indicated in the kit. The wells were washed five times with 300 µl of wash solution. Following the final washing, the plate slapped vigorously, well down on a bench top which covered with paper towels. Then, 100 µl of anti-bovine HRP conjugated was loaded into all the wells and incubated for 30 minutes at room temperature. The plate was washed as described above to remove the excess conjugate. For colour development, 100 µl of TMB was added to each well as a substrate and incubated for 10 minutes at room temperature at darkness. The reaction was terminated by the addition of 100 µl of stop solution to each well. The absorbance at 450 nm was monitored in ELISA reader.

Detection of BVDV E^{rns} antigen **by antigen-capture ELISA**. All samples were tested using commercial BVDV Antigen Test Kit /Serum Plus (HerdChek, IDEXX Laboratories, Westbrook, ME, USA), in which microtitre plates were coated with anti- E^{rns} monoclonal antibodies. The kit is based on the detection of the E^{rns} (gp44-48) glycoprotein of the BVD virus. The sensitivity and specificity of the test as manufacture instruction were mentioned 100% and 100%, respectively. The serum samples were diluted (1:1) by wash solution. Fifty µl of sera was loaded into wells and incubated for 2 hours at 37º C. The rest of the test was followed as mentioned in serum antibody assay and finally the absorbance at 450 nm was monitored in ELISA reader.

Calculation and statistical analysis. The result could be read visually where the OD was measured at 450 nm. Calculations for test samples were analyzed as follow for BVDV antibody:

The presence or absence of BVDV antibodies in the sample is determined by S/P ratio for each sample.

$$S/P = \frac{\text{Sample A450} - \text{NCx}^{-}\text{ A450}}{\text{PCx}^{-}\text{ A450} - \text{NCx}^{-}\text{ A450}}$$

PCx^{-} and NCx^{-} represent positive and negative control mean respectively. According to manufacture instructions, samples with S/P values less than 0.2 were classified as negative and samples with S/P values equal or greater than 0.3 were classified as positive for BVDV antibody. For BVDV antigen, the presence or absence of BVDV antigen in the sample is determined by the corrected OD value (S-N) for each sample as follow: S-N= Samples A450 - NCx^{-}

Samples with S-N values less or equal to 0.3 were classified as negative and samples with S-N values higher than 0.3 were classified as positive for BVDV antigen.

Statistical analysis. Proportion of seropositivity was compared between aborted and healthy cows using Chi-square test.

RESULTS

For antibody detection, 89 (74.17%) out of 120 serum samples were interpreted BVDV seropositive ($S/P \geq 0.3$) (Table 1). From these positive samples, 12(13.48%), 54 (60.68%) and 23 (25.84%) samples were associated to the first, second and third trimester of pregnancy, respectively (Table 1). 31 (25.83%) of serum samples of aborted cows had $S/P < 0.2$ values and were interpreted BVDV seronegative. In control

group, 20 samples (66.66%) were antibody positive and 10 samples(33.34%)were negative (Table 1).

Table 1. The numbers of positive samples to BVDV antibody in first, second and third trimester of pregnancy and also in control group.

			Distribution of Positive cases in 3 Trimesters		
Total number of samples	Positive samples	Negative samples	First trimester	Second trimester	Third trimester
Aborted cows (120)	89 (74.17%)	31 (25.83%)	12(13.48%)	54 (60.68%)	23 (25.84%)
Control cows (30)	20 (66.66%)	10 (33.34%)	-------	-------	--------

The observed difference between aborted cows and control group is not significant (P=0.41). From 89 seropositive samples in aborted cows, 12 (13.48 %) samples were related to stillbirth. From these, 2 (16.67%), 7 (58.33%) and 3(25%) samples were related to first, second and third trimester of pregnancy. Also, 8 (8.99%) samples associated with mummified fetus. From these samples, 3 (37.50%), 3 (37.50%) and 2 (25%) were associated to the first, second and third trimester of pregnancy (Table 2).

Table 2. Distribution of stillbirth and mummified fetus in 3 trimester of pregnancy.

Fetus characteristic	Total n. of positive samples	First trimester	Second trimester	Third trimester
Stillbirth	12 (14.28%)	2 (16.67%)	7 (58.33%)	3 (25%)
Mummified	8 (8.99%)	3 (37.50%)	3 (37.50%)	2 (25%)

From 89 positive samples, 71 (79.78%) were related to cattle between 2-5 years old and 18 (20.22%) were associated to cattle more than 5 years old. Also the presence of BVD antigen in serum samples was investigated by ELISA. From 120 sera samples, 2 samples were positive (1.67%), which were belongs to the second period of pregnancy and in control group, none of the samples were antigen positive (Table 3).

DISCUSSION

In this study we showed the prevalence of BVDV infection among cows with the history of abortion in industrial dairy cattle herds of Mashhad area of Iran. Our results showed that BVDV infection present widely (74.17%) in aborted cows in these herds. The rate of seropositive cows was also high in the control group (66.66%), but lower than the cows with no history of abortion. However the difference was not significant. Therefore we can not conclude that the abortion is a direct consequence of BVDV infection in the herds studied. Although no significant association was found between BVDV infection and abortion, BVDV infection could be related to other reproductive parameters (such as infertility or embryonic death) which were not studied in this research. Other recent studies in Mashhad area of Iran have shown that the BVDV seroprevalence is 72.25% (Talebkhan Garoussi *et al* 2009). All of the herds in this study were antibody positive against BVDV and the prevalence ranged from 66 to 100% within the herds of Mashhad area of Iran.

Table 3. Distribution BVDV antigen in second trimester of pregnancyin 2 aborted cases.

Total number of samples	Positive samples	Negative samples	First trimester	Second trimester	Third trimester
Aborted cows (120)	2 (1.67%)	118 (98.33%)	0	2 (1.67 %)	0
Control cows (30)	-------	30 (100%)	-------	-------	--------

Our results are in agreement with this study and since vaccination against BVDV is not practiced in the cattle herds of Iran, serological response reflected natural infection. Most probably, these herds have had a recent or an ongoing infection most likely due to the presence of PI animal(s) (Houe & Meyling, 1991). BVDV infection can cause abortion at any time during gestation, but only in dams not previously exposed to the infection (Grooms 2004). Thus, in our study, BVDV seroprevalence of 74.17% among cows with the history of abortion reflects previous exposure to BVDV infection. Our data showed that seroprevalence of 66.66% in control group with no history of abortion. Therefore, we could not attribute this slight difference between aborted and control group to BVDV infection.

Bovine viral diarrhea virus contributed significantly and substantially to economic loss of dairy herds in many parts of the world and associated to increased abortion rates, extended calving-to-conception intervals, and reduced milk production (Heuer *et al* 2007). Therefore, it must be studied more for the prevalence and different epidemiological aspects and risk factors of BVDV in Mashhad as an important pole of dairy production in Iran. Research studies based on the BVDV antibodies detection, either in individual animals or bulk milk, have shown that the prevalence of infected herds ranged 70% to 100% in many parts of the world (Edwards *et al* 1987, Reinhardt *et al* 1990, Niskanen *et al* 1993, Obando *et al* 1999). It was shown that the herds with high cattle population density had higher prevalence of infection than the herds which were smaller (Loken *et al* 1991). In samples from PI calves, BVDV-specific maternal antibodies may block viral infectivity or detection of viral antigens, usually up to an age of around three months (Palfi *et al* 1993), and this is why the percentage of positive samples in our antigen test is low. There is a clear relationship with the maternal antibody titre; the higher the average antibody titre, the lower the frequency of virus isolation test positives. Thus, in the presence of high levels of maternal antibodies, the virus isolation and the antigen ELISA tests were shown to be unreliable indicators of the presence of persistent infections with BVD virus. These findings confirm the results of the experiment published by Palfi *et al* (1993). Adherence of antibodies to the virus surface may also explain the false negative results using the antigen ELISA test in the presence of high levels of antibodies. In our study, only 2 out of 120 samples were BVDV antigen positive in aborted cows and at the same time BVDV antibody titers were high in these cows. To make most of the information obtained from laboratory diagnostic investigations, a thorough understanding of both the epidemiology of BVD as well as the performance of diagnostic tests is essential. For example, typical values for the sensitivity and specificity of an excellent Ag ELISA for BVDV may be 97% and 99%, respectively, which means that 3% of the PI animals in a population are not detected **(**Sandvik 1999). The Sandvik،s results, clearly showed that in a high proportion of the PI animals, the virus isolation test and the antigen ELISA test, when run at day 7 after the ingestion of colostrum, were both negative. Taken together our results clearly demonstrate a high prevalence rate of BVDV infection in dairy cattle herds with the history of abortion in Mashhad area of Iran. The results presented in this study confirm previous reports of high incidence of BVDV infection in this region of Iran. Therefore preventive measures should be taken in consideration in order to control the level of infection and subsequently reduce the economic impact of BVDV infection.

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RESEARCH ARTICLE

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The Role of Pestiviruses (BDV and BVDV) in Ruminant Abortion Cases in the Afyonkarahisar Province

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ABSTRACT

Pestiviruses are important viral agents that can cause abortion in ruminants. In this study, roles of Border Disease Virus (BDV) and Bovine Viral Diarrhoea Virus (BVDV) were investigated in ruminant abortion cases. Aborted foetal tissue samples were collected from 101 animals (74 sheep foetuses and 27 bovine foetuses), each from epidemiologically different farms, during the months of January 2016 and December 2017 in the Afyonkarahisar Province. One step real-time duplex RT-PCR was used for the detection of BDV and BVDV RNA. Genetic characterization of the field isolates of pestiviruses was conducted by sequencing 5' untranslated region (5' UTR). BDV RNA was detected in 9 (12.16%) of the 74 aborted sheep foetuses, whereas BVDV RNA was detected in 6 (22.2%) of the 27 bovine foetuses. Phylogenetic analysis based on the 5' UTR region indicated that BDV isolates in the present study belong to BDV-7 genotype whereas BVDV isolates belong to BVDV-1 genotype. The results of this study showed that pestivirus infections play important role in ruminant abortion cases in Afyonkarahisar province.
Keywords: Border disease virus, bovine viral diarrhoea virus, abortion, sheep, cattle

Afyonkarahisar İlinde Ruminant Abort Vakalarında Pestivirusların (BDV ve BVDV) Rolleri

ÖZ

Pestiviruslar ruminantlarda abortlara neden olan önemli viral ajanlardır. Bu çalışmada, ruminant abort vakalarında Border Disease Virus (BDV) ve Bovine Viral Diarrhoea Virus (BVDV)'un rolleri araştırılmıştır. Abort olmuş fötus doku örnekleri 101 hayvandan (74'ü koyun fötusu, 27'si sığır fötus), her biri epidemiyolojik olarak farklı çiftliklerden, Ocak 2016 ve Aralık 2017 ayları arasında Afyonkarahisar ilinden toplanmıştır. BDV ve BVDV RNA'sının tespiti için tek adımlı real-time dubleks RT-PCR yöntemi kullanılmıştır. Sahadan izole edilen pestivirus'ların genetik karakterizasyonu 5' translate olmayan bölge sonunun (5' UTR) sekansı ile gerçekleştirilmiştir. BDV RNA'sı, 74 aborte koyun fötusunun 9 (%12.16)'unda, BVDV RNA'sı ise 27 sığır fötusunun 6 (%22.2)'sında tespit edilmiştir. 5' UTR bölgesinin filogenetik analizi bu çalışmada izole edilen BDV izolatlarının BDV-7 genotipine, BVDV izolatlarının ise BVDV-1 genotipine ait olduğunu göstermiştir. Bu çalışmanın sonuçları, pestivirus enfeksiyonlarının, Afyonkarahisar ilindeki ruminant abort vakalarında önemli rol oynadığını göstermektedir.
Anahtar Kelimler: Border disease virus, bovine viral diarrhoea virus, abort, koyun, sığır

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INTRODUCTION

Border Disease Virus (BDV), Bovine Viral Diarrhoea Virus 1 (BVDV-1) and Bovine Viral Diarrhoea Virus 2 (BVDV-2) belong to the *Pestivirus* genus of the *Flaviviridae* family, together with Classical Swine Fever Virus (CSFV). Pestiviruses are enveloped, single-stranded, positive-sense RNA viruses genome of 12.5 kb in length. Based on the genetic analysis, BDV isolates have been segregated into seven clusters (BDV-1 to BDV-7) whereas BVDV has two genotypes: BVDV-1 and BVDV-2 (Simmonds et al. 2012). Pestivirus infections have been associated with abortions, mummified foetuses, infertility, diarrhoea, respiratory disease and persistent infection (PI) of the offspring (Nettleton et al. 1998; Munoz-Zanzi et al. 2004).

It has been reported that pestiviruses are not host specific. Both BDV and BVDV can infect sheep, goat, cattle and swine (Nettleton et al. 1998; Passler and Walz 2010). Main route of transmission of pestiviruses is horizontal via transiently infected and PI animals. Furthermore, vertical transmission occurs in all host species (Van Campen and Frolich 2001).

Pestivirus infection has a worldwide distribution. Previous studies of abortion cases in ruminants in different regions of Turkey identified pestiviruses as the cause of abortion (Hasircioglu et al. 2009; Azkur et al. 2011; Avci et al. 2013; Berber and Sozdutmaz 2013; Tuncer-Goktuna et al. 2016; Ural and Erol 2017; Bulut et al. 2018). Small ruminants and cattle are important livestock in Afyonkarahisar province. Abortion in ewes and heifers causes serious economic losses in the livestock industry. Therefore, the aim of the present study was to investigate the role of BDV and BVDV in abortion cases of ruminants in the Afyonkarahisar Province.

MATERIAL and METHOD

Sample collection

During January 2016 and December 2017, foetal tissue samples (lung, liver, spleen, kidney and brain) were collected from 74 aborted sheep foetuses and 27 aborted bovine foetuses from flocks and herds where abortion cases occurred in the Afyonkarahisar province. Details of the sampled flocks and herds given in Table 1. Farmers reported that animals were not vaccinated against pestivirus infection in sampled flocks and herds.

RNA extraction and one step real-time duplex RT-PCR

Foetal tissue samples were homogenised in PBS using the TissueRuptor (Qiagen, Hilden, Germany). Viral RNA extraction was carried out from tissue homogenates using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. One step real-time duplex RT-PCR was performed with primers and probes that targeting 92 bp and 103 bp conserved regions of the 5'-UTR of BDV and BVDV, respectively (Table 2). The protocol described by La Rocca and Sandvik (2009) was used for detection of pestiviruses. One step real-time duplex RT-PCR reaction was carried out with one step RT-PCR kit (Cat No. 210212, Qiagen, Hilden, Germany) in a final volume of 25 µl reaction mix which contained 5 µl 5 x RT-PCR buffer, 200 µM of each dNTP, 1 µL enzyme mix, 0.4 µM of forward primer, 0.6 µM of reverse primers, 0.5 µM of each probes and 2.5 µL of sample RNA. Amplification was performed using LightCycler 2.0 real time PCR machine (Roche Applied Science, Indianapolis, IN, USA) with the following conditions: reverse transcription step of 10 min at 50 °C and 5 min at 95 °C, followed by 45 cycles at 95 °C for 15 s and 60 °C for 30 s. The samples that had a Ct value <35 were considered positive.

One-step RT-PCR amplification and sequencing of 5' UTR region

Samples that were positive by real-time duplex RT-PCR were subjected to one-step RT-PCR amplification using primers 324 and 326 which amplify a 288 bp region of the 5' UTR region (Vilcek et al. 1994). The protocol described by Vilcek et al. (1994) was used for detection of pestiviruses. RT-PCR reaction was carried out with one step RT-PCR kit (Cat No. 210212, Qiagen, Hilden, Germany) in a final volume of 25 µl reaction mix which contained 5 µl 5 x RT-PCR buffer, 400 µM of each dNTP, 1 µL enzyme mix, 1 µM each primer, and 2.5 µL of sample RNA. Amplification was performed using MJ Research thermal cycler with the following conditions: reverse transcription step of 30 min at 50 °C and 15 min at 95 °C, followed by 40 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s and final extension step in 72ºC for 5 min. The PCR products were analysed on 1.5% agarose gel stained with Gelred (Biotium, USA) after electrophoresis at 90 V for 60 min (Fig. 2). Amplified PCR products were sequenced both the forward and reverse directions on the ABI 3500XL DNA Analyser (Applied Biosystems, USA) with the

BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) by Intron Saglik Urunleri (İzmir, Turkey). Primers 324 and 326 were used in sequence analysis. Phylogenetic tree was constructed, via the neighbour-joining method using MEGA software version 6, for the 5' UTR region of pestiviruses with additional sequences from GenBank. Kimura two-parameter model was used to describe the evolutionary distances between sequences.

Nucleotide sequence accession numbers

The 5' UTR region sequences reported in this paper are available in the GenBank under accession numbers MH395751 to MH395754.

Statistical analysis

The difference in the detected rate of BDV and BVDV was compared with Fisher's exact test. $P<0.05$ was considered to be statistically significant.

RESULTS

Detection of BDV and BVDV RNA by one step real-time duplex RT-PCR

BDV RNA was detected in 9 of the 74 aborted sheep foetuses whereas BVDV RNA was detected in 6 of the 27 aborted bovine foetuses (Table 1). Positive samples had Ct values between 20.17 and 34.06 (Fig. 1). There was no significant difference between the detected rate of BDV and BVDV ($P = 0.2193$). Furthermore, no significant differences were found between the districts where pestiviruses were detected ($P = 0.5294$).

Table 1. Districts where samples were collected
Tablo 1. Örneklerin toplandığı ilçeler

Districts	No. of examined flocks	No. of positive flocks	No. of examined herds	No. of positive herds
City Center	8	1	3	1
Çay	7	2	2	1
Çobanlar	5	-	3	1
Dazkırı	8	1	4	-
Dinar	7	-	3	1
Emirdağ	12	2	6	1
Hocalar	6	-	2	-
İhsaniye	7	-	1	-
Sinanpaşa	4	1	2	-
Sultandağı	10	2	1	1
Total	**74**	**9**	**27**	**6**

Table 2. Details of the primers and probes used for detecting pestiviruses by one step real-time duplex RT-PCR.
Tablo 2. Pestivirusların one step real-time dubleks RT-PCR ile saptanmasında kullanılan primerler ve problar

Primers and Probes	Sequence (5' - 3')	Target pestiviruses	Reference
106-F	CCATRCCCDTAGTAGGACTAGC	BDV-BVDV	La Rocca and Sandvik (2009)
190-R	GYGTCGAACCAYTGACGACT	BVDV	
179-R	GYGTYGAACTACTGACGACT	BDV	
Probe-162	FAM-TGGATGGCYKAABCCCTGAGTACAG-EDQ	BVDV	
Probe-128	YY-ACTAGCYDTCGTGGTGAGATCCCTG-EDQ	BDV	

Sequence and phylogenetic analyses of the 5' UTR region

Nucleotide sequences were obtained for two BDV and two BVDV field isolates. The analysis of the 5' UTR region sequences revealed that the homology between two BDV field isolates was 82.7% whereas the similarity with previously characterized BDV isolates ranged from 60.5% to 87%. The highest nucleotide homology was observed with

previous Turkish isolate (BDV-Aydin-04). The analysis of the 5' UTR region sequences revealed that the homology between two BVDV field isolates was 88.8% whereas the similarity with previously characterized BVDV isolates ranged from 70.2% to 96.5%. The highest nucleotide homology was observed with previous Germany isolate (BVDV CP7 strain).

The phylogenetic tree based on 5' UTR region sequences revealed that BDV field isolates in this study belonged to BDV-7 cluster whereas BVDV field isolates were typed as BVDV-1 (Fig. 3).

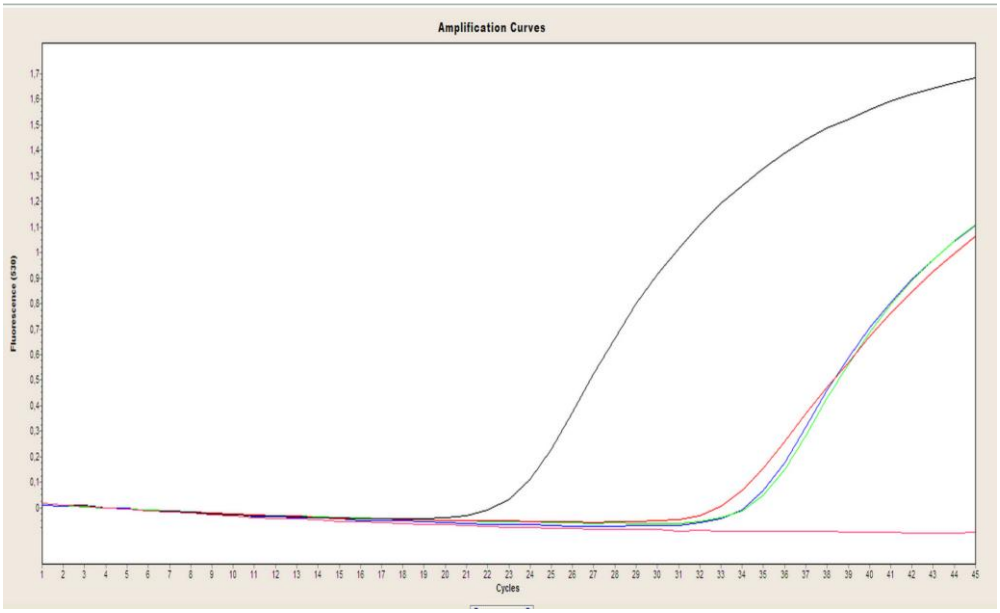

Figure 1. One step real-time duplex RT-PCR based on 5' UTR region of pestiviruses. Black line: positive control, pink line: negative control, other colourful amplification curves: positive pestivirus samples.

Şekil 1. Pestivirusların 5' UTR bölgesine dayalı one step real-time duplex RT-PCR. Siyah çizgi: pozitif kontrol, pembe çizgi: negatif kontrol, diğer renkli amplifikasyon eğirileri: pozitif pestivirus örnekleridir.

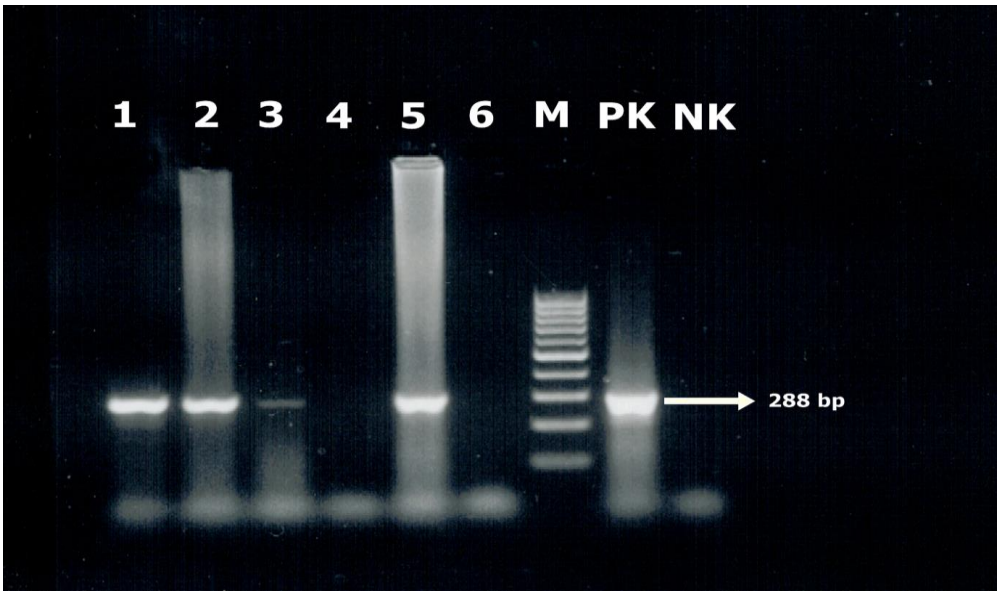

Figure 2. Agarose gel electrophoresis of RT-PCR product based on 5' UTR region of pestiviruses, M: Molecular marker of 100 bp, Lane 1-6: Samples, Lane PK: Positive control, Lane NK: Negative control.

Şekil 2. Pestivirusların 5' UTR bölgesine dayalı RT-PCR ürünlerinin agaroz jel elektroforezi, M: 100 bp moleküler marker, 1-6: Örnekler, PK: Pozitif kontrol, NK: Negatif kontrol.

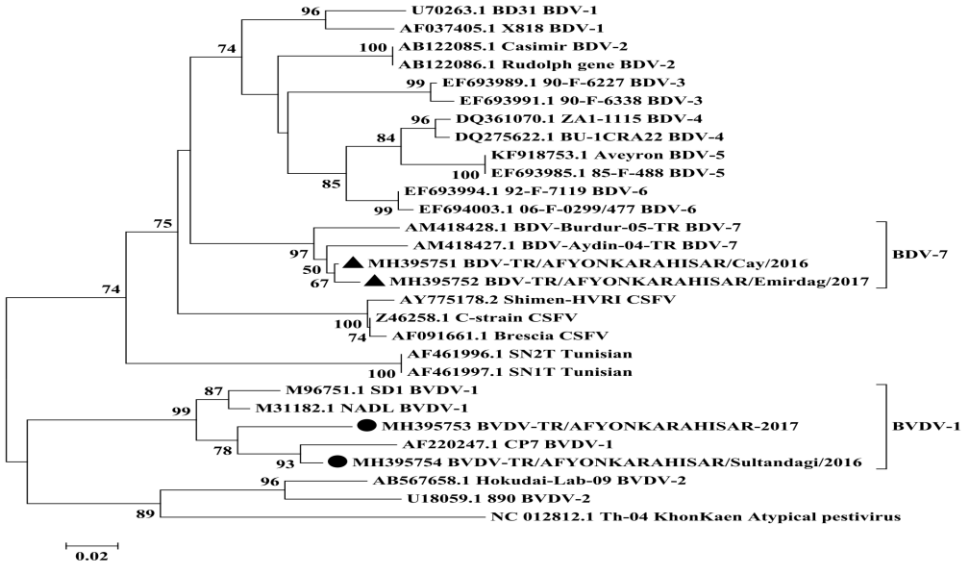

Figure 3. Phylogenetic tree constructed based on the 5' UTR region sequences using the Kimura two-parameter model. The BDV sequences obtained in this study are marked with black triangle (▲), and BVDV sequences are marked with round black spot (●).
Şekil 3. Kimura 2 parametre yöntemi kullanılarak oluşturulan 5' UTR bölgesi sekanslarının filogenetik ağacı. Bu çalışmada elde edilen BDV sekansları siyah üçgen ile (▲), BVDV sekansları ise siyah yuvarlak spotla (●) işaretlenmiştir.

DISCUSSION

Pestiviruses are distributed worldwide, and cause significant economic losses due to their impact on health and reproduction (Nettleton et al. 1998; Munoz-Zanzi et al. 2004). Pestiviruses are not highly host-specific (Nettleton et al. 1998; Passler and Walz 2010). Numerous studies have shown that both BDV and BVDV strains infect sheep, goat, cattle, swine and deer (Paton et al. 1995; Strong et al. 2010). However, in the study BDV RNA was only detected from aborted sheep foetuses, and BVDV RNA was from aborted bovine foetuses. Bulut et al. (2018) reported that prevalence of BVDV in sheep abortion cases in the Marmara and Eastern Anatolia regions in Turkey was 10.10% (40/396), and they suggested that the cause of BVDV infection in sheep may be pasture which contaminated with nasal drifts and saliva of persistently infected cattle. Furthermore, a previous study reported that close contact between small ruminants and cattle increases the risk of pestivirus transmission (Braun et al. 2013). In this study, BDV positive aborted sheep foetuses were from flocks which had only sheep for breeding, and according to farmers' report sheep and cattle were not use same pastures. Therefore there was no contact between sheep and cattle in BDV positive flocks. This could explain why BVDV RNA was not detected from aborted sheep foetuses.

The rate of pestiviruses in ruminant abortion cases in this study was 14.9% (15/101). This finding is in agreement with previous reports. Reported rates of pestiviruses in ruminant abortion cases in different regions of Turkey were between 0.93% and 66.6% (Cokcaliskan 2002; Hasircioglu et al. 2009; Albayrak et al. 2012; Avci et al. 2013; Tuncer-Goktuna et al. 2016; Bulut et al. 2018).

In this study, BDV RNA was found in 9 (12.16%) of the 74 aborted sheep foetuses. This result in agreement with previous report (Hasircioglu et al. 2009), but was lower than previous field studies that reported rates of the presence of pestiviruses in aborted sheep foetuses were 24.7%, 47.3% and 66.6% in the Marmara region, west part of Marmara region and Northern region of Turkey, respectively (Albayrak et al. 2012; Tuncer-Goktuna et al. 2016; Bulut et al. 2018). Possible explanations for this result may be the detection method, number of sampled animals and farm management. In this study, BVDV RNA was found in 6 (22.2%) of the 27 aborted bovine foetuses. This result in agreement with previous report (Albayrak et al. 2012), but was higher than previous study that detected BVDV antigen in 2.2% (2/92) of the aborted calves (Ozturk et al. 2012). Furthermore, Tuncer-Goktuna et al. (2016) detected pestivirus antigen in 31 (51.6%) of the 60 aborted calves in west part of Marmara region of Turkey. Possible explanations for these discrepancies may be the

number of sampled animals and number of sampled farms, and detection methods.
Serological and virological studies have been performed in the Afyonkarahisar province for pestiviruses (Gur 2009; Gur et al. 2009). However, molecular detection and genetic characterisation of pestiviruses in ruminant abortion cases in the Afyonkarahisar province has not been previously reported.

In previous studies, pestivirus isolates obtained from small ruminants in Turkey were classified into BDV-3, BDV-7 and BVDV-2 (Oguzoglu et al. 2009; Toplu et al. 2012; Yesilbag et al. 2014). Phylogenetic analysis of partial 5' UTR revealed that BDV field isolates in this study were of the BDV-7 genotype with the previous Turkish isolates (BDV-Burdur-05-TR and BDV-Aydin-04-TR). This result indicates that BDV-7 genotype is in circulation in the sheep population in Turkey.

The 5' UTR genetic analysis using sequences for pestiviruses revealed that BVDV field isolates in this study belonged to the BVDV-1 genotype (Fig. 3). The circulation of BVD-1 genotype in Turkey was also reported in previous studies (Yesilbag et al. 2008; Aslan et al. 2015). Furthermore, BVDV-2 genotype was detected from cattle in Turkey (Oguzoglu et al. 2010; Sarikaya et al. 2012; Yilmaz et al. 2012). It seems that both BVDV-1 and BVDV-2 are in circulation in cattle in Turkey.

In conclusion, a control programme for pestiviruses has not been applied in Turkey. Therefore, pestivirus infections are still animal welfare problem. Infection with pestiviruses causes serious economic losses in the livestock industry due to abortion problems, death and reduced reproductive performance. The results of this study showed that pestivirus infection play important role in ruminant abortion cases in Afyonkarahisar Province. A control programme for pestivirus infection will be beneficial to prevent economic losses.

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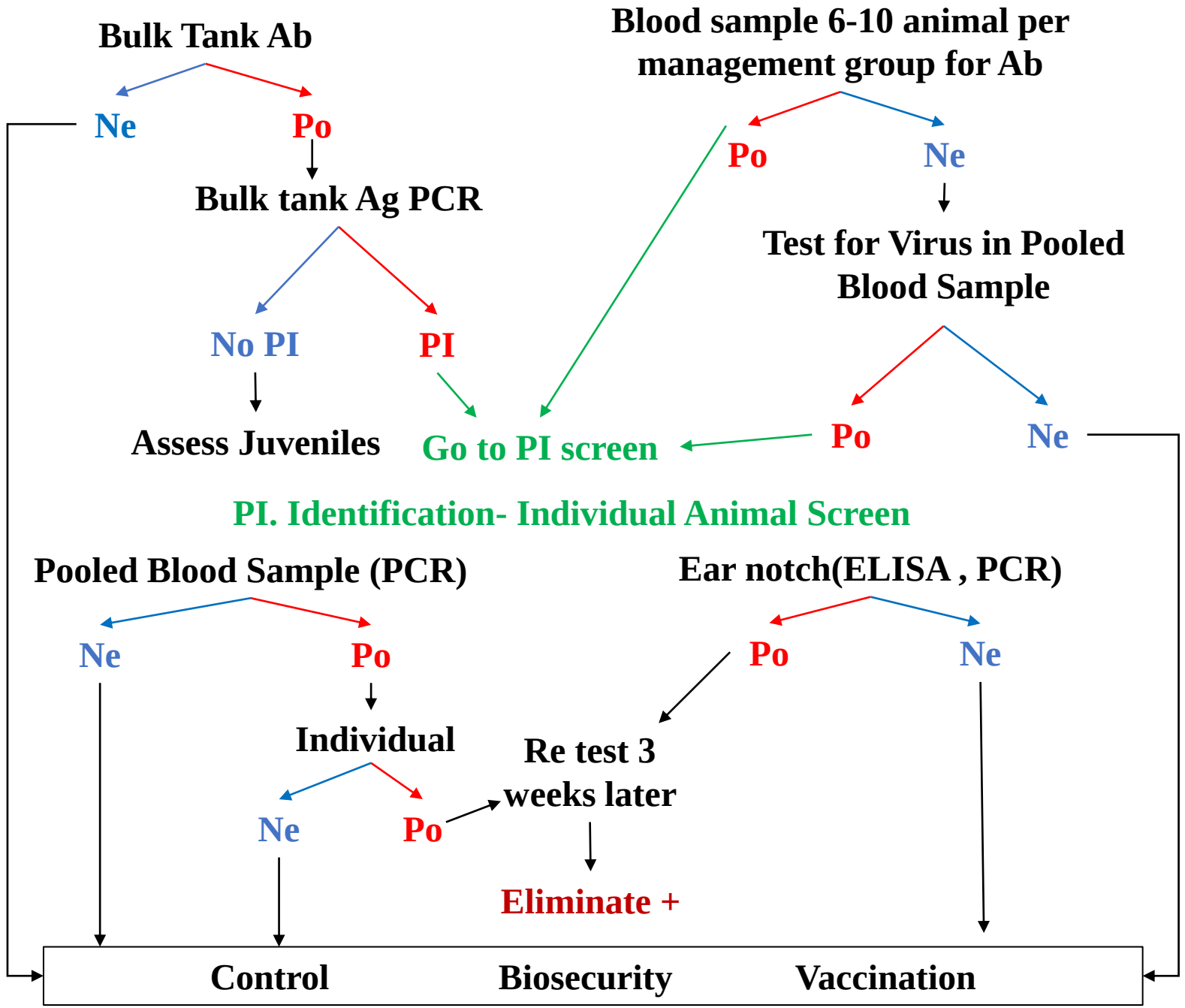
Diagnosis of BVD



Dr. Armin Pourabbas

"BVD FOETAL PROTECTION INDUCED BY VACCINATION IN CATTLE"

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INTRODUCTION

Bovine Virus Diarrhoea Virus (BVDV) is a pestivirus with worldwide distribution. Infections are common in New Zealand with an estimated 85% of herds infected (Horner, 1996). BVDV infection leads to a range of disease problems but reproductive failure is the principal cause of economic loss. There are two ways to protect heifers from reproductive failure:

1. Exposure to natural infection. This produces a strong, long-lasting immunity (Potgeiter, 1995).

2. Vaccination. This offers the advantage of controlled, safe and strategic protection against BVDV (Galletti, 2007).

The following trial was undertaken to determine if vaccination of sero-negative heifers with a multivalent viral vaccine (HIPRABOVIS®, Hipra; Spain) containing a 1a BVD strain (NADL) was able to protect the foetus from infection with a field strain of BVDV.

This study was conducted by: Animal Health Services Centre, Massey University, ESTENDART LTD, Palmerston North, New Zealand.

MATERIALS AND METHODS

48 non-pregnant heifers seronegative to BVDV, Neospora caninum and Enzootic Bovine Leukosis were randomly allocated to two groups of 24. Heifers in one group were vaccinated with HIPRABOVIS®, followed by a booster 21 days later. Heifers in the second group were unvaccinated controls. All 48 heifers were oestrus-synchronized in a CIDR programme then mated using artificial insemination. Ten heifers from each group shown to be pregnant were selected 118 days after vaccination and grazed together with 2 persistently infected (PI) calves for the rest of gestation period. Because of the low survivability of PIs, two PI calves were used for this challenge although it did mean that this provided an unusually high level of infective pressure. All calves born alive were blood sampled at birth prior to suckling colostrum and their BVD status determined by SNT ELISA Ag.

RESULTS

Three heifers had early foetal re-absorptions (early abortion); two from the HIPRABOVIS® group and one from the controls.

Six heifers (one from the HIPRABOVIS® group and 5 from the controls) had late abortions (calves at term). All 6 calves were positive for BVDV by ELISA Ag and antibody.

Table 1. Foetal abortions of pregnant cattle vaccinated with HIPRABOVIS® versus untreated control.

Group	n	Early abortions	Late abortions	Successful calving
HIPRABOVIS®	10	2	1	7
Control	10	1	5	4

Eleven calves were born alive: 7 in the HIPRABOVIS® group and 4 in the controls.

Two of 7 live calves in the HIPRABOVIS® group were identified as PIs (28.5%). The four live calves in controls were all identified as PI animals at birth (100%) by SNT titres (<1:4) and ELISA Ag positive.

When late abortion and PI calf numbers were combined, the protective effect of vaccination with HIPRABOVIS® was significant compared with the unvaccinated group (p=0.009).

Table 2. Comparative protection of pregnant cattle vaccinated with HIPRABOVIS® versus untreated control.

Group	LATE ABORTION		PERSISTENTLY INFECTED (PI)		LATE ABORTION + PERSISTENTLY INFECTED (PI)	
	Number of animals affected/ susceptible	% Protection HIPRABOVIS®	Number of animals affected/ susceptible	% Protection HIPRABOVIS®	Number of animals affected/ susceptible	% Protection HIPRABOVIS®
HIPRABOVIS®	1/8	87,5% Late Abortion	2/7	71,5% Foetal Protection	3/8	62,5% Global Protection
Control	5/9		4/4		9/9	

CONCLUSIONS

The results demonstrate that the use of Hiprabovis® BVD 1a strain in seronegative heifers provided significant protection against foetal infection when the herd was subsequently exposed to a high level of natural BVD challenge during gestation.

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Vaccination Failure in Eradication and Control Programs for Bovine Viral Diarrhea Infection

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Vaccination against bovine viral diarrhea (BVD) is one of the key elements to protect cattle herds from this economically important disorder. Bovine viral diarrhea virus (BVDV) is a pestivirus infecting animals at all ages with significant impact on reproductive, digestive, and respiratory systems. Financial burden caused by this pathogen prompts many farmers to introduce vaccination as the control and prophylactic measure especially when persistently infected (PI) individuals, being the main source of the virus in the herd, are removed after test-and-cull approach. The aim of the study was to compare the serological response in cattle herds where new PI calves were identified without prior removal of PI animals or despite their removal and after the introduction of whole herd vaccination against BVDV infection. Overall seroprevalence in 5 vaccinated herds was 91.7 and 83.3% using ELISA and virus neutralization test, respectively. Despite high titers for both vaccine and field strains of BVDV in analyzed herds the analysis of comparative strength of neutralization indicated that 41.4% of positive samples did not have a predominant titer against one specific subtype of BVDV. In 3 herds BVDV-1b subtype was identified while in 2 others it was BVDV-1d, while the vaccine used was based on BVDV-1a which was never identified in Poland so far. To increase the success of the BVDV eradication program, a careful approach is suggested when planning herd vaccination. Comparison of existing field strains and their similarity with vaccine strains at antigenic and genetic levels can be a useful approach to increase the effectiveness of vaccination and efficient protection of fetuses from persistent infection.

Keywords: bovine viral diarrhea, BVDV, vaccination, control, genetic diversity, cross neutralization

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INTRODUCTION

Bovine viral diarrhea (BVD) is one of the most important infectious viral diseases of cattle, caused by bovine viral diarrhea virus (BVDV), with an enormous economic and animal welfare impact on beef and dairy industries. This pathogen has a worldwide distribution and infects livestock and wildlife ruminants. BVDV belongs to the growing *Pestivirus* genus, within the family *Flaviviridae*. Based on the latest classification of the International Committee on Taxonomy of Viruses, genus *Pestivirus* is composed of 11 recognized species with 2 species of BVDV, namely *Pestivirus A* (according to former nomenclature: Bovine viral diarrhea virus species 1 – BVDV-1) and *Pestivirus B* (Bovine viral diarrhea virus species 2 – BVDV-2). Molecular typing allowed distinction of at least 23 subtypes within BVDV-1 and 4 within BVDV-2 (1, 2). Additionally, both virus species occur as two biotypes, i.e., cytopathic (cp) and non-cytopathic (ncp), according to their ability to induce cell

5′UTR sequences were obtained from a total of 6 BVDV-1 positive samples. For 4 of them, the sequence of the N^{pro} region was also generated. BLAST search and analysis with reference strains from GenBank showed that identified isolates belonged to BVDV-1b (herds A, OS1, and OS2) and BVDV-1d (herds K1, K2, and L).

A neighbor-joining tree was constructed which confirmed the subtyping obtained by sequence analysis, clustering the strains inter alia with the same subtypes detected earlier in Poland. To confirm the grouping within the 5′UTR region, sequences of the partial N^{pro} region of 4 viruses were analyzed. Representative strains from all farms are presented in **Figure 3A** for the 5′UTR region and in **Figure 3B** for the N^{pro} region, both along with vaccine strains available in the GenBank and subtype specific strains from earlier studies in Poland (identified by 2–3 digits and followed by two letters identifying the herd of origin). The GenBank accession numbers of sequences of virus strains used in phylogenetic analyses are shown in the figures.

The nucleotide identity, calculated with BioEdit (version 7.2.5), for BVDV-1b and BVDV-1d strains detected in this survey was in the range of 99.6–100 and 99.2%, respectively. Such a high similarity of the analyzed sequences may indicate one strain introduction into the herd.

DISCUSSION

Our study identified 5 herds where PI animals were detected despite ongoing vaccination against BVD. Field strains from PI individuals were of different subtypes from vaccine strain of BVDV. In three herds (marked as K, A, and L) vaccination followed the identification and removal of PI animals. In remaining 2 herds (OS1 and OS2) PIs were not identified and removed before the vaccination. The owners of those two herds expected that natural pressure from vaccine strain of BVDV will allow to get rid of virus source in a longer run so the vaccination was continued for 6 years before testing the whole herd for persistently infected animals. Despite different strategies, in both types of herds the vaccine did not protect the fetuses from intrauterine infection with BVDV subtypes different from the vaccine strain leading to the birth of virus shedders.

Extensive genetic variability of different strains of BVDV-1 (23 subtypes) and BVDV-2 (4 subtypes) hampers the success of vaccination worldwide. According to VIOLIN database (35), currently almost 130 licensed vaccines for BVD are available commercially and despite their common use many herds are not free from the virus and reinfections occur frequently.

In two retrospective phylogenetic studies of BVDV positive samples collected in Poland in years 2004–2011 and 2015–2018, which were based on 5′-untranslated region (5′-UTR) and N^{pro} coding sequences, 4 and 7 subtypes of BVDV were identified, respectively, but not BVDV-1a (13, 14). In the latter study predominant subtypes were BVDV-1b, BVDV-1g (27% each of all subtypes identified), and BVDV-1f (24%). BVDV-1d, which was second predominant subtype in Poland in years 2004–2011 (37% compared to 48% of BVDV-1b) was identified in 9% of all positive samples detected in 2015–2018. In this study two

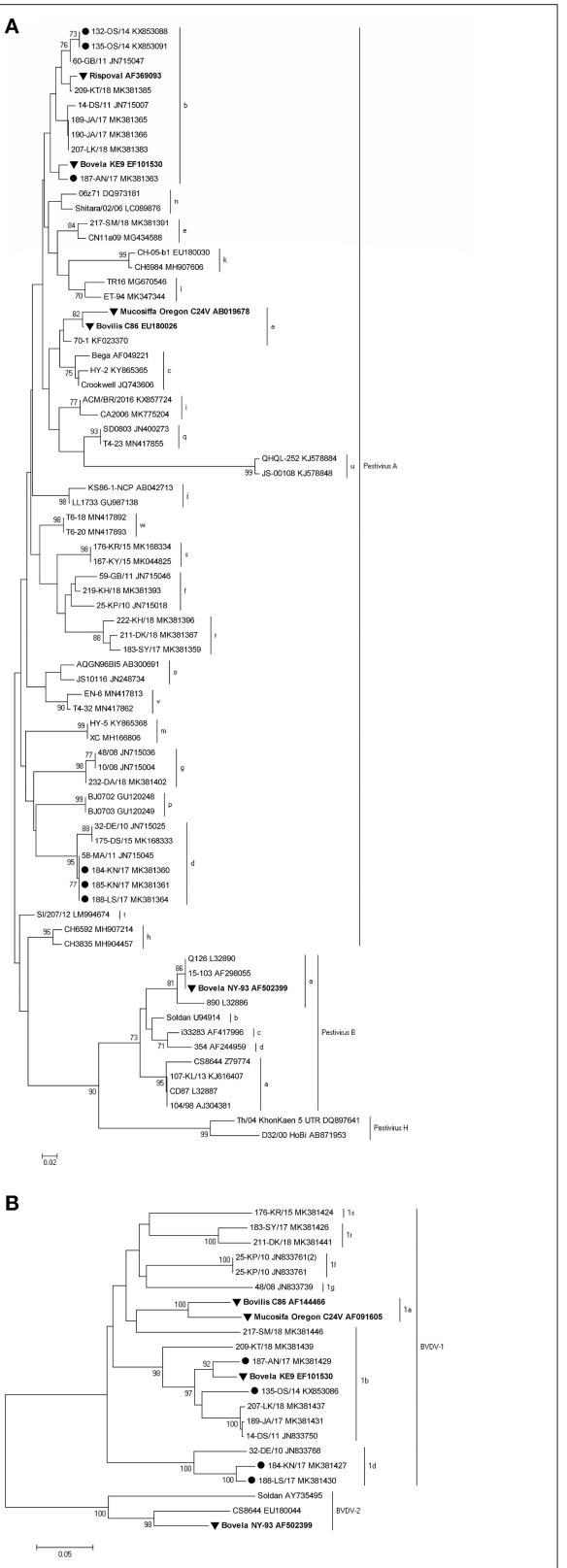

FIGURE 3 | Phylogenetic trees based on the partial **(A)** 5′UTR and **(B)** N^{pro} sequences obtained from vaccinated herds. Strains reported in this study are marked with a black circle, and vaccine strains are labeled in bold and marked *(Continued)*

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RESEARCH ARTICLE Open Access

Genetic diversity of Bovine Viral Diarrhea Virus from cattle in Chile between 2003 and 2007

Astrid Donoso, Felipe Inostroza, María Celedón and José Pizarro-Lucero*

Abstract

Background: *Bovine Viral Diarrhea Virus* causes significant economic losses in cattle. BVDV has high genomic diversity, with two species, BVDV-1 and BVDV-2, and at least twenty-one subgenotypes for BVDV-1 and four subgenotypes for BVDV-2. Vaccines are important tools to reduce the economic losses caused by this virus. However, vaccine strains must correspond to the antigenic profile of the viruses present in the region where the vaccine is applied. A restricted phylogenetic study with 14 viruses isolated from cattle between 1993 and 2001 showed that the genetic profile of BVDV in Chile consisted of viruses of both species and sub-genotypes 1a, 1b, 1c (currently 1j) and 2a. To determine more accurately the genetic profile of BVDV in Chile, in this study a larger number of viruses obtained from bovines between 2003 and 2007 were typed.

Results: The study was performed using partial sequences from the 5′ noncoding region (5′UTR) and E2 coding region of the viral genome of thirty-five Chilean viruses isolated from geographic regions that have 84.6% of the Chilean cattle. All tested viruses belonged to species BVDV-1. Eighteen viruses belonged to BVDV-1j subgenotype (51.4%), twelve belonged to BVDV-1b (34.3%) and five belonged to BVDV-1a (14.3%). The Chilean BVDV-1j viruses showed low genetic diversity, both among themselves and with the BVDV-1j present in other regions of the world. This could be explained by a relatively recent introduction of this viral subgenotype in cattle, which agrees with its low geographical distribution worldwide. Otherwise, Chilean BVDV-1b viruses grouped into a single cluster, different even than the viruses present in Argentina and Brazil, countries geographically close to Chile, a process of local evolution that could generate antigenic differences between the Chilean viruses and the viruses used as vaccine strains.

Conclusions: The high presence of viruses of the BVDV-1j subgenotype, which show major antigenic differences with BVDV-1a and BVDV-1b subgenotypes used in the commercial vaccines, suggest that BVDV-1j viruses could be an emergent subgenotype of BVDV in cattle in South America and suggest evaluating an update of the vaccines used in Chile.

Keywords: *Bovine viral diarrhea virus*, *Pestivirus*, BVDV, Genetic diversity, 5′UTR, E2

Background

The *Bovine Viral Diarrhea Virus* (BVDV) is a virus of worldwide distribution, which causes a wide variety of clinical symptoms in cattle, being recognized as the viral agent that causes the main economic losses in the global cattle industry [1, 2].

In immunocompetent animals, BVDV usually is associated with respiratory and gastrointestinal diseases of different severity, hemorrhagic syndrome, and reproductive problems, such as infertility. BVDV also causes immunosuppression, which increases the severity of diseases caused by other pathogens. The virus is able to cross the placenta infecting the fetus, causing embryonic reabsorption, fetal mummification, abortion and congenital malformations, especially of the central nervous system. Fetal infection with a non-cytopathic virus in the first trimester of pregnancy causes persistently infected

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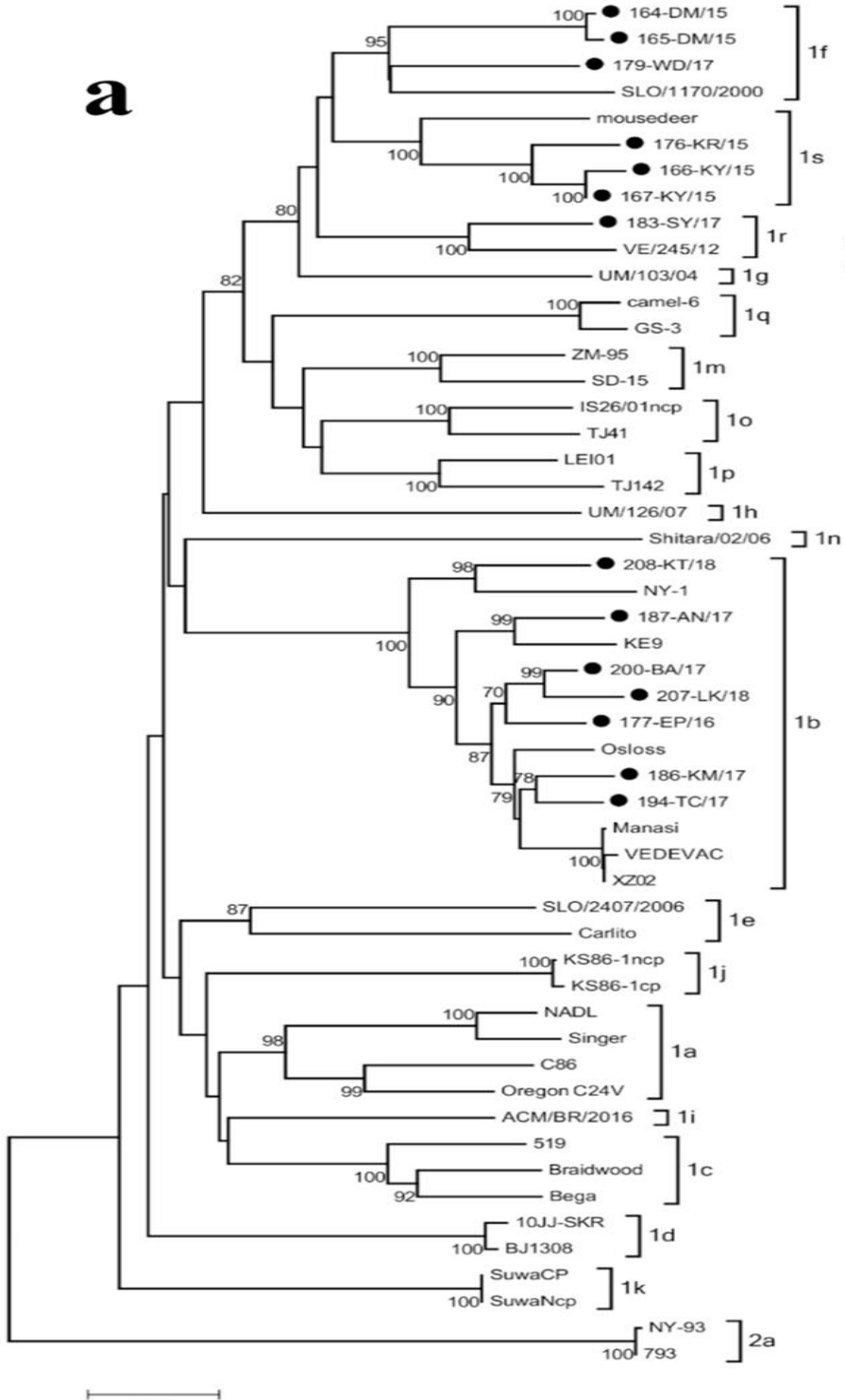
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183-SY/17
VE/245/12
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UM/103/04
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camel-6
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1q
ZM-95
SD-15
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IS26/01ncp
TJ41
1o
LEI01
TJ142
1p
UM/126/07
1h
Shitara/02/06
1n
208-KT/18
NY-1
187-AN/17
KE9
200-BA/17
207-LK/18
177-EP/16
Osloss
186-KM/17
194-TC/17
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Research Article

Effect of Vaccination Against Ibr/Bvd on The Reproductive Performances of Brava Dos Açores -A Bovine Lidia Breed

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Abstract

From February 2012 to March 2018, 94 females aged more than 24 months were randomly divided in two groups in which 43 were vaccinated and considered the experimental group and 51 were not vaccinated and used as control. For the characterization of the reproductive indexes, reproductive registers made by the animal's owners as well as data obtained through the sanitary visits and reproductive consulting of our team allow to determine the follow parameters: number of parturitions; the mean age at first calving; calving to calving interval and calving to conception interval. The first service conception rate, heat intervals as well as the percentage of cows pregnant at first pregnancy diagnosis examination was also recorded. Data obtained from all animals clearly demonstrated that all parameters studied were better in the vaccinated group when compared to unvaccinated animals. The results where the benefits were most pronounced were in the calving to calving interval, where values decreased from 889 ± 25 to 471 ± 35 days respectively to unvaccinated and vaccinated females. This great improvement led that the real fertility raised from 28.6% of the unvaccinated group to 71.1% for the vaccinated group.

Keywords: Brava dos Açores; IBR/BVD; Vaccination; Reproduction; Bullfighting bull

Introduction

Lydia cattle breed usually called "Brava" is produced extensively and therefore the clinical manifestations of any pathological process go unnoticed to those responsible for the herd. The production system in this breed is determined not only by its characteristics, but also for its productive objective [1]. The "Brava" bovine breed, due to its rusticity and peculiarity in management, develops in large land surfaces, about 500 hectares in the Alentejo and central region, 300 hectares in the Lisbon region, and 30 hectares in the Azores [2] Reproductive management is one of the main key factors in the profitability of this breed, so breeding is an essential task on farms: cows and heifers are divided into several groups, known as batting lots [3], being the calf's birth of central importance in this type of bovine production [4]. The copulation periods are from November to April/May and 90% of the delivery distribution occurs between August and March.

Productivity, in suckling cows' herds, depends on reproductive efficiency and it is often measured by the number of offspring per breeding animal per unit of time [5] and collaborators [6] postulated that keep a suckler cow for a year costs between 500 and 900 €, being essential the production of a viable calf every 365 days, which cover this expenditure and give profits The reproductive failure is considered the main economic loss for beef cow-calf operations worldwide. The economic impact due to of embryonic and fetal losses in the US beef industry to be more than $1.2 billion yearly, with approximately 40 106 cattle exposed to breeding [7]. Several factors affect the length of the calving interval and nutrition, management and animal health are considered the most relevant [8].

The lifetime productivity of the beef-bred female commences from the onset of puberty and will be dictated by subsequent critical events including age at first calving, duration of the postpartum interval after successive calvings, conception and pregnancy rate, and ultimately manifested as length of inter-calving intervals [9]. Reproductive efficiency is key to the biological and economic

sustainability of suckled beef enterprises as well as is a major factor determining production and ultimately the profitability of beef cow enterprises. Reproductive control of the herd is only one component of the entire farm management system. Communication to the farmer of the cost benefit of veterinary services is a key feature for the success of health of the herd [10] Reproductive efficiency is measured by the timeliness of getting a cow bred back and producing a healthy calf within a 12-month period (Table 1).

Table 1: Reproductive parameters in suckling cows (Ifende et al., 2014).

Parameter	Goal
Copulation season length	< 90 days
Pregnancy rate (35 days after the end of the copulation season)	> 90%
Percentage of calves born alive	> 93%

How well this is done determines the number of calves that will be marketed each year, thus directly influencing upon the gross income of a cow/calf operation [10]. A long interval between deliveries results directly from the increase of the calving conception interval and is expressed by the number of "Open days". Calving and calving-to-conception intervals are commonly used as indicators of reproductive efficiency [11] Calving interval describes the number of days between successive calving's [9]. Calving intervals of 365 days would be ideal, resulting in one calving per cow per year, but intervals of 13-13.5 months are considered acceptable [11]. Optimal productivity should be the goal of each cow-calf producer and that productivity begins with cows producing at least one calf per year. This way, with proper management, including a good health and nutrition program, that goal is easily attainable [12]. This practice will result in a maximal cash flow: as the lowest calving interval, corresponds to the maximal number of calves produced [13].

Wikse [14] assumed that reproductive diseases are the greatest illness threats to the production and profitability of beef cattle herds. Infections by reproductive tract result in a wide array of losses including embryonic deaths, abortions, stillbirths and weak calves [14] These infectious can also create losses all throughout the reproductive cycle by decreasing ovulation rates, fertilization rates, embryonic, and fetal survival rates [15].

The prevalence of infectious agents in cattle herds may be due to several factors: animal and herds health management, diagnostic method, quality of the samples to be analyzed and the production system [16]. Health stress, reducing the conception rate, can be, however, restricted by a complete vaccination program, meeting mineral and nutritional requirements, and deworming young and thin cows [17]. Moreover, viral reproductive diseases, such as infectious bovine rhinotraechitis (IBR), bovine herpesvirus type 4 (BHV-4), and bovine viral diarrhea virus (BVDVV), can affect cattle all over the world, causing significant abortion losses and infertility [18] and up to 50% of pregnancy losses in cattle are associated with these infectious diseases. On the other hand, Bovine Herpesvirus Type 1 (BHV1) and bovine viral diarrhea virus (BVDVV) are two of the most important viruses of cattle, causing significant diseases. Both viruses induce a state of persistence in carrier animals, which is life-long, although the state of persistence is quite different between BHV1 and BVDVV infections [19].

Infectious bovine rhinotracheitis caused by the BHV-1, which negatively affects the production performance of infected cattle herds, results in considerable economic losses on cows [20]. BHV-1 leads to the IBR respiratory disease presenting the animals genital diseases in females or males such as infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). Other clinical syndromes, such as: conjunctivitis, metritis, mastitis, encephalitis, abortion, and enteritis can also be also very commonly observed [20]. The BHV-1 that causes IBR is known to directly impair ovarian function and embryo quality.

Regarding BVDV, this is the pathogen that most affects the reproductive system, in cattle, leading to poor conception rates, abortions and congenital defects, and also reducing the animal's resistance to other respiratory and enteric pathogens [21]. BVDV virus (BVDVV) infects reproductive tissues and interferes with follicular and embryo development [22,23]. Vaccinations as an integral tool for preventing disease and for maintaining herd health, may improve reproductive efficiency by reducing infertility, embryonic and fetal deaths, and abortions [24].

Up to now, there are no conclusive studies on the efficiency of vaccination against IBR/BVDV in the prevention of reproductive losses caused by these diseases in cattle. In addition, there is an evident concern that BVDV modified live vaccines may cause fetal losses, so decision-making on whether or not vaccination should be carried out is an important dilemma for practicing veterinarians working in the field [25]. Routinely, vaccination is commonly used to control of BHV1 and BVDV. For BVDV, removal of PI animals from herds and avoiding the reintroduction of PI animals into the herd (biosecurity) are also recognized as important control measures [19]. O´Connor and collaborators suggest that monitoring of IBR, and BVDV titers may be important in identifying causes of poor herd reproductive performance [26]. In view of the above exposed, and the lack of consensus on the effect of IBR/BVD vaccination on reproductive parameters in cattle, the aim of the work presented here is to study the effect of a vaccination against these two viruses on the reproductive parameters of bovine Lidia breed called "Brava dos Açores".

Materials and Methods

The present study was developed between February 2012 and March 2018 in Terceira Island using 94 females aged more

than 24 months in a geographical area with high concentration of fighting cattle, where animals are raised using an extensive system. The color, which is not an important trait in the selection process, were usually black or grey, avoiding brindled, roan red or chestnut animals. In the same way as timid and docile animals of both sexes are culled out after special tests and sold for beef, they were excluded from the study. After selection, animals were, randomly, divided in two groups in which 43 were vaccinated and considered the experimental group and 51 were not vaccinated and used as control. Animal's belonging to the experimental group were vaccinated until 2015 with one dose, of HIPRABOVIS 4 being each time revaccinated 21-30 days later and then a booster vaccination was administrated twice a year, in the neck muscles. Heifers were vaccinated using the same protocol, in which the first vaccination was administered one month before the first mating.

Then from February 2016 to March 2018, experimental animals were vaccinated using the same protocol as afore described, with HIPRABOVIS IBR MARKER LIVE® and HIPRABOVIS BALANCE ®, to allow for IBR the identification between infected or vaccinated animals, which it could not be possible with non marker conventional IBR vaccines IBR vaccines. Data, for the characterization of the reproductive indexes was obtained using the reproductive registers made by the animal's owners as well data obtained through the sanitary visits and reproductive consulting of our team. Moreover, for each animal, all data inserted in the National Bovine Register System (SNIRB), namely parturitions and dead animals, were also considered, and the reproductive parameters were calculated according Potter and Anderson [27]. Briefly, the data were processed to determine the mean age of the breeding herd, the number of parturitions.

The average age at first calving as well as the calving to calving interval, calving to conception interval, the first service conception rate, heat intervals as well as the percent of cows pregnant at first pregnancy diagnosis. Moreover, data of the mean age of the reproductive herd was calculated including the age of all females in the right time for breeding, confirming their ovarian cyclicity by their estrous behavior or by echography made during the reproductive visits. In case of pathologic problems such as cystic ovaries, animals were treated. Concerning fertility rate, defined as the number of cows that gave birth to those placed on mating, the fertility rate considered was the annual fertility rate, many times confused with the apparent fertility rate that is often referred to by the owner. In fact, if the farm's calving interval is 450 days, for example, the cow will produce a calf every 450 days (not 365 days) and for that example the annual fertility value can be adjusting by dividing the 365 days by 450 days, which corresponds to a correction factor of 0.81 [20]. The apparent fertility rate was consequently obtained by multiplying the fertility rate by the correction factor, which has been calculated by dividing 365 by the interval between parturitions. Since the only direct contact with the animals was on the days they were vaccinated. As this is a general management situation for cattle farms in general, the protocol of work reported in this manuscript has not been previously evaluated by any ethics committee. Therefore, at no stage of the work were animal rights ignored. Statistical analysis was performed using the software SPSS and the results are presented as mean ± standard deviation.

Results and Discussion

In the present study the effect of bovine Lidia breed called "Brava dos Açores" vaccination against IBR/BVD on the reproductive parameters, has been studied. Animals (n=94) were divided in two groups in which animals belonging to group A (n= 43) were vaccinated with HIPRABOVIS® IBR MARKER LIVE and HIPRABOVIS® BALANCE and animals belonging to group B (n=51) were used as control. The reproductive parameters studied was the age at the first calving, the calving to calving interval between calves, the apparent and the real fertility.

In the Table 2 can be observed the reproductive results, obtained the vaccinated and non-vaccinated with the Hiprabovis® BVD animals. When comparing the interval between calvings for the animals belonging the two groups, we noticed that this value influences the calculation of the real fertility of each group of animals (vaccinated and not vaccinated). Our research group demonstrated that, on average, the birth interval of the Bovine Lidia Called "Brava Dos Açores" was 680 ± 75 days, which in Spain it has been presented of 432.69±28.16 days [28] and in Portugal mainland this interval is, on average of 535.08±196 [19]. Calving and calving-to-conception intervals are commonly used as indicators of reproductive efficiency [29]. The calving interval comprises the interval from calving to first heat (postpartum interval), number and length of services of the cow until pregnancy (conception length), and the length of gestation [30] (Table 2).

Table 2: Reproductive results obtained for both groups, vaccinated and non-vaccinated with the HIPRABOVIS® RANGE vaccines

Reproductive Parameters	Vaccinated Group (43 animals)	Non-vaccinated group (51 animals)
Mean Age	90.07 months	109.5 months
Age first delivery	40.8 months	41.8 months
Interval Between calvings	471 ± 35 days	889 ± 25 days
Apparent fertility	91.80%	69.60%
Real Fertility	71.10%	28.60%

When comparing the interval between calvings for the animals belonging the two groups, we noticed that this value influences the calculation of the real fertility of each group of animals (vaccinated and not vaccinated). Our research group saw that In the that the mean value of the interval between of the Bovine Lidia Called "Brava Dos Açores" births for is 680 ± 75 days, and in Spain it has been presented of 432.69±28.16 days [28], and in Portugal mainland this interval is, on average of 535.08±196 days [19] Calving and calving-to-conception intervals are commonly used as indicators of reproductive efficiency [17].The calving interval comprises the interval from calving to first heat (postpartum interval), number and length of services of the cow until pregnancy (conception length), and the length of gestation [30].

In general, but particularly for a grass-based production system with seasonal calving's, fertility is of major economic importance. A delay in conception due to poor fertility prolongs inter-calving's interval causing a shift in calving pattern, which lead to the culling of the animals [31] Different factors contribute to the variation of the calving interval. Some of them depend largely upon the genetic material and others only on the environment or the interaction between genetic and environmental factors [32] and other factors like nutritional management and BCS influence pregnancy maintenance. Moreover, diseases, such as mastitis, metritis and retained placenta may influence negatively the reproductive performance in dairy herds. Possible causes include infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), leptospirosis, neosporosis, salmonellosis and venereal campylobacter can influence the fertility and increase the calving interval in herds [33].

BVDVV transmission is by nasal-pharyngeal secretions, urine, aerosols and by venereal route. Faeces are a weak source of infection. The PII calf (persistently infected immunotolerant) occurs when the fetus is infected before the 120th day of gestation; this is the time in which its immune system is mature and functional. The presence of PII animals in a group is key to the appearance of acute outbreaks of BVDV, as it is highly infective throughout its lifetime [37].

It nearly always presents slight or unapparent clinical symptoms, except in two situations: when a gestating cow is infected or when there is a co-infection with another virus, for example, those to tropism of the respiratory tract (BRSV, IBR, PI-3, Adenovirus, etc.); when cow is in heat: breeding and infertility (of 6 to 8 weeks). Early resorptions, and in pregnant cow: infection in the first four months of gestation may lead to still births, mummification and early fetal death. If the fetus becomes infected and is not aborted, it will be born a lifetime carrier of BVDV (PII). Infection after 120 days may harm the fetus (cerebral Hypoplasia) but it does not become immunotolerant. Concomitant infections: BVDV induces a marked immunosuppression that exacerbates concomitant infections and, more commonly, those that are respiratory in nature [38]. BVDV infection leads to a range of disease problems but reproductive failure is the principal cause of economic loss. Vaccination offers the advantage of controlled, safe and strategic protection against BVDV [39].

Richter and collaborators (2017) showed the ability of the virus to replicate and affect the cells of the ovarian follicles in cows at any time during follicular development in animals experimentally infected with the virus. The virus can have a detrimental impact on the developing fetus at all stages [40], but the ability of BVD to directly affect ovarian tissue is clear. Indeed, if a PI cow becomes pregnant, the result is always a PI calf, probably due to viral replication in the ovarian and reproductive tissues [41]. Therefore, Fulton postulated that it could be assumed that BVD is related to fertility problems and greater season repetition in animals that have or have had [42], detected a lower conception rate during viral circulation phases in the herd, thus confirming that BVDV infection temporarily reduced the conception rate and found clearly negative effects with more AI (artificial insemination) and pregnancy [43]. Early embryo death has been found in experimental studies about the effects of experimentally induced BVD infection in study animals [44].

The effect of fetal BVD infection depends, once again, on the type of virus, the virulence of the strain and the moment during pregnancy when the pregnant animal are infected and present viraemia [45]. It actually depends on the foetus's immune capacity to fight off the infection (Identification of cell membrane proteins linked to susceptibility to bovine viral diarrhea virus infection [50].

Infectious bovine rhinotracheitis (IBR) also known as INFECTIOUS PUSTULAR VULVOVAGINITIS, IBR, IPV, RED NOSE, has the ability of reaching the trigeminal ganglion provoking thus a latent infection. These latent infections can possibly reactivate, with or without clinical symptoms [37]. It is transmitted by direct contact and aerosol. Venereal transmission. The entire group may be affected within a period of 2 to 5 weeks. The incubation period is 3 to 7 days. Once infected, the animal will be a carrier of this herpes virus throughout its lifetime. The reproductive symptoms in the cow may include pustules reproductive symptoms in the cow may include pustules in the vaginal and vulval mucosa, clear to purulent discharge. On the other hand bulls may present pustules on the penis and prepuce, balanoposthitis (named as venereal form). Also, low reproductive indices of the farm, low fertility, endometritis and irregular returns to estrum (named as reproductive form) and miscarriages at 6 and 8 months of gestation as well as stillbirths, can be identified at herd level. Usually the reproductive form (named as the abortive form) is the most important clinical condition observed of this disease [51].

Carried in the bloodstream via white blood cells, the virus is able to gain access to the placenta and eventually the developing

fetus. After arriving at the placental tissue, BHV-1 causes the condition placentitis, which is a general term used to describe any inflammation of the placenta. This disease is often accompanied by vasodilation (blood vessel expansion), which increases the permeability and blood flow to the placenta and thereby the developing fetus. The virus is particularly drawn to the fetus, as it prefers actively growing tissue. The time from infection to ultimate abortion varies between animals, although once the virus begins replication in the developing fetus, death can occur in as little as 24 hours [52].

Abortion may not occur immediately after death as the fetus often goes through a period of autolysis. Though embryonic death can occur early on, most loss is associated with abortions that occur in animals greater than five months in gestation. Subsequent necrosis of the fetal liver and placental tissue are often identified post-mortem. Additionally, the expelled fetus will have a dark red coloration due to the blockage of hemoglobin. The tricky part in identifying an IBR-related abortion is that often the abortion occurs without any other clinical signs of IBR. Rypula and collaborators (2017) determined that for the success in the eradication of BoHV-1, the following conditions must be fulfilled: vaccination of all animals in a timely manner constant animal movement control (article30). In fact, systematic prophylactic vaccination, live or inactivated vaccines are a way to control this disease in cow herds.

Abortion rates associated with IBR have been reported to range from 5 to 60 percent in herds without a vaccination program. [37]. Management techniques to prevent pregnancy loss in dairy herds, such as hormonal manipulation, thermal comfort, and nutritional management are increasingly being implemented into dairy systems worldwide [53]. Conversely, immunization strategies developed to reduce the impact of reproductive diseases, such as vaccination against IBR, and BVD, do not receive proper attention. The target animals and vaccination regimes for vaccines against the bovine rhinotracheitis (IBR) and the bovine viral diarrhea virus (BVDV) are very similar. The two vaccines can be applied at the same day for the first or second dose of the BVD basic vaccination and then at the booster vaccinations [54].

Vaccination is therefore widely applied to control these viruses. The target animals and vaccination regimes for BHV-1 and BVD vaccines are very similar. In general, multivalent cattle vaccines are the preferred choice of farmers and veterinarian because they simplify animal handling and therefore reduce costs of vaccination and animal stress. In general, multivalent cattle vaccines are the preferred choice of farmers and veterinarian because they simplify animal handling, reducing costs of vaccination and animal stress. (Compatibility of a live infectious bovine rhinotraheitis (IBR) markervaccine and an inactivated bovine viral diarrhea virus (BVDV) vaccine). In compliance with the BHV-1 eradication programs, it is essential that vaccines allow differentiation between vaccinated and infected animals (so-called IBR marker vaccines) [55]. In order to respond to the market requirements to reduce animal handling to a minimum, one live IBR marker vaccine and one inactivated BVD vaccine with a proven fetal protection claim can be applied on the same day. the safety of the simultaneous (inactivated vaccine serves as solvent for the live vaccine) [55] and the concurrent (vaccines injected at two different sites) use of the two vaccines had been tested under field conditions with no local or general reactions [56].). In order to respond to the market requirements and to improve the animal health in extensive cow calf herds, the protocol using an IBR marker live vaccine and a tetravalent which contains BVD inactivate, PI3 inactivate and BRSV live not only cover the reproductive viruses, but also the respiratory producing a broad immunity response in the herds.

There are some studies that demonstrated the benefit of vaccination against BVD and IBR. Casademunt et al. (2016) emonstrated that the use of HIPRABOVIS® BALANCE with BVD 1a strain in seronegative heifers provided significant protection against fetal infection when the herd was subsequently exposed to a high level of natural BVD challenge during gestation. In a meta-analysis by Newcomer et al. (2015) demonstrated a quantitative benefit in BVD vaccination, with a 45% decrease in the number of abortions, an 85% decrease in fetal infections, and a 5% increase in pregnancy risk when compared vaccinated animals with unvaccinated co-inhabitants.

Regarding IBR, Newcomer (2017) demonstrated a 60% decrease in the risk of abortions in vaccinated cattle supporting the benefit of bovine herpesvirus 1 vaccination in the prevention of abortions.

Pereira and collaborators [27] demonstrated that vaccination against IBR, BVD using a commercial vaccine improved reproductive efficiency parameters in herds without a history of vaccinating the cow herd against these reproductive pathogens, Moreover, cows should receive [57-60] both doses of the vaccine prior to AI to ensure maximum antibody response and optimal reproductive outcomes during conception, as well as early- and mid-gestation. In our study, it was demonstrated that an herd health management, in which the use of commercial vaccines for IBR and BVD, PI3 and BRSV there was a substantial reduction of the interval between births, influenced the actual fertility rate. The group of cows vaccinated presented a value of 471 days± 35 days whereas in the group of unvaccinated cows the value increased for 889± 25 days. Pereira et alin their experiment saw that cows vaccinated with BVD and IBR vaccine had greater pregnancy rate groups also non vaccinated cows and pregnancy loss differ between groups also [27]. Accordingly, the incidence of pregnancy losses was reduced, as well as statistically insignificant, in ranches that vaccinated cattle against IBR, BVD, compared with ranches that did not vaccinate. Beginning the vaccination program before estrus synchronization further increased its benefits on pregnancy rates on Days 30 and 120, whereas it reduced, to some extent, pregnancy

losses. This outcome was attributed to the profile and timing of antibody responses upon vaccination using the vaccine.

Conclusion

Results of the present research clearly demonstrated that the vaccination of the Brava dos Açores bullfighting animals with a broad vaccination protocol against IBR, BVD, BRSV and PI3 substantially improved the reproductive events of these animals [61]. The results where the benefits were most pronounced were in the interval between calvings, where values decreased from 889 ± 25 to 471 ± 35 days respectively to unvaccinated and vaccinated females. This great improvement led that the real fertility raised from 28.6% of the unvaccinated group to 71.1% for the vaccinated group [62].

Acknowledgments

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Conflict of Interests

The authors declare the absence of any economic or conflict of interest related to this research.

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Hiprabovis 3 - Protection from transient viral infection (BVD and IBR challenge study)

Objective

To evaluate the efficacy of Hiprabovis® 3 for protecting calves against a challenge from the viruses Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhoea (BVD).

Study Design

Two groups of 10 three month old Friesian calves were enrolled in the study.

Within each group half of the calves were vaccinated with Hiprabovis 3 and the remainder left unvaccinated (negative control).

Two doses of Hiprabovis 3 were administered by intramuscular injection, with an interval of 21 days between doses.

The two groups were challenged 42 days after vaccination with either IBR or BVD viruses as outlined in table 1.

Calf treatment	Trial 1	Trial 2
	Challenge Virus: IBR	Challenge Virus: BVD
Hiprabovis 3 Vaccinated	5	5
Unvaccinated control	5	5

Table 1: Challenge programme

Challenge was via the intranasal route. The infective dose administered for each of the viruses is shown in table 2.

Virus	Strain	Titre $TCID_{50}$/mL
IBR	FM	10^7
BVD	NADL	10^5

Table 2: Infective dose of challenge viruses

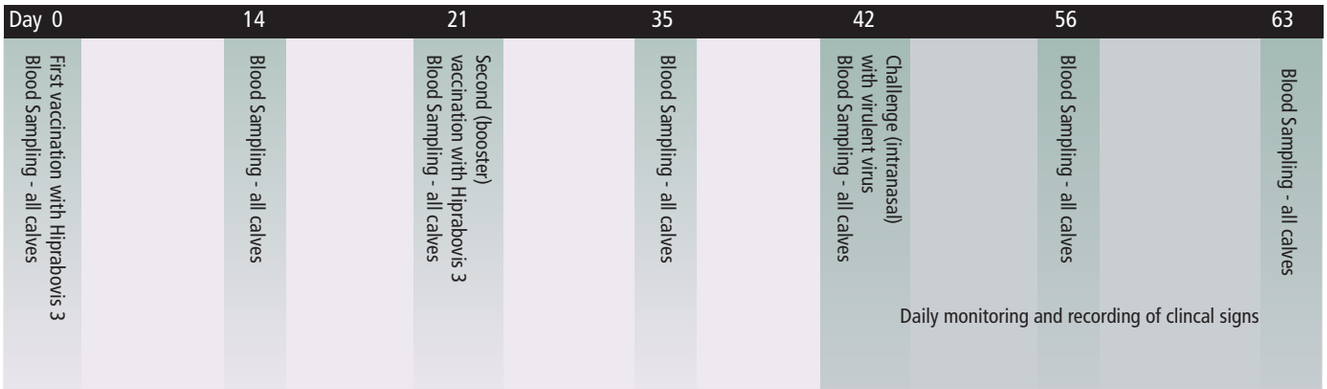
Measurements of interest were serological response (to vaccination, and following challenge) and clinical signs of bovine respiratory disease. Calves were monitored for 21 days following the challenge.

The clinical signs recorded daily following challenge infection included elevated temperature, cough, anorexia, nasal discharge and watery eyes.

Throughout the study serological response was measured using ELISA Relative Indexes (RI) for IBR and ELISA Inhibition Percentages (IP) for BVD.

Calves were housed in stables with straw bedding throughout the study. The IBR infected and BVD infected groups were kept separate. Vaccinated and unvaccinated animals within a group were housed together.

Study Timeline



Results

Virus	Group	Calf	Days after Challenge													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14 - 21
IBR	Vacc	1	H, A	-	-	-	-	-	-	-	-	-	-	-	-	-
		2	H, A	A	-	-	-	-	-	-	-	-	-	-	-	-
		3	H	-	-	-	-	-	-	-	-	-	-	-	-	-
		4	H	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	6	H, A	H, A	-	-	-	-	-	-	-	-	-	-	-	-
		7	H, A	H, A, T	T, H, SN	T, H, SN	T, H, SN	T, H, SN	T, H, SN	SN	SN	SN	-	-	-	-
		8	H, A	H	H, A	H, A, SN	H, SN	T, H, SN	T, SN	SN	SN	-	-	-	-	-
		9	H	H, A	H, A, SN	H, A, SN	A, SN	H, SN, A,T	L, T, H, SN	L, T, H, SN	L, T, H, SN	L, T, H, SN	L, T, H, SN	L, SN	L, SN	L, SN
		10	H	H, A	H	-	-	-	-	-	-	-	-	-	-	-

Key: A: Anorexia T: Cough SN: Nasal Discharge I: Watery eyes H: Hyperthermia Vacc: vaccinated with Hiprabovis 3

Table 3: Clinical Signs after challenge with virulent IBR Virus

Virus	Group	Calf	Days after Challenge													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14 - 21
BVD	Vacc	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Control	16	-	SN	SN	SN	SN	-	-	-	-	-	-	-	-	-
		17	-	-	SN	SN	SN	SN	SN	SN	SN	SN	-	-	-	-
		18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		19	-	-	SN	SN	SN	SN	SN	SN	SN	SN	-	-	-	-
		20	-	SN	SN	SN	-	-	-	-	-	-	-	-	-	-

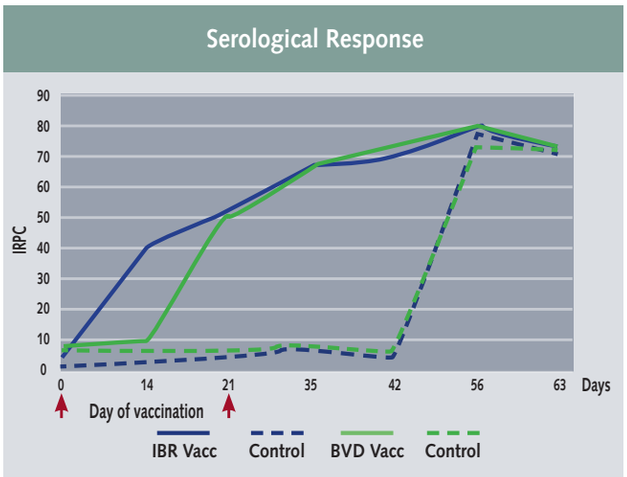
Key: A: Anorexia T: Cough SN: Nasal Discharge I: Watery eyes H: Hyperthermia Vacc: vaccinated with Hiprabovis 3

Table 4: Clinical Signs after challenge with virulent BVD Virus

Clinical signs were mild or absent in all vaccinated calves.

In contrast, moderate to severe clinical signs were observed in many of the control animals after challenge and half of these animals continued to show clinical signs for a week or more. The clinical observations are shown in tables 3 and 4.

The study animal's serological response, throughout the study, is shown in graph 1 (below).



Graph 1: Serological Response in trial calves

Conclusions

Hiprabovis 3 stimulated an active immune response in vaccinated animals. Antibody levels rose as expected following vaccination and titres were protective shortly after the booster vaccination.

Disease challenge resulted in a serological response in the control animals. This group suffered from clinical signs of bovine respiratory disease (BRD) following intranasal challenge with virulent IBR or BVD virus. In contrast, clinical signs were mild and transient or non-existent in the Hiprabovis 3 vaccinated group.

It was concluded that Hiprabovis 3 provided vaccinated calves significant protection from clinical signs following intranasal infection with virulent IBR and BVD.

Regulatory Study; data on file.
Hiprabovis 3 is a trademark of Laboratorios Hipra S.A. and is distributed in New Zealand by AgriHealth NZ Ltd.

Hiprabovis 3 is a NZ Restricted Veterinary Medicine, registered pursuant to the ACVM Act, 1997 No A07140.

Age of Calf Vaccination - Effect of Maternal Antibodies on Serological Response to Vaccination with Hiprabovis 4#

Objective

To investigate the effect of maternal antibodies on the immunity of calves when vaccinated with Hiprabovis® 4 at different ages.

Study Design

Thirty healthy Friesian two year old cows were enrolled in the study. Eighteen of these animals were seropositive for Infectious Bovine Rhinotracheitis (IBR), Parainfluenza Virus 3 (PI-3) and Bovine Viral Diarrhoea (BVD). These animals were divided into three groups (A, B and C), each with six cows. The remaining twelve were seronegative for IBR, PI-3 and BVD. These animals were divided into two groups of six cows (D and E).

The cows were inseminated and retained in the trial facility throughout their pregnancy. Their immunological status remained unchanged during this period.

When the calves were born, they were separated from their dam. Colostrum from each of the trial cows was obtained for the first 48 hours. Calves were fed every 3 – 4 hours with 750mL of their own mother's colostrum. From day three onward, calves were fed cow's milk.

At 8 days of age calves were blood sampled and colostral antibody levels measured.

The trial groups were as follows:

Group	Status of Dam	Vaccination of Calf
A	Seropositive	Not vaccinated (seropositive control group)
B	Seropositive	Vaccinated at 1 month of age and revaccinated 21 days later
C	Seropositive	Vaccinated at 2 months of age and revaccinated 21 days later
D	Seronegative	Not vaccinated (seronegative control group)
E	Seronegative	Vaccinated at 2 months of age and revaccinated 21 days later

Calves were blood sampled on days 8, 30, 51, 60, 81, 109, 137, 165, 193, 221 and 249. The level of antibodies for each antigen was tested (IBR, PI-3 and BVD).

The serological methods used were ELISA Relative Indexes (RI) for IBR, Haemagglutination Inhibition (HI) for PI-3 and ELISA Inhibition Percentages (IP) for BVD.

Groups A and D were kept separate from the vaccinated animals and from each other.

Results

Antibody levels on day 8 in calves fed colostrum from seropositive dams were considered protective. Conversely calves fed colostrum from seronegative dams were themselves seronegative on day 8.

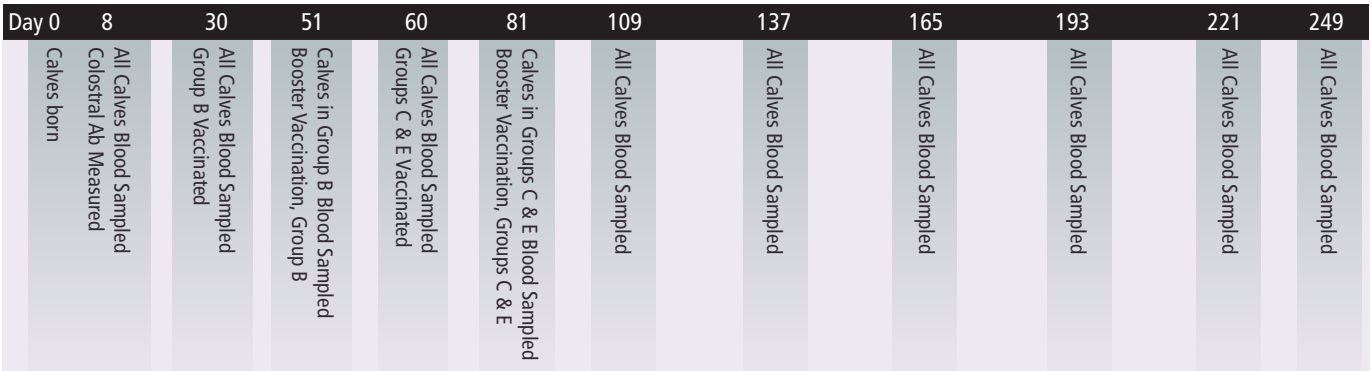
	RI ELISA IBR	HI TITRE PI-3	IP ELISA BVD
Group A	65	1/128	89
Group B	70	1/256	93
Group C	73	1/128	79
Group D	2	<1/4	2
Group E	1	<1/4	3

Table 2: Calf antibody levels on day 8.

Antibody levels declined over time in Group A, and by 60 days of age maternal antibodies were no longer considered protective.

Group B were vaccinated at 1 month of age and booster vaccinated 21 days later. Nonetheless, the antibody levels in this group followed the same kinetics as Group A. The titre of maternal antibody was too high to allow the calves to mount a humoral response to vaccination. This demonstrates interference of maternal antibodies in the active immune response of the calves in this group.

Study Timeline

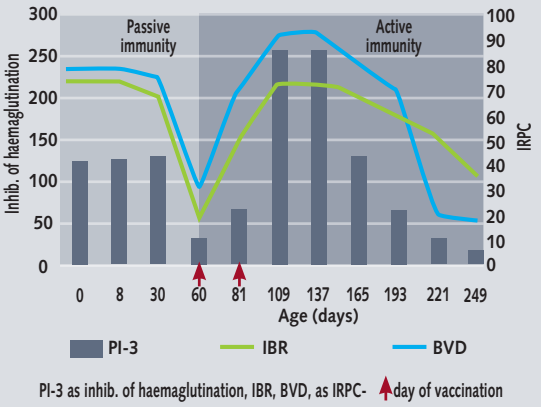


Calves vaccinated at 2 months of age (with booster vaccination 21 days later) (Group C) demonstrated a satisfactory humoral response. Antibody levels increased substantially after vaccination and were protective one month after the booster vaccination. These levels remained high for the duration of the study (> 8 months). The serological results from this group can be seen on graph 1.

Antibody levels in Group D remained the same throughout the study. This group verified that there was no infection challenge on the study farm for the duration of the study.

The serological response in group E was similar to that in group C.

Antibody response in young calves (with maternal antibody) vaccinated with Hiprabovis®4



Graph 1: Serological response to the three Hiprabovis 3 antigens in calves first vaccinated with Hiprabovis 4 at two months of age.

Conclusions

Calves from both seronegative and seropositive dams were vaccinated with Hiprabovis 4 to evaluate the serological response.

From two months of age, maternal antibodies from seropositive dams did not interfere with the calf's response when compared with calves born to seronegative cows.

Vaccination of one month old calves in the face of maternal antibody demonstrated maternal interference, and the calves did not mount an acceptable immune response following vaccination.

Beginning a vaccination program with Hiprabovis 4 in calves from two months of age results in a protective serological response to all three antigens. This is the case whether or not antibody positive colostrum has been consumed.

This Study provides information for NZ Vets to assess when considering whether to recommend "off-label" administration of Hiprabovis 3 to calves younger than four months of age.

Hiprabovis 3 – NZ Label Claims

Hiprabovis 3 is a trivalent inactivated vaccine that stimulates active immunity against respiratory and genital conditions in cattle caused by Bovine Herpes Virus 1 (BoHV-1); i.e. IBR / IPV, Parainfluenza Virus 3 (PI-3); and Bovine Viral Diarrhoea (BVD).

DOSAGE AND ADMINISTRATION:

Calves: 3mL per calf by intramuscular or subcutaneous injection.
Initial vaccination should be given at four months or older to avoid interference by maternal antibodies.
Give booster vaccination three weeks after first (sensitiser) vaccination.

Cattle: 3mL per animal by intramuscular or subcutaneous injection.
First vaccination: administer two vaccine doses three weeks apart.
Annual revaccination with a single dose.

Previously Unvaccinated Heifers: Administer two vaccine doses three weeks apart, the last injection occurring one month before the first mating.
Annual revaccination with a single dose.

Breeding Females: Annual booster vaccination one month before mating.
Pregnant Animals: For enhancing maternal antibodies, administer a booster vaccination 2 to 6 weeks before calving. Safe for use in pregnant cows.

INDICATIONS:

Calves: Reduce clinical signs of respiratory and genital disease associated with bovine herpesvirus 1 (infectious bovine rhinotracheitis (IBR)), parainfluenza virus 3 (PI-3). Stimulate active immunity against and aid in the control of bovine viral diarrhoea virus (BVD)

Cattle: Reduce clinical signs of respiratory and genital disease associated with bovine herpesvirus 1 (infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginits (IPV)), parainfluenza virus 3 (PI-3). Stimulate active immunity against and aid in the control of bovine viral diarrhoea virus (BVD)

This Study was conducted to support registration of Hiprabovis 4 in the EU.

#Hiprabovis 4 is comprised of two fractions – a liquid containing inactivated BVD, IBR and PI-3 antigens and a freeze dried component containing live bovine respiratory syncytial virus. The liquid vaccine is used as the diluent for the freeze-dried fraction. Hiprabovis 3 is the liquid fraction of the Hiprabovis 4 product. Hiprabovis 4 is not registered in NZ.

Hiprabovis 3 is a Restricted Veterinary Medicine, registered pursuant to the ACVM Act, 1997 No A07140.
Registered to: Hipra NZ Ltd. Manufactured by: Hipra S.A.