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Original Article

Risk factors analysis applied to antibodies to Bovine Herpesvirus Type 1, Bovine Viral Diarrhea Virus, Bovine Leukemia Virus and *Brucella abortus* among cattle: a cross-sectional study

Fatores de risco associados às infecções causadas por Herpesvírus Bovino Tipo I, Vírus da diarreia viral bovina, Vírus da leucose bovina e *Brucella abortus* em rebanhos bovinos: um estudo transversal

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ABSTRACT

The aim of the current cross-sectional study is to identify risk factors associated with infections caused by BoHV-1, BVDV, BLV and *Brucella abortus* in beef cattle herds bred under semi-intensive production system in São Luis Island - MA. Sixteen (16) cattle herds were selected and 160 serum blood samples from female animals in these herds (older than 24 months) were analyzed. An epidemiological questionnaire was answered by owners of each assessed property in order to identify risk factors associated with the studied infections; evaluations were made by means of multivariate statistical analysis. The prevalence of cattle positive to BoHV-1, BVDV, BLV and *B. abortus* was 68.12%, 66.87%, 62.50% and 3.12%, respectively. Variable 'cattle purchase' was a risk factor for infections caused by BoHV-1, BVDV and *B. abortus*. Pasture sharing and pig breeding in the same property were factors associated with the occurrence of BoHV-1 and *B. abortus* in the assessed herds. Variables 'miscarriage' and 'diarrhea' were indicative of *B. abortus*. Based on the current results, further attention should be given to the herein investigated region, where epidemiological investigations and control interventions should be implemented in order to avoid infections caused by BoHV-1, BVDV, BLV and *B. abortus*.

RESUMO

O objetivo do presente estudo transversal foi identificar os fatores de risco associados às infecções causadas por BoHV-1, BVDV, BLV e *Brucella abortus* em rebanhos bovinos de corte sob sistema de produção semi-intensiva na Ilha de São Luis - MA. Para isso, dezesseis (16) rebanhos bovinos foram selecionados e analisados sorologicamente 160 amostras de sangue de fêmeas bovinas (com idade superior a 24 meses). Em cada propriedade avaliada aplicou-se um questionário epidemiológico para identificar os fatores de risco que poderiam estar associados às infecções em estudo, por meio de análise estatística multivariada. As prevalências de bovinos positivos ao BoHV-1, BVDV, BLV e *B. abortus* foram de 68,12%, 66,87%, 62,50% e 3,12%, respectivamente. No estudo dos fatores de risco, a variável compra de bovinos foi identificada como fator de risco simultaneamente para as infecções pelo BoHV-1, BVDV e *B. abortus*. Compartilhamento de pasto e presença de suínos estiveram associadas ao mesmo tempo à ocorrência do BoHV-1 e *B. abortus* entre os animais avaliados. E ainda para a *B. abortus*, foram identificadas como fatores de risco as variáveis, ocorrência de abortamento e diarreia. Com base nos resultados desse estudo pode-se concluir que maior atenção deve ser dada à região estudada onde investigações epidemiológicas e intervenções de controle para as infecções pelo BoHV-1, BVDV, VLB e *B. abortus* devam ser implementadas.

Palavras-chave:
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INTRODUCTION

Bovine Herpesvirus type 1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), Bovine Leukosis Virus (BLV) and *Brucella abortus* are microorganisms that must be investigated because they affect the productive and reproductive efficiency of cattle herds in Brazil and worldwide. Moreover, *Brucella abortus* has strong impact on public health; therefore, it requires high investments in control and eradication campaigns. Infections caused by this microorganism hamper the trade of animals and of animal-origin products, such as semen, embryos, meat, milk, among others. The incidence of *Brucella abortus* also requires investments in laboratory diagnostic techniques and in technologies for epidemiological monitoring.

BoHV-1 is a virus belonging to order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae, genus *Varicellovirus*, which is the causative agent of miscarriage in females of infected herds. Infection caused by this microorganism is also associated with clinical respiratory and genital manifestations such as infectious pustular vulvovaginitis - IPV and infectious pustular balanoposthitis - IPB, as well as with ocular, gastrointestinal and neurological manifestations (ALVES DUMMER; PEREIRA LEIVAS LEITE; VAN DRUNEN LITTEL-VAN DEN HURK, 2014).

Infections caused by BVDV, which belongs to family Flaviviridae, genus *Pestivirus*, cause a wide variety of clinical symptoms associated with respiratory, digestive and reproductive diseases, mucosal disease (MD), hemorrhagic syndrome (HS) and with immunosuppression. However, greater losses come from infected pregnant females who end up generating stillbirths, malformed fetuses and persistently infected (PI)/weak calves immunotolerant to the virus (LANYON et al., 2014).

BLV, which belongs to family Retroviridae, subfamily Oncovirinae, genus *Deltaretrovirus,* causes highly contagious infectious diseases in cattle; among them, a chronic disease responsible for significant economic losses. Furthermore, in the long run, this disease allows the proliferation of infected lymphocytes due to the formation of lymphosarcomas (LEITE et al., 2013).

Brucellosis remains a serious disease affecting humans and domestic and wild animals worldwide. The etiological causative agent of brucellosis is an optional intracellular bacterium belonging to genus *Brucella*. This genus comprises nine species : *Brucella abortus*, *B. melitensis, B. suis, B. neotomae, B. ovis, B. canis, B. pinnipediae, B. cetaceae, B. microti*. *Brucella abortus* is a flat, highly pathogenic species, which causes serious illness, particularly in cattle (GOMES, 2013). This species also has clinical and epidemiological importance, since it is pathogenic for humans, as well.

Losses caused by bovine brucellosis are countless; infected herds face decreased milk and meat productivity. Moreover, the presence of the disease leads to animal price devaluation in the market, as well as to devaluation in the price of animal-origin products from endemic regions; longer calving intervals in the herds, miscarriages in infected females, sterility, sacrifice of reagent animals and consequent extra expenses due to the purchase of new animals (CARVALHO et al., 2016).

Based on previous studies, BoHV-1, BVDV, BLV and *B. abortus* are widely disseminated in dairy herds in Maranhão State (BEZERRA et al., 2012; CARVALHO et al., 2016; CHAVES et al., 2012a; PRAZERES et al., 2014); however, only few studies identify risk factors associated with the presence of these agents in beef herds in the State. Therefore, the aim of the current study was to identify risk factors associated with infections caused by BoHV-1, BVDV, BLV and *Brucella abortus* in beef cattle herds bred under semi-intensive production system in São Luis Island, Maranhão State.

MATERIALS AND METHODS

Ethics Statement

The current study involves a questionnaire-based survey applied to farmers, as well as blood samples collected from females in the selected herds. The study protocol was submitted to, and approved by, the Ethics Committee on Animal Experimentation – CEEA, of the Veterinary Medical School of the State University of Maranhão, Protocol 037/2011.

A meeting was held in each property in order to explain the aims of the study. Property owners provided their verbal informed consent for animal blood sampling, and for questionnaire answering. Professional veterinarians collected the blood samples, they also followed the regulations and guidelines set for animal husbandry.

Study Site and Sampling Design

Sampling was conducted in two stages; first, a predetermined number of properties/herds was randomly selected. These properties represented the primary sampling units. A predetermined number of animals from the primary unit formed the secondary units. Animals in the secondary unit were randomly sampled in order to determine the health condition of animals in the selected herds. Confidence level, desired precision level and expected prevalence were used to determine the number of herds by applying the simple random sampling formula.

The random selection of herds was based on records of rural properties under higher epidemiologic risks found in the corresponding Local Veterinary Unit. The existing properties were numbered, and their data were stored in Microsoft Excel 2000® spreadsheets.

The sampling design applied to the secondary units aimed at estimating the least number of animals to be examined in each property. The number of selected animals in each herd was determined in the Herdacc®

software, version 3 (University of Guelph) and lied on the least acceptable prevalence of 30% of each disease and on 95% probability of detecting at least one positive animal in the herd, based on Frandoloso; Anziliero; Spagnolo (2008).

The number of selected animals in each herd was similar; 10 animals were sampled per property because herds were composed of at most 99 females (PRAZERES et al., 2014). Simple random sampling was used to select animals in the assessed properties.

In total, 160 serum samples were analyzed. They were collected from female beef cattle in the age group 24 months´, and older, who were bred under semi-intensive production system. The selected females came from 16 herds (4 herds per county), they were not vaccinated against the assessed diseases. The present research did not include males and postpartum females - fifteen days prior to, or fifteen days, after delivery. This procedure followed the National Program for the Control and Eradication of Brucellosis and Tuberculosis.

Blood Sample Collection

Blood collection was performed by puncturing the jugular vein with a sterile disposable needle connected to vacuum blood collection tubes. Serum samples were stored in plastic microtubes and frozen at -20°C until the serological tests were complete.

Serological Testing

The qualitative detection of anti-BVDV and anti-BoHV-1 antibodies was performed based on the ELISA method by using the commercial indirect ELISA kit (CHEKIT IBR – SERO - Dr. BOMMELI AG/Liebefeld – Bern – Swiss).

Serum samples were assessed through Ouchterlony-LEB-AGID Double Radial Immunodiffusion, which is a method globally known for its accuracy to detect anti-BLV specific serum antibodies in gelatinous diffusion substrate. The method uses glycoprotein antigen (gp 51) extracted from the enzootic bovine leukemia virus envelope – the herein used extract was produced by the Technology Institute of Paraná - TECPAR. Readings were conducted 72 hours after the system was set.

The Buffered Acidified Antigen (BAA) test was performed to screen anti-*Brucella abortus* antibodies by using the antigen produced by TECPAR. Samples reactive to BAA were subjected to both 2-Mercaptoethanol (2-ME) and Slow Serum Agglutination in tubes (SSA) filled with the antigen produced by TECPAR, at ratios 1:25, 1:50, 1:100 and 1:200.

Questionnaire Design and Risk Factor Analysis

An epidemiological questionnaire was answered by the owner of each selected property in order to collect information about the management and about the health conditions of the selected animals. Analyzed variables were purchase of animals, presence of sheep/goat and pigs in the properties the selected cattle came from, veterinary care, digestive symptoms, diarrhea, reproductive symptoms, miscarriage rates, pasture rental, pasture sharing and calving paddocks.

The risk factor analysis was divided into univariate and multivariate analysis. Each independent variable was crossed with the dependent variable (animals' health conditions) in the univariate analysis. Animals recording $P \leq 0.2$ in the Fisher's exact test (PRAZERES et al., 2014) were selected and used in the multivariate analysis, which was based on a multiple logistic regression that was substantiated by data input in the final model. The multivariate analysis adopted the 5% significance level; all analyses were performed in the Statistical Package for Social Sciences (SPSS) Software (version 22.0) for Windows.

RESULTS

Prevalence rates 68.12% (n = 109/160), 66.87% (n = 107/160), 62.50% (n = 100/160) and 3.12% % (n = 05/160) were observed in infections caused by BoHV-1, BVDV, BLV and *Brucella abortus*, respectively. BoHV-1, BVDV and BLV antibodies were detected in 100% of the selected herds; *B. abortus* antibodies were diagnosed in 31.25% of the herds.

The univariate analysis applied to detect risk factors associated with the occurrence of animals positive to BoHV-1 counted on the following variables: purchase of animals ($P < 0.001$), presence of pigs in the properties the cattle came from ($P < 0.001$), diarrhea ($P = 0.17$), miscarriage ($P = 0.14$) and pasture sharing ($P < 0.001$) (Table 1).

Based on results of the final model applied to the multiple logistic regression, purchase of animals ($P < 0.001$), pasture sharing ($P < 0.001$) and presence of pigs ($P < 0.001$) were the major risk factors associated with the incidence of BoHV-1 in the assessed animals (Table 2).

Variables selected for the univariate analysis of risk factors associated with BVDV were purchase of animals ($P = 0.02$), miscarriage ($P = 0.14$) and pasture sharing ($P = 0.08$) (Table 3). Based on the multiple logistic regression analysis, purchase of animals ($P = 0.03$) was the most relevant risk factor for infections caused by this virus (Table 2).

Variables selected in the univariate analysis to assess factors associated with BLV occurrence were diarrhea ($P = 0.02$), reproductive symptoms ($P = 0.18$) and miscarriage ($P = 0.18$) (Table 4). However, the multiple logistic regression analysis did not identify risk factors associated with infection caused by this microorganism in the assessed animals.

Table 1 – Potential risks associated with BoHV-1 seropositivity in each of the 160 assessed animals.

Variables		Positive Animals (BoHV-1) N	%	P-value
Purchase of animals	Yes	102	63.75	< 0.001*
	No	7	4.37	
Presence of sheep/goat	Yes	94	58.75	0.30
	No	15	9.37	
Presence of pigs	Yes	85	15.00	< 0.001*
	No	24	53.12	
Veterinary care	Yes	24	15.00	1.00
	No	85	53.12	
Digestive symptoms	Yes	77	20.00	0.81
	No	32	48.12	
Diarrhea	Yes	99	61.87	0.17*
	No	10	6.25	
Reproductive symptoms	Yes	83	51.87	1.00
	No	26	16.25	
Miscarriage	Yes	65	40.62	0.14*
	No	44	27.50	
Pasture rental	Yes	06	3.75	0.47
	No	103	64.37	
Pasture sharing	Yes	77	48.12	< 0.001*
	No	32	20.00	
Presence of calving paddocks	Yes	06	3.75	0.47
	No	103	64.37	

* Variables selected to the multiple logistic regression analysis

Table 2 – Results of the final risk factor model applied to detect BoHV-1, BVDV and *B. abortus* seropositivity in the assessed animals.

BoHV1			
Risk Factors	*Odds ratio*	CI 95%	P-Value
Purchase of animals	6.47	2.32-18.03	< 0.001
Pasture sharing	2.88	1.50-5.94	< 0.001
Presence of pigs	0.07	0.38-2.84	< 0.001
BVDV			
Risk Factors	*Odds ratio*	CI 95%	P-Value
Purchase of animals	2.84	1.20-6.69	0.03
B. abortus			
Risk Factors	*Odds ratio*	CI 95%	P-Value
Diarrhea	72.70	3.87-1365.5	< 0.001
Purchase of animals	7.11	1.13-44.64	0.03
Presence of pigs	7.11	1.13-44.64	0.03
Miscarriage	7.11	1.13-44.64	0.03
Pasture sharing	7.11	1.13-44.64	0.03

Variables selected in the univariate analysis to assess risk factors associated with the incidence of *B. abortus* were purchase of animals (P = 0.04), presence of goats/sheep (P = 0.11) and pigs in the properties (P = 0.04), lack of veterinary care (P = 0.11), diarrhea (P < 0.001), miscarriage (P = 0.04) and pasture sharing (P = 0.04) (Table 5).

Based on the multiple logistic regression analysis, purchase of animals (P = 0.03), presence of pigs (P = 0.03), diarrhea (P < 0.001), miscarriage (P = 0.03) and pasture sharing (P = 0.03) were the major risk factors associated with *B. abortus* (Table 2).

DISCUSSION

The current study addresses risk factors associated with infections caused by BoHV-1, BVDV, BLV and *B. abortus*. However, there were no previous epidemiological data about the simultaneous occurrence of the four different infections in beef cattle herds.

According to several studies, the mean global prevalence of BoHV-1, BVDV, BLV and *Brucella abortus* antibodies ranges from 10% to 50% or more, from 50% to 90%, 27.60%, and from 0.8% to 10.5%, respectively (BEZERRA et al., 2012; SHIRVANI et al., 2012; LINDAHL et al., 2014; LØKEN; NYBERG, 2013). These values are lower than, or close to, those recorded in the current study.

Table 3 – Potential risks associated with BVDV seropositivity in each of the 160 assessed animals.

Variables		Positive Animals (BVDV)		P-value
		N	%	
Purchase of animals	Yes	95	59.37	0.02*
	No	12	7.50	
Presence of sheep/goat	Yes	95	59.37	0.42
	No	12	7.50	
Presence of pigs	Yes	82	51.25	1.00
	No	25	15.62	
Veterinary care	Yes	19	11.87	0.81
	No	88	55.00	
Digestive symptoms	Yes	75	46.87	0.69
	No	32	20.00	
Diarrhea	Yes	94	58.75	1.00
	No	13	8.12	
Reproductive symptoms	Yes	82	51.25	1.00
	No	25	15.62	
Miscarriage	Yes	64	40.00	0.14*
	No	43	26.87	
Pasture rental	Yes	06	3.75	0.47
	No	101	63.12	
Pasture sharing	Yes	75	46.87	0.08*
	No	32	20.00	
Presence of calving paddocks	Yes	06	3.75	0.47
	No	101	63.12	

* Variables selected to the multiple logistic regression analysis.

Table 4 – Potential risks associated with BLV seropositivity in each of the 160 assessed animals.

Variables		Positive Animals (BLV)		P-value
		N	%	
Purchase of animals	Yes	93	58.12	0.74
	No	7	4.38	
Presence of sheep/goat	Yes	88	55.00	0.80
	No	12	7.50	
Presence of pigs	Yes	75	46.88	1.00
	No	25	15.62	
Veterinary care	Yes	21	13.12	0.40
	No	79	49.37	
Digestive symptoms	Yes	72	45.00	0.58
	No	28	17.50	
Diarrhea	Yes	83	51.87	0.02*
	No	17	10.62	
Reproductive symptoms	Yes	71	44.37	0.18*
	No	20	12.50	
Miscarriage	Yes	71	44.37	0.18*
	No	20	12.50	
Pasture rental	Yes	07	4.37	0.74
	No	93	58.12	
Pasture sharing	Yes	65	40.62	0.40
	No	35	21.87	
Presence of calving paddocks	Yes	07	4.37	0.74
	No	93	58.12	

* Variables selected to the multiple logistic regression analysis.

Table 5. Potential risks associated with *Brucella abortus* seropositivity in each of the 160 assessed animals.

Variables		Positive Animals (*Brucella abortus*)		P-value
		N	%	
Purchase of animals	Yes	3	1.87	0.04*
	No	2	1.25	
Presence of sheep/goat	Yes	3	1.87	0.11*
	No	2	1.25	
Presence of pigs	Yes	3	1.87	0.04*
	No	2	1.25	
Veterinary care	Yes	2	1.25	0.11*
	No	3	1.87	
Digestive symptoms	Yes	4	2.50	0.27
	No	1	0.62	
Diarrhea	Yes	5	3.12	< 0.00*
	No	0	0.00	
Digestive symptoms	Yes	4	2.50	0.27
	No	1	0.62	
Diarrhea	Yes	3	1.87	0.04*
	No	2	1.25	
Reproductive symptoms	Yes	4	2.50	0.27
	No	1	0.62	
Miscarriage	Yes	3	1.87	0.04*
	No	2	1.25	
Pasture rental	Yes	1	2.50	0.27
	No	4	0.62	

* Variables selected to the multiple logistic regression analysis.

The high prevalence of BoHV-1 (68.12%), BVDV (66.87%) and BLV (62.50%) in cows living in herds clearly indicates the possible outbreak of these viruses in beef cattle properties in São Luis and in other cattle-breeder counties that use sanitary management procedures similar to those used in the sampled properties. Therefore, these microorganisms represent a significant health issue in Maranhão State.

Five (5) of the 16 examined properties tested positive for *B. abortus*; thus, such number indicate that 31.25% of the herds could be focus of the disease. Based on this result, brucellosis remains a common and worrisome matter, despite the official health actions against it taken over the last decades in almost all Brazilian regions. Therefore, the epidemiological behavior acquired by brucellosis is similar to its behavior at national and international level (PRAZERES et al., 2014). Greater relevance is given to when herds are the epidemiological unit, because the disease outbreak has low potential when the unit is a single animal.

The univariate analysis highlighted the explanatory variables associated with the occurrence of BoHV-1, BVDV, BLV and *B. abortus* in the sampled animals (Tables 1, 3, 4 and 5). Some of these variables were previously described in other studies as important risk factors for infections affecting cattle. Among the aforementioned variables, one finds purchase of animals, presence of sheep/goats and pigs, diarrhea, miscarriage and pasture sharing (BEZERRA et al., 2012; CHAVES et al., 2012; OLIVEIRA et al., 2013; PRAZERES et al., 2014; CARVALHO et al., 2016).

The multiple logistic regression model identified that the purchase of animals was a risk factor associated with infections caused by BoHV-1, BVDV and *B. abortus*. Pasture sharing and the presence of pigs were selected for infections caused by BoHV-1 and *B. abortus*. Diarrhea and miscarriage were characterized as risk factors associated with the incidence of B. *abortus*.

Animal purchasing proved to be a risk factor associated with simultaneous infections caused by BoHV-1, BVDV and *B. abortus* in the assessed animals. All assessed herds were composed of animals purchased in other properties in the region. However, the traded animals were not diagnosed for infectious and contagious diseases prior to join the new herd, including the herein assessed females; moreover, the health condition of the acquired animals was also not previously evaluated. Oliveira et al. (2013) described the same situation in family farms that live on cattle breeding in wild Paraíba, and it suggests that infection sources could be circulating within the herds in the studied location, as well as that new infection cases were not caused by the acquisition of animals from other States.

Based on variable 'purchasing of animals', some factors may act in separate, or in combination, for example, purchase frequency, animal origin and history of serological tests applied to assess the incidence of the diseases (OLIVEIRA et al., 2013). The introduction of new animals in the herd is not the core issue, since it is a common practice in Brazilian cattle herds, but the acquisition of animals without sanitary care, i.e., lack of previous tests or of knowledge about the health condition of the original herd.

Cattle purchasing for rearing or fattening often counts on animals with low genetic potential and lower commercial value. These animals are kept in pasture until the time to be slaughtered. Some of them reproduce within the herd and reinforce the risk of developing infections caused by BoHV-1, BVDV and *B. abortus*.

Pasture sharing was identified as risk factor associated with infections caused by BoHV-1 and *B. abortus* in the assessed animals, since it may favor the contact of the herd with previously contaminated environments. In this case, the main risk associated with infections caused by BoHV-1 and *B. abortus* is related to contamination via contact with secretions and excretions such as those resulting from miscarriages. Depending on the environment, these secretions and excretions can make the survival of infectious agents feasible in outdoor environments. In addition, current management practices in the assessed region allow disease-free populations to have contact with infected animals. This variable was also taken into consideration as risk factor in studies conducted by Dias et al. (2009).

Because BoHV-1 and *B. abortus* samples isolated from cattle can infect pigs, it is relevant taking such possibility into consideration in studies focused on assessing these infections in farms where different animal species are bred together. Contact between cattle and pigs proved to be a risk factor associated with infections caused by these two agents in the current study. Possibly, swine species play an important role in the epidemiology of these infections, since infected pigs can become asymptomatic (KEUSER et al., 2004).

Variables 'diarrhea' and 'miscarriage' were statistically significant in the multivariate analysis applied to infections caused by *B. abortus* in the sampled animals. Diarrhea emerged as risk factor associated with the incidence of this infection. International studies have shown that the greatest losses resulting from infections caused by *B. abortus* resulted from infections in pregnant females after miscarriages (ANKA et al., 2013; OLIVEIRA et al., 2013).

Although the multiple logistic regression analysis did not identify risk factors associated with BLV in the current study, some variables were noteworthy given their frequency in positive properties and in animals, and their similarity to variables such as diarrhea and miscarriage, which were identified in the univariate analysis applied to infections caused by *B. abortus*. Additionally, studies have shown that this virus compromises the integrity of the immune system by penetrating and indefinitely incorporating itself to the lymphocytic genome. This virus is also associated with immunocitological evidences that BLV infects circulating monocytes and increases hosts' susceptibility to other infections such as brucellosis (MENDES et al., 2011).

It is worth identifying risk factors associated with infectious and contagious diseases in order to develop adequate health policies to control and, eventually, to eradicate a certain disease. Accordingly, it is possible inferring that the epidemiological data obtained in the current study can help structuring control strategies based on findings associated with infections caused by BoHV-1, BVDV, BLV and *B. abortus* in Maranhão State.

CONCLUSIONS

Based on the present results, beef cattle herds are highly prone to develop infections. The herein identified risk factors (purchase of animals, pasture sharing, presence of pigs, diarrhea and miscarriage) should be taken into consideration in studies about the manifestation of these infections, together or in separate. Based on the collected data, measures such as (i) gradually removing the infected animals; (ii) performing quarantine at the time new animals enter the properties; (iii) purchasing animals with adequate sanitary control; (iv) avoiding the use of probably contaminated pastures; and (v) performing serological tests and vaccinations must be adopted in order to prevent and control BoHV-1, BVDV, BLV and *B. abortus*.

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