



Seroprevalence and associated factors of infectious bovine rhinotracheitis and bovine viral diarrhoea in dairy cows in the Caparaó region, Espírito Santo, Brazil

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ABSTRACT: *This study evaluated the seroprevalence and associated factors of Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhoea (BVD), and to analyze the possible relationship between IBR, BVD, and the occurrence of mastitis. For this purpose, 854 crossbred dairy cows were evaluated from 69 properties allocated in the 12 municipalities that make up the Caparaó region, Espírito Santo (ES), Brazil. The seroprevalence of IBR and BVD was determined using the indirect ELISA test. Associations between variables were estimated using the prevalence ratio (PR) with a 95% confidence interval. The chi-square test was used to verify the significance of the associations ($P < 0.05$). The average prevalence of IBR and BVD was 48.59% and 26.46%, respectively. Animals seroreactive for IBR were more likely to develop subclinical mastitis ($P < 0.01$; PR: 1.27), and cows that were seroreactive for BVD were more likely to develop clinical mastitis ($P < 0.01$; PR: 2.24). Mechanical milking was considered a factor associated with IBR ($P < 0.01$; PR: 1.36) and BVD ($P < 0.01$; PR: 1.25). Reproductive management by natural breeding was considered a factor associated with IBR ($P < 0.01$; PR: 1.22). Animals seroreactive for BVD were more likely to develop reproductive problems ($P < 0.01$; PR: 1.34). It was concluded that the agents that cause IBR and BVD are widely disseminated in dairy cattle herds in the municipalities of the Caparaó region, ES, Brazil. The presence of IBR and BVD increased the chances of cows developing subclinical mastitis and clinical mastitis, respectively, and the cows that were mechanically milked were more likely to be seroreactive for IBR and BVD.*

Key words: BVD, crossbred cow, dairy cows, indirect ELISA, IBR, mastitis.

Seroprevalência e fatores associados à rinotraqueíte infecciosa bovina e diarreia viral bovina em vacas leiteiras na região do Caparaó, Espírito Santo, Brasil

RESUMO: *O objetivo do presente estudo foi avaliar a soroprevalência e os fatores de risco associados à Rinotraqueíte Infecciosa Bovina (IBR) e Diarreia Viral Bovina (BVD), e analisar a possível relação entre IBR, BVD e à ocorrência de mastite. Para tanto, foram avaliadas 854 vacas leiteiras mestiças de 69 propriedades localizadas nos 12 municípios que compõem a região do Caparaó, Espírito Santo (ES), Brasil. A soroprevalência de IBR e BVD foram determinadas pelo teste ELISA indireto. As associações entre variáveis foram estimadas pela razão de prevalência (PR) com intervalo de confiança de 95%. O teste do qui-quadrado foi utilizado para verificar a significância das associações ($P < 0,05$). A prevalência média de IBR e BVD foi de 48% e 26%, respectivamente. Os animais sororreagentes para IBR foram mais propensos a desenvolver mastite subclínica ($P < 0,01$; PR: 1,27), e as vacas sororreagentes para BVD foram mais propensas a desenvolver mastite clínica ($P < 0,01$; PR: 2,24). À ordenha mecânica foi considerada um fator associado a IBR ($P < 0,01$; PR: 1,36) e BVD ($P < 0,01$; PR: 1,25). O manejo reprodutivo por monta natural foi considerado um fator associado IBR ($P < 0,01$; PR: 1,22). Os animais sororreagentes para BVD foram mais propensos a desenvolverem problemas reprodutivos ($P < 0,01$; PR: 1,34). Concluiu-se que os agentes causadores de IBR e BVD estão amplamente disseminados em rebanhos leiteiros nos municípios da região de Caparaó, ES, Brasil. A presença de IBR e BVD aumentaram as chances das vacas desenvolverem mastite subclínica e mastite clínica, respectivamente, e as vacas que foram ordenhadas mecanicamente apresentaram maior probabilidade de serem sororreagentes para IBR e BVD.*

Palavras-chave: BVD, ELISA indireto, IBR, mastite, vacas mestiças, vacas leiteiras.

INTRODUCTION

The Caparaó region, located in the southern part of the Brazilian state of Espírito Santo, is an important milk production region and is home to approximately 14% of the total dairy production in

Espírito Santo (IBGE, 2019). Despite the expansion of the dairy industry in the region, there are still few official animal health programs, and large productive losses can be related to infectious diseases, such as Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhoea (BVD), caused by the agents Bovine

Herpesvirus Type 1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDV), respectively (CAN et al., 2016). Even though these diseases are better known due to the reproductive disorders observed in affected animals (STRAUB, 2001; WERNICKI et al., 2015), they affect cattle worldwide, causing significant economic losses due to decreased performance and milk yield, as well as increased risk of morbidity and mortality (CAN et al., 2016).

The seroprevalence of IBR can vary drastically according to geographic region, with a prevalence of 35% in Belgium (BOELAERT et al., 2000), 77% in Italy (RINALDI et al., 2007), 69% in England (PATON et al., 1998), 37% in Uruguay (GUARINO et al., 2008), and 51% in Peru (STAHL et al., 2002). In Brazil, various studies have reported a seroprevalence of IBR of 83% in Goiás (VIEIRA et al., 2003), 68% in São Paulo (JUNQUEIRA et al., 2006), and 58% in Minas Gerais (ROCHA et al., 2001). Only one previous study exists in the state of Espírito Santo, finding an average prevalence of 66%, evaluating neutralizing antibodies (SANTOS et al., 2014).

BVD is distributed worldwide, and its seroprevalence is estimated to range between 46% and 78% (RICHTER et al., 2019). In Brazil, several serological studies have demonstrated a wide distribution and high prevalence rate in several states, as follows: Bahia, 56% (NORONHA et al., 2001); Paraíba, 39% (FERNANDES et al., 2016); Goiás, 54% (BRITO et al., 2010); Minas Gerais, 61% (MINEO et al., 2006); São Paulo, 56% (SAMARA et al., 2004); Paraná, 61% (ALFIERI et al., 2004); and Rio Grande do Sul, 58% (SCHERER et al., 2002). However, until now, no data were reported for the state of Espírito Santo.

Economic losses due to decreased milk production are associated with IBR and BVD infection. Losses of 2.6 kg of milk per day in cows with subclinical IBR compared to seronegative cows have been reported in England (STATHAM et al., 2015). A study conducted in Turkey reported milk production reductions of up to 10% for animals that had detectable BHV1 (seropositive) compared with animals seronegative (CAN et al., 2016). For BVD, losses of \$ 40 to \$ 95 were estimated per infected cow in Danish herds (HOUE, 2003), and a reduction of up to 19€ per thousand liters of produced milk has been reported in France (FOURICHON et al., 2005). There was a decrease of up to 10% in milk production in cows with BVDV antibodies compared to negative cows (MOERMAN et al., 1994). There are reports that the immunosuppressive properties of BHV-1 and BVDV may play a secondary role in the etiology of

bacterial diseases, especially mastitis (POTGIETER et al., 1984; HUTCHINGS et al., 1990; KOPPERS-LALIC et al., 2001).

The objective was to conduct a cross-sectional study on the seroprevalence of IBR and BVD in the Caparaó region, Espírito Santo, Brazil, and to analyze the possible relationship between these viral diseases and mastitis, as well as identifying the factors associated with management that can contribute to the transmission of IBR and BVD.

MATERIALS AND METHODS

Study population

A total of 854 clinically healthy crossbred (*Bos taurus taurus* × *Bos taurus indicus*) dairy cows in different lactation phases were used. The farms were located in the 12 municipalities that compose the Caparaó Capixaba micro-region (20° 26' 5" S and 41° 47' 2" W), in the southern part of Espírito Santo (Alegre, Bom Jesus do Norte, Divino de São Lourenço, Dolores do Rio Preto, Guaçuí, Ibatiba, Ibitirama, Irupi, Iúna, Jerônimo Monteiro, Muniz Freire, and São José do Calçado), Brazil, and were chosen at random in the period from February 2015 to December 2015. Of the 72 farms evaluated at random, through registration with a dairy company in the region, animals from 69 farms were used (with no history of vaccination for IBR and BVD), 5 farms per municipality, except Alegre and Jerônimo Monteiro, where 9 and 10 farms were evaluated, respectively.

The sample size calculation was performed according to the following formula (DEAN et al., 2013):

$$n = deff \times \frac{Npq}{1.96^2 (n-1) + pq}$$

where n is the sample size, $deff$ is the design effect, N is the population size, p is the estimated proportion, $q = 1 - p$, and d is the absolute precision level.

Using an anticipated frequency of 50% for the diseases (IBR and BVD), a sampling error of 5%, confidence interval of 95%, and herd size of 2,223,531 in Espírito Santo in 2015 (IBGE, 2019), we calculated the minimum sample size as 385 animals. A total of 854 animals were evaluated.

Serological tests for infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea (BVD)

Blood samples were collected from all lactating cows, with no history of vaccination for IBR or BVD, by puncturing the coccygeal vein in a vacuum collection system. The collected blood was stored in tubes without anticoagulant, and the samples were

centrifuged at 200 G for 5 minutes, and the obtained serum was aliquoted in 1.5 mL polyethylene tubes and stored at -80°C . The diagnostic examination for IBR and BVD was performed via an indirect enzyme-linked immunosorbent assay (ELISA) using the CIVTEST® BOVIS IBR kit (sensitivity, 85.4%; specificity, 99.7%; Amer, Catalonia, Spain), effective at detecting antibodies to specific antigens of Infectious Bovine Rhinotracheitis (IBRv), and the CIVTEST® BOVIS BVD/BDP80 kit (sensitivity, 96.9%; specificity, 97.3%; Amer, Catalonia, Spain), designed to detect antibodies against a specific protein present in all strains of BVDV (non-structural protein P80).

Test for mastitis

The presence of clinical mastitis was determined by identifying the clinical signs of inflammation of the mammary gland and by the black background mug test. In turn, subclinical mastitis was diagnosed by the California mastitis test (CMT). The animals were considered positive for clinical or subclinical mastitis when there was an alteration in the mug or California test, respectively, in at least one of the teats (COLE et al., 1965).

Data analysis

A questionnaire regarding the reuse of disposable needles, testing for brucellosis, milking type (manual/mechanical), reproductive management (artificial insemination/natural breeding), and whether the cow presented reproductive problems (abortion,

estrus repetition, placental retention, and uterine infection) was answered by the owners. The farms were classified into small (up to 53 L/day), medium (from 54 to 133 L/day), and large (>133 L/day) milk producers (BARROS et al., 2001). The result of the serology for the IBR and BVD tests (positive and negative) was considered as a dependent variable and the information regarding the epidemiological questionnaire was the independent variable.

The statistical results were demonstrated by descriptive analysis. Associations between variables (univariable analysis) were estimated by the cross-product ratio (prevalence ratio, PR) and 95% confidence interval. The chi-square test was used to verify the significance of the associations ($P < 0.05$). The calculations were performed using Epi Info 7.2 software.

RESULTS

The prevalence of IBR and BVD varied in the studied municipalities of Espírito Santo (Table 1). For IBR, we reported a mean prevalence of 48% (95% CI: 47.72 – 49.45), ranging from 20% to 65%, and a mean prevalence of 26% (95% CI: 47.74 – 49.43) for BVD, ranging from 7% to 45%.

Table 2 shows the percentage distribution of the possible variables associated with the seroprevalence of Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhea in crossbred dairy cows in the Caparaó region, Espírito Santo, Brazil.

Table 1 - Seroprevalence of Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhea (BVD) analyzed with specific indirect ELISA tests on crossbred dairy cows in the municipalities of the Caparaó region in Espírito Santo, Brazil (February 2015 to December 2015).

Municipality	Total number of animals	-----IBR-----		-----BVD-----	
		Number of positives	Prevalence (%)	Number of positives	Prevalence (%)
Alegre	143	91	63.63	58	40.55
Bom Jesus do Norte	38	21	55.26	3	7.89
Divino de São Lourenço	63	13	20.63	17	26.98
Dores do Rio Preto	84	44	52.38	8	9.52
Guaçuí	36	12	33.33	10	27.77
Ibatiba	57	19	33.33	10	17.54
Ibitirama	72	35	48.61	7	9.72
Irupi	35	17	48.57	7	20.00
Iúna	69	45	65.21	9	13.04
Jerônimo Monteiro	137	66	48.17	55	40.14
Muniz Freire	75	27	36.00	34	45.33
São José do Calçado	45	25	55.55	8	17.77
Total	854	415	48.59	226	26.46

Table 2 - Percentage distribution of possible variables associated with Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhea (BVD) seroprevalence in crossbred dairy cows in the municipalities of the Caparaó region in Espírito Santo, Brazil (February 2015 to December 2015).

Variables	n Farms	n animals	+ IBR	% IBR	+ BVD	% BVD
-----Milk producers-----						
Small (up to 53 L/day)	7	41	20	48.78	10	24.39
Medium (54 to 133 L/day)	22	167	87	52.09	35	20.95
Large (>133 L/day)	40	646	308	47.67	181	28.01
-----Disposable needles-----						
Yes	31	467	238	50.96	123	26.33
No	38	387	177	45.73	103	26.61
-----Reproductive problems-----						
Yes	33	401	194	48.37	131	32.66
No	36	453	221	48.78	95	20.97
-----Testing for brucellosis-----						
Yes	32	432	197	45.60	103	23.84
No	37	422	218	51.65	123	29.14
-----Milking type-----						
Mechanical	55	750	381	50.80	215	28.66
Manual	14	104	34	32.69	11	10.57
-----Reproductive management-----						
Artificial insemination	40	521	233	44.72	128	24.56
Natural breeding	29	333	182	54.65	98	29.42

n Farms = number of farms evaluated; n animals = number of animals tested; + IBR / BVD = animals seroreagent for Bovine Infectious Rhinotracheitis (IBR) or Bovine Viral Diarrhea (BVD); % IBR / BVD = percentage of animals seroreagent for IBR or BVD.

We found an association ($P < 0.01$; PR: 1.27) between IBR and the presence of subclinical mastitis, as well as an association ($P < 0.01$; PR: 2.24) between BVD and the presence of clinical mastitis (Table 3). In this context, animals that were

seroreactive for IBR had a higher prevalence ratio for the development subclinical mastitis, animals that were seroreactive for BVD had a higher prevalence ratio for clinical mastitis (Table 4). However, there was no association between IBR and clinical mastitis

Table 3 - Univariable analysis for factors associated with seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in crossbred dairy cows in the municipalities of the Caparaó region in Espírito Santo, Brazil (February 2015 to December 2015).

	Prevalence ratio	Confidence interval (95%)	p-value
Clinical mastitis	1.10	0.63 – 1.92	0.84
Subclinical mastitis	1.27	1.08 – 1.48	0.002*
Natural breeding	1.22	1.06 – 1.40	0.005*
Mechanical milking	1.36	1.17 – 1.59	0.0007*
Reproductive problems	0.99	0.85 – 1.14	0.96
Needle reuse	1.11	0.96 – 1.28	0.14
Brucellosis test	0.88	0.76 – 1.01	0.08
Production level (small)	1.00	0.55 – 1.83	0.98
Production level (medium)	1.12	0.88 – 1.41	0.38
Production level (large)	0.96	0.90 – 1.03	0.35

* ($P < 0.05$) using the chi-square test.

Table 4 - Univariable analysis for factors associated with seroprevalence of Bovine Viral Diarrhea (BVD) in crossbred dairy cows in the municipalities of the Caparaó region in Espírito Santo, Brazil (February 2015 to December 2015).

	Prevalence ratio	Confidence interval (95%)	p-value
Clinical mastitis	2.24	1.28 – 3.90	0.006*
Subclinical mastitis	1.07	0.91 – 1.27	0.42
Natural breeding	1.06	0.98 – 1.16	0.13
Mechanical milking	1.25	1.15 – 1.35	0.0001*
Reproductive problems	1.34	1.16 – 1.55	0.0001*
Needle reuse	0.98	0.79 – 1.23	0.98
Brucellosis test	0.81	0.65 – 1.02	0.09
Production level (small)	0.89	0.44 – 1.79	0.89
Production level (medium)	0.76	0.57 – 1.02	0.08
Production level (large)	1.07	0.99 – 1.14	0.08

*($P < 0.05$) using the chi-square test.

nor was there an association between BVD and subclinical mastitis.

Natural breeding ($P < 0.01$; PR:1.22) and mechanical milking ($P < 0.001$; PR: 1.36) were considered factors associated with animals seroreactive for IBR (Table 4). In this context, animals that go through natural breeding and are mechanically milked have higher prevalence rates for IBR when compared to animals from artificial insemination and manual milking. The presence of reproductive problems, reuse of disposable needles, brucellosis tests, and production levels was not significant ($P > 0.05$).

The presence of antibodies against BVD increases the prevalence ratio of reproductive problems, and mechanical milking increases the prevalence ratio of cows being seroreactive for BVD (Table 4). There was no association between BVD and natural breeding, disposable needle reuse, the brucellosis test, or production level ($P > 0.05$).

DISCUSSION

In the Brazilian state of Paraná, the prevalence of IBR in dairy cattle ranged from 47% to 99% (DIAS et al., 2013). In Espírito Santo, a prevalence of IBR of 49%, 62%, 67%, and 80% was reported for the metropolitan, southern, northern, and northwestern regions, respectively. The authors also reported a high prevalence of seroreactive samples for the municipality of Alegre (90%) (SANTOS et al., 2014), with values higher than those found in the present study. Higher seroprevalence of BVD was reported when compared to the present study. In the state of Paraíba, with

39% (FERNANDES et al., 2016), Rio Grande do Sul (57%; FRANDOLOSO et al., 2008), Maranhão (65%; CHAVES et al., 2012), and Goiás (64%; BRITO et al., 2010), as well as in beef cattle in Maranhão (66%; BEZERRA et al., 2019).

The lack of knowledge on the part of producers regarding IBR and BVD, as well as the lack of measures that contribute to their control and elimination, favor the appearance of new cases in the herd. Factors such as the age of the animals, absence of tests at purchase, and reuse of rectal palpation gloves are measures that can contribute to the spread of the studied conditions (BARBOSA et al., 2019). Another factor that can contribute to the different seroprevalence of IBR and BVD in Espírito Santo and Brazil, is that these diseases do not yet have specific legislation, despite notification to the official veterinary service being mandatory, with vaccination being optional. This is unlike foot-and-mouth disease and bovine brucellosis, where there is specific legislation for the control and eradication, and vaccination of animals.

One factor that could be related to IBR, BVD, and the presence of mastitis (Table 3 and 4) would be the immunosuppressive properties of BHV-1 and BVDV, which play a secondary role in the etiology of bacterial diseases (POTGIETER et al., 1984; HUTCHINGS et al., 1990; KOPPERS-LALIC et al., 2001). Previous studies have isolated BHV-1 from mastitis milk samples, which were negative for bacterial culture; however, the evidence that the virus caused mastitis was only circumstantial (ROBERTS et al., 1974). Similarly, there have been reports of increased cases of mastitis in herds with increased

titers of antibodies for BVDV, but the role played by BVDV in these mastitis cases is unclear (NISKANEN et al., 1995; WAAGE, 2000; WELLENBERG et al., 2002). That is, there is a lack of knowledge regarding the actual impact of the virus on cow udder health, and there is insufficient evidence on the effect of BHV-1 and BVDV infection on mastitis. However, immunosuppression caused by viral diseases can be considered a triggering factor for the onset of mastitis since there is no specific treatment for animals with IBR and BVD, and the animals will be virus carriers for the rest of their productive life.

In the present study, mechanical milking was considered a factor associated with IBR (Table 3) and BVD (Table 4). Corroborating the present study, a similar result was reported in Minas Gerais for both diseases (BARBOSA et al., 2019). It is widely reported that BHV-1 and BVDV are excreted in the milk of infected cows (WAAGE, 2000; WELLENBERG et al., 2002) and therefore deficiencies in management, udder health, and inadequate disinfection and maintenance of mechanical milking equipment can be considered factors that help in the transmission of IBR and BVD.

Animals from reproductive management by natural breeding increased the prevalence ratio for IBR (Table 3). Similar results were also found by other authors (ROCHA et al., 1999; DIAS et al., 2013; BARBOSA et al., 2019). The foreskin mucosa and urethra are sites of viral replication, meaning that a seroreactive bull may be responsible for spreading the disease within the herd (VAN DER ENGELBURG et al., 1993). Therefore, it is important that the entire herd be tested for IBR in farms where natural breeding occurs (29 of the 69 farms evaluated), especially when bulls are used for breeding, in order to prevent the transmission of the disease to healthy animals.

There was no association between reproductive management by natural breeding and BVD (Table 4). A similar result was also reported by other authors (TADESSE et al., 2019). Unlike in the present study, in a previous study, an association between natural breeding and BVD was reported in which the bull used for breeding was considered a factor associated with the disease. The same authors reported that the use of artificial insemination could be considered a form of infection control (CHAVES et al., 2012).

There was no association between the presence of reproductive problems and IBR (Table 3). Of the 69 evaluated farms, 33 (47.82%) reported reproductive problems (Table 2), with abortion (16), estrus

repetition (8), placental retention (5), and uterine infection (4) being the most cited. Corroborating the present study, no associations were found between IBR and reproductive signs or cases of abortion (BARBOSA et al., 2005; BEZERRA et al., 2012). However, the presence of BVD was considered a factor associated with reproductive problems, that is, in farms with animals seroreactive for BVD, here was a higher prevalence rate of reproductive problems (Table 4). The association between estrus repetition and BVDV has also been reported by other authors (TADESSE et al., 2019). In this context, an association was also reported between the occurrence of abortion and BVD, but the authors could not state whether it was an associated risk factor since the confidence interval ranged from 0.88 to 1.22 (BRITO et al., 2010). However, unlike in the present study, no association was found between the presence of reproductive problems (abortion) and BVDV, but the authors reported a higher prevalence of BVD in properties where abortion or reproductive problems occurred (CHAVES et al., 2012; MARQUES et al., 2016; BEZERRA et al., 2019).

There was no association between IBR and BVD and the test for brucellosis, production level, and reuse of disposable needles. It was expected that the reuse of disposable needles would be a factor associated with IBR and BVD since this has been reported to be associated with other infectious, contagious diseases such as Bovine Enzootic Leukosis (ALMEIDA et al., 2021; VÁSQUEZ et al., 2016). However, these same authors also reported no association between the reuse of disposable needles and the presence of IBR.

It is important to highlight that studies that evaluate the incidence are more reliable in the association of risk factors, because the prevalence, assessed in the present study, is a combination of incidence and duration of the disease, being, therefore, more difficult to determine the causes of the risk factors. However, actions such as diagnosis, prevention, and reduction of herd exposure to infectious diseases, such as IBR and BVD, contribute to animal health and the proper functioning of the immune system, thus minimizing economic losses in the production system. However, several factors affect the productive and reproductive efficiency of cattle, especially the occurrence of such diseases in the herd. Therefore, the exposure of the herd to infectious diseases should be minimized so that they can provide greater profitability to the producer. Additional studies are needed to verify the progress of BHV-1 and BVDV in the region, as well as the molecular diagnosis for

the detection of persistently infected animals for BVDV, one made that the ELISA test performed is not able to detect the infection in such animals.

CONCLUSION

It can be concluded that the prevalence of IBR and BVD are high in dairy cattle herds in the municipalities of the Caparaó region in Espírito Santo, Brazil, and measures for the control of IBR and BVD must be considered since an association was found between such viral diseases and mastitis and reproductive problems.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The project was approved by the Committee for Ethics in Animal Use of the Federal University of Espírito Santo (UFES), under protocol number 008/2018.

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DECLARATION OF CONFLICT OF INTEREST

We certify that there is no conflict of interest with any organization regarding the material discussed in the manuscript.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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