PREVALENCE OF ANTIBODIES AND RISK FACTORS TO BOVINE VIRAL DIARRHEA IN NON-VACCINATED DAIRY CATTLE FROM SOUTHERN ECUADOR[1]

[PREVALENCIA DE ANTICUERPOS Y FACTORES DE RIESGO PARA LA DIARREA VIRAL BOVINA EN GANADO LECHERO NO VACUNADO DEL SUR DE ECUADOR]

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SUMMARY

The aim of this work was to determine the prevalence of antibodies and risk factors of bovine viral diarrhoea virus (BVDV) in non-vaccinated dairy cattle at the South of Ecuador. A cross-sectional study was carried out to identify risk factors for BVDV infection in 394 randomly selected dairy cows from 75 farms, which were tested for antibodies in milk samples using a commercial Kit ELISA (IDEXX). Epidemiological survey was conducted to determine the risk factors and signs associated with BVDV. Results of this test revealed that the BVDV herd prevalence was 63.5% and the BVDV individual prevalence was 27%. The utilization of artificial insemination (AI) was significantly associated with BVDV status (P > 0.001) where the use of AI increased 2.35 the odds of BVDV positivity (95% CI: 1.46 – 3.38). The cows with clinical signs (diarrhoea, abortions, and ocular and nasal discharge) were not predominantly positive to BVDV antibodies.

Keywords: BVDV, epidemiology, risk factor, prevalence, artificial insemination, antibodies.

RESUMEN

El objetivo de este trabajo fue determinar la prevalencia de anticuerpos y factores de riesgo del virus de la diarrea viral bovina (BVDV) en bovinos lecheros no vacunados en el sur de Ecuador. Se llevó a cabo un estudio transversal para identificar los factores de riesgo de infección por BVDV en 394 vacas lecheras seleccionadas al azar de 75 granjas, que se analizaron en busca de anticuerpos en muestras de leche utilizando un Kit ELISA comercial (IDEXX). Se realizó una encuesta epidemiológica para determinar los factores de riesgo y los signos asociados con BVDV. Los resultados de esta prueba revelaron que la prevalencia del rebaño de BVDV fue del 63,5% y la prevalencia individual del BVDV fue del 27%. La utilización de inseminación artificial (IA) se asoció significativamente con el estado de BVDV (P> 0,001), donde el uso de IA aumentó 2,35 las probabilidades de positividad para BVDV (IC 95%: 1,46 – 3,38). Las vacas con signos clínicos (diarrea, abortos y descarga ocular y nasal) no fueron predominantemente positivas a los anticuerpos contra el BVDV.

Palabras clave: BVDV, epidemiología, factor de riesgo, prevalencia, inseminación artificial, anticuerpos.

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INTRODUCTION

Bovine Viral Diarrhea (BVD) is caused by a small single-stranded RNA virus of positive polarity belonging to the genus Pestivirus of the family Flaviviridae. This virus affects cattle compromising their health and milk production that leads to important economic impairment (Pellerin *et al.,* 1994; Fourichon *et al.,* 2005; Weldegebriel *et al.,* 2009). The bovine viral diarrhea virus (BVDV) is a worldwide spread cattle pathogen. The genus contains a number of species including the two genotypes of bovine viral diarrhea virus (BVDV) (types 1 and 2) and the closely related classical swine fever and ovine border disease viruses (Ridpath *et al.,* 1994). The first way of transmission of BVDV-1 is through nasal, ocular, and genital secretion, and by semen from infected cattle (Guarino *et al.,* 2008).

The BVDV infection mainly affects pregnant cows causing abortions, stillborns, foetus mummification and calves birth with immune-tolerance to BVDV (Terpstra, 1985; Houe, 1995; Paton, 1995; Nettleton *et al.,* 1998). The cows infected with noncytopathic BVDV during the early gestational period are very likely to produce infected calves, which are mainly responsible for spreading BVDV via continuous viral shedding from all mucosal surfaces in herds (Bauermann *et al.,* 2014). Furthermore, Pestivirus infection occurs with leukopenia and immunosuppression because BVDV mostly attack the immune system cells, making these animals susceptible to other pathogens (Potgieter, 1995; Thabti *et al.,* 2002). This infection can be indirectly detected by antibody analysis of serum or milk from animals surrounding the infected groups (Houe, 1992; Niskanen, 1993; Beaudeau *et al.,* 2001).

In many countries the information about prevalence, incidence and associated risk factors have been the baseline for designing and implementing effective regional control actions that minimizes the adverse effects of BVDV infection on herd health and productivity (Rush *et al.,* 2001). Moreover, factor such as type of reproduction, elevation of daily herd, age of cows, and livestock production system have been associated with BVDV infection (Mainar-Jaime *et al.,* 2001; Talafha *et al.,* 2009; Saa *et al.,* 2012).

In the south region of Ecuador there are some reproductive problems that can be associated with BVD. A previous study on seroprevalence of BVDV infection was performed in the Central and North region of Ecuador (Saa *et al.,* 2012), but the risk factors related to this infection have not been clearly defined. Therefore, the aims of the present study were to know about the distribution of BVDV prevalence and to determine their risk factors in a population of non-vaccinated dairy herds from the South of Ecuador. In this region cattle have many reproductive problems that can be associated with BVD.

MATERIALS AND METHODS

This cross-sectional study was performed in the district of Loja to investigate prevalence and risks associated with the presence of antibodies against BVDV in the milk in dairy cows. The information of dairy herds was collected in the three peri-urban (El Valle, San Sebastian y Sucre) and 10 rural sub-districts (Chuquiribamba, El Cisne, Gualel, Jimbilla, Malacatos, San Lucas, Santiago, Taquil, Vilcabamba and Yangana) from February to April 2015, where the vaccinated cows were excluded from the study.

Sample size

Sample size was calculated as Aguilar-Barojas (2005) described. Due to the lack of updated information, the number of adult bovine units was taken from a projection for 2013 made by the National Institute of Statistics and Census (ESPAC, 2013). This projection estimates that the district of Loja will have approximately 49.829 adult bovine units. Sixty percent of them are categorized as dry cows and dairy production cows, 50% of which are related to our study due to the milk sample analysis. Therefore, the sample size was 394 cows from 75 dairy farms.

Data collection

The epidemiological survey was conducted before collecting the milk sample. The cattle farmers were interviewed using "close-ended" questions. For identification purposes, each cattle farmer and milk cow was assigned a unique identification code. The variables included in this study were:

1) Serological status: the presence or absence of BVDV antibodies were determined by sample and positive control (S/P), where the values ≥ 0.30 were considered positives and values ≤ 0.20 were considered negatives.
2) Livestock production systems: semi-intensive and extensive system
3) Quarantine: application and not application of quarantine
4) Breeding methods: artificial insemination and natural mating.
5) Clinical signs: diarrhea, ocular discharge, abortions, infertility, and runny nose.
6) Elevation of dairy herd: it was classified into Groups: 1) from 1400 to 2100 meters above sea level and Group 2) more than 2100 meter above the sea level.
7) Number of calving: it was considered from the first birth onwards.

8) Biosecurity: footbath, wheel-dip or neither was considered.
9) Breeds: all breeds that are exploited in the district of Loja were chosen. It comprises Holstein, Brown Swiss, Jersey and creole cattle.

Sample collection and serological examination

Aseptic teat end preparation (cleaned with ethanol 70%) and discard of the first 4 jets of milk were performed before taking the sample. A single milk sample (~10 mL) per cow was collected. Then, it was held on ice until it arrived to the investigation facilities, this took at maximum 8 hours. Next, the samples were stored at −20°C. To start the analyses in the laboratory, the samples were gradually defrosted to reach room temperature. The presence of BVDV antibodies was tested using the commercial Kit ELISA (IDEXX HerdChek® ELISA kit for BVDV-Ab, IDEXX laboratories, Westbrook, Maine, USA). Following the manufacturer's instructions for undiluted milk, the samples were tested and expressed as sample-to-positive (S/P) ratio where the cut point was set to ≥18U/ul. Antibody concentration was measured at 450 nm using a photometer Biotek ELx800. For measuring the optical density (D.O.), the GEN 5 software and a photometer were employed. The data obtained were calculated by applying the following formulas:

Negative Control Mean.

$$NC\bar{x} = \frac{NC1\ A450 + NC2\ A450}{2}$$

where:
$NC\bar{x}$: Negative control mean
NC1 A450: Negative control_1 has been read in optical densities at 450 nm
NC2 A450: Negative control_2 has been read in optical densities at 450 nm

Positive Control Mean

$$C\bar{x} = \frac{PC1\ A450 + PC2\ A450}{2}$$

where:

$PC\bar{x}$: Positive control mean
PC1 A450: Positive control_1 has been read in optical densities at 450 nm
PC2 A450: Positive control_2 has been read in optical densities at 450 nm

Test Sample.

$$S/P = \frac{Sample\ A450 - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$$

where:

S/P: Sample/Positive ratio
$NC\bar{x}$: Negative control mean
$PC\bar{x}$: Positive control mean

Statistical Analysis

An ELISA sensitivity (Se) of 96.3% and a specificity (Sp) of 99.5% (IDEXX HerdChek® ELISA kit for BVDV-Ab, IDEXX laboratories, Westbrook, Maine, USA) were used to adjust the apparent prevalence (AP) by using the equation for true prevalence (TP) = (AP + Sp)/(Se + Sp) (Thrusfield, 2007). The 95% confidence interval (CI) for the prevalence was based on the normal approximation to the binomial distribution. These tests were performed utilizing R software [R Development Core Team (2014)]. The prevalence and CI were calculated using the Clopper-Pearson exact method (Clopper and Pearson, 1934) of the 'epi.prev' function from the Package epiR version 0.9-62 (Stevenson *et al.*, 2013). Then, these data were presented using ArcGIS 10, version 10.4 software.

The observation (cow) is independent from each other. Then, logistic regression was used to analyze the association between the BVDV antibody status and the predictors. Univariable analysis was performed using all preselected variables. Logistic regression was made using SAS PROC LOGISTIC (SAS Studio version 3.4, Institute Inc, Cary, NC, USA). Multivariable model was not built because the significant variables ($p < 0.1$) selected for inclusion in the multivariable analysis were correlated ($r > 0.8$) between them. Next, the risk was calculated as odds ratio (OR). The OR's standard error and 95% confidence interval were calculated according to Altman (1991).

RESULTS

Three hundred and ninety-four cows were sampled across thirteen sub-districts from 75 dairy farms. These cows had diverse number of calving: 71 cows (18.0%) had one calving, 190 (48.2%) had two to three calving, and 133 (33.8%) had more than three calving. The percentage of farms with semi-intensive and extensive livestock production systems were 11% (8/75 farms) and 89% (67/75 farms), respectively. Most of the subjects were naturally bull serviced (74%) and only 26% of the cows were artificially inseminated. The true herd prevalence of BVDV in Loja and their sub-districts are presented in Figure 1. From the 394 examined milk samples, one hundred and four (26.4%) were BVDV positive by antibody ELISA. The true individual prevalence of BVDV was 27.0% (95% CI: 22.5 - 31.9%).

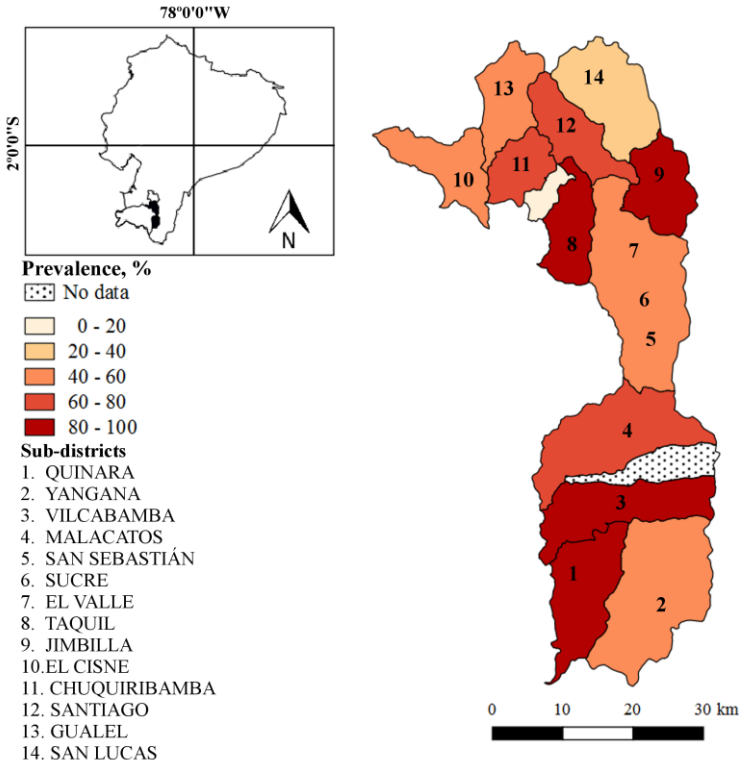

Figure 1. Map of Southern Ecuador indicating the true prevalence of each sub-district of Loja. True prevalence (%) of each sub-district of Loja, Southern Ecuador.

The overall prevalence of antibodies against BVDV in cattle ranged from 6.93% to 86.4% among the thirteen sub-districts studied. The BVDV antibodies occurrence in the peri-urban sub-districts was lower than that of the rural sub-district (12.1% vs. 29.8% respectively). The true herd prevalence was 63.5% (95% CI: 51.0 – 75.0%).

Intrinsic and extrinsic factors associated with the prevalence of antibodies against BVDV are showed in Tables 1, 2 and 3. Herd antibodies against BVDV tended to be associated with the livestock production systems and the use of some biosecurity measures ($P = 0.10$; OR: 5.02). The use of AI is an intrinsic risk factor associated with BVDV in the South Ecuador ($P < 0.001$; OR: 2.35). The AI is strongly related with Semi-intensive livestock production system and application of quarantine ($P < 0.001$).

A linear relationship with the parameter (beta) for estimating the elevation of daily herd (as continuous variable) was observed ($P = 0.05$; Table 3). The number of calving and the breeds of cow (as categorical variables) were not significant related to BVDV ($P > 0.40$). Animals that didn't present the clinical signs showed the highest BVDV prevalence (Table 1). No clinical evidence was found in positive animals to BVDV antibodies. The vesicular stomatitis, nasal discharge and abortions were not related with BVDV prevalence ($P > 0.35$). The cows without diarrhea or ocular discharge showed a higher BVDV antibodies prevalence ($P < 0.01$) than the animals with these clinical signs.

DISCUSSION

As the cows with vaccination against BVDV were not sampled in this study, the presence of antibodies indicates a natural exposure to BVDV at some point of its life. Herd prevalence in this study was higher (63.5%) than those reported in other regions of Ecuador (36.2%; Saa *et al.*, 2012) and lower than that of Peru (96%; Stahl *et al.*, 2002). However, there is an important variation of the BVDV prevalence among different sub-district of Loja (from 6.93% to 86.4%). This prevalence variation could be attributable to factors such as population density and different management practices (Houe, 1995).

Table 1. Descriptive results for explanatory variables and clinical sings with BVDV status among 394 daily cattle from 75 farmers surveyed in Loja from February to April 2015

Variable	Category	N[1,2]	DVB true prevalence, %	
			Positive	% (CI 95%)
Extrinsic factors[1]				
Livestock production systems	Semi-intensive	8	7	90.8 (48.9 – 100)
	Extensive	67	39	60.2 (47.0 – 72.7)
Biosecurity measures	yes	8	7	90.8 (48.9 – 100)
	no	67	39	60.2 (47.0 – 72.7)
Quarantine	yes	6	5	86.5 (36.9 – 100)
	no	69	41	61.5 (48.4 – 73.7)
Intrinsic factors[2]				
Parity	1	71	21	30.3 (19.6 – 42.9)
	2, 3	190	52	28.0 (21.6 – 35.3)
	≥4	133	41	31.6 (23.6 – 40.6)
Artificial insemination	yes	103	44	44.1 (33.9 – 54.6)
	no	291	70	24.6 (19.6 – 30.1)
Breed	Brown Swiss	28	6	21.8 (8.14 – 42.2)
	Brown Swiss mestizo	22	6	27.9 (10.7 – 51.9)
	Holstein Friesian	104	47	46.6 (36.4 – 57.2)
	Holstein Friesian mestizo	230	63	28.1 (22.2 – 34.6)
	Jersey	1	0	0 (0 – 100)
	Creole	9	2	22.7 (2.42 – 62.1)
Clinical Sings				
Sings	yes	161	63	40.3 (32.4 – 48.7)
	no	233	51	22.3 (17.0 – 28.4)
Stomatitis	yes	3	0	0 (0 – 73.3)
	no	391	114	29.9 (25.2 – 34.9)
Diarrhea	yes	31	4	12.9 (3.26 – 30.6)
	no	363	110	31.1 (26.2 – 36.3)
Aborts	yes	3	0	0 (0 – 73.3)
	no	391	114	29.9 (25.2 – 34.9)
Nasal discharge	yes	44	12	27.9 (15.1 – 44.1)
	no	350	102	29.9 (25.0 – 35.2)
Eye discharge	yes	187	40	21.8 (15.9 – 28.7)
	no	207	74	37.0 (30.0 – 44.0)

[1]Number=75 dairy farming. [2]Number = 394 cows

The results show that the semi-intensive livestock production system and application of quarantine tended to be a risk factor in the sampled herds. The semi-intensive livestock production system can be associated with a great herd size and herd density or the use of AI. This also consistent with previous studies (Houe *et al.*, 1995; Valle *et al.*, 1999), who have shown that the important risk factors for BVDV infection are herd size and herd density. Additionally, the infection prevalence tends to increase with the increment in the cattle density in the area.

The AI was the biggest risk factor related with the prevalence of antibodies against BVDV. In the same way, Saa *et al.* (2012), found the same relation on the north Ecuador. Moreover, several studies have found association between BVDV and bovine reproductive management such as contaminated semen and use of infected bulls (Houe, 1999; Lindberg and Alenius, 1999; Gard *et al.*, 2007; Saa *et al.*, 2012). Additionally, common risk factors in epidemiological studies about BVDV are the acquisition of new animals or being in contact with animals from other farms (Solis-Calderon *et al.*, 2005; Luzzago *et al.*, 2008; Talafha *et al.*, 2009; Saa *et al.*, 2012).

On the other hand, our identification of AI utilization as a BVDV risk factor could be indirectly associated with the spread of infection. This could be explained by the indirect transmission through the materials used during AI process or transmission by fomites from farm to farm transported by the AI technicians, who usually inseminate a number of cows in numerous farms per day. Several ways of indirect

transmission of BVDV have been demonstrated such as plastic gloves used in rectal palpation (Lang-Ree et al., 1994), needles and nose tongs or contaminated vaccines (Houe, 1999)

Table 2. Odds ratio analysis for risk factors associated with BVDV status of dairy herds in Loja

Variable	Category	Odds ratio	95% CI	*P-value*
Extrinsic factors[1]				
Livestock production systems	Semi-intensive	5.02	0.585 – 43.2	0.10
	Extensive	1	Reference	
Biosecurity measures	yes	5.02	0.585 – 43.2	0.10
	no	1	Reference	
Quarantine	yes	3.42	0.378 – 30.8	0.25
	no	1	Reference	
Intrinsic factors[2]				
Parity	1	0.942	0.503 – 1.77	0.85
	2, 3	0.845	0.519 – 1.38	0.49
	≥4	1	Reference	
Artificial insemination	yes	2.35	1.46 – 3.78	<0.001
	no	1	Reference	
Breed	Brown swiss	0.954	0.156 – 5.85	0.96
	Brown swiss mestizo	1.31	0.211 – 8.18	0.77
	Holstein Friesian	2.89	0.572 – 14.6	0.20
	Holstein Friesian mestizo	1.08	0.219 – 5.39	0.92
	Jersey	1.00	0.030 – 33.3	0.99
	Creole	1	Reference	

[1]Number=75 dairy farming. [2]Number = 394 cows

Nevertheless, the altitude of daily farm was detected as a risk factor when this variable was considered as a continuous variable. This can be explained by the relation between the altitude of farms and the livestock system production. For instance, farms in Loja with semi-intensive livestock production system are located below 2000 m above sea level. Meanwhile, in central and north region from Ecuador the most intensive dairy production farms are located over 2000 m above sea level.

Table 3. Results of univariable logistic regression analysis for risk factors associated with BVDV status of dairy herds in Loja

Variables	Level	Estimate (ß)	P-value
Artificial insemination			
	Yes	0.366	0.003
	No		
Elevation	Continues	-0.0131	0.050

The cows with clinical signs (diarrhea, abortions, and ocular and nasal discharge) were no predominantly positive to BVDV antibodies. This further support the idea of these clinical signs as cofactors of BVDV infection (Pfeiffer *et al.,* 2002; Park *et al.,* 2006) and a high occurrence of subclinical BVDV infections (between 70% to 90% of BVDV infections) occur without manifestation of clinical signs (Ames, 1986).

Although, the exact mechanism of immunotolerance is unidentified, it is due that circulation of virus during of gestation is when immunocompetence is developing and is a prerequisite for persistence. Viral proteins are recognized as self-antigens resulting negative to antibody-antigen test (Grooms, et al., 2004).

CONCLUSIONS

The results suggest that the natural exposure to BVDV in the Southern Ecuadorian dairy cattle is common and the main risk factors associated with the BVDV infection are the artificial insemination and the livestock production system.

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