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Frequency of reproductive impact diseases in dual-purpose cattle located in Oaxaca, Mexico

Frecuencia de enfermedades de impacto reproductivo en bovinos de doble propósito ubicados en Oaxaca, México

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ABSTRACT

Brucellosis, leptospirosis, bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) are abortive diseases that compromise productive efficiency in cattle. In different municipalities of Oaxaca, Mexico, reproductive problems suggestive of these diseases have been observed. This study determined the frequency of BVD, IBR, brucellosis and leptospirosis in dual-purpose cattle herds in different regions of Oaxaca, Mexico. A total of 2,691 blood samples were collected from 127 bovine herds to diagnose brucellosis serologically using the Rose Bengal and Rivanol tests; microscopic agglutination test (MAT) with a battery of six *Leptospira* serovars; ELISA by blocking and indirect for BVD and IBR respectively. The apparent overall frequency of herd was brucellosis: 2.3%, leptospirosis: 86.6%, IBR: 65.4% and BVD: 56.7%. The real frequency for brucellosis, leptospirosis, IBR and BVD was -9.1% (CI_{95}=-10.1, -7.9%), 64.3% (CI_{95} = 62.5, 66.1%), 40.1% (CI_{95} = 38.2, 41.9%) and 33.2 % (CI_{95} = 31.4, 35.0%) respectively. In Costa region, the highest percentage of animals with antibodies against *Leptospira*, IBR and BVD was observed. The present study showed serologically the presence of antibodies against brucellosis, IBR, BVD and six serovars of *Leptospira* in bovine herds' double purpose of Oaxaca State, Mexico.

Keywords: brucellosis, leptospirosis, BVD, IBR, frequency

RESUMEN

La brucelosis, leptospirosis, diarrea viral bovina (DVB) y rinotraqueítis infecciosa bovina (IBR), son enfermedades abortivas que comprometen la eficiencia productiva en hatos bovinos. En diferentes municipios de Oaxaca, México, se han observado problemas reproductivos sugestivos a estas enfermedades. El objetivo del estudio fue determinar la frecuencia de DVB, IBR, brucelosis y leptospirosis en bovinos de doble propósito criados en diferentes regiones de Oaxaca. Se colectaron 2,691 muestras sanguíneas a partir de 127 hatos bovinos para diagnosticar serológicamente brucelosis mediante las pruebas de tarjeta al 8% y Rivanol; aglutinación microscópica (MAT) con una batería de seis serovariedades de *Leptospira*; ELISA por bloqueo e indirecta para DVB e IBR respectivamente. La frecuencia general aparente de hato fue brucelosis: 2.3%, leptospirosis: 86.6%, IBR: 65.4% y DVB: 56.7%. La frecuencia real para brucelosis, leptospirosis, IBR y DVB fue de -9.1% (IC_{95}= -10.1, -7.9%),

64.3% (IC_{95}= 62.5, 66.1%), 40.1% (IC_{95}= 38.2, 41.9%) y 33.2% (IC_{95}= 31.4, 35.0%) respectivamente. En la región Costa se observó el mayor porcentaje de animales con anticuerpos contra *Leptospira*, IBR y DVB. El presente estudio evidenció serológicamente la presencia de anticuerpos contra brucelosis, IBR, DVB y seis serovariedades de *Leptospira* en hatos bovinos doble propósito del estado de Oaxaca, México.
Palabras clave: Brucelosis, leptospirosis, DVB, IBR, Frecuencia.

INTRODUCTION

Oaxaca ranks sixth in the national livestock census with 1,741,741 bovines, although meat and milk production of this species is in position 12 and 17 respectively (SIAP, 2018). This state is characterized by a humid tropical climate, where dual purpose herds predominate, and family dairy type of 30 animals; grazed in native rainfed species. In this region of Mexico, there are reproductive diseases that put calf production at risk. These sanitary problems increase production cost due to treatments, a low calving rate and a lower volume of milk due to pregnancy losses.
In Mexico, more than 70% of abortions are considered of unknown origin, coupled with these problems are added health problems, which compromise the efficient productivity of animals (Escamilla *et al*., 2007). Among the most important diseases are those that affect reproduction, putting the availability of calves at risk; they also increase production cost due to treatments (Rojo *et al.,* 2009). Infectious agents associated with reproductive disorders in ruminants include abortifacient viral agents, such as Bovine Viral Diarrhea (BVD) (Brodersen, 2014; Larghi, 2018) and Infectious Bovine Rhinotracheitis (IBR) (Baillargeon *et al*., 2017; Valas *et al.,* 2019) of bacterial origin to *Brucella abortus* (Zakia *et al.,* 2016; Poester *et al.,* 2013) and *Leptospira* (Martins and Lilenbaum., 2017; Lilenbaum and Martins., 2014). Therefore, the objective of the study was to determine the frequency of antibodies against brucellosis, leptospirosis, BVD and IBR in dual-purpose cattle raised in different regions of Oaxaca State, Mexico.

MATERIAL AND METHODS

Geographic location and study animals

The study was carried out in fourteen municipalities located in the Sierra Norte, Itsmo and Costa, located in Oaxaca State (Table 1). 2,691 female bovine animals of different breeds were used between heifers with reproductive age (1.5 - 3 years) and adults (3 to 12 years). These animals represent 217 dual-purpose cattle herds, family dairy type. The herds sampled had no history of vaccination against brucellosis (BRU), bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR) and leptospirosis (LEP).

Study design and sampling

The study design was descriptive, observational, and cross-sectional. A non-probabilistic sampling (for convenience) was carried out in herds of cooperating producers. In each sampled bovine 10 ml of blood were collected (disposable tube without anticoagulant), by venipuncture of the coccygeal vein; using Vacutainer® extraction equipment. The samples were identified and placed at rest for approximately

20 min at room temperature to detach the clot, and then stored at 5 °C for transport to the laboratory for small ruminant diseases, INIFAP, Palo Alto. Blood samples were centrifuged at 20 x g for 10 min to obtain serum and stored at -5 °C until serological analysis.

Serological tests

For the brucellosis diagnosis, the 8% Rose Bengal test (Aba test, PRONABIVE, Mexico) was used; the positive samples were confirmed with the rivanol test (Aba test PRONABIVE, Mexico), considering a positive sample, when the titers were ≥ 1:50 (Mexican Official Standard NOM-041-ZOO-1995).

The diagnosis of leptospirosis was carried out using the microscopic agglutination test (MAT), using six serovars and three reference strains: Sejroe (Wolffi serovar), Sejroe (Hardjo serovar) and Tarassovi (Tarassovi serovar); as well as three of national isolation: Icterohaemorrhagiae (serovar Icterohaemorrhagiae), Sejroe (Hardjo prajitno) and Canicola (serovar Portland-vere); considering a positive sample when the titers were ≥ 1: 100 (OIE, 2004).

For the detection and quantification of antibodies against BVD, a commercial test was used: CIVTEST® BOVIS BVD/BD P80 from Hipra, SA, Laboratories following the manufacturer's instructions, including the procedures for determining the relative index (determined from the Optical density (OD) of samples and controls This is a blocking ELISA that detects antibodies against a specific protein (p80) present in all vBVD strains.

The plates were read with an ELISA reader, with a wavelength of 450 nm. The results were expressed as an inhibition percentage, according to the following formula:

% IN = (mean OD of negative control-OD sample/mean OD of negative control) * 100.

An inhibition percentage less than 50% is seronegative.

An inhibition percentage of equal to or greater than 50% is seropositive.

For the diagnosis of IBR, a commercial test was used, CIVTEST® BOVIS IBR from Hipra, SA, laboratories following the manufacturer's instructions, including the procedures for determining the relative index (determined from the optical density (OD) of the samples and controls. The test is based on an indirect ELISA, which detects specific antibodies against the IBR virus.

The plates were read with an ELISA reader, with a wavelength of 450 nm. The results are expressed in relative index x 100 according to the following formula:

IRPC = (OD sample- mean OD negative control/mean OD positive control-mean OD negative control) * 100

A Relative Index per 100 (IRPC) less than or equal to 9 is a negative result.

An IRPC greater than 9.0 and less than 15.0 is a suspicious result.

An IRPC greater than 15 is a positive result.

Statistical analysis

Contingency tables were constructed to calculate the frequency of seropositive animals by disease and region. From the number of positive samples to each of the serological tests, the apparent frequency at herd (p_{AH}) and apparent individual (p_{IA}) levels was estimated. With the sensitivity value and specificity of each test used, the number of seropositive animals within each disease, bovine population size in Oaxaca state (N), sample size (n) by region and total number of sampled animal, the real prevalence (p_{re}) and respective confidence intervals (CI_{95}%) were calculated for each disease within each region (Noordhuizen *et al*., 1997). To calculate the data, they were processed in the WinEpi program (Working in Epidemiology, http://www.winepi.net/) according to the following formula: P_{re} = Apparent Ind Prevalence - (1- Esp)/1- [(1-Esp) + (1- Sens)]

Because the sampling was not proportional to the size of the herd, the prevalence (p) and respective standard error ($S.E_p$) was corrected for herd size using this formula: $p = \sum Nipi/N$ and $S.E_p = \sqrt{D} * S.E._S$, where Ni is herd size; pi herd prevalence, $N = \sum Ni$ is the total number of cattle in the sampled herds; D= the design effect (D=1.96) (Bennett *et al*., 1991; Otte and Gumm, 1997); $S.E._S$ the standard error for a simple random sample $\sqrt{pq/n}$; n is the total number of animals sampled (n=2691). D was calculated as $D = 1 + (k - 1)\ r_e$; where k is the number of animals in the herd and r_e is the correlation within the herd, estimated from the components of a one-way analysis of variance that included the random herd effect. The approximate standard error of r_e and D was obtained according to (Solis *et al*., 2003).

RESULTS

Brucellosis

Table 1 shows the frequency of brucellosis. p_{AH} = 2.3% of positive herds for brucellosis was observed with the rivanol test, which corresponds to a p_{re} = -9.1% (CI_{95} -10.1, -7.9%). The above is equivalent to a total of four positive animals, being Costa region where the highest frequency of animals was observed and the Sierra Norte where there were no reactors.

Leptospirosis

86.6% of the sampled herds had at least one leptospirosis positive animal, for any of the six serovars that were included in this study (Table 1), equivalent to a p_{re} = 64.3% (CI_{95} 62.5, 66.1%) in the animals sampled. The Itsmo and Costa regions had a p_{AH} of 100%; however, the Sierra Norte region was the highest p_{re} (76.5%). The serovars that had the highest frequency were Hardjo prajitno (49.09%) and Icterohaemorrhagiae (34.89%); both of national isolation (Table 2).

Table 1. Frequency of diseases at the herd and individual level calculated in four regions of Oaxaca state

		HERDS			INDIVIDUAL				
Disease	Region	n	(+)	p_{AH}	n	(+)	p_{IA}	p_{re}	CI_{95}%
Brucellosis (Rivanol)	*Sierra Norte*	120	0	0.0%	1031	0	0.0%	-0.09	(-10.9, -7.4%)
	Itsmo	57	2	3.5%	964	1	0.10%	-9.07%	(-10.9, -7.3%)
	Costa	40	3	7.5%	696	3	0.43%	-8.8%	(-10.9, -6.72%)
	Oaxaca	217	5	2.3%	2691	4	0.15%	-9.1%	(-10.1, -7.9%)
Leptospirosis	*Sierra Norte*	120	91	75.8%	1031	783	75.9%	76.5%	(73.9, 79.1%)
	Itsmo	57	57	100%	964	628	65.1%	65.1%	(62.0, 68.1%)
	Costa	40	40	100%	696	323	46.4%	45.3%	(41.5, 48.9%)
	Oaxaca	217	188	86.6%	2691	1734	64.4%	64.3%	(62.5, 66.1%)
IBR	*Sierra Norte*	120	50	41.7%	1031	420	40.7%	46.4%	(43.4, 49.5%)
	Itsmo	57	54	94.7%	964	262	27.2%	30.6%	(27.7, 33.5%)
	Costa	40	38	95.0%	696	268	38.5%	43.8%	(40.1, 47.5%)
	Oaxaca	217	142	65.4%	2691	950	35.3%	40.1%	(38.2, 41.9%)
BVD	*Sierra Norte*	120	48	40.0%	1031	430	41.7%	40.8%	(37.8, 43.8%)
	Itsmo	57	38	66.7%	964	307	31.8%	30.3%	(27.4, 33.2%)
	Costa	40	37	92.5%	696	194	27.9%	26.0%	(22.7, 29.3%)
	Oaxaca	217	123	56.7%	2691	931	34.6%	33.2%	(31.4, 35.0%)

For the calculation of the P_{re} (real prevalence), N = 1,766,208 animals were considered for Oaxaca state (SIAP, 2018), a sensitivity and specificity by test of 83% and 93% for brucellosis (Rivanol); 98.2% and 96.4% for leptospirosis (MAT); 86.59% and 99.10% for IBR (ELISA); 96.94% and 97.84% for BVD (ELISA)

Table 2. Frequency of seropositive animals for each of the *Leptospira* serovars identified by region

Serovars	REGION			
	Sierra Norte	**Costa**	**Istmo**	**Total**
Canicola*	2.03% (21/1031)	32.76% (228/696)	6.95% (67/964)	12.00% (316/2691)
Hardjo	25.80% (266/1031)	19.68% (137/696)	35.17% (339/964)	27.60% (742/2691)
Hardjo prajitno*	53.34% (550/1031)	37.64% (262/696)	52.80% (509/964)	49.00% (1321/2691)
Icterohaemorrhagiae*	57.13% (589/1031)	35.78% (249/696)	10.48% (101/964)	34.90% (939/2691)
Tarassovi	4.07% (42/1031)	6.18% (43/696)	7.26% (70/964)	5.80% (155/2691)
Wolffi	33.66% (347/1031)	3.16% (22/696)	7.57% (73/964)	16.00% (442/2691)

*National strains

Infectious bovine rhinotracheitis

A p_{AH} = 65.4% was observed in the herds sampled in Oaxaca state, and a p_{re} = 40.1% (CI_{95} 38.2, 41.9%) of animals with antibodies against IBR; although the pAH was higher in the Coastal region (95%) and the p_{re} was higher in the Sierra Norte (46.4%).

Bovine viral diarrhea

56.7% of the herds had antibodies against BVD, with a p_{re}=33.2% (CI_{95} 31.4, 35.0%) in the sampled bovines. A higher percentage of affected herds were located on the coast (92.5%); however, it was the region of the Sierra Norte where the highest p_{re} (40.8%; CI_{95} 37.8, 43.8%) of antibodies against BVD was detected.

DISCUSSION

Despite the economic importance that cattle represent for Oaxaca State and the impact that reproductive diseases have, the frequency of these diseases was unknown. However, several studies have been carried out in other country´s regions on the frequency of the four diseases studied (Rosete *et al.,* 2018; Segura *et al.,* 2010; Segura *et al*., 2003).

In this work, serological evidence was found, although with a low frequency of the presence of *Brucella*, in these Oaxaca´s regions. The results are similar to those reported by the National Service for Agrifood Health, Safety and Quality (SENASICA, 2014), of 0.09% for bovine brucellosis in that state. In contrast, the prevalence reported by some authors in intensive dairy herds, located in endemic areas of this disease, are much higher, influenced by overcrowding, lack of exclusive calving areas, among others that favor the transmission of the bacterium (Milián *et al*., 2016).

The results of this work show a high serological frequency of leptospirosis, BVD and IBR; This may be due to the purchase and introduction of cattle from infected herds, the lack of physical barriers, the contact of cattle with other productions, or the failure to quarantine or vaccinate to avoid the appearance of clinical manifestations of the disease (Miyama *et al.,* 2017*;* Milián *et al*., 2016; Gates *et al.,* 2013; Lilenbaum and Martins., 2014; Muylkens *et al*., 2007; Nandi *et al*., 2009). In addition, the humid conditions that prevail in these areas favor the survival of *Leptospira* outside the hosts, causing other animals to acquire the infection. With serological tests, it is not possible to determine whether the presence of antibodies is due to a recent or long-standing infection; that can be concluded is that the vast majority of animals are exposed or live in conditions that allow infections with these agents, since in most herds there was at least one seropositive animal.

In the studies carried out in bovines, the Hardjo serovar is the one with the highest frequency, because bovines are reservoirs of this serovar and transmission between them is facilitated by direct contact and does not depend on environmental factors (Carmona *et al.,* 2011; Olmo, 2019). Segura *et al*., 2003 report a seroprevalence of 62.8% in Yucatán; the Hardjo and Tarassovi serovars had the highest seroprevalence 54.1% and 53.3% respectively. The results obtained in these studies coincide with those obtained in the present, with the serovars Hardjo, Wolffi and Icterohaemorrhagiae being the most frequently diagnosed (Carmona *et al.,* 2011; Escamilla *et al.,* 2007).

IBR results are similar to those reported in states in the southern part of the country; Solís *et al.,* 2003 obtained a seroprevalence of 54.4% in Yucatán state; Milián *et al*., 2016 report a seroprevalence between 57-83% in dairy cattle in Mexico. In studies carried out in the center of the country, the reported prevalences differ from our results. Ojeda *et al.,* 2016 in a study they carried out to estimate IBR prevalence in different municipalities of Mexico State, reported 18% of seropositive animals, which coincides with Magaña *et al*., 2005 who report a rate of 22% in backyard cattle in Michoacán.

The frequency of the disease varies in each region, so it cannot be attributed that close contact is the only factor that determines the high prevalence of the disease, since high prevalences of BVD and IBR have been reported in dairy herds and dairy herds. dual purpose, where the animals are grazing (Milián *et al*., 2016).

The BVD frequency in the country also presents variable results. In this study a frequency of 35.3% was obtained, Moles *et al.,* 2002 reported 72.3% for BVD in cattle from the central zone of Mexico, also finding the presence of antibodies against IBR and leptospirosis; Romero *et al.,* 2013 with 76.5% in Veracruz; Segura *et al.,* 2016 with 47.8% in Tamaulipas; Escamilla *et al.*, 2007 with 70% in Querétaro; Meléndez *et al.,* 2010 with 32.8% in Aguascalientes; Segura *et al.,* 2010 with 16.4% in Michoacán, Milián *et al*., 2016 report a prevalence of 79% in different production systems in various states of the Mexican Republic. Rosete *et al.,* 2018 in a study carried out in Veracruz, Puebla and Tabasco; report that 100% of the herds presented antibodies against BVD, thus suggesting that the BVD virus is widely distributed in the three states.

Based on the results of this study, the presence of antibodies against IBR, BVD and six serovars of *Leptospira* was serologically evidenced in the study population. In the Sierra Norte region, the highest percentage of animals with antibodies against leptospirosis, bovine viral diarrhea and infectious bovine rhinotracheitis was detected; but it was the coastal region where the greatest distribution of the four diseases in the study was detected. The causative agents must be isolated and identified so that recommended prevention and control strategies are implemented in each case.

CONCLUSIONS

The study determined the presence of antibodies against leptospirosis (86.6%), IBR (65.4%), BVD (56.7%) and brucellosis (2.3%) in dual-purpose cattle herds, located in different municipalities of Oaxaca State, Mexico. The distribution, frequency and reproductive level and economic impact of these diseases suggests the implementation of sanitary measures to prevent and control them.

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