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Seroprevalence and risk factors associated with bovine herpesvirus 1 and bovine viral diarrhea virus in North-Eastern Mexico

J.C. Segura-Correa[1], C.C. Zapata-Campos[2,*], J.O. Jasso-Obregón[2], J. Martinez-Burnes[2] and R. López-Zavala[2]
[1]*Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Km. 5 Carretera Mérida-Xmatkuil, Mérida, Yucatán, C.P. 97315, México*
[2]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Km. 5 A.P. No. 263 C.P. 87000, Mexico*

Abstract
Bovine herpesvirus 1 (BoHV-1) and bovine viral diarrhea virus (BVDV) are well known etiological agents of cattle that produce important economic losses due to reproductive failures and calf mortality, as well as enteric and respiratory disease. Tamaulipas is located northeast of Mexico, an important cattle production and the principal exporter of calf and heifer to the United States. The objectives of this study were to estimate the seroprevalence of BoHV-1 and of BVDV, and to determine the effects of risk factors on these infections. Blood samples of cattle from 57 farms from rural districts of Tamaulipas were collected. The samples were tested for antibodies against BoHV-1 and BVDV using commercial ELISA kits. Data on potential risk factors were obtained using a questionnaire administered to the farmer at the time the blood samples were taken. The seroprevalences for BoHV-1 and BVDV were 64.4% and 47.8%, respectively. In the logistic regression analysis, the significant risk factors were rural district, herd size and cattle introduced to the farm. This study confirms the high seroprevalence of BoHV-1 and BVDV in unvaccinated cattle in Tamaulipas, Mexico. The results of this study could be used for the development of BoHV-1 and BVDV prevention and control program in North-Eastern, Mexico.
Keywords: Bovine, Bovine herpesvirus 1, Bovine viral diarrhea virus, Risk factor, Seroprevalence.

Introduction

Bovine herpesvirus 1 (BoHV-1) and bovine viral diarrhea virus (BVDV) are viruses of cattle that can result in economic losses due to reproductive failures, calf mortality, enteric and respiratory disease. BoHV-1 is a virus of the family *Herpesviridae,* subfamily *Alphaherpesvirinae*, the causative agent of infectious bovine rhinotracheitis, a highly contagious, infectious disease (King *et al*., 2012; Newcomer and Givens, 2016). Typical clinical signs associated with BoHV-1 infection include respiratory disease, but the virus can also be associated with conjunctivitis, vulvovaginitis, abortions, encephalitis and balanoposthitis. The transition from primary manifestations of infection to a latent stage of persistency is often the source of spread after virus reactivation (Viu *et al*., 2014). BVDV is a *Pestivirus* from the family *Flaviviridae*, etiological agent of bovine viral diarrhea/mucosal disease (King *et al*., 2012). Clinical signs include pyrexia, diarrhea and reduced production; it is a highly morbid disease but cause low mortality of infected animals (Grooms, 2004; Nardelli *et al*., 2008). Both BVDV type 1 and 2 are present in Mexico. Infection of pregnant cows can result in transplacental fetal infection. Fetuses may be aborted, mummified, stillborn or born with severe anomalies. In many cases, immunotolerant (persistently infected) calves are born (Van Oirschot *et al*., 1999). Also, BVDV can have immunosuppressive effects, which predispose animals to infection by other microorganisms (Reggiardo and Kaeberle, 1981). The use of vaccines may reduce the economic losses caused by clinical disease, but does not seems to reduce the prevalence of either BVDV or BoHV-1 infections (Xue *et al*., 2011). It is difficult to accurately estimate the real economic impact due to infected animals that often have no clinical signs of these infections. BoHV-1 and BVDV are widespread in Mexico as indicated by previous studies (Solis-Calderon *et al*., 2003, 2005; Magaña-Urbina *et al*., 2005; Segura-Correa *et al*., 2010). However, state differences may exist within a country and between regions. Risk factors effects may also varied from region or farm to farm because microclimatic changes, management differences, stock densities, along with other factors (Almeida *et al*., 2013). BVDV is spread between herds mainly by cattle movement, live vaccines use, semen and embryos, visitors, including veterinaries and artificial insemination technicians (Lindberg and Alenius, 1999). Some European studies report several risk factors associated to infection with BoHV-1 such as animal age, vaccination status, herd size, production system (dairy or beef), season and introduction of animals to the farm (Boelaert *et al*., 2005; González-Garcia *et al*., 2009). Several reports associated risk factor to BVDV infection such as density of cattle farms, altitude, more than six calves aged ≤ 12 months, animal purchasing

***Corresponding Author:** Cecilia C. Zapata-Campos. Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Km. 5 A.P. No. 263 C.P. 87000, Mexico. E-mail: cezapata@uat.edu.mx

and presence of veterinary assistance (Saa *et al*., 2012; Fernandes *et al*., 2016). Therefore, information on the epidemiology of BoHV-1 and BVDV is important to establish if tailored prevention and control programs are required for specific regions. The purpose of this study was to estimate the seroprevalence of BVDV and BoHV-1 in cattle and evaluate risk factors in northeast of Mexico.

Materials and Methods

Area of study

The state of Tamaulipas is located at northeast Mexico between 22º 13´ and 27º 40´ N and 97º 09´ and 99º 58´ W. The climate in the State varies from humid to semi-dry. Cattle production is an important activity in Tamaulipas which has a population of approximately 1,366,489 cattle and is the principal exporter of calf and heifer to the United States (SIAP, 2015). According to SAGARPA (Mexican Agricultural Department), Tamaulipas is divided into nine rural districts: Jaumave, Matamoros, Mante, Victoria, Gonzalez, Abasolo, San Fernando, Laredo and Díaz Ordaz.

Animals and sample collection

To estimate seroprevalence, the total cattle population of Tamaulipas (985, 896 heads) was taken into account (INEGI, 2007). The number of animals sampled (n=385 heads of more than 6 months) in the study was calculated considering an expected prevalence of 50%, a confidence level of 95% and a precision of 5% (Segura and Honhold, 2000). Farms and animals within farms were randomly selected. The smallest farm sampled had at least 50 animals. One to 17 heads were sampled per farm. Blood samples were collected from cattle of reproductive age (both sexes). All included animals were not vaccinated against BoHV-1 or BVDV. To identify possible risk factors associated to those diseases, a questionnaire was provided to farm owners, to collect information on putative herd and animal level risk factors. Most of the animals sampled belonged to the Zebu type crosses. There were no clinical signs in the animals recorded at sampling, conducted between May 2010 and December 2011.

Blood samples (10 mL) were collected from the coccygeal vein of each animal, using disposable needles (21 mm 1.5 mm) and vacutainer tubes. The samples were identified and transported on ice to the Diagnostic Laboratory of the Veterinary Medicine Faculty of University Autonomous from Tamaulipas. The blood samples were centrifuged at 1,500 g at 4°C for 10 min and the serum was transferred into disposable microcentrifuge tubes (Eppendorf®) and stored at –20°C until testing.

Laboratory analysis

Blood samples were tested for antibodies against BoHV-1 and BVDV using HerdCheck IBRgB Ab and HerdCheck BVDV p80 Ab ELISA kits (IDEXX laboratories Inc., Westbrook; Maine 04092 USA). The tests were performed according to manufacturer's instructions. A blocking ELISA assay was used for the detection of IgG antibodies against BVDV in serum o plasm, and an indirect ELISA assay for the detection de antibodies anti BoHV-1 using monoclonal antibodies. The results were read in a microplate photometer, where the optical density (OD) was measured at 450 nm. The cut off OD was calculated as A = OD (corrected negative control) 2.0. All samples with an OD greater or equal than 0.25 were considered positives. The sensitivity and specificity of these tests were 100% and 99.5% respectively.

Potential risk factors

Data on potential risk factors were obtained using a questionnaire provided to the farmer at the time the blood samples were collected. The factors evaluated were rural district; herd size (50–200, 201–500, >500 animals), production system (dairy, beef), cattle introduced to the farm (no, yes), replacement origin (own farm; purchased), water origin (Tube water, reservoir, stream, well), age of cattle (6–36, 37-69 and 70-216 months) and sex (female, male).

Data analysis

Descriptive statistics were used to calculate the frequency of seropositive animals for antibodies against BoHV-1 and BVDV. A primary screening test to identify risk factors significantly related to BoHV-1 and BVDV seropositivity was performed using chi-square tests. Only those factors associated ($P < 0.10$) with the response variable were offered to the logistic binomial regression models. All statistical analyses were carried out using the SAS package (SAS, 2008).

Results

Overall seroprevalence values for BoHV-1 and BVDV were 64.4% and 47.8%, respectively. Among 385 cattle sampled, 142 animals were detected to have antibodies against both viruses and 93 were free of antibodies to both viruses. Seroprevalence and chi-square test results for BoHV-1 and BVDV are shown in Tables 1 and 2, respectively. Preliminary chi-square tests showed associations ($P < 0.10$) between the presence of antibodies to BoHV-1 and rural district, herd size, cattle introduced to the farm, replacement origin and water origin (Table 1); whereas the presence of antibodies against BVDV were associated with rural district, production system, herd size, and cattle introduced to the farm (Table 2).

In the logistic regression analyses, the significant risk factors were rural district, herd size and cattle introduced to the farm for BVDV (Table 3); and rural district and herd size for BoHV-1 (Table 4). The lowest seroprevalences for BoHV-1 and BVDV were observed in the rural district of Matamoros and the highest in Laredo and Abasolo, respectively (Tables 1 and 2). Seroprevalences of BoHV-1 and BVDV were significantly higher in large and middle herds, respectively ($P<0.05$). Farms that introduced animals to their herds showed higher odds of antibodies against BVDV.

Table 1. Seroprevalence by risk factor for bovine herpesvirus 1 in Tamaulipas, Mexico (n=385).

Risk factor	*Number of animals*	*Positive*		*p value*
		Number	*%*	
Rural district				<0.0001
Matamoros	10	2	20.00	
Mante	26	13	50.00	
Victoria	67	39	58.21	
Gonzalez	106	58	54.72	
Abasolo	72	46	63.89	
San Fernando	48	37	77.08	
Laredo	56	55	98.21	
Production system				0.6947
Beef	330	213	64.55	
Dairy	55	37	67.27	
Introduction of animals to the herd				<0.0001
Yes	113	90	80.53	
No	272	158	58.19	
Sex				0.9085
Female	346	225	65.03	
Male	39	25	64.10	
Age group (months)				0.3896
6-36	134	82	61.19	
37-69	139	96	69.06	
70-216	112	72	64.29	
Management				0.2910
extensive	324	214	66.05	
Intensive	61	36	59.02	
Herd size (head)				0.0170
50-200	176	101	57.39	
201-500	92	65	70.65	
>500	117	84	71.79	
Replacement origin				<0.0001
Outside	100	46	46.00	
Own herd	285	204	71.58	
Water origin				0.0064
Tube water	126	74	58.73	
Reservoir	165	101	61.21	
Stream-river	37	29	78.38	
Well	57	46	80.70	

Table 2. Seroprevalence by risk factor for bovine viral diarrhea virus in Tamaulipas, Mexico (n=385).

Risk factor	*Number of animals*	*Positive*		*p value*
		Number	*%*	
Rural district				0.0007
Matamoros	10	1	10.00	
Mante	26	11	42.31	
Victoria	67	34	50.75	
Gonzalez	106	44	41.51	
Abasolo	72	45	62.50	
San Fernando	48	15	31.25	
Laredo	56	34	60.71	
Production system				0.0503
Beef	330	151	45.76	
Dairy	55	33	60.00	
Introduction of animals to the herd				0.0685
Yes	113	63	55.75	
No	272	122	44.85	
Sex				0.6453
Female	346	164	47.40	
Male	39	20	51.28	
Age group (months)				0.7295
6-36	134	66	49.25	
37-69	139	68	48.92	
70-216	112	50	44.64	
Management				0.7473
extensive	324	156	48.15	
Intensive	61	28	45.90	
Herd size (heads)				<0.0001
50-200	176	67	38.07	
201-500	92	66	71.74	
>500	117	51	43.59	
Replacement origin				0.3776
Outside	100	44	44.00	
Own herd	285	140	49.12	
Water origin				0.1879
Tube water	126	58	46.03	
Reservoir	165	85	51.52	
Stream-river	37	12	32.43	
Well	57	29	50.88	

Table 3. Results of the logistic regression for bovine viral diarrhea virus seroconversion.

Risk factor	*b*	*EE*	*OR*	*95% CI$_{OR}$*
Rural district				
Matamoros	-1.640	1.017	0.05	0.01, 0.43
Mante	2.324	0.602	2.54	0.48, 13.4
Victoria	0.670	0.421	0.49	0.10, 2.27
Gonzalez	-1.586	0.462	0.05	0.01, 0.26
Abasolo	0.465	0.416	0.40	0.08, 1.94
San Fernando	-1.626	0.433	0.05	0.01, 0.19
Laredo	0	-	1	-
Introduce animals				
Yes	0.913	0.2700	6.21	2.15, 17.9
No	0	-	1	-
Herd size (heads)				
50-200	-1.534	0.2675	0.20	0.09, 0.46
201-500	1.474	0.2541	4.11	1.90, 8.89
>500	0	-	1	-

b: regression coefficient; EE: standard error of b; OR: odds ratios; 95%CI$_{OR}$: 95% confidence interval of OR.

Table 4. Results of the logistic regression for bovine herpesvirus 1 seroconversion data.

Risk factor	*b*	*EE*	*OR*	*95% CI$_{OR}$*
Rural district				
Matamoros	-1.1151	0.9511	0.017	0.001, 0.254
Mante	0.4730	0.5554	0.084	0.007, 0.990
Victoria	-0.4559	0.4033	0.033	0.003, 0.358
Gonzalez	-1.5031	0.4405	0.012	0.001, 0.130
Abasolo	0.2386	0.4060	0.041	0.004, 0.466
San Fernando	-0.1133	0.4136	0.047	0.005, 0.462
Laredo	0	-	1	-
Herd size (heads)				
50-200	-0.7886	0.2172	0.415	0.199, 0.869
201-500	0.6989	0.2018	1.839	0.926, 3.652
>500	0	-	1	-

Discussion

BoHV-1 and BVDV are involved in the respiratory disease complex. BVDV can induce a variety of clinical manifestations which may vary from clinically in apparent infection to acute or chronic severe disease (Baker, 1995). However, the most important economical consequence of BVDV infection is reproductive losses (De Vries, 2006). Clinical signs of BoHV-1 include symptoms of inflammatory processes in both respiratory and genital organs, and abortion (OIE, 2010).

The seroprevalence to BoHV-1 here found (64.4%) is higher than that of cattle in Yucatan, (54.4%) (Solis-Calderon *et al*., 2003) and Michoacán, Mexico (22%) (Magaña-Urbina *et al*., 2005). However, the prevalences in this study are lower than those reported by Córdova-Izquierdo *et al*. (2007) in humid tropics of Mexico (90%). Seroprevalences of BoHV-1 in the literature range from 7.5 up to 70.89 % (Solis-Calderon *et al*., 2003; Eiras *et al*., 2009; Gupta *et al*., 2010; Cedeño *et al*., 2011; Raizman *et al*., 2011; Yousef *et al*., 2013; Saravanajayam *et al*., 2015). The seroprevalence found in this study for BoHV-1, indicates that it is a widely distributed infection in the region.

The seroprevalence to BVDV determined in this study was 47.8% which is within the range 6.3 to 75% of seroprevalences reported in Mexico and other countries in Latin-America (Orjuela *et al*., 1991; Obando *et a*l., 1999; Moles *et al*., 2002; Solis-Calderon *et al*., 2005; Ramirez-Vazquez *et al*., 2016). Differences in antibody prevalence between regions and countries could in part be explained by factors such as production system, herd size, disease-control measures, type of breeding, and age of the animal, this is important because indicates the permanence in the environment of both diseases (Orjuela *et al*., 1991; Mainar-Jaime *et al*., 2001). The BVDV infection could be controlled in the region by not allowing the introduction of persistently infected animals from infected herds. Lindberg and Alenius (1999) reported BVDV infection eradication without any other intervention than controlled introduction of new animals.

The high seroprevalences found in this study, indicates that BoHV-1 and BVDV are common in all rural districts of Tamaulipas. High seroprevalence for those infections have been reported in other parts of Mexico (Solis-Calderon *et al*., 2003, 2005; Magaña-Urbina *et al*., 2005). Animals having antibodies to BoHV-1 and BVDV may be infected by respiratory or via reproductive tract. Therefore, control measures should be installed to prevent contagion between animals of the same region and between other regions.

Risk factors

Rural district

There were significant differences between seroprevalence of a given virus in different rural districts. This heterogeneity may be related to the density of farms in each rural district; differences in prevalence between districts and by factors such as herd size, disease control measures, type of breeding and age of the animal (McDermott *et al*., 1997).

Herd size

Detection of significant differences in the seroprevalence among herd sizes indicates that this is an important risk factor for BoHV-1 and BVDV infections. Orjuela *et al*. (1991) reported in Colombia a high seroprevalence for BVDV in middle-sized farms (9.1%) when compared to small (6.2%) and large farms (3.4%); which agree with the results of this study (Tables 3 and 4), where the odds of infection was 4.11 times the level of infection in large herds. McDermott *et al*. (1997) and Van Wuyckhuise *et al*. (1998) reported; however, that large herds or herds with high stock density are associated with high odds for IBR.

Introduction of animals or cattle origin

The lack of differences in the seroprevalence of BoHV-1 between introduced or not introduced animals to the herd agrees with the results of Solis-Calderon *et al.* (2003). However, the introduction or not of animals was an important risk factor for BVDV (OR=6.21), which suggest the purchasing of persistently infected animals. Solis- Calderon *et al*. (2005) reported that purchase of cows (introduction of animals to the herd) in small herds increased the prevalence and risk of BVDV infection as compared to middle and large herd sizes. Mainar-Jaime *et al*. (2001) in dairy cattle in Spain found that the seroprevalence of purchased cows was much higher than for cows whose origin was the farm. However, seropositive animals are not the main risk of infection of BVDV, but the presence of persistently infected (seronegative) animals in the herd.

Age group of animals

Age group is a frequent reported risk factor for BoHV-1 seropositivity. De Quevedo *et al*. (1978), Orjuela *et al*. (1991), McDermott *et al*. (1997), Hage *et al*. (1998) and Solis-Calderon *et al*. (2003) reported higher seroprevalence in old animals. However, in this study, age was not a significant risk factor. The similar seroprevalence of BVDV observed among the three age-groups suggest the dissemination of persistently infected animals in the herds studied. The distribution of virus and risk factors identification are important in order to establish prevention and control programs against economically important diseases such as BVD and IBR.

In conclusion, this study confirms the high seroprevalence of BoHV-1 and BVDV in non-vaccinated cattle in Tamaulipas, Mexico. The fact that animals were not vaccinated and that all age-groups had high seroprevalence indicates that the BoHV-1 and BVDV are naturally circulating in this population. So is urgently needed to establish measures for the epidemiological control and prevention of these diseases to decrease their incidence.

Conflict of interest

The authors declares that there is no conflict of interest.

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