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Full Length Research Paper

# Seroprevalence and risk factors associated with infectious bovine rhinotracheitis in unvaccinated cattle in southern Veracruz, Mexico

Dora Romero-Salas<sup>1</sup>\*, Concepción Ahuja-Aguirre<sup>1</sup>, Felipe Montiel-Palacios<sup>1</sup>, Zeferino García-Vázquez<sup>2</sup>, Anabel Cruz-Romero<sup>3</sup> and Mariel Aguilar-Domínguez<sup>1</sup>

<sup>1</sup>Universidad Veracruzana, Facultad de Medicina Veterinaria y Zootecnia. Veracruz, México. <sup>2</sup>INIFAP, Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria. Morelos, México. <sup>3</sup>Colegio de Postgraduados Campus Veracruz, Veracruz, México.

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A cross-sectional study was conducted to determine the seroprevalence and risk factors associated with infectious bovine rhinotracheitis (bovine herpesvirus-1 [BHV-1]) in unvaccinated cattle in southern Veracruz, Mexico. The study was conducted from September 2007 to March 2009. A total of 968 serum samples from 83 herds were analyzed through ELISA test to determine the presence of antibodies against BHV-1. Descriptive statistics was used to calculate the specific seroprevalence with the SPSS software, and the Vassar Stats software was used to calculate the confidence intervals of the specific prevalence and to determine the risk factors. Overall seroprevalence was 64.5%. The dairy cattle showed the highest seroprevalence (72.4%) and the dual-purpose cattle the lowest (61.7%). The 7-year-old cattle showed the highest seroprevalence (91.9%) and the 1-year-old females (11.8%) the lowest. The cows with  $\geq$  6 calvings showed the highest seroprevalence (82.4%) and the pubertal heifers the lowest (19.4%). Seroprevalence in bulls was 100%. In cows with or without history of abortions, seroprevalence was 76.5 and 72.1%, respectively. The risk factors associated with BHV-1 infection were the dual-purpose cattle, 6-year-old cattle, and all the reproductive stages, except pubertal heifers. In conclusion, BHV-1 infection is present in cattle in southern Veracruz, Mexico.

Key words: IBR, BHV-1, seroprevalence, risk factor, beef cattle, dairy cattle, dual-purpose cattle.

# INTRODUCTION

Bovine herpesvirus type 1 (BHV-1) is an important pathogen of cattle worldwide that causes respiratory disease, reproductive failure, abortion, and increased calf mortality (Anderson, 2007; Nandi et al., 2009). This virus is responsible for infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis, infectious pustular balanoposthitis, and encephalitis (Ackermann et al., 1990a; Rissi et al., 2008). BHV-1 generally infects cattle older than 6 months of age (Bennett and Ijpelaar, 2003). The main route of transmission is by direct contact with nasal, ocular, and genital secretions from infected animals, through infected semen (Ackermann et al., 1990a; Nuotio et al., 2007), or indirectly through contaminated material and wind borne particles (Wentink et al., 1993). Pathogenicity of BHV-1 infection can vary from mild to severe (Cowley et al., 2011). Clinical manifestations include conjuntivitis, nasal discharge, fever, loss of appetite, milk drop, abortion, vulvovaginitis, and even death (Ackermann et al., 1990a; Miller et al., 1991; van Oirschot, 1995). Subclinical infection has been observed after introduction of

\*Corresponding author. E-mail: dromero@uv.mx. Tel: +52 (229) 9344053. Fax: +52 (229) 9342075.

Municipality	Number of herds	Sample size	Number of positive	Seroprevalence (%)	Confidence interval (95%)
Acayucan	3	37	28	75.7	58.4-87.6
Agua Dulce	4	48	29	60.4	45.3-74.0
Cosoleacaque	4	47	30	63.8	48.5-77.0
Hidalgotitlán	7	80	61	76.3	65.2-84.7
Ixhuacán del Sureste	4	47	34	72.3	57.1-83.1
Jesús Carranza	13	116	85	73.3	64.1-81.0
Las Choapas	17	204	141	69.1	62.2-75.3
Mecayapan	4	47	13	27.7	16.0-43.0
Minatitlán	15	196	104	53.1	45.8-60.2
Moloacán	5	49	18	36.7	23.8-51.7
San Juan Evangelista	4	57	43	75.4	62.0-85.4
Sayula de Alemán	3	40	38	95.0	81.8-99.2
Total	83	968	624	64.5	61.3–67.4

 Table 1. Seroprevalence of BHV-1 by municipality in cattle herds in southern Veracruz, Mexico.

of the virus to naive herds (van Oirschot et al., 1993; Pritchard et al., 2003).

After initial infection and disease, cattle become carriers of the virus for life, as it remains latent in the trigeminal and sciatic ganglia, tonsils (Ackermann et al., 1982), and probably other organs (Bilge-Dağalp et al., 2008). Reactivation of the virus may occur under physiological stress (Ackermann et al., 1990a) or by administration of immunosuppressive hormones (Whetstone et al., 1989); once reactivated; the BHV-1 is excreted and can be transmitted to susceptible cattle (Ackermann et al., 1990a; Wentink et al., 1993). Animals with latent infections can be identified through the detection of antibodies against BHV-1 in serum (Lemaire et al., 2000). Latent infections make it difficult to control transmission to unexposed animals, since they cannot be diagnosed by clinical examination or quarantine (Guarino et al., 2008).

Seroprevalence of BHV-1 in cattle has been reported worldwide in dairy and beef herds, and some risk factors for the presence of BHV-1 antibodies in cattle include large herd size, older age, dairy herds with presence of beef cattle, high density of herds within an area, purchased cattle, and herds located close to BHV-1 positive herds (van Schaik et al., 1998; Boelaert et al., 2005). However, information from developing countries is limited (Kampa et al., 2004). In Mexico, seroprevalence of BHV-1 has been reported in dairy (Escamilla et al., 2007; Segura-Correa et al., 2010) and beef (Solis-Calderon et al., 2003; Cordova-Izquierdo et al., 2009) cattle throughout the country, but no information is available on the seroprevalence of this virus in dual-purpose (Bos taurus / Bos indicus) cattle, commonly found in the tropical regions of Mexico. Veracruz is a state located in the Mexican tropics, and has one of the largest cattle inventory in the country, hence its importance in bovine production. Therefore, this study determined the seroprevalence and risk factors associated with BHV-1 infection in dairy, beef and dual-purpose cattle herds in southern Veracruz, Mexico.

#### MATERIALS AND METHODS

#### Type of study and experimental animals

A cross-sectional study was conducted from September 2007 to March 2009 in 83 herds of dairy (Holstein, Brown Swiss, Simmental), beef (Zebu breeds) and dual-purpose (undefined Bos taurus / Bos indicus crosses) cattle from 12 municipalities (Table 1) in the southern region of the state of Veracruz, Mexico. This region was selected because it harbors a great proportion of the total of cattle found in the state. The herds included in the study were selected by convenience sampling. No herd had ever been vaccinated against BHV-1. Sample size by municipality was determined with the Win Episcope ver. 2.0 software, for confidence level and error of 95 and 5%, respectively, assuming an expected seroprevalence of 30% as previously reported in Mexico (Segura-Correa et al., 2010). The sampling method was stratified random sampling. A total of 968 cattle were included in the study, from which 960 were females with age ranging from 1 to 7 years (pubertal heifers, first-calf heifers, cows), and 8 bulls used as sires.

#### **Risk factors**

The risk factors considered in the study were purpose of the herd (dairy, beef, or dual-purpose), age, reproductive stage and history of abortions.

#### Management practices in the farms

At the start of the study, a questionnaire was applied to the manager of each herd to obtain information on age of the animals, occurrence of abortions within the last three years and vaccination status.

Herd purpose	Sample size	Number of positive	Seroprevalence (%)	Confidence interval (95%)
Beef	168	116	69.0	61.4-75.8
Dairy	134	97	72.4	63.8-79.6
Dual-purpose	666	411	61.7	57.8-65.4
Total	968	624	64.5	61.3-67.4

Table 2. Seroprevalence of BHV-1 by purpose of the herd in cattle in southern Veracruz, Mexico.

Table 3. Seroprevalence of BHV-1 by age in cattle in southern Veracruz, Mexico.

Age (year)	Sample size	Number of positive	Seroprevalence (%)	Confidence interval (95%)
1	51	6	11.8	5.0-24.5
2	102	36	35.3	26.2-45.4
3	158	87	55.1	47.0-63.0
4	159	101	63.5	55.4-71.0
5	224	162	72.3	66.0-78.0
6	138	107	77.5	69.5-84.0
7	136	125	91.9	85.6-95.7
Total	968	624	64.5	61.3-67.4

#### **Blood samples**

From each of the 968 animals, one blood sample was collected via jugular venipuncture into sterile vacutainer<sup>TM</sup> tubes with no anticoagulant. Samples were transported in ice to the laboratory, where they were centrifuged at 1000 *g* for 15 min to separate the serum, which was stored at -20°C until analysis.

#### Detection of antibodies against BHV-1

All samples were tested for the presence of BHV-1 antibodies through ELISA test using a commercial kit (HerdChek® Infectious Bovine Rhinotracheitis [IBR]/ Bovine Herpesvirus-1 [BHV-1] gB; IDEXX Laboratories, Inc., The Netherlands). The sensitivity and specificity of the assay were 100 and 98.9%, respectively. For the determination of positive and negative samples, the software XCheck 3.3<sup>®</sup> (IDEXX Laboratories, Inc.) was used, and those samples showing an absorbance above the cut-off point of 0.3 were considered as positive.

#### Statistical analysis

Data were stored in Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics was used to calculate the specific seroprevalence (by municipality, purpose of the herd, age, reproductive stage, and history of abortions) with the SPSS® ver. 19.0 software. The Vassar Stats® software was used to calculate the confidence intervals (set at 95%) of the specific prevalences, and to determine the risk factors. Results were statistically different when P<0.05.

# RESULTS

#### Seroprevalence

Of the 968 animals sampled, 624 were positive to the

presence of antibodies against BHV-1, for an overall seroprevalence of 64.5%. The seroprevalence by municipality ranged from 95 to 27.7% (Table 1).

Regarding the purpose of the herd, the dairy cattle showed the highest seroprevalence (72.4%) and the dual-purpose cattle the lowest seroprevalence (61.7%) (Table 2).

With respect to the age of cattle, the lowest seroprevalence was obtained in the group of 1-year-old females (11.8%), and the highest in the group of 7-year-old cattle (91.9%) (Table 3).

When the reproductive stage of the female was considered, the pubertal heifers had the lowest seroprevalence (19.4%), whereas the cows with  $\geq$  6 calvings showed the highest seroprevalence (82.4%) (Table 4). All the bulls tested positive for BHV-1 antibodies, for a prevalence of 100% (Table 4).

In cows with a history of abortions, seroprevalence of BHV-1 was 76.5%, while in the cows with no history of abortions it was 72.1% (Table 5).

# **Risk factors**

Table 6 shows the risk factors associated with BHV-1 infection in the study herds. The risk factors obtained in the present study were purpose of the herd, age of cattle, and reproductive stage of cattle. With respect to purpose of the herd, the dual-purpose cattle were associated with an increased risk of infection with BHV-1, whereas the beef cattle were not a risk factor. Regarding the age of the animals, 6-year-old cattle were highly associated with the risk of infection with the virus, whereas 1- and 2-year-

Reproductive stage	Sample size	Number of positive	Seroprevalence (%)	Confidence interval (95%)
Bull (sire)	8	8	100	60.0-100.0
Pubertal heifers	103	20	19.4	13.0-28.1
Pregnant heifers	80	35	43.8	33.0-55.2
1st-calving heifers	170	94	55.3	47.4-62.8
2nd-calving cows	208	151	72.6	66.0-78.4
Cows with 3 to 5 calvings	348	274	78.7	74.0-83.0
Cows with $\geq$ 6 calvings	51	42	82.4	68.6-91.1
Total	968	624	64.5	61.3-67.4

Table 4. Seroprevalence of BHV-1 by reproductive stage in cattle in southern Veracruz, Mexico.

Table 5. Seroprevalence of BHV-1 considering the history of abortions in cattle in southern Veracruz, Mexico.

History of abortions*	Sample size	Number of positive	Seroprevalence (%)	Confidence interval (95%)
Yes	17	13	76.5	49.7-92.2
No	760	548	72.1	68.7-75.2
Total	777	561	72.2	68.8-75.3

\*The females considered were 1st-calving heifers and cows with 2 to  $\ge$  6 calvings.

 Table 6. Risk factors associated with seropositivity to BHV-1 in cattle in southern Veracruz, Mexico.

Variable	OR	CI 95%	P-value
Purpose of the herd			
Dairy	1	-	-
Dual-purpose	1.6	1.07-2.45	0.02
Beef	1.2	0.71-1.93	0.05
Age (years)			
2	1	-	-
3	2.2	1.32-3.69	<0.01
4	3.3	1.99-5.61	<0.01
5	4.6	2.84-7.69	<0.01
6	6.2	3.54-10.99	<0.01
7	3.8	2.01-7.24	<0.01
Reproductive stage*			
Pubertal heifers	1	-	-
Pregnant heifers	3.0	1.58-5.93	<0.01
1st-calving heifers	5.1	2.89-9.11	<0.01
2nd-calving cows	2.1	1.42-3.37	<0.01
Cows with 3 to 5 calvings	3.0	2.02-4.48	<0.01
Cows with $\geq$ 6 calvings	3.7	1.72-8.23	<0.01

\*Because of the small sample size for bulls, this variable was found non-significant and therefore not included in the table.

old cattle were not risk factors. Finally, with respect to the reproductive stage of cattle, all the reproductive stages, with the exception of pubertal heifers, turned out to be risk factors. The history of abortions was not a risk factor of BHV-1 infection.

# DISCUSSION

Since none of the herds included in the study had been vaccinated against BHV-1, the seroprevalence obtained indicate that the study herds had been exposed to the

virus, assuming that the presence of antibodies can only be caused by exposure to the pathogen (van Schaik et al., 1998; Kampa et al., 2004). To this respect, several authors indicate that considering the persistence of BHV-1 specific antibodies and the phenomenon of self-clearance (Kaashoek et al., 1996; Fredriksen et al., 1999), serosurveys may give positive results years after last exposure to the virus, thus positive cattle may not have been recently exposed to the BHV-1(Kampa et al., 2004), but they can be latent carriers of the pathogen, which might be the case of the present study.

In Mexico, seroprevalence of BHV-1 has been reported in unvaccinated cattle in several states, such as Michoacan, where Magaña-Urbina et al. (2005) and Segura-Correa et al. (2010) reported individual seroprevalence of 22 and 3.4%, respectively, both in dairy cows. In beef cattle, seroprevalence was reported in Yucatan at 54.4% (Solis-Calderon et al., 2003), and in the humid tropical region at 89.8% (Cordova-Izquierdo et al., 2009).

The presence of antibodies against BHV-1 has also been reported in unvaccinated cattle around the world. For example, in Canada, Waldner (2005) indicates 20.4% seroprevalence in beef heifers and cows. In Costa Rica, Raizman et al. (2011) reported individual seroprevalence of 48% in dual-purpose, dairy and beef cattle. In South America, prevalence of antibodies against BHV-1 in beef cattle was reported at 67% in Venezuela (Obando et al., 1999) and 35% in Uruguay, with the highest seroprevalence in cows (44%) and bulls (88%), and the lowest in heifers (11%) (Guarino et al., 2008). In Ecuador, Carbonero et al. (2011) indicate individual seroprevalence of 43.2%, and herd prevalence of 82.1% in dairy and dual-purpose cattle. In Peru, in crossbred native cows and heifers managed extensively, Zacarías et al. (2002) pointed out overall seroprevalence of 67.6%, ranging from 75.8 to 62.1%: these authors indicate that since the cattle had not been vaccinated, the antibodies detected were induced by the virus in the field. These results are comparable to those obtained in the present study, and could be due to the extensive management of cattle, since although some of the study herds were dairy cattle; their management and that of the rest of the herds was mainly extensive. This assumption can be supported by the lower prevalences reported by Rosadio et al. (1993) also in Peru, who indicate 22.6 and 36.5% in intensively managed dairy and beef cattle, respectively. Nonetheless, also in Peru, Villacagui et al. (2006) mention seroprevalence of 0.6% in unvaccinated crossbred and dairy cattle managed extensively, which was much lower than the prevalences reported by other authors in that country and in other countries, where extensive and intensive management is practiced; these authors explain the low prevalence due to absence of spreading agents of the virus and the management practiced in that region that keeps animals from close contact with each other. In herds with a history of reproductive disorders such as repeat breeding and abortions, seroprevalences reported

were 39 and 86% in Holstein herds in Brazil (Mineo et al., 2006), and 74.7% in cows and in bulls used as sires, of crossbred, Zebu and European genotype in Colombia (Betancur et al., 2006). This suggests that the bulls are a main factor of infection in the herds, similar to the result in the present study, where 100% of the bulls used as sires tested positive for antibodies against BHV-1; this is particularly important because in Mexico, mainly in dual-purpose cattle herds, artificial insemination of cows is rarely used, thus natural breeding could be contributing to the dissemination of the disease.

In Europe, although some countries have controlled BHV-1 since long ago (Ackermann et al., 1990b), the virus is still present in some other countries, with the infection tending to be endemic in most populations, but with national and regional variations (Kampa et al., 2004; Gonzalez-Garcia et al., 2009). In unvaccinated cattle, Boelaert et al. (2000) in Belgium indicate herd prevalence of 84, 89 and 53% in dairy, mixed and beef herds, respectively, whereas in Spain, Gonzalez-Garcia et al. (2009) in dairy, beef and crossbred cattle reported individual and herd seroprevalence of 45.7 and 70.4%, respectively, with prevalence by province ranging from 50 to 83.3%. In unvaccinated cattle, O'Grady et al. (2008) indicate seroprevalence of 20% in beef cattle in Ireland, and Woodbine et al. (2009) mention seroprevalence of 83.2% in dairy and suckler cattle herds in England. In Estonia, Raaperi et al. (2010) reported herd prevalence of 22% in dairy cattle.

In Asia, in unvaccinated dairy cattle, seroprevalence reported was 67 and 61% in Thailand (Kampa et al., 2004, 2009), and 72.1% in Turkey (Bilge-Dağalp et al., 2008), which are comparable to the results of the present study. However, there are other reports also in unvaccinated dairy catle that indicate lower seroprevalences than the ones obtained in this study, such as 17.1% in Turkey (Yeşilbağ and Güngör, 2008), 35.8% ranging from 12.1 to 77.8% by province in China (Yan et al., 2008), and 38% ranging from 71.1 to 0% in India (Nandi et al., 2011). These lower results could be probably due to the different management practices of the herds.

In general, most of the seroprevalence values mentioned above are comparable with those obtained in the present study, which might indicate that BHV-1 is present in cattle around the world regardless of the different climate and management conditions of the herds in different countries.

Regarding the risk factors, in unvaccinated dairy herds in Michoacan, Mexico, Magaña-Urbina et al. (2005) and Correa-Segura et al. (2010) indicate as risk factors the size of the herd, the age of the cattle, and the purchase of animals from other locations; Magaña-Urbina et al. (2005) also mention that the history of abortions was not a risk factor. All these results are similar to those obtained in the present study. In unvaccinated beef cattle in Yucatan, Mexico, Solis-Calderon et al. (2003) indicate that animals in large herds or in production have higher odds of seropositivity than those in small herds or growing, while in the Mexican humid tropical region Cordova - Izquierdo et al. (2009) mention that dual-purpose cattle are a risk factor for BHV-1 infection, similar to the result of the present study.

In Ecuador, in unvaccinated dairy and dual-purpose cattle herds, Carbonero et al. (2011) reports that animals older than 4 years of age, BRSV infection, altitude above sea level and average slope are risk factors associated with BHV-1 infection, whereas good cleaning of the facilities is a protective factor. In Uruguay, Guarino et al. (2008) pointed out that the larger the size of the herd, the higher the seroprevalence of BHV-1. These authors agree with the observations made by Inui et al. (2000) also in Uruguay, that the large percentage of seronegative heifers suggest that exposure to the virus occurs at breeding, and they also mention that most of the positive cattle can be attributed to natural exposure to the agent, which could be the case of the present study. In Colombia, in unvaccinated crossbred, Zebu and European repeat breeding cows, cows with history of abortions, and bulls used as sires, Betancur et al. (2006) mention as risk factors cows older than 5 years of age, and bulls older than 7 years of age, and indicate that the history of abortions is not a risk factor, which agrees with the result of the present study.

European studies have also reported several risk factors associated with infection with BHV-1, such as animal age, vaccination, herd size, production type (dairy or beef), season of the year and purchase of cattle (van Schaik et al., 1998; Boelaert et al., 2005; Gonzalez-Garcia et al., 2009). In Ireland, Cowley et al. (2011) indicate the herd size as a risk factor, whereas in England, Woodbine et al. (2009) report the adult cattle in herds without grower cattle, homebred cattle, and herds that were totally restocked after the foot-and-mouth epidemic in 2001 as risk factors. In Estonia, Raaperi et al. (2010) indicate that the seroprevalence increased significantly with herd size, whereas the young stock were uninfected; these authors remark that the veterinarian or employees of the farm may play a major role in the spread of the virus. In nonvaccinated beef, dairy and crossbred cattle in Spain, Gonzalez-Garcia et al. (2009) indicate that the risk factors for BHV-1 infection are herd size, lack of specific cattle infrastructure, beef crossbreeding, history of reproductive disorders, purchase of replacements and proximity to an urban area. In the Netherlands, van Schaik et al. (1998) indicate as risk factors for BHV-1 infection in dairy herds the purchasing and participation in cattle shows, more professional visitors in the barn not using protective clothing, and location closer to other cattle farms.

In the present study, the presence of BHV-1 in the cattle might have affected the respiratory airways and been misdiagnosed as parasitic pneumonia, particularly in young cattle, as pointed out previously (Zacarías et al., 2002). Since the BHV-1 infection in adult cattle is usually

subclinical, these animals may be acting as reservoirs and therefore as source of infection for younger animals (Rivera et al., 1994).

The fact that in the present study cattle in all the age groups tested positive for BHV-1 antibodies suggests that animals might have started to seroconvert during their first year of life. However, some authors indicate that the antibodies may first appear at six months of age (Woodbine et al., 2009), and might remain for at least five years (Chow, 1972); if that is the case, it could explain the seroprevalence found in all groups of age.

Since no reports on seroprevalence of BHV-1 in dualpurpose cattle in Mexico are available in the literature, the present study is the first to report the presence of the virus in this type of cattle, commonly found in the Mexican tropics.

In conclusion, there was evidence of the presence of BHV-1 in dairy, beef and dual-purpose cattle in southern Veracruz, Mexico. Therefore, seroprevalence of BHV-1 should be also determined in other regions of the state of Veracruz, as well as in other states of the country, to have more accurate data on the presence of the disease in Mexico, and to take preventive measures to avoid its dissemination.

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