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Seroepidemiology of Bovine Herpes Virus-1 Infection in Water Buffaloes from the state of Veracruz, Mexico

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Abstract. Water buffaloes (*Bubalus bubalis*) were introduced to Mexico at the end of the last century. In Mexico, buffaloes are commonly pastured together with cattle; however few studies have been done on buffalo herd health in Mexico. We hypothesized that a better knowledge of the epidemiology of infections shared between cattle and buffaloes may improve herd profitability and promote buffalo production in areas unsuitable to cattle farming. This study aimed to determine the prevalence of antibodies against bovine herpes virus – 1 (BoHV-1) in water buffaloes raised on six farms from the state of Veracruz, Mexico. Of 368 buffaloes sampled, 217 (59%) were seropositive for BoHV-1. Age was identified as a risk factor for BoHV-1 infection with buffaloes older than 5 years being the most likely to be infected. Animals more than 7 years old had the highest prevalence (86.0%). Females and males had similar seroprevalence rates. Females with history of abortion had higher prevalence of anti-BoHV-1 antibodies than those with no record of abortion. Buffaloes and cattle were raised together in only one of the six farms under study. Interaction with cattle was not a risk factor for BoHV-1 seropositivity. This study showed that BoHV-1 is prevalent among buffalo herds in the state of Veracruz, Mexico. Buffaloes appear to play an important role in the epidemiology of BoHV-1 infection in parts of Mexico when there is no apparent risk of interaction with cattle. Animal health programs established to mitigate the burden caused by BoHV-1 must take into consideration buffaloes when this bovid species is part of the agroecosystem shared with cattle.

INTRODUCTION

Since its domestication in Asia approximately 3,000–6,000 years ago, the water buffalo (*Bubalus bubalis*), herein referred to as buffalo, has been economically important as dairy, meat, and draught livestock in various parts of the world (Michelizzi *et al*., 2010; Czerniawska-Piatkowska *et al*., 2010). The global population includes around 181 million buffaloes, which represents approximately 12% of the world bovine stock (Soliman & Bassiony, 2011; Zhang *et al*., 2011). Asia is the continent with the largest proportion (~97%) of the world buffalo population (Pasha & Hayat 2012; Deb *et al*., 2016). In some Asian countries buffalo is the most important livestock species (Magsi *et al*., 2017; Wang *et al*., 2017). The buffalo population in America is constituted of approximately 4.12 million animals, and Brazil has the largest herd with approximately 3.5 million buffaloes (Zava, 2010).

Cattle and buffaloes are closely related and form part of the tribe Bovini within the family Bovidae (Czerniawska-Piatkowska *et al*., 2010; Zava, 2010). Although buffaloes are considered resistant to many diseases, they are susceptible to several of the infectious agents and parasites that affect cattle (Fagiolo *et al*., 2005; Czerniawska-Piatkowska *et al*., 2010). Many of the infectious diseases of buffaloes and cattle are transmissible between these species. Infectious diseases that affect buffaloes and cattle are of socio-economic relevance because of the impact they can have on the international trade of livestock and livestock products (Caruso *et al*., 2016; Deb *et al*., 2016; Qui *et al*., 2017).

Bovine herpes virus 1 (BoHV-1) is an economically important pathogen that can infect buffaloes and cattle (Nandi *et al*., 2009; Graham, 2013; Majumder *et al*., 2015). BoHV-1 is a double-stranded DNA virus classified in the family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Varicellovirus*. It causes clinical syndromes such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), abortion, infertility, conjunctivitis and encephalitis, primarily in cattle (Nandi *et al*., 2009; Graham, 2013; Majumder *et al*., 2015; Thiry *et al*., 2006). BoHV-1 has been isolated from naturally infected buffaloes (Fusco *et al*., 2015), and buffalo bull semen (Lata *et al*., 2009). Enzyme-linked immuno-sorbent assays (ELISA) are used to assess individual or herd status of BoHV infections (Puentes *et al*., 2016). Seroepidemiological data documented the exposure of buffaloes to BoHV-1 (Scicluna *et al*., 2007; Mahmoud *et al*., 2009; Ferreira *et al*., 2010; Nandi *et al*., 2011; Harun *et al*., 2012; Waffa *et al*., 2015; Viana *et al*., 2016), as well as to the buffalo herpesvirus 1 (BuHV-1) (European Safety Authority, 2006; Sasso *et al*., 2010), which is more closely related to BoHV-5 (De Carlo *et al*., 2004).

There were no observable clinical signs consistent with the syndromes ascribed to BoHV-1 in young male buffaloes experimentally infected by intranasal inoculation with a field strain virulent to cattle, which suggested that BoHV-1 infection in buffaloes is generally subclinical (Scicluna *et al*., 2010). However, it was reported that samples collected from dead newborn and euthanized buffalo calves were positive for BoHV-1 (Fusco *et al*., 2015), which indicated that the virus is as pathogenic to buffaloes as it is to cattle. BuHV-1 caused diarrhea and nasal discharge after pharmacological reactivation in buffaloes, and infection with this virus has been associated with abortion (De Carlo *et al*., 2004; Amoroso *et al*., 2013).

Buffaloes were introduced in Mexico at the end of the last century (Dominguez-Aguilar *et al*., 2013; Romero-Salas & Pérez de León, 2014). The state of Veracruz is located in the Mexican tropics. Veracruz has one of the largest cattle herds in the country, and some ranchers also raise buffaloes (Romero-Salas *et al*., 2013). Our group initiated serosurveys of buffalo herds in the state of Veracruz, Mexico, to better understand the epidemiology of infections shared between cattle and buffaloes (Suazo-Cortez *et al*., 2012; Romero-Salas *et al*., 2013; Romero-Salas *et al*., 2016), because some of those infections can cause severe animal health problems, diminish animal production, and be of public health importance (Munchow & Pisarz, 1994; Sharma & Kumar, 2003). The knowledge provided by the serosurveys will help develop disease management and control programs allowing ranchers to maximize herd profitability and promote buffalo production in tropical agroecosystems of Mexico unsuitable to cattle farming.

We documented that buffaloes in Mexico are infected with BoHV-1 (Romero-Salas *et al*. 2017). However, some epidemiological aspects of BoHV-1 infection remained to be determined. This study explored further the seroepidemiology of BoHV-1 infection by testing a larger sample from a different population of water buffaloes in the state of Veracruz, Mexico.

MATERIALS AND METHODS

Study area

The present study was conducted on six buffalo farms located in the municipalities of Isla, Juan Rodriguez Clara, Las Choapas and Sayula de Aleman, in the central and southern regions of the state of Veracruz, Mexico, from January to June 2014. The state of Veracruz is located between 17°03' and 22°27' north latitude, and 93°36' and 98°38' west longitude. Veracruz borders the Gulf of Mexico to the east and has an average annual temperature of 23°C. The average maximum temperature is around 32°C, which occurs in April and May. The average minimum temperature is 13°C and occurs in January. Humidity ranges from 74% to 80%, and the average annual precipitation is 1500 mm. In the central region, rainfall occurs from June to September; while in the southern region it rains throughout the year. There is no official census on the total population of buffaloes in the state of Veracruz because the species was recently introduced. However, our previous studies indicate that the population includes around 4,000 individuals (Dominguez-Aguilar *et al.*, 2013; Romero-Salas & Pérez de León, 2014; Romero-Salas *et al.*, 2016). In the farms studied, buffaloes were pastured together with cattle and usually found resting near large puddles or mud holes. The buffaloes did not receive any nutritional supplementation and were not part of a health or reproductive program.

Sample size

The sample size for estimating the seroprevalence of anti-BoHV-1 antibodies in buffaloes of the state of Veracruz was calculated using simple random sampling and was set at 351, considering a population of 4,000 buffaloes, assuming an expected seroprevalence of 50% and a desired absolute precision of 5% for 95% confidence interval. One to seven-year-old animals were included in the study.

Questionnaires

A series of questions used previously for the initial documentation of BoHV-1 infection among buffaloes in the state of Veracruz formed part of the questionnaire prepared for this study (Romero-Salas *et al.*, 2017). The manager of each farm was interviewed using a questionnaire to yield basic data about the buffalo herd including animal age, gender, breed (Carabao, Murrah, or Jafarabadi), and history of abortions.

Serological assay

Three hundred and sixty eight buffaloes from six buffalo farms were randomly chosen for blood collection. Blood samples (approximately 5 ml) were collected into vacutainer tubes through jugular venipuncture. Serum in the blood samples was separated by centrifugation at 3,500 rpm for 15 min, aliquoted and kept frozen at -20°C at the Parasitology Laboratory in the Diagnostic Unit of the Faculty of Veterinary Medicine at the State University of Veracruz (Universidad Veracruzana) until further analysis. All samples were tested for the presence of antibodies against BoHV-1 by ELISA using the commercial kit HerdChek® Infectious Bovine Rhinotracheitis [IBR]/ Bovine Herpesvirus-1 [BHV-1] gB (IDEXX Laboratories, Inc., The Netherlands), which also detects BuHV-1 antibodies. The specificity and sensitivity of this indirect gB ELISA to detect BoHV-1 antibodies are reported to be 99.8 and 98%, respectively (Puentes *et al.*, 2016; Bertolotti *et al.*, 2015). Seropositivity in the samples was determined using the software XCheck 3.3® (IDEXX Laboratories, Inc., The Netherlands).

Statistical analysis

Descriptive statistics were used to calculate seroprevalence. Multivariate analyses were applied to assess the association between the buffaloes' characteristics and BoHV-1 seropositivity. The dependent variable was seropositivity to BoHV-1 by ELISA. Independent variables in the bivariate analysis included age, gender, and history of abortions. Odds ratio (OR) and 95% confidence interval (CI) were calculated by multivariate analysis using the software STATA 11.0 (Stata Inc., College Station, Texas), and Vassar Stats® (http://vassarstats.

net/vsclin.html). Fisher's exact test was used with smaller samples for abortion events (CI=95%). Results were considered to be statistically significant at $p < 0.05$.

RESULTS

Table 1 summarizes epidemiological data for the three hundred and sixty-eight buffaloes from 6 farms located in 4 municipalities of the state of Veracruz, Mexico that were sampled in this study. Buffalo coexisted with cattle only in the farm located in the municipality of Isla. All farms had positive animals and the overall seroprevalence of anti-BoHV-1 antibodies was 59% (217/368). However, according to the sensitivity and specificity of the test the true prevalence was 80%. The highest (83.3% [$CI_{95\%}$ 68.0-92.5]) and lowest (49.2% [$CI_{95\%}$ 36.0-62.4]) seroprevalence rates were observed in farms located in the municipalities of Sayula de Aleman and Juan Rodriguez Clara, respectively (Table 1).

Of the 368 animals sampled, the majority were females (89.40%), and males comprised 10.60% of the population (Table 1). Only 2.43% (8/329) of the females had a history of abortion (Table 1). One to two-year-old animals had the lowest prevalence of anti-BoHV-1 antibodies (36.4% [$CI_{95\%}$ 27.8-45.6]), and those over seven years old had the highest seroprevalence (86.0% [$CI_{95\%}$ 73.3-94.2]) (Table 1). The seroprevalence between females and males was equivalent. Buffalo cows that had a history of abortion showed a higher prevalence of anti-BoHV-1 antibodies (75.0% [$CI_{95\%}$ 35.6-95.5]) than those that had no abortion (60.7% [$CI_{95\%}$ 55.2-66.1]) (Table 1).

The seroepidemiological data were used to evaluate potential risk factors for BoHV-1 infection (Table 2). Regarding age, 5-6 (OR = 3.35; $CI_{95\%}$ 1.15-9.76, p = 0.02), and > 7-year-old buffaloes (OR = 7.67; $CI_{95\%}$ 2.25-26.16, $p < 0.01$) had a higher risk of being seropositive than animals in the other age groups. Additionally, these analyses indicated that gender, animal category (buffalo calves, breeding bulls, heifers, females with one birth, females with $\geq$ 2 births), breed, shared water sources, interaction with cattle, interaction with canines, and history of abortion did not present a risk for BoHV-1 infection in buffaloes (Table 2).

DISCUSSION

Our preliminary epidemiological findings helped design this study to enhance our understanding of BoHV-1 infection among buffaloes in Mexico (Romero-Salas *et al.*, 2017). BoHV-1 is the etiological agent of IBR, IPV and IPB as well as other clinical syndromes such as abortions, infertility, conjunctivitis and encephalitis, which results in important economic losses which are of major concern to the livestock industry (Thiry *et al.*, 2006; Nandi *et al.*, 2009). This herpesvirus is distributed worldwide and its biology, epidemiology and control are well characterized in cattle (Raaperi *et al.*, 2014; Majumder *et al.*, 2015). However, the effects of BoHV-1 infection in buffaloes remain to be fully determined. The susceptibility to BoHV-1 infection was tested by inoculating buffaloes with a virulent BoHV-1 field strain isolated from cattle, but no clinical alteration ascribed to BoHV-1 infection was observed in the studied animals (Scicluna *et al.*, 2010). These results suggested the development of subclinical infection, and indicated the importance of buffaloes as host/reservoirs of BoHV-1.) The presence of BoHV-1 in naturally infected buffaloes correlates with pathogenicity to newborn buffaloes as well as its likely role in abortion (Albayrak *et al.*, 2012; Fusco *et al.*, 2015). Similar syndromes are also associated with BoHV-1infection in cattle (Graham 2013).

Serosurveys have shown that BoHV-1 infection can be relatively high in countries with a considerable buffalo population and where cattle are also raised. IBR is endemic in India where the prevalence of BoHV-1 in cattle and buffalo ranges from 8.56% to 76.70% and 2.75% to 81%, respectively, with large variation among states in that country (Majumder *et al.*, 2015; Samrath *et al.*, 2016). In Pakistan, a country with a buffalo population of approximately 23.4 million head (Abbasi *et al.*, 2017), the BoHV-1 sero-

Table 1. Prevalence of anti-BoHV-1 antibodies according to potential risk factors among water buffaloes from the state of Veracruz, Mexico

Potential Risk Factors	No. of animals	Positive	Sero-prevalence (%)	$CI_{95\%}$	Fisher test	X^2 value	p-value
Farm/Municipality							
Choapas	30	22	73.3	53.8–87.0		17.9	<0.01
Choapas	59	32	54.2	40.8–67.1			
Sayula de Alemán	42	35	83.3	68.0–92.5			
Isla	53	33	62.3	47.9–74.9			
Juan Rodríguez Clara	125	66	52.8	43.7–61.7			
Juan Rodríguez Clara	59	29	49.2	36.0–62.4			
Total	368	217	59	53.7–64.0			
Age (years)							
<1	18	8	44.4	21.5–69.2		49.51	<0.01
1–2	121	44	36.4	27.8–45.6			
3–4	109	71	65.1	55.4–74.0			
5–6	70	51	72.9	60.9–82.8			
≥7	50	43	86.0	73.3–94.2			
Gender							
Female	329	195	59.3	53.7–64.6		0.12	0.72
Male	39	22	56.4	39.8–71.8			
Type of animal							
Buffalo calves	22	9	40.9	20.7–63.6		46.01	<0.01
Breeding Bulls	17	13	76.5	50.1–93.2			
Heifers	134	54	40.3	31.9–49.1			
Females with one birth	86	53	61.6	50.5–71.9			
Females with ≥ 2 births	109	88	80.7	72.1–87.7			
Breed							
Murrah	207	122	58.9	51.9–65.7		0.35	0.83
Carabao	98	56	57.1	46.7–67.1			
Jafarabadi	63	39	61.9	48.8–73.9			
Shared water sources							
No	226	133	58.8	52.1–65.3		<0.01	0.95
Yes	142	84	59.2	50.6–67.3			
Interactions with Cattle							
No	151	133	88.1	81.8–92.8		<0.01	0.95
Yes	217	84	38.7	32.2–45.5			
Interactions with Canines							
No	151	126	83.4	76.5–89.0		0.11	0.73
Yes	217	91					
Abortions							
No	321	195	60.7	55.2–66.1	0.49		
Yes	8	6	75.0	35.6–95.5			
Total	368	217	59	53.7–64.0			

Table 2. Assessment of potential risk factors for Bovine Herpes Virus-1 infection based on seroepidemiological data in water buffaloes from the state of Veracruz, Mexico

Potential risk factors	No of animals	Sero-positive	OR	$CI_{95\%}$	$P > \|z\|$	Std error	Z
Farm/Municipality							
1 Choapas	30	22	1 (Ref)	–	–	–	–
2 Choapas	59	32	0.43	0.16–1.12	0.08	0.21	-1.72
3 Sayula de Alemán	42	35	1.81	0.57–5.71	0.30	1.06	1.02
4 Isla	53	33	0.6	0.22–1.60	0.30	0.30	-1.02
5 Juan Rodríguez Clara	125	66	0.40	0.16–0.98	0.04	0.18	2.0
6 Juan Rodríguez Clara	59	29	0.35	0.13–0.92	0.03	0.17	-2.14
Total	368	217					
Age (years)							
<1	18	8	1 (Ref)	–	–	–	–
1–2	121	44	0.71	0.26–1.94	0.51	0.36	-0.66
3–4	109	71	2.33	0.85–6.41	0.10	1.20	1.65
5–6	70	51	3.35	1.15–9.76	0.02	1.82	2.22
≥7	50	43	7.67	2.25–26.15	<0.01	4.80	3.26
Gender							
Female	329	195	1 (Ref)	–	–	–	–
Male	39	22	0.73	0.36–1.45	0.37	0.25	-0.89
Type of animal							
Buffalo calves	22	9	1 (Ref)	–	–	–	–
Breeding Bulls	17	13	4.69	1.15–19.16	0.03	3.36	2.15
Heifers	134	54	0.97	0.39–2.44	0.97	0.45	-0.05
Females with one birth	86	53	2.31	0.89–6.02	0.08	1.13	1.73
Females with ≥ 2 births	109	88	6.05	2.28–16.03	<0.01	3.0	3.62
Breed							
Murrah	207	122	1 (Ref)	–	–	–	–
Carabao	98	56	0.98	0.57–1.51	0.76	0.23	-0.30
Jafarabadi	63	39	1.13	0.63–2.02	0.67	0.33	0.42
Shared water sources							
No	226	133	1 (Ref)	–	–	–	–
Yes	142	84	1.01	0.66–1.55	0.95	0.22	0.06
Interactions with Cattle							
No	151	133	1 (Ref)	–	–	–	–
Yes	217	84	1.01	0.66–1.55	0.95	0.22	0.06
Interactions with Canines							
No	151	126	1 (Ref)	–	–	–	–
Yes	217	91	0.93	0.61–1.41	0.73	0.199	0.34
Abortions							
No	321	195	1 (Ref)	–	–	–	–
Yes	8	6	2.19	0.42–10.64	0.36	1.74	0.91
Total	368	217					

prevalence was reported to be 70.3% (Shabbir *et al.*, 2013). By comparison, in Brazil where there are more cattle than buffaloes, the BoHV-1 prevalence in states where buffaloes are raised can range from 56.1% to 85% (Viana *et al.*, 2016; Soares *et al.*, 2017). Although the true prevalence obtained according to the ELISA test we used was 80%, the observed seroprevalence reported here (59%) was similar to a previous report (57.6%) on BoHV-1 infection among buffaloes that were raised together with cattle in Mexico (Romero-Salas *et al.*, 2017). Epidemiological evidence indicates that buffaloes contribute to the maintenance of BoHV-1 infection in areas where cattle and buffaloes share the same pastures (Scicluna *et al.*, 2010; Nandi, *et al.*, 2011; Fusco *et al.*, 2015).

In the state of Veracruz the seroprevalence of BoHV-1 in cattle was reported to be 64.5% (Romero-Salas *et al.*, 2013), which is higher than the 59% observed prevalence for the buffaloes included in this study. The buffaloes we studied had not been vaccinated against IBR, so the presence of antibodies against BoHV-1 suggests that seropositive animals had been infected with the virus. Although cattle can be a reservoir of BuHV-1 (Maidana *et al.*, 2016), the prevalence between cattle and buffalo populations in a region can vary. Studies in India and Egypt also showed that BoHV-1 was more widespread in cattle than in buffalo (Mahmoud *et al.*, 2009; Verma *et al.*, 2014; Samrath *et al.*, 2016). However, in Pakistan and Italy, BoHV-1 seropositivity was proportionately higher for buffaloes than in cattle (Scicluna *et al.*, 2007; Shabbir *et al.*, 2013).

It is possible that the buffaloes we tested in the state of Veracruz were infected with BuHV-1. An ELISA specific for BuHV-1 was unavailable to us during this study. BuHV-1 is antigenically and genetically related to BoHV-1 and BoHV-5 (van der Kolk 2016). The genomes of BuHV-1 and BoHV-5 were shown to be 92.2% similar (Scheffer *et al.*, 2017). Buffaloes and cattle are susceptible to heterologous infection with BoHV-1 and BuHV-1 (Scicluna *et al.*, 2017; Caruso *et al.*, 2016). Goats are also susceptible to infection with BuHV-1 (Camero *et al.*, 2017). Serological tests can cross-react between BuHV-1 and BoHV-1 (Nogarol *et al.*, 2014). Thus, further studies are required to determine the extent of BuHV-1 infection in buffaloes in the state of Veracruz, Mexico. In the present study, animals over seven years of age had the highest seroprevalence of anti-BoHV-1 antibodies (86.0%). Animal age was identified as a risk factor for BoHV-1 infection. Buffaloes that were 5-6 years old, or older than seven years of age were more likely to be infected than animals of the other age groups. Similar results have been observed by other investigators in buffaloes (Ferreira *et al.*, 2010; Verma *et al.*, 2014; Viana *et al.*, 2016), cattle (Boelaert *et al.*, 2005; Jacevièius *et al.*, 2010; Romero-Salas & Pérez de León, 2014), and mithun (Rajkhowa *et al.*, 2004). Rajkhowa *et al.* (2004) suggested that this trend likely occurs because as animals grow older, they are more likely to be exposed to BoHV-1 by coming into contact with animals that have recovered from the disease and remain carriers. However, the explanation for higher seroprevalence in older animals may be due to increased susceptibility or repeated subclinical infection that keeps a high antibody titer in infected animals, or decreased immunity and increased stress, which may reactivate latent virus (Verma *et al.*, 2014). BoHV-1 virus infection in cattle and buffaloes tends to be mild and non-life threatening (Nandi *et al.*, 2009; Majumder *et al.*, 2015). Thus, a protracted interaction between infected buffaloes or infected cattle on the same farm could increase the risk of continued exposure, which may result in higher herd seroprevalence.

There was no observed difference in BoHV-1 seropositivity between male and female buffaloes, which may indicate that both sexes have the same chances of becoming infected. However, a higher prevalence of BoHV-1 in males than in females was reported for buffaloes in western parts of Uttar Pradesh, India (Verma *et al.*, 2014). A study on the risk factors associated with BoHV-1 seropositivity based on a cluster sample of the Belgium cattle population showed that bulls were more at risk to be positive than cows (Boelaert *et al.*, 2005). Jacevièius *et al.* (2010) showed a significantly higher seroprevalence of BoHV-1 in cows (34.64%) than in bulls (2.01%), heifers (10.01%), and calves (4.41%).

In contrast to results from a previous study exploring the seroepidemiology of infection with BoHV-1 in buffaloes of the state of Veracruz (Romero-Salas *et al*., 2017), in the present study interaction with cattle was not a risk factor for BoHV-1 seropositivity. Buffalo were raised with cattle only in the farm located in the Isla municipality. As noted above, all the buffaloes under study were unvaccinated against IBR. Although buffaloes are considered reservoirs of BoHV-1 (Scicluna *et al*., 2010), our studies on the epidemiology of BoHV-1 in livestock indicate the need to investigate the routes of BoHV-1 introduction to buffalo herds in the state of Veracruz (Romero-Salas *et al*., 2013; Romero-Salas *et al*., 2017). Some of the factors that can be studied include the movement between farms of buffaloes of undetermined BoHV-1 infection status, and the potential for viral airborne transmission (Mars *et al*., 2000). Buffaloes can be infected with BoHV-1 through the intranasal route (Scicluna *et al*., 2010). The knowledge generated through these studies could serve as the basis for BoHV-1 control measures in buffalo farms in the state of Veracruz.

Abortion is one of the main clinical syndromes caused by BoHV-1 in cattle (Thiry *et al*., 2006; Nandi *et al*., 2009; Graham, 2013; Majumder *et al*., 2015), which may also occur in buffaloes as a result of BoHV-1 infection (Fusco *et al*., 2015). Our results showed that anti-BoHV-1 antibodies were more prevalent in female buffaloes with a history of abortion than in those with no history of abortion. However, the multivariate analysis failed to demonstrate an association between seropositivity to BoHV-1 and a history of abortion. This suggests that abortion does not increase the risk for BoHV-1 infection in buffaloes. Similar results were reported from studies on risk factors associated with BoHV-1 infection in cattle performed in Mexico (Magaña-Urbina *et al*., 2005; Romero-Salas *et al*., 2013), and Colombia (Betancur *et al*., 2006).

CONCLUSIONS

By comparison to cattle, ELISA-based serosurveys can also be applied to check status of BoHV-1 infection in water buffalo individuals or herds. Serosurveillance results reported here showed for the first time that buffalo herds in the state of Veracruz, Mexico are infected with BoHV-1 when there is no apparent risk of interaction with cattle. Further research is needed to assess the development of clinical signs and symptoms of BoHV-1 infection in buffaloes. Animal health programs established to mitigate the burden of syndromes caused by BoHV-1 in cattle must take into consideration buffaloes when this bovid species is part of the same agroecosystem.

Ethics approval and consent to participate
This project was approved by the Bioethics and Animal Welfare Commission of the Veterinary and Animal Science School (Facultad de Medicina Veterinaria y Zootecnia) of the State University of Veracruz (Universidad Veracruzana). Consent was obtained from the buffaloes producers.

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Author Disclosure Statement
No competing financial interests exist.

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