

# SEROEPIDEMIOLOGY OF INFECTION WITH *NEOSPORA CANINUM*, *LEPTOSPIRA*, AND BOVINE HERPESVIRUS TYPE 1 IN WATER BUFFALOES (*BUBALUS BUBALIS*) IN VERACRUZ, MEXICO

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We aimed to determine the seroprevalence of infection with *Neospora caninum*, *Leptospira*, and bovine herpesvirus type 1 and risk factors associated with these infections in water buffaloes in Veracruz State, Mexico. Through a cross-sectional study, 144 water buffaloes (*Bubalus bubalis*) raised in 5 ranches of Veracruz were examined for anti-*N. caninum* and anti-bovine herpesvirus type 1 antibodies by enzyme immunoassays, and anti-*Leptospira interrogans* antibodies by microscopic agglutination test.

Of the 144 buffaloes studied, 35 (24.3%) were positive for *N. caninum*, 50 (34.7%) for *Leptospira*, and 83 (57.6%) for bovine herpes virus. The frequencies of leptospiral serovars in buffaloes were as follows: 18.7% for Muenchen ( $n = 27$ ), 10.4% for Hardjo LT ( $n = 15$ ), 9.0% for Pyrogenes ( $n = 13$ ), and 4.8% for Icterohaemorrhagiae ( $n = 7$ ). Seropositive buffaloes were found in all 5 ranches studied. Logistic regression showed that cohabitation of buffaloes with cows was associated with infection with *Leptospira* (odds ratio [OR], 2.2; 95% confidence interval [CI], 1.04–4.5;  $P = 0.03$ ) and bovine herpesvirus (OR, 12.0; 95% CI, 4.0–36.2;  $P < 0.01$ ).

This is the first study that provides serological evidence of *N. caninum*, *Leptospira*, and bovine herpesvirus type 1 infections in water buffaloes in Mexico. Our findings could be used to enhance preventive measures against these infections.

**Keywords:** *Neospora*, *Leptospira*, bovine herpesvirus, water buffaloes, seroprevalence, Mexico

## Introduction

*Neospora caninum* (*N. caninum*), *Leptospira*, and bovine herpesvirus type 1 are important infectious pathogens that lead to bovine abortion, neonatal mortality, and birth of weak calves [1]. The protozoan *N. caninum* is an obli-

gate intracellular parasite in the Apicomplexa phylum that causes Neosporosis [2, 3]. Neosporosis affects domestic and wild animals including the water buffalo (*Bubalus bubalis*) [4] and is recognized as an important cause of abortions and neonatal deaths among populations of susceptible species around the world [2, 5]. Tissue cysts of

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*N. caninum* have been found in aborted fetuses of water buffaloes with encephalitis and myocarditis [6]. Neosporosis in water buffaloes has economic importance in several countries including Brazil, India, Italy, and Vietnam [3]. Seroprevalence of *N. caninum* in water buffaloes varies among countries; for instance, 34.6% in southern Italy by the indirect florescence antibody test (IFAT) [6], 64% and 53% in female water buffaloes in Sao Paulo State, Brazil by IFAT and *Neospora* agglutination test, respectively [7], and 64% in northeast Argentina by IFAT [8].

Bacteria of the genus *Leptospira* cause leptospirosis, which is a zoonotic disease of global importance [9, 10]. Infection with *Leptospira* occurs in more than 160 species of domestic and wild animals [11]. Serological evidence of *Leptospira* infection has been reported in water buffaloes [12, 13], especially in those 3 to 5 years old [14]. Diverse seroprevalence rates of *Leptospira* infection in water buffaloes obtained by microscopic agglutination test (MAT) among countries have been reported, e.g., 22% in northeast Argentina [15], 29% in Egypt [16], 30.5% in Thailand [17], and 48% in Philippines [18].

Bovine herpesvirus type 1 is a pathogen that causes infectious bovine rhinotracheitis, abortion, infectious pustular vulvovaginitis, balanoposthitis, and neurological and systemic disease in cattle [19]. Water buffaloes are susceptible to this virus [20, 21]. Seroprevalence of bovine herpesvirus type 1 in water buffaloes obtained by microserum neutralization test varied from 14.7% in Brazil [22] to 85% in India [23].

Water buffalo raising is gaining popularity in Mexico as an alternative livestock production system [24]. In the Mexican state of Veracruz, water buffalo and cattle can share the agroecosystem [25, 26]. Preliminary findings highlighted the risk for infection with *N. caninum*, *Leptospira*, and bovine herpesvirus type 1 in water buffaloes in Mexico [24]. Therefore, this study aimed to: (1) determine the seroprevalence of infection with *N. caninum*, *Leptospira*, and bovine herpesvirus type 1 in water buffaloes (*Bubalus bubalis*) in Veracruz State, Mexico and (2) determine the risk factors associated with these infections in the water buffaloes studied.

## Materials and methods

### *Study design and place of the study*

A cross-sectional study was performed in five ranches, also known as bovine production units (BPU), where water buffaloes (*Bubalus bubalis*) were raised and located in the municipalities of Isla, Juan Rodríguez Clara y Sayula de Alemán in the south of Veracruz State, México, from August 2012 to July 2014. The Isla municipality (18° 01' 45" N; 95° 31' 35" W) has an altitude of 60 m above sea level, a warm-humid climate, an average annual temperature of 24.9 °C, and a mean annual precipitation of 2316 mm. The Juan Rodríguez Clara municipality (17° 59' 35" N; 95° 24' 06" W) has an altitude of 130 meters above

sea level, a warm climate, an average annual temperature of 25.0 °C, and a mean annual precipitation of 1266 mm. The Sayula de Alemán municipality (17° 52' 50" N; 94° 57' 34" W) has an altitude of 80 m above sea level, a warm climate, an average annual temperature of 27.0 °C, and a mean annual precipitation of 1650 mm.

### *Buffaloes studied and sampling*

One hundred and forty-four buffaloes (21 males and 123 females) were studied. All water buffaloes were raised under an extensive system. A convenience sampling was used in this study. Blood (10 ml) was drawn from buffaloes by direct jugular venipuncture. Sera samples obtained by blood centrifugation were kept frozen at -20 °C until tested. Data about ranches and buffaloes were obtained with the aid of a questionnaire. Information gathered included ranch identification, number of animals, handling of placentas and fetuses, presence of dogs, type of feeding, cohabitation with cows, and water supply. Additionally, data about age, weight, type of reproductive stage (bull calves, studs, heifers, female buffaloes), and reproductive history (abortions, deliveries) of buffaloes were obtained.

### *Laboratory tests*

Anti-*N. caninum* IgG antibodies were detected by a commercially available enzyme immunoassay (EIA) "IDEXX Neospora Ab test" (IDEXX® Laboratories, Westbrook, USA). This test has a sensitivity of 100% and a specificity of 98.9%. Anti-*Leptospira interrogans* antibodies were detected by MAT. A panel of nine leptospiral serovars was used: Canicola, Hardjo, Icterohaemorrhagiae, Pomona, Pyrogenes, Autumnalis, Ballum, Wolfi, and Muenchen. Anti-bovine herpesvirus type 1 antibodies were detected by a commercially available EIA (HerdChek IBRgB, IDEXX® Laboratories). This test has a sensitivity of 97.4% and a specificity of 92.4%.

### *Statistical analysis*

Results were analyzed with the aid of the software STATA version 11.0. Seroprevalence of each infection was calculated. The association of infections with general data of ranches and buffaloes was analyzed by  $\chi^2$  and by logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. *P* values of <0.05 were considered as statistically significant.

### *Ethics statement*

This project was approved by the Bioethics and Animal Welfare Commission of the Veterinary Medicine and Animal Husbandry School (Facultad de Medicina Veterinaria

y Zootecnia) of the State University of Veracruz (Universidad Veracruzana). Consent for the field research was obtained from the water buffalo ranchers.

## Results and discussion

Of the 144 buffaloes studied, 35 (24.3%; 95% CI, 17.7–32.2) were positive to anti-*N. caninum* antibodies. Buffaloes seropositive for *N. caninum* were found in all 5 BPU studied. Table 1 shows the seroprevalences of *N. caninum*, *Leptospira*, and bovine herpesvirus type 1 and how they correlated with the buffalo BPU. The seroprevalence of *N. caninum* found in the present study is higher than those reported in other countries using EIA. For instance, Huong et al. reported a 1.5% seroprevalence in water buffaloes in southern Vietnam [27], whereas other researchers found no cases of *N. caninum* infection in the herds studied in China [28] or in the Peruvian Amazon [29]. Flores et al. found a seroprevalence of 14.6% in water buffaloes in Rio Grande do Sul State, Brazil [30]. In the present study, the higher seroprevalences of *N. caninum* infection were observed in buffaloes aged less than 1 year (40%; 95% CI, 13.7–72.6) and in those aged  $\geq 7$  years (41%; 95% CI, 21.5–63.3). Table 2 shows the seroprevalences of infections and their correlation with age groups of buffaloes. The seroprevalence of *N. caninum* infec-

tions in water buffaloes increased with age [8, 31]. The high seroprevalence of *N. caninum* infection in the oldest buffaloes found in our study is consistent with finding of other reports, e.g., in an Italian study, Guarino et al. found a higher (43.1%) seroprevalence of *N. caninum* infection in water buffaloes aged  $>6$  years than in those 1–2 years (24.5%) [6]. Concerning the reproductive stage of buffaloes, female buffaloes with 1 delivery had the highest seroprevalence of *N. caninum* infection (33.3%; 95% CI, 18.6–52.0). The correlation of seroprevalence of infections and reproductive stage of buffaloes is shown in Table 3. Seropositive buffaloes were found in all reproductive stages of buffaloes. The high seroprevalence of *N. caninum* in female buffaloes with 1 delivery might be due to an old age of these animals. The older the animals, the longer the time for a likely expose to *N. caninum* [3]. In our study, none of the characteristics of the ranches examined including ranch, number of animals, handling of placentas and fetuses, presence of dogs, type of feeding, cohabitation of cows, and water supply, or characteristics of the water buffaloes examined including age, weight, reproductive stage, and reproductive history were associated with *N. caninum* infection by logistic regression. Coyotes and dogs were present in the ranches examined; however, their presence was not associated with *N. caninum* infection. Therefore, other infection routes may have contributed to infection in the water buffaloes, e.g., trans-

**Table 1.** Seroprevalences of infections in the water buffaloes and their correlation with each bovine production unit (BPU)

BPU	No. of buffaloes tested	Positive to anti- <i>N. caninum</i>		Positive to anti- <i>Leptospira</i>		Positive to anti-BHV*	
		No.	%	No.	%	No.	%
1	7	1	14.3	5	71.4	6	85.7
2	56	11	19.6	16	28.6	26	46.6
3	42	10	23.9	20	47.6	38	90.5
4	5	2	40.0	1	20.0	2	40.0
5	34	11	32.4	8	23.6	11	32.4
Total	144	35	24.3	50	34.7	83	57.6

\*Bovine herpesvirus type 1

**Table 2.** Seroprevalences of infections in the water buffaloes and their correlation with age

Age (years)	No. of buffaloes tested	Positive to anti- <i>N. caninum</i>		Positive to anti- <i>Leptospira</i>		Positive to anti-BHV*	
		No.	%	No.	%	No.	%
<1	10	4	40	1	10	6	60
1–2	45	8	17.8	8	17.8	9	20
3–4	42	10	23.8	17	40.5	24	57.1
5–6	25	4	16	13	52	23	92
>7	22	9	41	11	50	21	95.5

\*Bovine herpesvirus type 1

**Table 3.** Seroprevalences of infections in the water buffaloes and their correlation with reproductive stage

Stage	No. of buffaloes tested	Positive to anti- <i>N. caninum</i>		Positive to anti- <i>Leptospira</i>		Positive to anti-BHV*	
		No.	%	No.	%	No.	%
Bull calves	13	4	30.8	1	7.8	7	54.0
Studs	8	1	12.5	3	37.5	8	100.0
Heifers	48	7	14.6	14	29.2	12	25.0
Female buffalo with 1 delivery	33	11	33.3	11	33.3	17	51.5
Female buffalo with 2 deliveries	42	12	28.6	21	50.0	39	93.0

\*Bovine herpesvirus type 1

placental infection as reported by other researchers [3, 32].

With respect to *Leptospira* infection, 50 of the 144 water buffaloes examined had antibody titers of >1:100. Thus, the seroprevalence of *Leptospira* infection in the water buffaloes studied was 34.7% (95% CI, 27.1–43.2). Seroprevalence of *Leptospira* infection varied among the buffalo BPU (Table 1). The frequencies of leptospiral serovars in the 144 water buffaloes examined were as follows: Muenchen in 27 (18.7%; 95% CI, 13.1–26.3), Hardjo LT in 15 (10.4%; 95% CI, 6.1–16.9), Pyrogenes in 13 (9.0%; 95% CI, 5.1–15.2), and Icterohaemorrhagiae in 7 (4.8%; 95% CI, 2.1–10.1). We compared those seroprevalence results with data reported elsewhere using the same MAT. The seroprevalence of *Leptospira* infection found in water buffaloes in the present study is lower than the 67.2% reported in water buffaloes in Italy [33]. The leptospiral serovars found in the present study also differ from those reported in the Italian study where researchers found that serovars Sejroe, Hardjo bovis, and Icterohaemorrhagiae were predominant. In contrast, the seroprevalence found in water buffaloes in Mexico is higher than the 17.0% seroprevalence of *Leptospira* infection in water buffaloes in India [12]. The leptospiral serovars reported in the Indian study also differ from the ones found in the present study. The leptospiral serovars Hardjo and Andamana were predominant in the Indian study. On the other hand, our seroprevalence is similar to the 33.3% seroprevalence of *Leptospira* infection reported in water buffaloes in the sub-Himalayan Kumaon region [14]. In the present study, the seroprevalence of *Leptospira* infection increased with age in the water buffaloes. This observation agrees with findings reported by other researchers [14, 34]. Table 2 shows a correlation of *Leptospira* seropositivity and buffalo age groups. With respect to reproduction stage, we observed that female buffaloes with  $\geq 2$  deliveries had the highest seroprevalence of *Leptospira* infection (50%; 95% CI, 35.5–64.5) (Table 3). This finding might be due to increased age in these animals. Logistic regression showed that *Leptospira* infection was associated with cohabitation of water buffaloes with female bovines (OR, 2.2; 95% CI, 1.04–4.5;  $P = 0.03$ ). Other characteristics of the ranches examined including location, number of animals, handling of placentas and fetuses, presence of dogs, type of feeding,

and water supply, or characteristics of the water buffaloes examined including age, weight, reproductive stage, and reproductive history were not associated with *Leptospira* infection by logistic regression. The leptospiral serovars Muenchen and Icterohaemorrhagiae have been associated with infection in rats, and the serovar Hardjo with cattle [34]. Our findings highlight the importance of contact with cattle for *Leptospira* transmission and suggest the likely contributing role of rats for *Leptospira* transmission in the water buffaloes examined.

Concerning infection with the bovine herpesvirus type 1, we found a seroprevalence of 57.6% (95% CI, 49.1–65.7). This infection was more frequent than *N. caninum* and *Leptospira* infections in the water buffaloes studied. Infection with bovine herpes virus was found in all the buffalo BPU studied (Table 1). In a study in India, researchers found a 13.5% seroprevalence of this infection in buffalo bulls [35]. In the present study, stratification by age showed that water buffaloes  $\geq 7$  years old had the highest seroprevalence of bovine herpesvirus infection (95.5%; 95% CI, 75.1–99.8) (Table 2). This finding is in line with an increase of bovine herpes virus prevalence with age [36]. As to reproductive stage, the highest seroprevalence was found in studs (100%; 95% CI, 60–100) and female buffaloes with  $\geq 2$  deliveries (93%; 95% CI, 79.5–98.1) (Table 3). This is likely due to the increased age of these animals. Logistic regression showed that bovine herpesvirus infection was associated with cohabitation of water buffaloes with female bovines (OR, 12.0; 95% CI, 4.0–36.2;  $P < 0.01$ ). Other characteristics of the ranches and water buffaloes examined were not associated with bovine herpesvirus infection by logistic regression. All studs examined were seropositive to bovine herpesvirus, and sexual transmission might have contributed to infection in female buffaloes. However, secretions of the virus in nose but not in vagina have been demonstrated in experimental infections in buffaloes [37]. Therefore, infection acquired by contact with nasal secretion may be more efficient for virus transmission than the sexual route. Cohabitation with cattle has been reported as a transmission route for bovine herpesvirus infection in water buffaloes [36]. This route of transmission also predisposed buffaloes in Veracruz, Mexico to infection with bovine herpesvirus infection.

## Conclusions

This was the first seroepidemiological study on *N. caninum*, *Leptospira*, and bovine herpesvirus type 1 infection in water buffaloes in Mexico. Results provided serological evidence of these infections in water buffaloes in the southern Mexican state of Veracruz. Serovars of *Leptospira* were determined. Cohabitation with cattle was an important factor for the transmission of *Leptospira* and bovine herpesvirus in water buffaloes.

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## Competing interests

The authors declare that they have no competing interests.

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## References

1. Radostits OM, Gay CC, Blood DC, Hinchcliff KW (2002): Medicina veterinaria, tratado de las enfermedades del ganado bovino, ovino, porcino, caprino y equinos, 9th edition, Vol. 2, Editorial Mc Graw-Hill Interamericana
2. Dubey JP, Lindsay DS: A review of *Neospora caninum* and neosporosis. *Vet Parasitol* 67, 1–59 (1996)
3. Dubey JP: Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol* 41, 1–16 (2003)
4. Dubey JP, Romand S, Hilali M, Kwok OC, Thulliez P: Seroprevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) from Egypt. *Int J Parasitol* 28, 527–529 (1998)
5. Anderson M, Andrianarivo A, Conrad P: Neosporosis in cattle. *Anim Reprod Sci* 61, 417–431 (2000)
6. Guarino A, Fusco G, Savini G, Di Francesco G, Cringoli G: Neosporosis in water buffalo (*Bubalus bubalis*) in southern Italy. *Vet Parasitol* 91, 15–21 (2000)
7. Fujii TU, Kasai N, Nishi SM, Dubey JP, Gennari SM: Seroprevalence of *Neospora caninum* in female water buffaloes (*Bubalus bubalis*) from the southeastern region of Brazil. *Vet Parasitol* 99, 331–334 (2001)
8. Campero CM, Pérez A, Moore DP, Crudeli G, Benitez D, Draghi MG, Cano D, Konrad JL, Odeón AC: Occurrence of antibodies against *Neospora caninum* in water buffaloes (*Bubalus bubalis*) on four ranches in Corrientes province, Argentina. *Vet Parasitol* 150, 155–158 (2007)
9. Levett NP: Leptospirosis: a forgotten zoonosis? *Clin Appl Immunol Rev* 4, 435–448 (2004)
10. Pinto PS, Libonati H, Lilenbaum W: A systematic review of leptospirosis on dogs, pigs, and horses in Latin America. *Trop Anim Health Prod* 49, 231–238 (2017)
11. Torres-Castro M, Hernández-Betancourt S, Agudelo-Flórez P, Arroyave-Sierra E, Zavala-Castro J, Puerto FI: Current review of the epidemiology of leptospirosis. *Rev Med Inst Seguro Soc* 54, 620–625 (2016)
12. Ramani PR, Punya KB: Seroprevalence of leptospirosis in domestic animals. *Indian Vet J* 82, 670–671 (2005)
13. Priyantha MA, Gunawardana GA, Puvanenderan S, Wijemuni MI, Alwis PS: Serological detection of *Leptospira* serovars from aborted water buffaloes in Sri Lanka. In 9th World Buffalo Congress. Buenos Aires, Argentina 480–483 (2010)
14. Dwivedi HP, Kumar M, Srivastava SK: Risk factors analysis for prevalence of different *Leptospira* sp. in ruminants of sub-Himalaya Kumaon region. *Indian Vet Med* 26, 100–103 (2006)
15. Konrad JL, Moore DP, Crudeli G, Caspe SG, Cano DB, Leunda MR, Lischinsky L, Regidor-Cerrillo J, Odeón AC, Ortega-Mora LM, Echaide I, Campero CM: Experimental inoculation of *Neospora caninum* in pregnant water buffalo. *Vet Parasitol* 187, 72–78 (2012)
16. Horton KC, Wasfy M, Samaha H, Abdel-Rahman B, Safwat S, Abdel Fadeel M, Mohareb E, Dueger E: Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. *Vector Borne Zoonotic Dis* 14, 633–639, doi: 10.1089/vbz.2013.1525 (2014)
17. Suwancharoen D, Chaisakdanugull Y, Thanapongtharm W, Yoshida S: Serological survey of leptospirosis in livestock in Thailand. *Epidemiol Infect* 141, 2269–2277 (2013)
18. Villanueva MA, Mingala CN, Gloriani NG, Yanagihara Y, Isoda N, Nakajima C, Suzuki Y, Koizumi N: Serological investigation of *Leptospira* infection and its circulation in one intensive-type water buffalo farm in the Philippines. *Jpn J Vet Res* 64, 15–24 (2016)
19. Raaperi K, Orro T, Viltrop A: Epidemiology and control of bovine herpesvirus 1 infection in Europe. *Vet J* 201, 249–256 (2014)
20. Muylkens B, Thiry J, Kirten P, Schynts F, Thiry E: Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. *Vet Res* 38, 181–209 (2007)
21. Scicluna MT, Caprioli A, Saralli G, Manna G, Barone A, Cersini A, Cardeti G, Condoleo RU, Autorino GL: Should the domestic buffalo (*Bubalus bubalis*) be considered in the epidemiology of Bovine Herpesvirus 1 infection? *Vet Microbiol* 143, 81–88 (2010)
22. Lage AP, Castro RS, Melo MI, Aguiar PH, Barreto Filho JB, Leite RC: Prevalence of antibodies to bluetongue, bovine herpesvirus 1 and bovine viral diarrhoea/mucosal disease viruses in water buffaloes in Minas Gerais State, Brazil. *Rev Elev Med Vet Pays Trop* 49, 195–197 (1996)
23. Nandi S, Kumar M, Manohar M, Chauhan RS: Bovine herpes virus infections in cattle. *Anim Health Res Rev* 10, 85–98 (2010)

24. Romero Salas D, Pérez de León AA (2014): Bubalinocultura en Mexico: retos de industria pecuaria naciente. In: Logros y Desafíos de la Ganadería Doble Propósito, 6th ed, eds. González Stagnaro C, Madrid Bury N, Soto Bellozo E, Fundación GIRARZ: Maracaibo, VN, 707–715
25. Alvarado-Esquivel C, Romero-Salas D, García-Vázquez Z, Cruz-Romero A, Peniche-Cardena A, Ibarra-Priego N, Aguilar-Domínguez M, Pérez-de-León AA, Dubey JP: Seroprevalence of *Toxoplasma gondii* infection in water buffaloes (*Bubalus bubalis*) in Veracruz State, Mexico and its association with climatic factors. *BMC Vet Res* 10, 232 (2014)
26. Romero-Salas D, Mira A, Mosqueda J, García-Vázquez Z, Hidalgo-Ruiz M, Vela NA, Pérez de León AA, Florin-Christensen M, Schnittger L: Molecular and serological detection of *Babesia bovis*- and *Babesia bigemina*-infection in bovines and water buffaloes raised jointly in an endemic field. *Vet Parasitol* 217, 101–107 (2016)
27. Huong LT, Ljungstrom BL, Uggla A, Bjorkman C: Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes in southern Vietnam. *Vet. Parasitol* 75, 53–57 (1998)
28. Yu J, Xia Z, Liu Q, Liu J, Ding J, Zhang W: Seroepidemiology of *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes (*Bubalus bubalis*) in the People's Republic of China. *Vet Parasitol* 143, 79–85 (2007)
29. Jara VJ, Chávez VA, Casas AE, Sánchez PN, Moreno-López J, Merza M: Determinación de anticuerpos contra *Neospora caninum* en búfalos de agua (*Bubalus bubalis*) en la amazonia peruana. *Rev Inv Vet Perú* 22, 61–65 (2011)
30. Flores F, Arenhart S, Vicoso F: Anticorpos anti-*Neospora caninum* em bovinos, ovinos e bubalinos no Estado do Rio Grande do Sul. *Ciencia Rural* 36, 1948–1951 (2006)
31. Moore DP, Odeón AC, Venturini MC, Campero CM: Neosporosis bovina: conceptos generales, inmunidad y perspectivas para la vacunación. *Rev Argent Microbiol* 37, 217–228 (2005)
32. Chryssafidis LA, Soares MR, Rodrigues AA, Carvalho TN, Gennari SM: Evidence of congenital transmission of *Neospora caninum* in naturally infected water buffalo (*Bubalus bubalis*) fetus from Brazil. *Parasitol Res* 108, 741–743 (2011)
33. Ciceroni L, D'Aniello P, Russo N, Picarella D, Nese D, Lauria F, Pinto A, Cacciapuoti B: Prevalence of *Leptospira* infections in buffalo herds in Italy. *Vet Rec* 137, 192–193 (1995)
34. Céspedes ZM: Leptospirosis: enfermedad zoonótica re-emergente. *Rev Peru Med Exp Salud Pública* 22, 290–307 (2005)
35. Nandi S, Kumar M, Yadav V, Chander V: Serological evidences of bovine herpesvirus-1 infection in bovines of organized farms in India. *Transbound Emerg Dis* 58, 105–109 (2011)
36. Ferreira RN, Ribeiro HF, Vale WG, Rolim-Filho ST, Barbosa EM: Prevalence of infectious bovine rhinotracheitis (IBR) in buffalo bulls in Amapá State and Marajo Island, Amazon Basin, Brazil, in 9th World Buffalo Congress. Buenos Aires, Argentina, 475–477 (2010)
37. De Carlo E, Re GN, Letteriello R, Del Vecchio V, Giordanelli MP, Magnino S, Fabbri M, Bazzocchi C, Bandi C, Galiero G: Molecular characterization of a field strain of bubaline herpesvirus isolated from buffaloes (*Bubalus bubalis*) after pharmacological reactivation. *Vet Rec* 154, 171–174 (2004)