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Full Length Research Paper

First notification on the presence of brucellosis in water buffalo (*Bubalus bubalis*) in Mexico by serological tests

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A cross-sectional study was conducted to determine the seroprevalence of brucellosis in water buffaloes (*Bubalus bubalis)* of three farms located in the south of Veracruz, México. Card test and rivanol were used in serial for detection of antibodies against *Brucella* spp. From a total of 565 buffaloes, 99 were tested and the overall seroprevalence of brucellosis was 13% by card test and 7% by rivanol. By farm, seroprevalence was 2.94% for farm 1. 4% for farm 2, and 12.5% for farm 3. Brucellosis seroprevalence showed an increasing trend with age, with adult cattle (>6 years) recording the highest seroprevalence (11.1%), but differences were not statistically significant (p>0.05). In two farms, buffaloes shared grazing land and water sources with bovine cattle, so 75 head were tested for brucellosis resulting negative. Because clinical signs suggestive of brucellosis were not observed, isolation was not attempted. This is the first known report on the presence of brucellosis in water buffalo in Mexico; thus, public awareness and further epidemiological studies of the disease in wildlife, livestock, and humans in the study area are of great importance.

Key words: Brucellosis, domestic water buffalo, epidemiology, Mexico.

INTRODUCTION

Brucellosis, a zoonosis of worldwide importance, is caused by Gram-negative bacteria of the genus Brucella (Moreno et al., 2002; López and Contreras, 2004; Cutler et al., 2005). Brucellosis in domestic water buffalo (*Bubalus bubalis*) is generally caused by infection with *Brucella abortus* (Mohan, 1968; Godfroid, 2002). Brucellosis is primarily a reproductive disease of cattle, characterized by late-term abortions, retained placentas, epididymitis, and orchitis (Nicoletti, 2001). Clinical signs in buffaloes include abortion, decreased fertility and milk production, and testicular degeneration in the bull, as a result of epididymitis - orchitis (Acha and Szyfres, 2003); because of this, it is considered one of the most damaging diseases to livestock and disturbing rates of productivity in buffalo herds (Borriello et al., 2006; Martínez et al., 2006), since prevention and control involves a high economic impact.

Water buffalo is well adapted to tropical and subtropical regions, particularly to flooded areas where bovine cattle thrive with difficulty, while buffalo uses efficiently pasture resources (Bhat, 1992; Mahadevan, 1992; Borghese and Mazzi, 2005; Borghese, 2006). The introduction of buffaloes to tropical states of Mexico like Veracruz and Tabasco is a relatively recent phenomenon and there is still a lot unknown about this species. In some farms, the interaction of water buffalo with other domestic ruminant species leads to the possibility of cross- species infections (Bengis et al., 2002).

Brucellosis in the water buffalo generally is caused by *B. abortus*, however, since little of its epidemiology has been studied; it is unclear how the species transmission may occur (Fosgate et al., 2011). In Mexico, health status in regard to brucellosis in water buffalo remains unknown. Therefore, it is necessary to conduct proper studies, which should start with determining the presence of

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brucellosis.

Mexico uses the card test as the official screening procedure for brucellosis, with rivanol test for confirmation (SAGDR, 1996). These tests have been studied in cattle (Alton et al., 1988; Abdoel et al., 2008) and subsequently evaluated for use in water buffalo and other species (Nicoletti, 1992; Fosgate et al., 2002, 2003; Godfroid et al., 2010). Card test is fast, easy to perform and allows processing a large numbers of samples per day (Aricapa, 2006). This test allows to classify animals as positive or negative, and then perform rivanol test to positive samples (Acha and Zyfres, 2003), which functions as complementary and confirmatory, as it allows to differentiate infected animals from those who have been vaccinated (OIE, 2004).

As far as the authors are aware, no investigation on the presence of brucellosis in water buffalo in Mexico has hitherto been attempted. This study was carried out therefore to investigate the presence of brucellosis in water buffalo (*B. bubalis*) in three farms located in the south of the state of Veracruz, Mexico.

MATERIALS AND METHODS

Study site and animal population

The study was conducted in the contiguous municipalities of Isla and Juan Rodríguez Clara, located at the south of the state of Veracruz, Mexico. Climate is hot humid and annual rainfall range from 1200 to 2300 mm. Three buffalo's commercial farms were identified in the area, showed willingness to participate in the study, and were surveyed for brucellosis (Table 1).

Sample collection techniques

Population in the three farms was 565 head. At each farm, systematic random sampling (that is, 1/6 animals interval) was used to select individual animals. In order to minimize possible false positive reactors due to maternal antibodies in younger animals, only those older than six months were sampled. Blood samples for the detection of antibodies against *Brucella* spp. were collected from all sampled animals. The survey covered the period from May to June 2011 and a total of 99 serum samples were collected in the three farms from animals older than six months. Information about each animal such as sex and age was collected and entered into a data sheet. A second data sheet containing information from each farm was also constructed.

Testing for brucellosis

Antibodies to *Brucella* spp. were detected by using the card test as a screening test, and the rivanol test as a confirmatory test, according to the official Mexican regulations (SAGDR, 1996). Card test was performed on plates where 25 µl of the serum was mixed with equal amounts of a stained, buffered, whole cell suspension of *B. abortus* strain 1119-3 antigen (pH 3.6, concentration of 8%). The samples were mixed on a rocker for 5 min. Depending on the presence of agglutination; the test is interpreted as either negative or positive without a "suspicious" category. Rivanol test was performed by mixing 0.5 ml of serum with 0.5ml of a 1% solution of rivanol. The tube was then allowed to stand for at least five minutes for allowing the pack to precipitate. The tube was centrifuged and the supernatant tested by pipetting 0.08, 0.04, 0.02 and 0.01 ml onto a glass plate and mixing with 0.03 ml of rivanol plate test antigen, consisting of inactivated *B. abortus* strain 1119-3 stained with brilliant green and crystal violet (pH 5.8 to 6.2, concentration of 4%). The mixtures were incubated for 12 min and sera from non vaccinated animals showing a complete agglutination reaction at any dilutions from 1:25 to 1:400 were considered positive.

Data analysis

The overall number of seropositive animals was calculated from the total number of samples tested over the study period, expressed as a percentage. Seropositive animals were examined in relation to age, sex, and farm. Vassarstats software was used to analyze data to evaluate differences in seroprevalence and to calculate confidence intervals. Chi-square test was used to measure differences between categories. Values of $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Seropositivity by card test

For the test card, the overall seropositivity was 13%. All three farms had animals testing positive, but with varying proportions, as shown in Table 2. According to the farms' veterinarians, none of the cattle from the studied areas had been vaccinated against brucellosis, implying that the antibodies detected were more likely to be due to natural infection with *Brucella* spp. rather than by *B. abortus* S19. Furthermore, the rivanol test used for confirmation of seropositive animals has been reported to differentiate vaccinal antibodies from those of natural infection (Nielsen and Yu, 2010). Since *Yersinia enterocolitica* is assumed to be rare or absent in the tropics, cross-reactions with *B. abortus* were unlikely to have an impact on the results (Nielsen et al., 2004). Moreover, card test for screening has a high sensitivity (>90%), thus reducing the possibility of false negative reactors (OIE, 2004). Hence, it is unlikely that the tested animals in the present study were wrongly classified as false negative or false positive due to cross-reactions with other *Brucella* spp.

Overall seropositivity by card test was 13% in this study, which is close to the 12% reported in the municipality of Lorica, Department of Córdoba, Colombia by card test, ELISA (Calderón et al., 2010), but higher than the 2% observed in four farms in the Departments of Antioquia and Cordoba, Colombia (Aricapa, 2006), and closer to the 9.38% obtained in Pakistan (Hussain et al., 2008), and the 9.59% reported in Gujarat, India (Ghodasara et al., 2010), all by the test card. In turn, Nowroozi et al. (2007) in Khoozestan province, Iran determined in 400 buffaloes a seroprevalence of 20.5 % by card test, 19.5% by agglutination, and 11 % by 2-mercaptoethanol. In Egypt, in a study that also included bovine, sheep, and goats, Samaha et al. (2008) reported a seroprevalence in buffaloes of 3.52% by card test, 3.44%

Table 1. Characteristics of buffalo's commercial farms enrolled in brucellosis study. Veracruz, Mexico. 2011.

Item	Farm 1	Farm 2	Farm 3
Municipality	**Isla**	Juan Rodriguez Clara	Juan Rodriguez Clara
Farm objective	Beef production	Beef production	Beef production
Farm size, hectare	700	300	500
Terrain	Gentle hills and lowlands with ponds and a stream	Mainly low hills with some small ponds.	Sloppy hills, but there are some ponds and a stream.
Herd	60 head Murrah breed. The initial herd came from another farm in the same municipality, but still some 75 % of the animals were not born in this farm.	70 head, dominated by the Murrah breed. The Initial herd came from the neighboring municipality of Acayucan. Herd is mostly composed by females three to five year-old.	435 head, Murrah breed is dominant, but there is also a small number of animals of Mediterranean and Carabao breeds. The initial herd came from the nearby municipality of Las Choapas in 2006. Most animals were born in farm, but bulls came from other farms.
Feeding	Continuous grazing. Animals are kept year-round in a restricted area	Extensive management conditions. No further protein or mineral supplementation is done.	Animals graze under pasture rotation but are not supplemented.
Animal management	Minimum. Neither deworming nor vaccination is performed. Animals are unidentified and regular records are not kept. Except for the presence of sick animals, the herd remains unchecked. Every four months females are inspected by rectal palpation for pregnancy diagnosis. Calf delivery is unattended so personnel are unaware of abortions. A bull that came with the initial herd is still used for reproduction.	Most animals remain unidentified and records are not regularly kept. A bull that arrived with the initial herd is still used for natural mating. Herd monitoring is occasional, veterinary services are only required if an animal is sick. Females are not followed up by rectal palpation; hence the situation on pregnancies or abortions is unknown. Animals are not dewormed or vaccinated against brucellosis or any other disease.	Records and animal inventory are kept, but not all animals are identified. Because some diarrhea cases were observed in calves, now internal and external parasites control is routine. Scheduled vaccination is only carried out against brucellosis. Only if an animal is sick the veterinary is called for care. Some abortion and difficulties for pregnancy had been observed in the herd.
Interaction with bovine	During a short period every year, a 600 bovine herd shares common grazing and watering sources with buffaloes.	Pasture grazing is shared with a 150 head dual purpose bovine herd, but unlike the buffalo, bovine herd is rotated through the paddocks.	None

by agglutination,and 3.37% by rivanol.

Seropositivity by rivanol test

Herd seroprevalence obtained by rivanol test was 100%, because in the three herds at least one seropositive animal was found, however, within each herd seroprevalence is different, so it was 12.5% in herd 3.4% in herd 2, and 2.94% in herd 1. Overall seropositivity by rivanol test was 7% in this study. One out of four water buffalo farms tested in the province of Corrientes, Argentina by 2-mercaptoethanol test was affected by brucellosis with a seroprevalence of 30%; however, the overall seroprevalence for the animals was 4.8% (Martínez et al., 2006). Hussain et al. (2008) in Pakistan found a seroprevalence in buffaloes of 9.38% by card test, and 6.9% by ELISA; for bovine cattle, the seroprevalence was 0.1 and 8%, and for humans, 14 and 11%, respectively. Another study in Baluchistan, Pakistan, found that the seroprevalence of brucellosis in buffaloes was 1.7% milk ring test and 0% by indirect ELISA, whereas in bovine cattle, the seroprevalence was 4.6 and 20%, respectively (Shafee et al., 2011). Ghodasara et al. (2010) in Gujarat, India sampled 73 buffaloes and found a brucellosis seroprevalence of 9.59% by card test, 12.33% by agglutination, and 14.45% by indirect ELISA.

Seroprevalence by age

Age-specific seroprevalence through rivanol confirmatory

Table 2. Seropositivity (%) to *Brucella abortus* by card test and rivanol in water buffalo on farms in Veracruz, Mexico.

Farm	Total sampled	Card test positives, No (%)	C. I. 95%	Rivanol test positives, No (%)	C. I. 95%
1	34	3 (8.82)	2 – 24	1 (2.94)	0 - 17
2	25	5 (20)	7 – 41	1 (4)	0 - 22
3	40	5 (12.5)	4 – 27	5 (12.5)	4 - 27
Total	99	13 (13)	7 – 21	7 (7)	3 - 14

Table 3. Seroprevalence (%) of antibodies to *Brucella abortus* by age in water buffalo in Veracuz, Mexico.

Age (year)	Positive No. (%)	I.C. 95%	Animals (Total)
0.5	1 (11)	0 - 49	9
1	0	0	12
2	1 (2.63)	0 - 15	38
3	4 (11.43)	3 - 27	35
4	0	0	3
5	1 (50)	2 - 97	2
Total	7 (7)	3 - 14	99

test was found ranging from 0%, in one and four year-old animals, to 50% in those of five year-old (Table 3). There are some reports claiming that older animals had increased chances of testing Brucella positive (Muma et al., 2007; Matope et al., 2010), what seems logic because as an animal ages its chances of contact with an infectious agent may increase. Also, the onset of sexual maturity is associated with a significant increase in the risk of infection with Brucella spp. and such animals are likely to seroconvert. Even though age is not clearly precised, Nowroozi et al. (2007) in Khoozestan province, Iran found a variation in seroprevalence according to age group and sex; in females, the seroprevalence was 12.9% for adults, 10.7% in subadult, and 3% for the youngest animals; however, the seoprevalence in males was 15% in adults, 10.6% in subadults and 5.3% in younger ages.

In this study, because of the small number of males, animals were not analyzed by sex, but Kubuafor et al. (2000) state that sex and brucellosis risk association can vary with different cattle populations.

Prevalence in bovine herds

Because two out of three herds have bovine cattle sharing the habitat with water buffaloes, it was decided to determine their health status in relation to brucellosis. Forty head were sampled in herd 1 and 35 in herd 2 to research herd status, but all animals were negative to *Brucella abortus.* Two recent studies in the region agree with this finding. Martínez (2008) found a seroprevalence of 0% for bovine cattle from the municipalities of Juan Rodríguez Clara, Tierra Blanca, and Tres Valles. Torres (2010), in the neighboring municipalities of Minatitlán, Mecayapan, and Agua Dulce reports a seroprevalence of 0.3% in bovine cattle. Finding buffalo herds positive whereas bovine herds remain negative seems to contradict Adesiyun et al. (2011) suggestion of water buffalo being more resistant to infection than bovine cattle.

The epidemiology of *Brucella* infection has not been studied extensively in domestic water buffalo, but differences between the epidemiology of brucellosis in water buffalo and bovine may complicate control (Borriello et al., 2006; Fosgate et al., 2011). Transmission of *B. abortus* is mainly by direct and mucosal contact with fluids or tissues associated with the birth or abortion of infected fetuses (World Health Organization, 2006).

Probably the most important spread of brucellosis takes place from animal to animal, when those infected contaminate the pasture and uninfected animals become infected by ingestion when grazing. Bovine cattle sharing grazing pastures and watering points with buffaloes are likely to facilitate transmission of the disease in both directions (Nicoletti, 1980; Bengis et al., 2002; Muma et al., 2007). Fosgate et al. (2011) demonstrated that the ingestion of *B. abortus* causes infection in buffaloes and their natural behavior of commingling in a small area facilitates disease transmission, and congregation of water buffalo in wallows may be an important factor for spread of brucellosis. In our study, two farms have buffaloes sharing ponds and pasture land with bovines, but not evidence of infection in the latter was found, probably due to the fact that the final prevalence rate is determined by the intensity of contacts within and

between herds and with infected pasture and water, or because bovine herds are subjected to a vaccination program for brucellosis.

The spread of *Brucella* spp. from one herd and one area to another is often due to the movement of an infected animal into a non-infected susceptible herd (Crawford et al., 1990). The purchase of unknown Brucella-status animals for the purpose of restocking herds can be suspected as the source of spread of brucellosis into the herds. In our study that may happen even though herds are closed if some of the animals in the starter herd were infected. A number of other factors may be associated with the outcome of infection in cattle such as age, reproductive and immunological status, natural resistance, route of infection, infectious challenge, and virulence of the strain (Borriello et al., 2006; Carvalho Neta et al., 2010).

Bovine brucellosis control programs have effectively reduced and eliminated the prevalence of diseases in livestock, but spillover of the disease from domestic livestock to wildlife has complicated regulatory efforts. It is difficult to eradicate brucellosis in cattle without resolution of the disease in wildlife (Olsen and Tatum, 2010).

The milk produced by infected females is the most important source of spread of Brucella spp. for animals to man (Corbel, 2006). Infected animals shed viable brucellae in milk, but dam-to-calf transmission has not been evaluated directly. However, buffalo calves born to seropositive dams on an infected farm are more likely to become seropositive themselves compared with calves born to seronegative dams (Akhtar and Mirza, 1995). Even though in our study buffaloes are not milked and contact with personnel is minimal, the results of the present study established the presence of brucellosis in buffaloes and hence as a potential threat to public health as brucellosis could be a serious zoonosis (Lucero et al., 2008; Selem et al., 2010). Although no human brucellosis information was available when this study was conducted, we strongly suggest further studies to investigate the impact of this zoonosis on human populations. Follow-up studies will be necessary to confirm the possible presence of brucellosis in these populations.

The source or origin of brucellosis in the present study area could not be accurately ascertained as there have been no previous studies on the disease in the area. Also, because no sick animals were observed during the study, isolation of Brucella sp. was not attempted. It is suggested to monitor the reproductive performance of buffaloes, particularly older females, in order to identify reproduction abnormalities suggestive of the presence of brucellosis. Infected water buffalo expel the bacterium during abortion, and this may serve as a source of infection for herd mates. Experimental studies have demonstrated that ingestion of virulent *B. abortus* causes infection in female water buffalo (Mohan, 1968). Also, although buffalo are not included within the Mexican law on brucellosis (SAGDR, 1996), it is suggested to start monitoring buffalo herds in the country by the implementation of approved serological tests to identify and remove reactors preventing the spread of disease inside and outside their herds. Finally, although there is no history of brucellosis vaccine application in the water buffalo in Mexico, other nations have chosen to follow the same vaccination protocol performed in cattle with a favorable outcome, based on this argument it would be possible to apply the vaccination schedule used in cattle based on the official regulation (SAGDR, 1996), with the use of strain 19 classical vaccine doses to prevent disease in females three to six month-old (Afzal et al., 2000), and reduce dose strain 19 vaccine for those over six month-old, even in pregnant animals. Apparently, the RB51 vaccine does not adequately protect against brucellosis infection in water buffalo (Fosgate et al., 2011).

Conclusions

Brucellosis was present in three water buffalo herds in the state of Veracruz, Mexico, but apparently not in the bovine herds, as determined by card test and rivanol test. Brucellosis in the sampled animals had its higher prevalence in individuals between three and five year-old. The source of brucellosis in buffaloes in the study area could not be accurately ascertained. Future studies should be directed to determining factors affecting susceptibility to brucellosis among different domestic animal species including water buffalos.

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REFERENCES

Abdoel T, Dias IT, Cardoso R, Smits HL (2008). Simple and rapid field tests for brucellosis in livestock. Vet. Microbiol., 130: 312–319.

Acha PN, Szyfres B (2003). Zoonoses and Communicable Diseases Common to Man and Animals. Third ed., Panamerican Animal Health Organization, Scientific Publications No 580. Washington, DC.

Adesiyun AA, Fosgate GT, Seebaransingh M, Brown G, Stoute S, Stewart-Johnson A (2011). Virulence of *Brucella abortus* isolated from cattle and water buffalo. Trop. Anim. Health Prod., 43: 13-16.

Afzal M, Mirza MA, Jahangir M (2000). Immune response of buffaloes to vaccination with *Brucella abortus* strain 19. Rev. Sci. Tech. Off. int. Epiz., 19: 867–870.

Akhtar S, Mirza MA (1995). Rates of seroconversion in the progeny of

Brucella abortus seropositive and seronegative cattle and buffalo. Rev. Sci. Tech. Off. int. Epiz., 14: 711–718.
Alton G, Jones LM, Angus RD, Verger JM (1988). Techniques for the brucellosis laboratory. National Institute of Agronomic Research, Paris.
Aricapa HJ (2006). Brucellosis in buffaloes. Proceedings of the Second Symposium of European and American Buffalo and Third in the Americans. Medellín, Colombia, pp. 170-174. Available at: http://www.unne.edu.ar/Web/cyt/cyt2006/04-Veterinarias/2006-V-046.pdf.
Bengis RG, Kock RA, Fischer J (2002). Infectious animal diseases: The wildlife/livestock interface. Rev. Sci. Tech. Off. int. Epiz., 21: 53–65.
Bhat PN (1992). Genetics of river buffaloes. In: Buffalo Production, Tulloh NM, Holmes JHG (Eds). Elsevier, New York, pp. 13–58.
Borghese A (2006). Production and morphology in dairy buffalo. 2° Buffalo Symposium of Europe and America and 3 BuffaloSymposium of the Americans. Medellin, Colombia, pp. 56-65.
Borghese A, Mazzi M (2005). Buffalo population and strategies in the world. In: Buffalo production and research, Borghese A (Ed.). Food and Agriculture Organization of the United Nations. Rome, Italy. pp. 1–40.
Borriello G, Capparelli R, Bianco M, Fenizia D, Alfano F, Capuano F, Ercolini D, Parisi A, Roperto S, Iannelli D (2006). Genetic Resistance to *Brucella abortus* in the Water Buffalo (*Bubalus bubalis*). Infec. Immun., 74: 2115–2120.
Calderón A, Tique V, Ensuncho CF, Rodríguez V (2010). Seroprevalence of *Brucella abortus* in water buffalo (Bubalusbubalis) in the municipality of Lorica, Cordoba, Argentina. Rev. UDCA Actual. & Divulg. Cient., 13 (Suppl 2): 125-132.
Carvalho Neta AV, Mol JPS, Xavier MN, Paixão TA, Lage AP, Santos RL (2010). Pathogenesis of bovine brucellosis. Vet. J., 184: 146–155.
Corbel MJ (2006). Brucellosis in humans and animals. World Health Organization, Geneve, Suitzerland, p. 89.
Crawford RP, Huber JD, Adams BC (1990). Epidemiology and surveillance. In: Animal brucellosis. Nelson KE, Duncan JR (eds), CRC, Boca Raton, FL, `pp. 131–151.
Cutler SJ, Whatmore AM, Commander NJ (2005). Brucellosis – New aspects of an old disease. J. Appl. Microbiol., 98: 1270–1281.
Fosgate GT, Adesiyun AA, Hird DW, Johnson WO, Hietala SK, Schurig GG, Ryan J (2002). Bayesian comparison of brucellosis serologic tests without a gold standard in cattle and water buffalo (*Bubalus bubalis*) of Trinidad. Am. J. Vet. Res., 63: 1598–1605.
Fosgate GT, Adesiyun AA, Hird DW, Johnson WO, Hietala SK, Schurig GG, Ryan J, Diptee MD (2003). Evaluation of brucellosis RB51 vaccine for domestic water buffalo (*Bubalus bubalis*) in Trinidad. Prev. Vet. Med., 58: 211-225.
Fosgate GT, Diptee MD, Ramnanan A, Adesiyun AA (2011). Brucellosis in domestic water buffalo (*Bubalus bubalis*) of Trinidad and Tobago with comparative epidemiology to cattle. Trop. Anim. Health. Prod., 43: 1479-1486.
Ghodasara SN, Ashish R, Bhanderi BB (2010). Comparison of rose bengal plate agglutination, standard tube agglutination and indirect ELISA tests for detection of *Brucella* antibodies in cows and buffaloes. Vet. World, 3: 61-64.
Godfroid J (2002). Brucellosis in wildlife. Rev. Sci. Tech. Off. int. Epiz., 21: 277–286.
Godfroid J, Nielsen K, Saegerman C (2010). Diagnosis of Brucellosis in Livestock and Wildlife. Croatian Med. J., 51: 296-305.
Hussain I, Arshad MI, Mahmood MS, Akhtar M (2008). Seroprevalence of Brucellosis in Human, Cattle and Buffalo Populations in Pakistan. Turk. J. Vet. Anim. Sci., 32: 315-318.
Kubuafor DK, Awumbila B, Akanmori BD (2000). Seroprevalence of brucellosis in cattle and humans in Akwapim-South district of Ghana: Public health implications. Acta Tropica, 76: 45–48.
López A, Contreras A (2004). Brucella. Scand. J. Infect. Dis., 36: 636-638.
Lucero NE, Ayala SM, Escobar GI, Jacob NR (2008). Brucella isolated in humans and animals in Latin America from 1968 to 2006. Epidemiol. Infect., 136: 496-503.
Mahadevan P (1992). Distribution, ecology and adaptation. In: Buffalo Production, Tulloh NM, Holmes JHG (Eds). Elsevier, Amsterdam, pp. 1–12.
Martínez DE, Jacobo RA, Cipolini MF, Martínez EI (2006). Buffalo brucellosis in northwest of the province of Corrientes. Northeastern University, Communications Science and Technology. summary: V-046.
Martínez O (2008). Prevalence of bovine brucellosis in the municipalities of Juan Rodriguez Clara, Tierra Blanca and TresValles in central Veracruz, Mexico. Veterinary degree's thesis. Faculty of of Veterinary Medicine, Universidad Veracruzana. Veracruz, Mexico. 38 pp. Available at http://cdigital.uv.mx/bitstream/12345678/166/2/Oscar%20Martinez%20Lara.pdf.
Matope G, Bhebhe E, Muma JB, Lund A, Skjerve E (2010). Herd-level factors for Brucella seropositivity in cattle reared in smallholder dairy farms of Zimbabwe. Prev. Vet. Med., 94: 213–221.
Ministry of Agriculture, Livestock and Rural Development (1996). Norma Oficial Mexicana NOM-041-ZOO-1995, Campaña Nacional contra la Brucelosis en los Animales. Diario Oficial de la Federación. 18 de julio de1996. México, DF. pp. 43-66. Available at: http://www.senasica.gob.mx/includes/asp/download.asp?iddocumento=506&idurl=1258.
Mohan RN (1968). Diseases and parasites of buffaloes. Vet. Bul., 38: 647–659.
Moreno E, Cloeckaert A, Moriyón I (2002). Brucella evolution and taxonomy. Vet. Microbiol., 90: 209–227.
Muma JB, Munyeme M, Samui KL, Skejerve E, Oloya BC (2007). Risk factors for brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. Prev. Vet. Med., 80: 306–317.
Nicoletti P (1980). The epidemiology of bovine brucellosis. Adv. Vet. Sci. Comp. Med., 24: 69–98.
Nicoletti P (1992). An evaluation of serologic tests used to diagnose brucellosis in buffaloes (*Bubalus bubalis*). Trop. Anim. Health. Prod., 24: 40–44.
Nicoletti P (2001). Control, eradication and prevention. In: Madkour's brucellosis. Madkour MM (editor). Springer; New York. pp. 280–285.
Nielsen K, Smith P, Widdison J, Gall D, Kelly L, Kelly W, Nicoletti P (2004). Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O: 9 and *Escherichia coli* O157: H7. Vet. Microbiol., 100: 25–30.
Nielsen K, Yu WL (2010). Serological diagnosis of brucellosis. Contributions, Sec. Biol. Med. Sci., MASA XXXI: pp. 65–89.
Nowroozi A, Oliaei A, Poormahmood M (2007). A serological survey of Brucella spp.in water buffalo in Khoozestan province, Iran. Ital. J. Anim. Sci., 6: 825-827.
OIE (2004). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organisation for Animal Health. Paris.
Olsen SC, Tatum FM (2010). Bovine Brucellosis. Vet. Clin. North. Am. 26(1): 15-27. RB51 vaccine for domestic water buffalo (*Bubalus bubalis*) in Trinidad. Prev. Vet. Med., 58: 211-225.
Samaha H, Al-Rowaily M, Khoudair RM, Ashour HM (2008). Multicenter Study of Brucellosis in Egypt. Emerg. Infect. Dis., 14: 1916-1918.
Selem MN, Boyle SM, Srirangathan N (2010). Brucellosis: A re-emerging zoonosis. Vet. Microbiol., 140: 392-398.
Shafee M, Rabbani M, Sheikh AA, Ahmad M, Razzaq A (2011). Prevalence of Bovine Brucellosis in Organized Dairy Farms, Using Milk ELISA, in Quetta City, Balochistan, Pakistan. Vet. Med. Intern., 11: 1-3.
Torres DA (2010). Seroprevalence of bovine brucellosis in the towns of Minatitlan, Agua Dulce and Mecayapan located in the southern state of Veracruz. Veterinary degree's Thesis. Faculty of Veterinary Medicine, Universidad Veracruzana. Veracruz, Mexico, p. 57.
World Health Organization (2006). Brucellosis in Humans and Animals. World Health Organization/Food and Agriculture Organization of the United Nations/ World Organization for Animal Health. Washington, DC.