SEROPREVALENCE OF BRUCELLOSIS AMONG HORSES IN TAMIL NADU, INDIA

Preena, P1*, Gishnu, S2, P.I. Ganesan1, B. Samuel Masilamoni Ronald1, S. Balakrishnan5 and Vibin V6
Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai- 07, Tamil Nadu Veterinary and Animal Sciences University

ABSTRACT

A cross-sectional study was conducted to determine the seroprevalence of brucellosis among horses in the state of Tamil Nadu, India. Serum samples from one hundred Indian thoroughbred horses were analyzed with indirect Enzyme Linked Immunosorbent Assay (i-ELISA) and Rose Bengal Plate Test (RBPT). An overall prevalence of six per cent was obtained by i-ELISA and two percent by RBPT. It was also found that the risk factors like sex and coat colour have no significance in the incidence of brucellosis whereas age has high significance. The results suggested transmission of Brucella from other livestock species, thereby adding equines species into the contributing species for the endemic status of the country. It is concluded that routine serosurveillance of equines should also be done so that proper control measures can be taken with respect to vaccination of equines especially in breeding animals, thereby preventing the spread of infection in stud farms and associated zoonotic implications from aborted animals.

Keywords: Prevalence, Equine brucellosis, i-ELISA, RBPT, Tamil Nadu

INTRODUCTION

Brucellosis is one of the most prevalent infectious bacterial zoonosis causing heavy economic loss due to reproductive losses among livestock in India. The data regarding occurrence of brucellosis among equines in India appears scanty unlike in other livestock, although conditions suspecting brucellosis such as abortions, fistulous withers etc. occur in stud farms occasionally. Infection in horses is uncommon, but if occurring, it is most frequently caused by Brucella abortus which is associated with infectious bursitis, osteomyelitis, osteoarthritis or tenosynovitis. Cases of vertebral osteomyelitis, abortion and infertility in stallions have been recorded occasionally due to brucellosis. Fistulous withers (septic supraspinous bursitis) and poll evil (atlantal bursitis) are the commonest clinical manifestations in horses. Serological surveys indicated that many horses may be exposed to B. abortus without developing clinical signs of disease (Mair and Divers, 2009).

None of the Brucella species are specifically adapted to the horse. Brucella abortus has been reported as the most common cause

* Corresponding author: preenalinesh@gmail.com
1 Division of Medicine, ICAR-IIVR, Bareilly, Uttar Pradesh- 243 122
2 MRC Equine hospital, Tamil Nadu, India
3 Centre for Animal Health Studies, TANUVAS, MNC, Chennai-51
4 Department of Veterinary Microbiology, VCRI, Orathanadu, Thanjavur
5 Department of Epidemiology and Public Health, VCRI, Orathanadu, Thanjavur
6 People for Animals (PFA), Bogadi, Mysore, Karnataka- 570026

of brucellosis in horses (Carrigan et al., 1987 and Cohen et al., 1992). Brucella suis has been isolated from horses with bursitis, abortion and internal organs of animals with no external signs of brucellosis (Cook and Kingston, 1988 and Cvetic et al., 2005). Polding (1947) has isolated B. melitensis from horses. Infection with Brucella canis has not been reported naturally or successful experimentally in equines (Hagler et al., 1982).

Bacteria of the genus Brucella are nonmotile, aerobic (B. abortus and B. ovis may need added CO₂), facultative intracellular, Gram-negative coco-bacilli or short rods. Brucella spp. is transmissible to a wide range of species and among the domesticated animals, cattle, sheep, goats and pigs are most commonly affected (Godfroid et al., 2004). Wild animal species such as bison, elk, feral swine, wild boar, fox, hare, African buffalo, reindeer, caribou etc. are also occasionally infected (Godfroid, 2002). Brucella spp. is facultative intracellular pathogens which establish infection by invading macrophages and evading macrophage-induced host defence mechanisms (Gorvel and Moreno, 2002).

Horses usually become infected by the ingestion of feed contaminated with B. abortus and most reported cases indicate a history of contact with cattle (Ocholi et al., 2004). Acosta-Gonzalez et al. (2006) found that there is no evidence suggesting that horses are reservoirs of brucellosis in endemic areas or as a source of infection to other animals in Mexico. Horses are relatively resistant to brucellosis; however, disease can occur and can be transmitted from horses to humans. The frequency of association of B. abortus with fistulous withers varies with geographic region (Cohen et al., 1992).

MATERIALS AND METHODS

A total of one hundred serum samples were collected from horses from different parts of Tamil Nadu, India during the period of February and March, 2015. The animals were categorized based on age (below and above 3 years), sex (male and female) and coat colour (Bay, Chestnut and Gray) as depicted in Table 1. All the horses under study belonged to Indian thoroughbred breed. At the time of sample collection, none of the animals had any history of clinical signs of brucellosis.

Among the 57 males, 20 were geldings (castrated males), 34 colts (young uncastrated males below four years old) and three horses (a young uncastrated male above four years old). Among the females, 34 were filly (young female below four years old) and rest nine was mares.

Detection of antibodies to Brucella was performed by i-ELISA with purified Brucella LPS coated antigen and Anti-multi-species-IgG-HRP conjugate (ID-VET company, France [BRUS-MS ver 0612 GB]). RBPT being the OIE recommended test for brucellosis was also performed as per standard methods. RBPT antigen was obtained from IVRI, Izatnagar, India.

Ethical consideration

The study was approved by the Institute Animal Ethics Committee. Adequate measures were taken to minimize pain or discomfort to animals in accordance with the Institute Animal Ethics Committee while collection of samples.

Statistical analysis

The data obtained in this study with respect to the age, sex and coat colour of the animals were analysed by Chi-square test (Snedecor and Cochran, 1994).

RESULTS

The overall seroprevalence of brucellosis among horses was assessed (TABLE 2). Out of one hundred sera samples from horses, six per cent (6.0%) were found positive by i-ELISA and two percent by RBPT. None of the horses below 3 years of age were found positive for brucellosis.
Based on the sex of equines, four males (4.0%) and two females (2.0%) were positive by i-ELISA respectively and one each (male and female) by RBPT. Of the four males, all were geldings and among two positive females, one was a filly and the other was a mare. Based on the coat colour of horses, the prevalence of brucellosis was highest in the bay coat colour (5.0%) and the remaining one per cent for chestnut coat colour. In RBPT, both the seropositive animals were with bay coat colour.

Statistical analysis revealed that there is no significant difference (P>0.05) in the prevalence of brucellosis among horses with different sex and coat colour. However, high significance exist between the two age groups (P<0.01) of horses in the prevalence of brucellosis.

**DISCUSSION**

The reports on equine brucellosis so far showed isolation of three Brucella species, namely *B. abortus* biovar 1 (De Massis et al., 2014), *B. suis* biovar 1 and 3 (Cook and Kingston, 1988 and Cvetnic et al., 2005) and *B. melitensis* (Polding, 1947). Inter-species transmission of Brucella spp. to horses from cattle and pigs has been documented (Forbes, 1990). Acosta-Gonzalez et al. (2006) reported that the horse can be considered as reservoir for *B. suis* but not *B. abortus* causing infection in other farm animals. Abo-Shehada (2009) stated the possible risk factor for *B. melitensis* infections in equines may be due to the association of equids with ruminant herds with a history of brucellosis in the study area. PD_ADMAS (2012) reported screening of 135 equine sera samples from different stud farms in the country during 2010-2012 for brucellosis and found 10 (7.4%), 13 (9.6%) and 4 (3%) sera samples were positive by RBPT, protein-G ELISA and genus specific PCR respectively.

In the study, all seropositive horses were above 3 years of age which implies that sexually mature animals are most susceptible to brucellosis. Similarly, Nicoletti (2007) stated that most cases have been reported in horses aged >3 years with no apparent age, gender or breed predisposition to infection in horses. All positive males were geldings and thus the possibility of spread of infection from these castrated animals by virtue of breeding is not possible. The positive females (2%) may remain as potential source of infection to other animals and human. These horses might have got infection from livestock species like ruminants, small ruminants or pigs due to the endemicity of the *Brucella* organism in these animals of Tamil Nadu as stated by Ocholi et al. (2004) and Cvetnic et al. (2005). Possible occurrence of brucellosis in horses could be due to ingestion of plants from contaminated fodder lands since most of the animals shared common pasture lands.

The two serological tests used in this test, RBPT and i-ELISA showed different prevalence rate, with the highest seroprevalence being observed with i-ELISA. As suggested by these differences could be due to the intrinsic factors of each tests and immunoglobulin classes that each tests targets. I-ELISA detects IgG1 and IgG2, which are present in the later stage of infection and persist over a long period of time, while the RBPT detects mainly IgG1. In this study, six per cent prevalence in i-ELISA suggests the chronic nature of brucellosis in equine population of Tamil Nadu.

The gold standard for brucellosis diagnosis is the isolation and identification of *Brucella* from suspected samples such as blood, vaginal swabs, hygroma fluids, abscess draining tracts, lymph nodes etc. which was not done in the study. Moreover, the serological tests can result in false positive reaction due to other gram negative bacteria like *Yersinia*. However, the serosurveillance is the easy way to assess the epidemiology of brucellosis in animals to a greater extent than the laborious isolation and
identification methods. Weese (2002) suggested that serologic testing should be performed in all horses with fistulous withers. Serocconversion takes approximately 2 weeks, so repeated serologic testing of acute lesions is warranted. Various molecular assays like polymerase chain reaction (PCR), multiplex PCR, real time-PCR etc., can be employed for detection of the pathogen. Recent field assays like loop mediated isothermal amplification assay and lateral flow assay can also be employed (Karthikeyan et al., 2014).

Due to the close association between horses, human and other livestock species, the epidemiology of brucellosis needs to be investigated in depths. Abo-Shehada (2009) suggested that equines should be included in the vaccination programmes against brucellosis especially in areas where they are part of the livestock farming, in spite of the fact that little is known about Brucella infection in horses. It is concluded that serosurveillance of equine species should be conducted routinely which will provide a limelight to the epidemiology of equine brucellosis. This would in turn call forth the adequacy of control measures like vaccination in equines especially in breeding animals preventing spread of infection in stud farms and the associated zoonotic implications.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


Seroprevalence of brucellosis among horses in Tamil Nadu


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<td></td>
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Table - 1
Sample size of 100 thoroughbreds horses and its details

Table - 2
I-Elisa results of $\chi^2$ analysis and probability depicted:

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<td>0.71\text{NS}</td>
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** - P value is less than 0.01, significant at 99%; NS - P value is more than 0.05, not significant.