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EQUINE BRUCELLOSIS: REVIEW ON EPIDEMIOLOGY, PATHOGENESIS, CLINICAL SIGNS, PREVENTION AND CONTROL

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LAMP

ABSTRACT

Brucellosis is one of the major zoonotic diseases that affect several domestic animals, wild animals and also marine mammals. Though there is no specific *Brucella* sp. that can affect horses, *B. abortus* and *B. suis* can affect horses naturally and *B. canis* experimental infection has also been reported in equines. Brucellosis in equines is characterized by two conditions namely Poll evil and fistulous withers. Organism has its predilection for joints, ligaments and tendons in case of equines and causes inflammatory conditions leading to formation of fistula. Equine brucellosis has been documented from several parts of the world and prevalence has been reported time to time mostly based on serological diagnosis. Diagnosis of brucellosis mainly depends on serological methods though isolation of the organism is the gold standard. Due to hazardous nature of the pathogen, tests like Rose Bengal plate agglutination test, Standard tube agglutination test and other serological assays are commonly employed. Isothermal amplification assay like LAMP are gaining momentum these years due to swiftness in diagnosis of the pathogen. LAMP with high specificity and sensitivity for detection of *Brucella* spp. and also *B. abortus* has been developed in the recent years. Prevention and control of brucellosis is of utmost important to halt the spread of the organism to other animals and human. Trauma is a major reason for predisposition of poll evil and fistulous withers hence proper fitting of saddle will help to prevent the disease. Housing and feeding the horses separately can prevent spread of disease from cattle. The present review discusses equine brucellosis, its epidemiology, pathogenesis, clinical signs along with appropriate prevention and control strategies to be adapted.

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1 Introduction

Brucellosis is a major zoonotic disease caused by the bacterium of the genus *Brucella* of which several species has been listed to cause disease in domestic and wild animals. History shows that the disease was first identified in Malta in the year 1887 by Sir David Bruce who isolated *Brucella melitensis*, the then *Micrococcus melitensis* from a soldier died due to Maltese fever (Godfroid et al., 2005). Recent years has seen this bacterium as a pathogen causing disease in sea mammals thus showing the long journey of this pathogen from Malta to Marine. There are eleven *Brucella* species namely *B. abortus* (cattle), *B. melitensis* (sheep and goats), *B. ovis* (sheep), *B. suis* (hogs), *B. canis* (dogs), *B. neotomae* (wood rats), *B. microti*, *B. maris*, *B. ceti* and *B. pinnipedialis* (marine mammals) and *B. inopinata* (isolated recently from women breast implant) (Verger et al., 1987; Foster et al., 2007; Scholz et al., 2008).

These eleven *Brucella* spp. do not follow the strict rule towards host specificity as they can infect other animals and also human beings hence this pathogen is considered as an important zoonotic organism. *B. abortus* and *B. suis* affects wild animals and reports regarding *B. melitensis* infection in wild animals are less (Rhyan, 2000; Godfroid & Kasbohrer, 2002). There is no specific *Brucella* sp. that can cause disease particularly in horses. All the documented reports of brucellosis in horse are mainly by the cattle pathogen, *B. abortus* and to a milder extent *B. suis* biovar 3 (Lucero et al., 2008; Colavita et al., 2016).

Members of the genus *Brucella* are facultative intracellular gram negative cocco-bacilli or short rods measuring around 0.6 to 1.5 µm long and 0.5 to 0.7 µm width. *Brucella* genus neither form capsules nor spores nor motile though carry the genes for motility (except the chemotactic system that helps in assembling a functional flagellum) (Fretin et al., 2005). *Brucella* genus is classified phylogenetically within the α_2 subdivision of *Proteobacteria* which comprises of *Agrobacterium*, *Bartonella*, *Ochrobactrum*, *Rhizobium*, *Rhodobacter*, and *Rickettsia* (Moreno et al., 1990). Genome of *B. abortus* has two circular chromosomes of 2.1 Mb and 1.5 Mb size without plasmids (Michaux et al., 1993; Michaux-Charachon et al., 1997). Recent years has seen the completion of genome of *B. abortus* (Sanchez et al., 2001), *B. melitensis* (GenBank NC 003317 and NC 003318) (DelVecchio et al., 2002) and *B. suis* (GenBank NC 002969) thus increasing the opportunity for understanding the pathogenicity of *Brucella*.

Brucellosis mainly causes reproductive diseases in various animals, and in horses the clinical manifestation is termed as "poll-evil" or "fistulous withers" due to the inflammation of supraspinous bursa and connective tissue, leading to abscess formation and fistulation in the affected region and occasionally abortions and other reproductive problems are also reported (Denny, 1972). *B. abortus* has been reported worldwide causing infection in domestic animals though some

countries like Australia, Canada, Japan, Israel and New Zealand have controlled the disease (Nicoletti, 2007). Reports shows that age, breed and sex specificity does not exist for brucellosis in equine though more cases are documented in horses above 3 years of age (Nicoletti, 2007).

2 Epidemiology

Three species of *Brucella* namely *B. abortus*, *B. suis* and *B. canis* have been incriminated to infect horses, of which *B. abortus* and *B. suis* have been reported with natural infection and *B. canis* infection has been reported with experimental infection (Hagler et al., 1982).

Brucella antibodies have been reported from several parts of the world at various time frames showing that horses get infected from other animals. A study in Brazil was conducted where 123 crossbred cart horse serum samples were collected from a period April 2005 to June 2006. These samples were subjected to Rose Bengal Plate agglutination Test (RBPT) which showed eight animals (6.5%) to be positive for brucella antibodies. This study did not report isolation of the pathogen and the seropositive animals did not show any clinical manifestation of the disease (Antunes et al., 2013). A multispecies farm in Nigeria comprising of different livestock species like cattle, horse, goat and sheep was investigated for *B. abortus* infection status. This study revealed that all the seven horses under the study were found positive by RBPT. Vaginal samples from 6 horses were subjected for isolation of *B. abortus* which showed that two samples were positive for *B. abortus*. This study is the first report of isolation of the pathogen from non clinical cases from horse (Bertu et al., 2015). The report of this study warrants attention as disease free horses can also play role in transmission of the pathogen to other animals and also to human. Several other studies have been conducted earlier in Nigeria to investigate the presence of brucellosis in horses which documented 8.4% (14 positive of 166 animals) and 14.7% (11 samples out of 75 horses) which were lesser compared to the recent survey documenting 100% positivity (Bale & Kwanashie, 1984; Ehizibolo et al., 2011). Multispecies housing can increase the possibility of disease spread. Already there are established reports that horse to horse or other animals is a less likely event and these animals do not excrete the organism (Corbel & Henry, 1983; Macmillan & Cockrem, 1985). Sadiq et al. (2013) reported 5.5% seropositivity of brucellosis by RBPT and Microtiter Serum Agglutination Test in donkeys from Borno and Yobe states of Nigeria.

A very recent study conducted on the Mambilla plateau of Taraba state, Nigeria showed 16% prevalence of brucellosis and adult horses were affected more than young animals (Ardo & Abubakar, 2016). Another study conducted in the same Taraba state of Jalingo region showed 7 animals out of 90 adult horses screened by RBPT (Ardo et al., 2016).

Studies conducted in Iran also reported isolation of *B. abortus* from infected horses and serological diagnosis by RBPT, standard tube agglutination test (STAT) and 2-mercapto ethanol (2ME) showed 2.5% to 12% prevalence (Tahamtan et al., 2010). Another study in the Hamadan region of Iran showed 0.5% prevalence by RBPT and STAT (Ghobadi & Salehi, 2013). Positive animal in this study was also in close contact with other animals hence there was a higher chance of infection. Earlier study in the Mashhad region of Iran also showed 2.5% positive cases (Tahamtan et al., 2010).

An epidemiological survey was conducted in Peshawar district of Pakistan employing 500 serum samples from horses (n= 196), donkeys (n= 267) and mules (n= 37) collected during a time period of January to December, 2012. Higher prevalence (71.93%) was reported in horses than donkeys (63.67%) and mules (5.4%). Study also reported females and animals of 5-11 years were affected most (Safirullah et al., 2014). Younger animals tend to clear the pathogen faster though there is possibility of latent infection which may be the reason that the study had higher infection rate among older animals (Quinn et al., 2004). Sex hormone and erythritol tend to increase as the age advances and this may be another reason brucella infection is seen in older animals after sexual maturity (Radostits et al., 2000).

Serum samples of 227 equines (178 donkeys, 43 horses and 6 mules) collected in the Mossoró, Rio Grande do Norte, Brazil were subjected to RBPT, STAT and 2 ME showed 1.76% prevalence of brucellosis in horse serum (Dorneles et al., 2013). A massive survey was conducted recently employing serum samples of 6,439 animals comprising samples from 5,292 horses, 110 donkeys and 1,037 mules from 1936 herds of Minas Gerais State, Brazil over a period of September 2003 and March 2004 (Junqueira et al., 2015). RBPT and STAT was performed which showed that 70 horses out of 5,292 animals to be positive (1.32%), 1 out of 110 donkeys (0.91%) and 14 out of 1,037 mules (1.35%). This study documented that males (52 out of 4106 males) had higher sero-positivity compared to female (33 out of 2333 females) (Junqueira et al., 2015).

A study in Western Sudan employing serum samples from 346 horses and 28 donkeys showed 4.9% and 3.6% prevalence of brucellosis in horses and donkey (Musa, 2004). A total of 1954 serum samples (horses 782 and donkeys 1172) were collected from Sanliurfa and Diyarbakir provinces of South-East Turkey and were subjected to RBPT. Prevalence was higher in horses (13.68%) than donkeys (6.05%) (Tel et al., 2011). Several other workers have earlier studied the prevalence of brucellosis in horse in various regions of Turkey. Solmaz et al. (2004) reported 60.59% prevalence of brucellosis by RBPT in horses in the Van province of Turkey. Göz et al. (2007) reported 9.5% horses to be positive by STAT in Hakkari region of Turkey.

Brucella organism is to an extent resilient which has been recovered from manure and fetal samples even after 2 months when kept in cool weather condition. Exposure to sunlight for

few hours kills the bacteria and they are also readily killed by commonly used disinfectants (Glynn & Lynn 2008).

3 Pathogenesis

The major route of entry of the pathogen into the host (equines) is through the consumption of feed contaminated with *B. abortus* and contact infection from cattle has been reported by several workers (Denny 1973; O'Sullivan 1981; Ocholi et al., 2004). There prevails mystery regarding the role played by horse in the transmission of infection to other horses or to other animals/ humans. Hence there is no clear evidence that supports that horse's act as a ware house/ reservoir for transmission of brucellosis in endemic areas (Acosta-Gonzalez et al., 2006).

Being a facultative intracellular pathogen, *Brucella* survives, multiplies and evades host immune mechanism simultaneously found developing inside phagocytic cells (Gorvel & Moreno, 2006). Ecological niche inside the phagosomal compartment of host macrophages is conducive for the survival of brucellae and maintaining chronic infections depends upon the ability of surviving and replicating within these phagocytic cells (Roop et al., 2004; Neta et al., 2010) (Figure 1). *Brucella* infection occurs through ingestion and the organism enters the oral and pharyngeal cavities (Brinley et al., 1990). The bacteria are transported following penetration of the mucosal epithelium either free or within phagocytic cells to the regional lymph nodes through macrophages leading to spread and multiplication of organism in lymph nodes, spleen, liver, bone marrow, reproductive organs, tendon sheath and bursae occur (Canning et al., 1986; Riley et al., 1984; Memish et al., 2000; Adams, 2002). The capacity of *Brucella* to hide inside the macrophages makes it difficult to diagnose the disease and also hinder in the treatment of the disease (Glynn & Lynn 2008).

4 Clinical signs

In equines, the clinical signs due to brucellosis are mostly noticed in the musculoskeletal system mainly as the organism localise in the bursae (causing septic bursitis), joints (causing septic arthritis) and tendon sheaths (causing septic tenosynovitis) (Denny, 1972, Denny, 1973; Carrigan et al., 1987; Ocholi et al., 2004). Few reports regarding abortion, vertebral osteomyelitis and infertility in male horses have also been documented (Collins et al., 1971; Denny 1973). Most classical clinical signs observed in horses due to brucellosis are poll evil (septic supra-atlantal bursitis) and fistulous withers (septic supraspinatous bursitis). Draining sinuses are seen as these problems are chronic in case of equines (Crawford et al., 1990). An experimental infection of *B. abortus* instillation into the conjunctival sac was carried out in a horse to know the clinical signs. Serum antibody level was evident after 7 to 12 days of infection and intermittent bacteraemia was observed for 2 months (MacMillan et al., 1982).

Figure 1 Pathogenesis and clinical signs of equine brucellosis.

Lesions of granulomatous type were observed in lung, liver, testes and metatarsophalangeal synovial membranes (Megid et al., 2010). No apparent clinical signs were noted and female animals bred normally yielding negative results to isolation of organism (MacMillan & Cockrem, 1986). *B. abortus* induced abortion in equines are not common though some documented reports state that mid to late term abortion may occur (McNutt & Murray 1924; McCaughey & Kerr 1967; Shortridge, 1967; Robertson et al., 1973; Hinton et al., 1977). Organism in the vaginal excretions does not last longer as compared to cattle.

Some authors reported the isolation of *B. suis* from aborted equine fetuses (McNutt & Murray, 1924), horses affected with septic bursitis (Portugal et al., 1971) and also from the reproductive organs of mares without apparent clinical signs (Cvetnic et al., 2005).

5 Diagnosis

The gold standard test for diagnosis of Brucellosis is isolation and identification of the organism which needs 5-10% carbon dioxide for its growth. Even with all the conditions conducive for the growth of *Brucella*, it will take around 3-5 days for a

colony to appear. At times it may even take longer; hence culturing work is laborious and time consuming. The major setback in culturing is that Brucellosis is zoonotic and the handlers are always at the risk of infection (Karthik et al., 2014a). Hence it needs level 3 biocontainment facilities and highly skilled technical personnel for handling live culture (Alton et al., 1988).

Serological tests like Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme Linked Immunosorbent Assay (ELISA) are commonly employed (Nicoletti, 2007). Other serological tests include complement fixation test (CFTs), 2-mercaptoethanol (2ME), buffered *Brucella* antigen tests (BBAT), Milk ring test (MRT), etc. (Acha & Szyfres, 2003; Godfroid et al., 2010). These tests are inexpensive, fast and sensitive but not necessarily highly specific, antibodies may cross react with *Yersinia enterocolitica* serotype O:9, *Escherichia coli* O: 157, *Francisella tularensis*, *Salmonella* urbana O: 30, *Vibrio cholerae*, and others (Radostits et al., 2000).

Molecular techniques like PCR has been employed with various samples like blood, serum tissues from aborted foetus, semen and milk for diagnosis of brucellosis (Fekete et al.,

1992; Leal-Klevezas et al., 1995; Queipo-Ortuno et al., 1997; Amin et al., 2001; Kanani, 2007). More recently, real-time PCR has been used for detection of *Brucella*, offering improvement in detection times and specificity (Queipo-Ortuno et al., 2005). Real-time PCR is the latest method used in which hybridization probes are used to increase specificity (Bricker, 2002; Probert et al., 2004; Elfaki et al., 2005).

Isothermal amplification assay has the advantage of employing a set temperature for amplification of DNA, reducing the time for amplification and also there is no need for post amplification protocol for result visualization (Dhama et al., 2013). LAMP for the *Brucella* was developed against *Brucella* cell surface protein (*bcs*p31) gene and *omp*25 gene and sensitivity of both LAMP assay was higher than PCR (Ohtsuki et al., 2008; Lin et al., 2011). LAMP with visual detection based on calcein has also been developed targeting the same *omp*25 (Pan et al., 2011). Reports regarding development of LAMP targeting *IS*711 genes and real time quantitative LAMP are available (Pérez-Sancho et al., 2013). Visual LAMP targeting *omp*25 gene including loop primers was developed recently that can detect all *Brucella* spp. Developed LAMP assay with loop primers was 10 fold more sensitive than commonly employed PCR (Karthik et al., 2016). LAMP assay has the intrinsic property of product carry over contamination hence a novel closed tube LAMP assay was also developed for detection of *Brucella* spp. (Karthik et al., 2014b). LAMP assay for specific diagnosis of *B. abortus* was also developed which was 100 fold more sensitive than the commonly employed PCR (Karthik et al., 2014c). Severity of bone damage in cases of poll evil and fistulous withers can be identified by radiography.

6 Poll evil and fistulous withers

Poll evil and fistulous withers are chronic inflammatory conditions affecting supra-atlantal bursa and supraspinatus bursa and its associated tissues respectively (Gaughan et al., 1988; Rashmir-Raven et al., 1990; Cohen et al., 1992). The term fistula refers to draining wound from a normally closed structure, through the skin and fistulous withers is an infection of the bursa overlying the spines of the withers by usually caused by *Brucella* sp. in horse. Though *B. abortus* has been incriminated as the major cause for this condition, there are other pathogens/ wound which also plays role in causing this condition in horses. Other pathogens like *Streptococcus zooepidemicus*, *Streptococcus equi*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium* spp. *Actinomyces bovis*, *Bacteroides fragilis*, *Proteus mirabilis*, *Escherichia coli* and *Pasteurella* spp. have also been isolated from horses suffering from fistulous withers (Cohen et al., 1992; Hawkins & Fessler, 2000). Signs of fistulous withers include single or multiple draining tracts and in some case disseminated swelling in the wither region without drainage. Walls of the bursa gets thickened carrying clear, thick fluid which is of straw coloured (Megid et al., 2010). Initial signs include pain, swelling, heat at the bursal region leading to stiffness of the neck. Later stages the bursa ruptures and pus

material gets discharged through one or more opening. These fistulas may heal slowly and there are always chances that they reappear (Cohen et al., 1992). Few years back researchers have found an Iron Age horse cranium from Tuva Republic, Central Asia and the cranium had occipital lesions, cavities around the nuchal ligament attachment site was noted in the skull which may be due to inflammation followed by necrosis (Bendrey et al., 2011). The researchers concluded that condition is poll evil originating due to bacterial cause. Authors discussed that this case may be due to *B. abortus* the common cause of poll evil in horses. Thus this study shows that the pathogen existed long back causing poll evil in horses (Bendrey et al., 2011).

Drainage of the infected tissue (poll evil and fistulous withers) and treatment with systemic antibiotics can be employed to treat brucellosis in horse. Chloramphenicol, tetracyclines, streptomycin and some sulphonamides are commonly used for treatment of Brucellosis but these antimicrobials cannot penetrate the infected tissues (Nicoletti, 2007). Clofazimine has been reported to have good effect in the treatment of brucellosis in equines (Knottenbelt et al., 1989). *B. abortus* S19 vaccine has also be used with good effect for treatment of brucellosis with the regimen ranging from one dose to three doses at 10 days interval (Denny, 1973; Gardner et al., 1983; Cohen et al., 1992; Nicoletti, 2007). Use of this vaccine for treatment also involves local and systemic reactions and death has been reported in a horse that received intra venous injection (Denny, 1973; Cohen et al., 1992). Periodical drainage, cleaning the region with antiseptics and dimethyl sulphoxide will aid to control further complications of poll evil and fistulous withers (Cohen et al., 1992).

7 Prevention and control

- In most of the instances horse gets brucellosis when they are housed or allowed for grazing together with cattle. Hence horse should be housed or allowed for grazing away from cattle suspected for brucellosis (Cramlet & Bernhanu, 1979).
- Trauma is a major cause of predisposition of fistulous withers in horses hence proper fitting of saddle has to be taken care.
- Parasitic problem *Onchocerca* spp. can also cause fistulous withers hence minimizing the parasitic load by proper hygienic measures needs to be practiced (Cramlet & Bernhanu, 1979).
- Many of the countries follow testing and quarantine the horses or euthanize the horse since brucellosis is zoonotic. Hence measures needs to be followed for early diagnosis of the disease so that horses can be segregated or culled to prevent further spread of the disease.

8 Conclusion

Brucellosis, an age old disease is one of the important zoonotic disease that can infect several domestic animals, wild animals and also marine mammals. Eleven brucella species have been

described till date of which many are host restricted though it can infect other animals and also human. There is no single species of brucella that can cause brucellosis in equines. *B. abortus* and *B. suis* have been implicated to cause natural disease in equines. Mostly brucellosis in domestic animals are oriented to reproductive tract infection but in equines it causes mainly two important conditions affecting the musculoskeletal system termed as Poll evil and Fistulous withers. Abortions are also noticed in some cases but shedding of bacteria through the vaginal discharge is not documented yet. Though the disease has been reported for several years in horses there are still several questions that need to be clarified like the pathogenesis of bacterium in horse, predilection of bacterium towards musculoskeletal system in horse while mainly reproductive tract in other domestic animals. Diagnosis of brucellosis at the early stage is important to identify the diseased animals so that prior segregation of the animals can be done to minimize its spread to other animals. Advances in the field of diagnostics have made it possible to identify the agent early though proper sampling procedures needs to be practiced to achieve the same. Similarly treatment and vaccination aspects needs to be strengthened in order to control and eliminate the disease from the animal population so that its spread to humans can also be prevented.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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