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LEPTOSPIROSIS IN HORSES: SPECIAL REFERENCE TO EQUINE RECURRENT UVEITIS

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KEYWORDS

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ABSTRACT

Leptospirosis is a bacterial zoonotic disease with worldwide distribution. The disease affects several domestic and wild animals. Leptospirosis has seasonal nature with high incidence in hot rainy season especially in tropical regions. The disease in horses has ocular and systemic manifestations. However, stillbirths and neonatal mortality due to this disease is also common. The ocular manifestation is equine recurrent uveitis (ERU), also known as periodic ophthalmia or moon blindness, where autoimmune mechanisms also play an important role. Several diagnostic assays are employed and microscopic agglutination test has been commonly employed in several parts of the world though isolation is the gold standard test. Recent diagnostic advances like PCR, real time PCR, LAMP also aid in early diagnosis of the disease so that the spread of disease to other animals and human can be prevented.

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1 Introduction

Leptospirosis is a major animal and human health problem worldwide. Leptospire belong to family Leptospiraceae, order Spirochaetales. Leptospire are about 0.1 µm in diameter and 6-20 µm in length. Their major antigenic component is lipopolysaccharide (LPS). The disease is prevalent in many parts of the world both in urban and rural settings (Vinetz, 1997; Bharti et al., 2003; Verma et al., 2013; Khurana et al., 2015). The leptospirosis primarily causes chronic kidney infection in several domestic and wild animals. The leptospira colonize in renal tubules and are shed in urine. These bacteria survive for prolonged periods in moist conditions and thus transmit the infection.

Rats and other rodents are natural reservoirs of leptospira with no apparent signs of disease. They clear infections from their bodies except the kidney tubules. Other animals which are not natural carriers of infection have mild to severe infection and even death. The leptospire may cause reproductive problems mainly abortions (Coghlan & Bain, 1969; Faine et al., 1984; Faine et al., 1999; Bharti et al., 2003; Hamond et al., 2015; Hamond et al., 2016). Mother to foetus transmission is also common (Vinetz, 1997; Bharti et al., 2003; Alder & de la Pena Moctezuma, 2010; Verma et al., 2013; Hamond et al., 2014).

Animal handlers and waste water/ recycle workers are highly susceptible (Campagnolo et al., 2000). *Leptospira* spp. are endemic to several tropical and subtropical areas affecting military personnel, aid workers, tourists and general public (Ko et al., 1999; Bharti et al., 2003). Leptospirosis is less common in temperate regions. There are different reservoirs of infection in rural and urban areas, domestic and wild animals act as reservoirs in rural settings, dogs and rats are reservoirs in urban areas (Vinetz et al., 1996; Levett, 2001; Meites et al., 2004). Natural disasters, like floods may be followed by leptospirosis outbreaks (Fuortes & Nettleman, 1994). The leptospirosis has been described as “an occupational disease of soldiers” as the soldiers fighting in adverse terrains and conditions during wars are at a greater risk of acquiring leptospirosis infection (Johnston et al., 1983).

Symptoms in human beings may vary, but commonly include fever, headache, muscular pain, uneasiness, vomiting, conjunctivitis, uveitis, meningitis and jaundice. About 5 and 10% of patients progress to icteric phase. Fatality rates at this stage may be more than 20%. Mortality is primarily due to acute renal failure, pulmonary haemorrhages, intracerebral haemorrhage and multisystem organ failure (Vinetz, 1997; Faine et al., 1999; Ko et al., 1999; Levett, 2001; Bharti et al., 2003).

Leptospirosis in horses has been considered a relatively uncommon infection. Most of the infections are asymptomatic. A specific outcome of equine leptospirosis is recurrent uveitis which appears to be mediated by autoimmune mechanisms.

2 Equine Leptospirosis

2.1 Disease occurrence

The incidence of leptospirosis in horses remains uncertain as systematic studies on leptospirosis in horses are scanty. Its importance and economic impact in equines is also not as accurately distinguished as for other species of animals. Most epidemiological studies are based on serology with highly variable incidence in different geographical regions. There is also variability in the serovars. A sero-prevalence of only 1.5% was reported in central Italy, for serovars Icterohaemorrhagiae, Bratislava or Pomona (Ebani et al., 2012). However, a Brazilian study of 119 race horses showed a much higher seropositivity rate of 71% against serovar Copenhageni (Hamond et al., 2012b). All these horses were apparently healthy with no clinical signs of leptospirosis. In another study by the same group, seropositivity of 48% was reported and 35% of urine samples were detected positive by PCR, however these were culturally negative (Hamond et al., 2013). Recently a prevalence study conducted in Brazil in 38 mares having reproductive problems examined for leptospira by examining the serum, urine and vaginal fluid by isolation and PCR. Seventeen serum samples were positive (44.7%) for leptospira and of which sero groups Australis accounts for 76.4% and Pomona 23.6%. PCR results were positive for 17 vaginal fluid samples and 10 urine samples and the PCR products were sequenced which showed that the samples belonged to *L. interrogans* (sv Bratislava and Pomona) and *L. borgpetersenii*. Thus this study implies the presence of leptospira in reproductive tract (Hamond et al., 2015). Sixty two cart horse samples were screened in Curitiba, southern Brazil by microscopic agglutination test (MAT) and real time PCR of which 80.8% of the samples were positive for Icterohaemorrhagiae serovar (Finger et al., 2014).

Sero-positivity of 25% in Korea with serovars Sejroe and Bratislava being prominent (Jung et al., 2010), 79% in The Netherlands, serovars Copenhageni and Bratislava (Houwers et al., 2011) and 25% in Sweden (Baverud et al., 2009) show marked differences in prevalence and varied serovars according to geographical region. It was also indicated that the majority of equine infections were asymptomatic. A North American report in aborting mares revealed 20 out of 21 as due to serovar Pomona subtype Kennewicki (Timoney et al., 2011), which is correlated to the presence of this serovar and subtype in local wildlife mainly raccoons. Serovar Bratislava has been implicated in horses in Northern Ireland, based on culture and serology (Ellis et al., 1983). Pikalo et al. (2016) examined the presence of leptospiral antibodies in horses in middle Germany by MAT. 54 out of 314 (17.2%) horses were positive for one or more of eight leptospiral serovars analysed. Icterohaemorrhagiae (11.1%) was most prevalent followed by Bratislava (9.6%) and Grippotyphosa (1.9%). Dorrego-Keiter et al. (2016) detected 57.5% (127/221) horses with ERU having antibodies against leptospira by MAT in Germany.

Figure 1 Transmission and clinical signs of equine leptospirosis.

The most frequent antibodies were against Grippotyphosa (79/127), followed by Icterohaemorrhagiae (34/127) and Bratislava (29/127). Tsegay et al. (2016) detected significant antibody titres in 184 of 418 carthorses to at least one of 16 serovars of *Leptospira* species in central and southern Ethiopia. Serovar Bratislava (34.5%) was found to be most prevalent.

National Reference Centre for Leptospirosis (NRCL) of Italy along with other units evaluated the occurrence and distribution of leptospirosis in Italy. Analysis of the data for the one year (2010-2011) revealed that Australis was common among horses in Italy (Tagliabue et al., 2016).

Khurana et al. (2003) studied the sero-prevalence of leptospirosis in 436 equines in India by ELISA for detection of antibodies against six leptospiral serovars including *Leptospira interrogans* serovars Canicola, Pomona, Australis, Autumnalis, Icterohaemorrhagiae and Grippotyphosa. These samples included 379 serum samples from apparently healthy equines, 12 from cases of abortion and 45 from equines in contact with aborted animals. Out of the 379 apparently healthy horses, 64 (16.89%) harboured antibodies against *Leptospira* spp. They further reported the mares that came into contact with aborted animals, 66.7% had positive titres indicative of leptospiral infection. Animals in contact with aborted animals had also showed higher sero-prevalence of 64.4%. Antibody titres in

apparently healthy animals suggest either a subclinical form of the disease in these animals or previous infection. Region-wise sero-prevalence of leptospiral antibodies showed that studs in southern and western part of India country had higher seropositivity irrespective of the group.

2.2 Clinical signs, symptoms and disease manifestations

Equine leptospirosis is accompanied with mild fever. Loss of appetite and lethargy are common in mild form of disease. Jaundice, haemorrhages on the mucosa and depression are predominant signs in the severe form. Renal failure is more common in foals in comparison to old horses. Classic icteric leptospirosis occurs mainly in foals and is comparatively rare in adult horses. Leptospirosis may cause placentalitis, abortions and stillbirths in pregnant mares (Figure 1) (Timoney et al., 2011). The leptospire could be seen in foetal and maternal tissues, microscopically (Poonacha et al., 1993). Leptospiral abortions in the late stage of gestation, with no apparent clinical signs are common. Weak and icteric foals are also born (Donahue et al., 1991; Donahue et al., 1995). Infected mares shed leptospire in the urine for prolonged periods and transmit the infection (Donahue & Williams, 2000; Newman & Donahue, 2007).

Microscopically placental lesions include vasculities, thrombosis, inflammatory cells in the stroma and villi, cystic adenomatous hyperplasia of allantoic epithelium. Foetal liver and kidneys are enlarged. Microscopic lesions in foetus include suppurative and nonsuppurative nephritis, leukocytic infiltration of the portal triads, giant cell hepatopathy, pulmonary haemorrhages, pneumonia and myocarditis (Wilkie et al., 1988; Poonacha et al., 1993). Histopathological findings in young horses are marked with petechiae and lymphocytic infiltration in renal proximal tubules and glomeruli (Bernard, 1993; Faine et al., 1999). Equine recurrent uveitis is a common sequel in equines, which is dealt separately in this review.

The pulmonary haemorrhage is not common in equines (Bharti et al., 2003). Recently this syndrome is being reported more commonly than previous information (Broux et al., 2012) and endoscopy revealing pulmonary haemorrhage in 35% of sero-positive adult horses (Hamond et al., 2012a).

2.3 Diagnosis of leptospirosis in equines

The diagnosis of leptospirosis in horses is similar to that for other species. The gold standard is the culture and identification of leptospira. PCR is a more convenient and rapid (Alder & de la Pena Moctezuma, 2010). Real time PCR assay was recently compared with fluorescent antibody test (FAT) and microscopic agglutination test (MAT) for effective diagnosis of equine leptospirosis from foetal specimens like placenta, kidney, liver and heart blood. Out of the 21 confirmed cases of equine abortion real time PCR could detect all the positives correctly while MAT and FAT detected only 19 and 18 samples respectively. Thus qPCR is a better assay compared to MAT and FAT for diagnosis of leptospiral abortion in equines (Erol et al., 2015). Silver staining and FAT may be used to demonstrate leptospire in the placenta or foetal kidney. FAT is more sensitive than silver staining and more specific than MAT (Donahue & Williams, 2000; Szeredi & Haake, 2006; Newman & Donahue, 2007). Enzyme-linked immunosorbent assay (ELISA) has also been developed using different proteins of leptospira and its efficacy has been assessed time to time. Recently a cocktail of recombinant proteins namely rLipL21, rLoa22, rLipL32, and rLigACon4-8 of *Leptospira interrogans* were analyzed for its potential as a diagnostic marker through ELISA. The assay was tested with 130 serum samples and the results were compared with MAT and it was found that ELISA was sensitive and specific yielding similar results with MAT assay (Ye et al., 2014).

MAT is the test of choice for serological diagnosis. In an endemic area the value of a single positive specimen is limited. The four-fold rise in titre in paired sera is important for accurate diagnosis. In cases of leptospiral abortion, MAT on foetal fluids and maternal serum gives a very high titre indicative of positive diagnosis (Donahue & Williams, 2000). Serological tests in different areas should include prevalent serovars of that area as test antigens. Commercial enzyme immunoassays incorporating locally prevalent serovars are available.

2.4 Prevention and control

Treatment regimens for horses have mostly been derived by extrapolation from other species, due to non availability of specific information for horses. Streptomycin and penicillin are most common antibiotics of choice. Tetracyclines are used as an alternative. The penicillin dose is related to titre of leptospiral antibodies. The streptomycin is has severe toxic effects in horses (Bernard 1993; Newman & Donahue, 2007).

Till now there were no leptospirosis vaccine for horses. Cattle vaccines were being occasionally used in horses, which is not advisable. In leptospiral uveitis molecular mimicry occurs between leptospiral proteins and ocular tissues, in this situation vaccination with whole-cell bacterin may prime equines with cross-reacting antigens resulting in stronger immunological responses and development of eye inflammation in subsequent exposures. Ideally, proposed leptospirosis vaccine should be free of cross-reacting antigens. Several leptospiral antigens have been tested for protective efficacy (Alder & de la Pena Moctezuma, 2010; Murray et al., 2013). No antigen has been tested in horses. Recently, Zoetis has introduced a licensed equine leptospiral vaccine for prevention of leptospirosis caused by *Leptospira Pomona*. Prevention must therefore revolve around normal husbandry and hygiene practices, vaccination of other animals on the farm, minimizing contact with rodents and other wildlife carriers and other infected horses.

3 Equine Recurrent Uveitis (ERU)

A major consequence of leptospirosis in horses is uveitis or moon blindness also called periodic ophthalmia (Verma et al., 2013; Malalana et al., 2015). The uvea consists of three components, the iris, ciliary body (anterior uvea) and choroid (posterior uvea). The uveal tract is highly vascular, usually pigmented (Samuelson, 2007; Gilger & Deeg, 2011; Hollingsworth, 2011). Direct proximity to the peripheral vasculature, makes the uveal tract vulnerable to any disease of the systemic circulation (Hughes, 2010; Leiva et al., 2010; Gilger & Deeg, 2011). A blood-ocular barrier exists between the peripheral vasculature and the inner structures of the eye, divided into the blood-aqueous barrier (iris and ciliary body) and blood-retinal barrier (choroid). These barriers make the eye a protected or immune-privileged site. Disruption of this barrier allows the leakage of blood products and cells into the eye and the activation of several immune responses. Leptospira-associated uveitis forms an important part of ERU cases (Halliwell et al., 1985; Hartskeerl et al., 2004; Witkowski et al., 2016). ERU is inflammation of uvea which occurs recurring episodes (Cook & Harling, 1983). It is reported to have a worldwide prevalence of around 10% and thought to be a major cause of blindness in horses (Schwink, 1992; Hartskeerl et al., 2004).

Pathogenesis of ERU is not exactly elucidated though several possible ways has been reported. Eye of horses affected with ERU shows infiltration of macrophages, lymphocytes and

plasma cells into the ciliary body and also the iris. This shows that immunologically privileged sites wall has been breached by the organism. There is huge flow of CD4⁺ T lymphocytes in the anterior uveal tract (Romeike et al., 1998). In these affected horses T cell response is mainly of Th1 based (Gilger et al., 1999). Kalsow et al. (1994) reported that T- and B-cells are highly organized in the germinal centres in the horses affected with ERU. This highly organized structure shows the antibody response towards leptospira antigen in the anterior uvea (Kalsow et al., 1994). *Leptospira* can directly cause damage to the eye leading to ERU but mainly it is caused by the autoimmune response due to the antigen (Verma et al., 2005). Two leptospiral proteins namely LruA and LruB were suggested to play a major role in ERU since IgG and IgA specific for these proteins has been identified in the fluid from the eye (Verma et al., 2005). Hence these proteins can also be used as a diagnostic marker for detection of leptospiral infection in equines. An evidence of a antigenic relationship between *Leptospira* and equine eye is established further cross reactivity as a mechanism of disease progression has been proposed (Parma et al., 1985; Parma et al., 1987; Parma et al., 1997). Verma et al. (2005) described that intraocular expression of two leptospiral proteins, LruA and LruB antibodies was significantly higher than in the sera, indicating local production and antibodies in uveitic eyes. The lens proteins cross-reacting with LruA antiserum were identified as crystalline B and vimentin, and cross reacting retinal protein was identified as crystalline B2 (Verma et al., 2010). Therefore cross reactivity between leptospiral and ocular proteins may be responsible for immunopathogenesis of ERU of leptospirai origin.

ERU has three distinct clinical forms: classic, insidious and posterior (Gilger & Michau, 2004; Gilger & Deeg, 2011; Malalana et al., 2015). Classic ERU is characterised by active intraocular inflammation followed by quiet periods, where subsequent inflammatory phases show increased severity. Insidious ERU is characterised by low grade persistent inflammation. The Vitreous, choroid and retina are primarily affected in posterior uveitis.

The signs associated with an acute episode of anterior uveitis are varied including ocular pain, blepharospasm, lacrimation, chemosis, photophobia, oedema of the eyelid, swollen conjunctiva and corneal oedema, aqueous flare, hypopyon, hyphaema, miosis, iris colour changes and low intraocular pressure (Cook et al., 1983; Wada, 2006; Gilger & Deeg, 2011). Posterior uveitis is characterised by vititis with liquefaction of the vitreous and retinal changes.

Changes associated with previous episodes of uveitis in an otherwise quiescent eye may give clues to previous episodes. Some of these changes may include depigmentation, corneal scarring, atrophy and fibrosis of iris, abnormalities of the iris margin, cataract, glaucoma and fundic changes etc. (Williams et al., 1971) but are not specifically pathognomonic (Cook et al., 1983; Barnett, 1987; Spiess, 2010; Mathes et al., 2012). The prognosis is dependent on an early diagnosis and

treatment. A period of low inflammation follows the acute phase (Cook & Harling, 1983). Secondary cataract, anterior or posterior attachment of iris, lens luxation, vitreous exudates and retinal detachment are also witnessed due to severe inflammatory reaction (Rebhun, 1979; Cook & Harling, 1983; Gilger et al., 2000; Gilger & Michau, 2004). A pathognomonic sign of ERU is thick hyaline membrane near posterior aspect of iris and eosinophilic linear cytoplasmic inclusion bodies in nonpigmented ciliary epithelial cells (Cooley et al., 1990; Dubielzig et al., 1997).

ERU has been associated with sulphonamides administration or vaccination (Matthews & Handscombe, 1983; Whitcup, 2010). Higher prevalence in geldings compared to mares and stallions has been reported (Szemes & Gerhards, 2000). No particular sex related differences in prevalence have been reported (Gilger & Deeg, 2011; Kulbrock et al., 2013). Age of presentation has been reported to vary in different studies (Dwyer et al., 1995; Szemes & Gerhards, 2000).

Diagnosis of *Leptospira* associated ERU is based on the presence of classical signs of uveitis, history of recurrence and seropositivity by MAT. No specific test is available for the diagnosis of leptospiral uveitis. Negative MAT titres are not always indicative of absence of leptospiral infection.

Reducing inflammation is of primary concern in ERU therapy. An intraocular device containing cyclosporine A is found effective in treatment of leptospiral ERU (Werry & Gerhards, 1991; Gilger & Michau, 2004). However, usefulness of antibiotics in treating ERU has not been fully explored. A recent review conducted in the North Carolina State University Veterinary Health Complex with the medical records of ERU from 1999 to 2014 showed that most cases had blindness, eye globe loss and loss of eye function. Several owners opted for euthanasia and some opted to sell the animals due to recurrent eye problem (Gerding & Gilger, 2016). Thus this problem of ERU has caused great financial loss to the horse owners.

Thus leptospiral infections result in reproductive and respiratory problems in equines. However, the most important manifestation of leptospiral infection in equines is ERU, which affects equine population by causing blindness, thus rendering them useless. Therefore more researches are needed to explore the pathogenesis and mechanism of occurrence of ERU with an aim of its prevention and control.

Conflict of interest

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

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