LYME DISEASE IN HORSES

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Summary

Lyme disease is a tick-borne infectious disease caused by the spirochete *Borrelia burgdorferi*. Infection in most horses appears to be subclinical. Clinical signs most commonly attributed to Lyme disease in horses include low-grade fever, stiffness and lameness in more than one limb, muscle tenderness, hyperaesthesia, swollen joints, lethargy and behavioural changes. Diagnosis is made on the basis of the horse being housed in an endemic area, compatible clinical signs, ruling out other causes for these signs and a high titre using kinetic enzyme-linked immunosorbent assay, other serological tests or positive western blot (WB) test results for anti-*B. burgdorferi* antibodies. Treatments include oral doxycycline, i.v. oxytetracycline or i.m. ceftiofur.

Introduction

Lyme disease is a global health concern in man and has been associated with numerous neurological, rheumatological and psychiatric manifestations (Cameron 2008). It is a tick-borne infectious disease that occurs in parts of North America, Europe and Asia (Hovius *et al.* 2007). Although *Borrelia* spp. have been associated with human diseases (such as louse-borne relapsing fever and tick-borne relapsing fever) for many years, scant attention was directed toward the study of these organisms in the latter half of the 20th century until an epidemic of arthritis was described in Lyme, Connecticut (Steere *et al.* 1977). In sequential fashion, this condition was associated with a typical rash previously described in Europe as *erythema migrans* and the bite of the blacklegged tick, *Ixodes scapularis* (Steere *et al.* 1978; Steere and Malawista 1979). In 1982, Burgdorfer *et al.* (1982) reported the discovery of a spirochete in *Ixodes scapularis*, and a few months later in *Ixodes ricinus* (Burgdorfer *et al.* 1983), that proved to be the aetiological agent of Lyme disease or Lyme borreliosis. This spirochete was subsequently named *Borrelia burgdorferi* (Johnson *et al.* 1984).

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Aetiology and epidemiology

Borrelia burgdorferi, the cause of Lyme disease, is a helical shaped gram negative spirochete (Burdorfer 1984). In Europe and Asia, 3 major *Borrelia* genospecies (*burgdorferi* sensu stricto, garinii and afzelii) can be the causative agents of Lyme disease (Hovius *et al.* 2007), whereas in North America only, *B. burgdorferi* sensu stricto causes the disease (Lo Re III *et al.* 2004). *B. burgdorferi* is maintained in a 2-year enzootic cycle involving *Ixodes* spp. ticks and mammals (Rosa 1997). *Ixodes scapularis*, *Ixodes ricinus* and *Ixodes persulacatus* are the most important vectors in North America, Europe and Asia respectively (Hovius *et al.* 2007). Deer and the white-footed mouse (*Peromyscus leucopus*) are the most common mammals involved in maintaining the life cycle in North America. *I. ricinus* feeds on an extraordinarily broad array of hosts, from small, medium and large-sized mammals to birds and reptiles (Anderson 1991) and is the tick species that most frequently bites human subjects in Europe. Several species of mice, voles, rats and shrews have been shown to be competent reservoirs of *B. burgdorferi* in Europe (Gern *et al.* 1998).

Infection in mammals generally results from larval or nymph bites in the spring and summer, or adult female ticks feeding in the summer, autumn or winter. In horses, it is not known whether larval and nymph bites play an important role in Lyme infection. In most instances, the ticks must be attached to the mammal for at least 24 h for

B. burgdorferi transmission (Norman *et al.* 1996). Once tick feeding begins, the *Borrelia* organism begins both up and down regulation of certain genes to allow transmission and survival. OspA may be down-regulated, while other surface proteins (OspE, OspF and Vis-E/C6) that are in low concentration in the tick gut are up-regulated during transmission to enhance immune evasion in mammals (Pal *et al.* 2001; Kraiczy *et al.* 2002).

The organism may also live in the host by residing in connective tissue and collagen, and having no requirement for iron (Nanagara *et al.* 1996; Posey and Gherardini 2000). A large percentage of adult horses in the more eastern parts of the northeast and mid-Atlantic USA are or have been infected with *B. burgdorferi* (Magnarelli *et al.* 2000; Divers 2007). Infection is also common in Wisconsin and Minnesota (Burgess 1988). Prevalence is confirmed by serological surveys up to 75% of adult horses in some of these areas are believed to be seropositive (Divers 2007). Seroprevalence in horses in many other parts of the USA has not been reported, but would be expected to fluctuate in a manner similar to that seen with the human form of the disease. In the USA, the geographic area of human Lyme disease appears to be increasing based upon the Center for Disease Control (CDC) maps from 2002 and 2006 (**Fig 1**). In horses, high seroprevalence is more widespread than reflected by the 2002 CDC map, as horses in northern and central Virginia are commonly seropositive. Infection in Central and South America appears rare with only one report of infection in a small number of horses in northeast Mexico (Salinas-Mélendez *et al.* 2001).

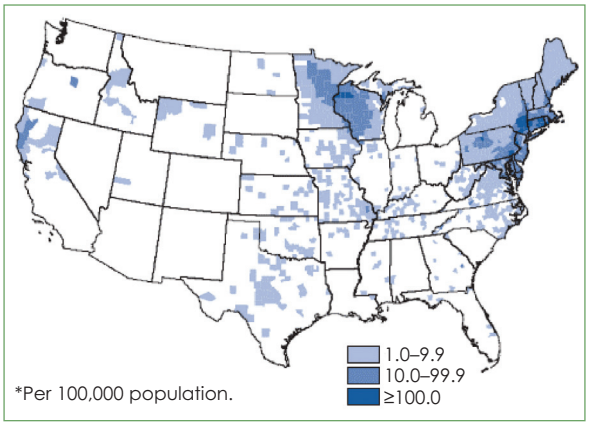

FIGURE 1: High-risk areas for Lyme Disease in the United States. From MMWR Surveill Summ. 2008 Oct 3;57 (10):1-9. Surveillance for Lyme disease-United States, 1992–2006. R.M. Bacon, K.J. Kugeler, P.S. Mead; Centers for Disease Control and Prevention (CDC).

Seroprevalence of infection in horses is reported in Sweden (Egenvall *et al.* 2001), Poland (Stefanciková *et al.* 2008), Austria (Müller *et al.* 2002), Japan and Turkey (Bhide *et al.* 2008), all having lower seroprevalence than in the northeast USA. Seropositive horses are also known to occur in the UK and Germany (Carter *et al.* 1994; Gerhards and Wollanke 1996). There are probably other areas of Europe and elsewhere in the world where seroprevalence may be moderately high. The incidence of human Lyme disease in Europe is unknown, but based on rates of serodiagnosis (and taking the limitations of seroprevalence methods into account) it is clear that Lyme disease shows a gradient of increasing incidence from west to east with the highest incidences in central-eastern Europe (**Table 1**). A gradient of decreasing incidence from south to north in Scandinavia and north to south in Italy, Spain and Greece has also been noted.

Clinical signs

A broad spectrum of clinical signs has been attributed to *Borrelia* infection in horses, but cause and effect have been difficult to document (Divers 2007). In fact, there is little doubt that most infected horses do not show obvious clinical signs. This seems to be agreed upon by most veterinarians regardless of the country

TABLE 1: Estimated human Lyme disease annual incidence in selected European countries (Anon 1995)

Country	Incidence per 100,000 population	Annual number of cases
UK*	0.3	200
Ireland	0.6	30
France	16.0	7,200
Germany*	25.0	20,000
Switzerland*	30.4	2,000
Czech Republic*	39.0	3,500
Bulgaria	55.0	3,500
Sweden (south)	69.0	7,120
Slovenia	120.0	2,000
Austria	130.0	14,000

* No published figures available.

of practice. Clinical signs most commonly attributed to Lyme disease in horses include low-grade fever, stiffness and lameness in more than one limb, muscle tenderness, hyperesthesia, swollen joints, lethargy and behavioural changes (Magnarelli *et al.* 2000). Neurological dysfunction and panuveitis have been reported in a horse and a pony (Burgess *et al.* 1986; Hahn *et al.* 1996).

Recently, one author (T.D.) has helped in the evaluation of 2 horses with ataxia, neck stiffness and behavioural changes that were attributed to Lyme infection. In one case, marked lymphocytic pleocytosis was found in the CSF and, upon necropsy, a marked cervical lymphocytic meningitis with thickened dura and nerve root involvement were found. Lymphocytes were of mixed size and type, ruling out lymphoma. In the other horse, there was a high CSF *Borrelia* antibody titre (ELISA of 393 units; 412 in the serum). Using the same samples, comparison of serum to CSF antibody to EHV1 (1:32 vs. 1:4), antibody to EPM (positive serum, negative CSF), and IgG (32 g/l serum vs. <2 g/l CSF) strongly suggested intrathecal production of *Borrelia* antibodies. The horse responded favourably to oxytetracycline and doxycycline treatments. The neurological signs, absence of other causes for the CNS signs, strong evidence of intrathecal antibody production, and response to therapy would meet the criteria for diagnosing neuroborreliosis in man (Lanska *et al.* 2008). Nine months after infection, one pony experimentally-infected with *Borrelia* had lymphocytic neuritis of the facial, tibial and fibular nerves only on the side that the infected ticks had been attached, suggesting that peripheral neuritis could be a possible clinical consequence of infection (Chang *et al.* 2000a).

Even more recently, an adult horse with bilateral uveitis was shown to have large numbers of *Borrelia* organisms in the ocular fluids of both eyes (observed on cytology and confirmed by PCR). Ocular involvement with *Borrelia* may be a rare occurrence as we have not seen this before and, in a study from Germany involving 79 horses with recurrent uveitis and controls, no association was found between seropositivity to *Borrelia* and the presence of ocular disease (Gerhards and Wollanke 1996). Why only a small number of horses might have clinical signs following infection is unknown, but could be related to an individual horse immune response to the infection. Fever and limb oedema frequently reported in association with recent *Borrelia* infection (proven by seroconversion) are most often the result of *Anaplasma phagocytophilum* infection, as many ticks are dually infected with both *Borrelia* and *A. phagocytophilum* in Lyme endemic areas in both the USA and Europe (Chang *et al.* 2000b; Engvall and Egenvall 2002).

Two retrospective clinical studies with control groups have been published in which an attempt was made to correlate seropositivity for *Borrelia* with commonly incriminated clinical signs. In one report from Connecticut, *Borrelia* infection, confirmed by serology, spirochetaemia or both was more common in horses with lameness or behavioural changes than in horses in the same region that did not have these clinical signs (Manion *et al.* 1998). A differently designed study (retrospective identification of seropositive horses examined for any illness followed up by owner questionnaire) was conducted in Sweden. In that study, no association was found between *Borrelia* seropositivity and the clinical signs commonly attributed to Lyme disease (Egenvall *et al.* 2001). Experimental infection in ponies causes disease in the skin, muscle, peripheral nerves and both perisynovial nerves and blood vessels, but clinical signs were not obvious (Chang *et al.* 2000a). After experimentally attaching infected ticks for 7 days followed by 9 months observation, *Borrelia* was consistently found in all synovial membranes and in skin and fascia near the site of previous tick attachment. There does seem to be a predilection for persistence of infection at these sites (Chang *et al.* 2000a).

The anatomical predilection for persistent infection and mild lymphocytic response in the synovial membranes may help explain the dramatic response to either doxycycline and/or oxytetetracycline observed in many suspect field cases (strongly seropositive horses with multiple limb stiffness/lameness that was not explained by other diseases) (Divers 2007). Oxytetracycline and doxycycline have their primary matrix metalloproteinase anti-inflammatory effect on the synovium as opposed to articular cartilage cells (L.A. Fortier, unpublished data). The common finding of *Borrelia* in the fascia of experimentally infected horses may explain the hyperaesthesia reported in many suspect cases and may give a clue as to potential movement and persistence of the organism (skin, fascia, nerves and synovial membranes).

Diagnosis

Lyme disease is diagnosed on the basis of the horse being housed in an endemic area, compatible clinical signs, ruling out other causes for these signs, and a high kinetic enzyme-linked immunosorbent assay (ELISA) (KELA) titre (generally >300 KELA units), other serological test or positive western blot (WB) test results for anti-*B. burgdorferi* antibodies (Divers 2007). If the ELISA titre is >300, there is a 99% probability that WB results will be positive (Divers 2007). The WB is most useful in horses with moderate ELISA titres (200–300) or when there is suspicion that the horse may have received a commercial canine Lyme disease vaccine. Time from infection to seroconversion appears to be 3–10 weeks (Chang *et al.* 2000a). One potential limitation of serological tests is that they do not distinguish between active infection and previous exposure. For this reason, tests that detect the spirochete directly are more conclusive of current infection.

Detection of *B. burgdorferi* DNA in a synovial membrane of a painful joint via polymerase chain reaction (PCR) assay is strongly indicative of infection. The sensitivity of PCR amplification of *Borrelia* in a synovial membrane biopsy specimen in the horse is currently unknown. It is also possible that many infected horses may be infected for a prolonged duration, even life, or are repeatedly re-infected since prior infection may not produce protective antibody. These possibilities are supported by the fact that field horses with high ELISA values rarely have a substantial numerical decline in ELISA titre when monitored for months or even years. Treatment of naturally-occurring cases may result in a decline in titre compared to nontreated horses but the mean magnitude of decline is small (20–30 KELA units). The small titre decline in field cases is quite different from the decline seen in treated experimentally infected ponies (experimentally infected ponies had dramatic decline in serum antibody to *Borrelia* immediately after 4 weeks of treatment with either oral doxycycline i.v. oxytetetracycline or i.m. ceftiofur). Antibodies rose again within another 4 weeks in ponies that were not cured of the infection (3/4 in both the doxycycline and ceftiofur treated ponies, while one in each group and all 4 in the oxytetetracycline treated group had continual decline of antibody to <110 KELA units and absence of organism on *post mortem* examination [both culture and PCR] [Chang *et al.* 2005]).

More recently, an on-site C-6 ELISA Snap test marketed for detection of *Borrelia*, heartworm, *Ehrlichia canis* and *Anaplasma phagocytophilum* infection in dogs was evaluated for detection of *Borrelia* infection in horses; sensitivity of the test is lower than that of ELISA performed at referral laboratories. It does not appear to detect antibodies during infection any earlier than the ELISA (when 110 is used as a cut off for positive) nor did it become negative more rapidly after successful treatment in the experimental ponies (Johnson *et al.* 2008). Evaluation of this assay in treated field cases has not been reported. An advantage of the C-6 snap test and WB test (in most cases) is that vaccination with OSPA antigen or whole cell does not cause positive results. Most infections do not cause a strong reaction of the P32 WB band (associated with OSPA antigen), but a small number of horses will produce antibody to this antigen following natural infection. A luminex assay is currently under evaluation and it may be further helpful in determining the infection status of seropositive horses (B. Wagner, personal communication).

Common disorders that may be confused with Lyme disease and should be ruled out include osteoarthritis of the hock, osteochondrosis, polysaccharide storage myopathy and other chronic myopathies, polysynovitis (suspect Lyme cases rarely have dramatic synovial effusion), thoracic spinous process osteoarthritis and equine protozoal myelitis. Diagnostic tests that may be useful in ruling out these diseases include thorough lameness and neurological examinations, radiography, scintigraphy, muscle biopsy, cerebrospinal fluid collection and appropriate testing and serum analysis for concentrations of muscle enzymes (both before and after exercise). Lyme disease seems to be most commonly diagnosed in nonracing performance horses such as dressage, stadium jumping and 3-day event horses. We are not sure why this may be the case but, if true, it could be explained by: 1) more turnout into pasture and woods, and therefore increased tick exposure in sport horses; and 2) trainer/rider ability to detect subtle gait or behavioural changes in those horses.

Treatment

The 2 most commonly used antibiotics for treating Lyme disease in horses are oxytetracycline, given i.v., and doxycycline, given *per os* (Divers 2007). Horses with what are often believed to be more typical signs of Lyme disease (e.g. chronic stiffness, lameness and hyperesthesia) are most frequently treated with doxycycline (10 mg/kg bwt, *per os*, every 12 h). Duration of treatment is often 1 month, but this duration is only empirical. Horses treated with doxycycline should be observed for a change in stool consistency, as diarrhoea develops in a low percentage of treated horses. A clinical response of less stiffness or lameness is often reported following doxycycline treatment, but this could be a nonspecific anti-inflammatory response because doxycycline inhibits metalloproteinase activity (Fortier *et al.* 2008).

Oxytetracycline (6.6–11.0 mg/kg bwt, i.v., every 24 h) may be more efficacious because of higher blood concentration and, therefore, higher tissue concentrations of i.v. oxytetracycline as opposed to poorly absorbed doxycycline given *per os*. In the experimental infection studies, oxytetracycline given i.v. was superior to doxycycline given *per os* (Chang *et al.* 2005). Oxytetracycline should not be administered in high doses or for prolonged periods if dehydration is present or there is pre-existing renal dysfunction. Acute renal failure may occur in those horses if oxytetracycline is administered. Ceftiofur (2–4 mg/kg bwt, i.v. or i.m. administration, every 12 h) has also been used in the treatment of horses with Lyme disease. Although antibody titres decreased in experimentally-infected ponies given the antibiotic treatments, similar declines in titre in association with those same treatments have been rare in naturally-infected horses. The reasons for this may be a longer duration of infection before beginning treatment in horses with naturally-occurring disease (which would decrease the effectiveness of the antimicrobials in clearing the infection), reinfection or antibody mimicry.

Because horses do not develop *erythema migrans* (a characteristic cutaneous manifestation of human Lyme disease [Mullegger and Glatz 2008]), recognition of early infection is nearly impossible except in cases when firmly attached infected female *Ixodes* ticks are removed. In the experimentally-infected ponies, the organism was eliminated from the body only in ponies that maintained ELISA titres below 110 units for 2 months after treatment was completed (Chang *et al.* 2005). Other treatments that can be considered supportive include chondroprotective agents, nonsteroidal anti-inflammatory agents, and acupuncture. Acupuncture may be especially valuable for management of hyperaesthesia-perineuritis syndromes that are often poorly responsive to treatment with nonsteroidal anti-inflammatory drugs.

Prevention

Prevention of Lyme disease in endemic areas would involve preventing tick exposure or prolonged (more than 24 h) attachment and/or early antimicrobial treatment after *Ixodes* exposure; however the efficacy of these techniques is unproven. Decreased tick infection may be accomplished by clipping tall grasses, clearing shrubs and bushes, and preventing the horse from entering forest and woodlands. Topical sprays could be used when exposure to ticks is expected. We are not aware of adverse effects from use of the more common canine tick sprays (e.g. fipronil)[1] in the horse. Spraying is most commonly used when adult ticks are noticeable in the late summer, autumn and the early part of winter, but infection with larval or nymphal stages earlier in the year should also be considered. If ticks are found on the horse, they should be identified to determine whether they are *Ixodes* spp., which is the only species of tick in North America known to transmit *B. burgdorferi*. Ponies have been protected by vaccination with a OPSA vaccine (Chang *et al.* 1999), but a vaccine approved for use in equids is not commercially available at present. Efficacy of administration of the canine vaccine in the horse is unknown, but based upon the experimental study, efficacy might be expected. Re-infection can occur following natural infection, so vaccination during antibiotic treatment is used by some veterinarians. In the future, vaccines that deter tick attachment may be feasible in the horse.

Manufacturer's address

[1]FrontLine, Merial Limited, Iselin, New Jersey, USA.

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