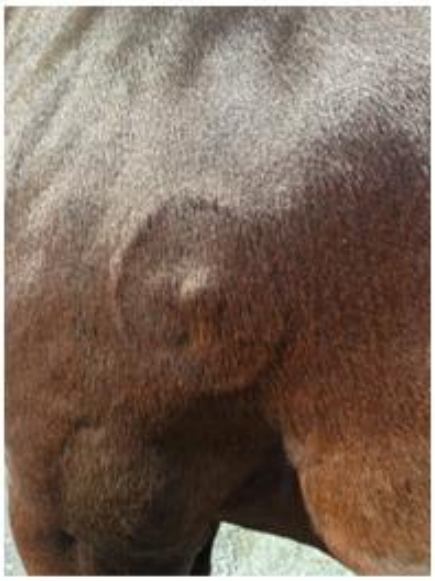
Lyme Disease in the Horse: Keeping up with Evolution

Dr. Susan Mende, DVM

Lyme borreliosis is caused by a bacterium that is a member of the family Spirochaetaceae. Lyme borreliosis is caused by the gram-negative spirochete organism ***Borrelia burgdorferi***. It gained recognition when implicated in 1982 by Dr. Will Burgdorfer as the causative agent of an epidemic of juvenile inflammatory arthritis in 39 children and 12 adults in Old Lyme, Lyme, and East Haddam, Connecticut in 1975 (Burgdorfer, 1982). Medical references commonly cite "**Lyme disease**" when referring to systemic infection with the spirochetal bacterium *Borrelia burgdorferi*. Borreliosis was discovered in dogs in 1984 and horses in 1985. Thus far, Lyme disease has been reported in humans, cats, dogs, sheep, goats, cattle, and horses, but to date it has been studied extensively in humans and dogs only. Dogs appear to be at greater risk than humans for Lyme disease (Eng, 1988).

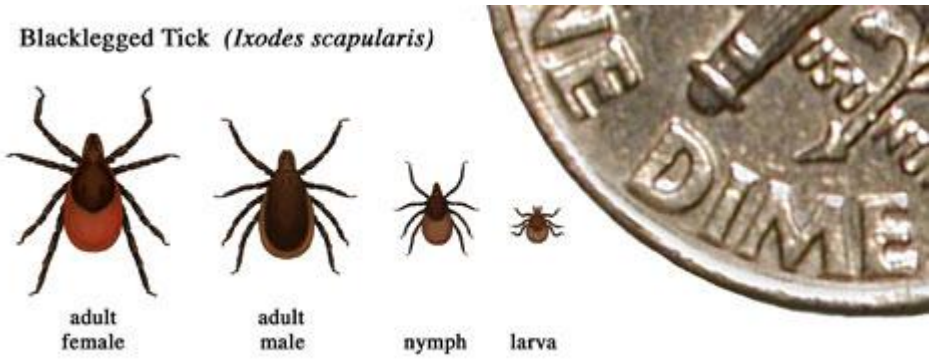
Lyme disease is a tick-borne infectious disease. The causative organism, *B. burgdorferi,* is widely distributed in the northern hemisphere. **Lyme borreliosis** has been reported extensively in Europe, England, the former Soviet Union, China, Japan, Southeast Asia, and South Africa (Schmid, 1985). *B. burgdorferi* is found in many arthropods, but the major route of transmission to animals and humans is ticks; no documented cases of flea transmission of *B. burgdorferi* have been reported. *B. burgdorferi* is transmitted from ticks belonging specifically to the ***Ixodes ricinus*** complex. Most transmissions of *B. burgdorferi* occur after the tick has been attached for more than 24 hours (Piesman, 1987).



Tick bite bullseye

Tick Description and Life-Cycle

Lyme disease only occurs in areas of the world where both the ticks and reservoir mammalian hosts coexist. On the east coast of the United States, *Ixodes scapularis* (**deer tick)** is the principal carrier, whereas on the west coast of the United States, *Ixodes pacificus* (**western black-legged tick)** is the main carrier identified. A much higher percentage of *I. scapularis* ticks (12-99%) carry the spirochete compared to *I. pacificus*, in which the maximum number of infected ticks is 4-5% (Anderson, 1989). *I. pacificus* sub-adults feed on the western fence lizard, which is an unsuitable host for *B. burgdorferi* and accounts for the low number of infected ticks on the west coast (Lane, 1989). *I.scapularis* is found in the east. The lower boundary of its region is Florida and westward into central Texas; the upper boundary is Maine and westward to Minnesota and Iowa. *I. scapularis* affects the greatest number of people because the infection rates are high in these ticks (Magnarelli 1986), their geographic distribution coincides with the greatest concentration of humans in the northeastern United States (Miller 1990), and the geographical range of the tick is spreading (Anderson, 1990). Distribution of *I. scapularis* is linked to the distribution and abundance of its primary reproductive host, the **white-tail deer** (Wilson, 1985). *Ixodes* adults are found most commonly on deer. In the northeastern United States, much of the landscape has been altered. Forests that were cleared for farming during colonial times were then abandoned in the late 1800s and 1900s causing succession of the fields to second-growth forests. These second-growth forests create "edge" habitats, which provide appropriate habitat for deer (Severinghaus 1956) and thus increased populations of deer ticks. Only deer or some other large mammal appears capable of supporting high populations of ticks (Duffy 1994).



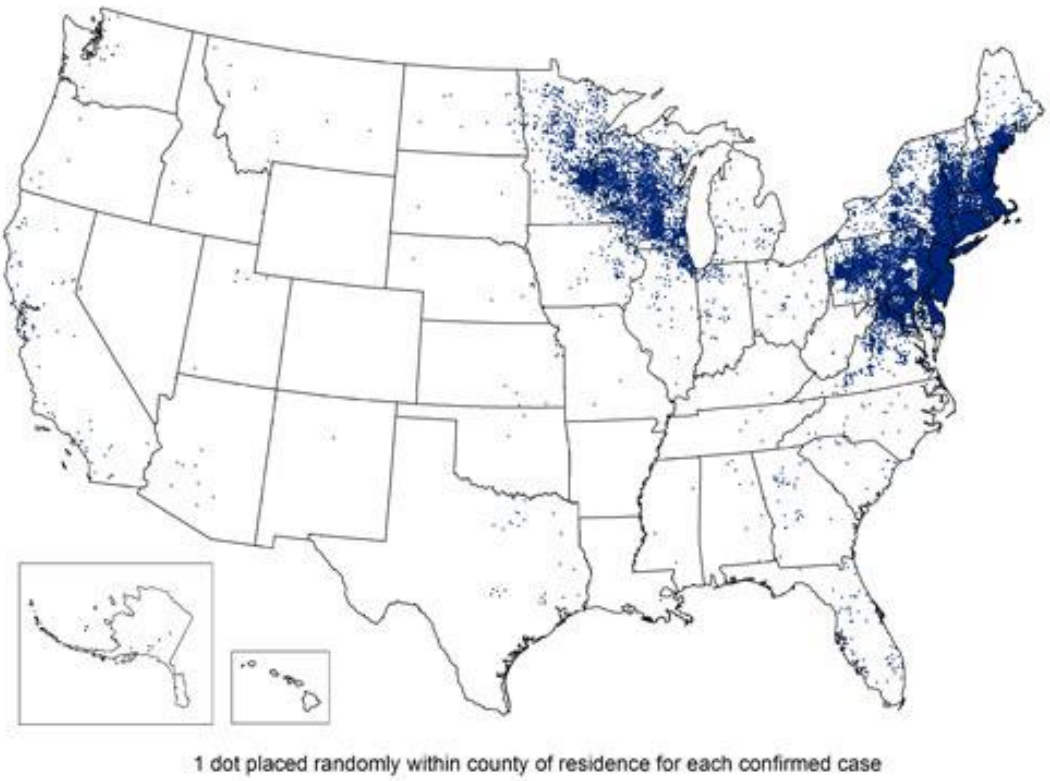
Ixodes scapularis is a three-host tick with a two-year life cycle. Each mobile stage feeds upon a different host animal. In June and July, eggs that were deposited earlier in the spring hatch into tiny six-legged larvae. Peak larval activity occurs in August, when larvae attach and feed on a wide variety of mammals and birds, primarily *Peromycus leucopus* (**white-footed mouse)** (Anderson 1980). After feeding for 3-5 days, engorged larvae drop from the host to the ground where they overwinter in leaf litter. In May of the following year larvae molt into nymphs that feed on a variety of hosts for 3-4 days. In a similar manner, engorged nymphs detach and drop to the forest floor where they molt into the adult stage. From May through October, adult *Ixodes* ticks feed on blood in order to reproduce. Adult ticks can remain active through the winter on days when the ground and ambient temperatures are above freezing. Adult female ticks feed for 5-7 days on large mammals, primarily white-tailed deer (Piesman 1987). Engorged adult females typically lay between 1,000-3,000 eggs each on the forest floor at the site where they detached from their host. They hatch the following June. Mortality rates for ticks are high; a variety of adverse climatic and microclimate conditions involving temperature and humidity have an impact on tick survival (Bertrand 1996). If the tick at any stage of development fails to find a host and take a blood-meal, it will die of starvation.

In **horses**, it is not known whether larval or nymph bites play the most important role in Lyme infection. Exposure to infected nymph stages is greatest during the summer months. Their small size, vastly greater abundance over the adult stages, and the difficulty in recognizing their bites (Berger 1989) tend to make nymphs the most important stage to consider for reducing disease risk. Many ticks are dually infected with both *Borrelia* and *Anaplasma phagocytophilium,* a tick-borne gram-negative rickettsial organism.

Laboratory testing, Diagnosis, and Disease

Lyme disease has not been studied as extensively in horses as in humans and dogs, but *B. burgdorferi* causes similar illness in many of our domestic animals. The Centers for Disease Control (CDC, 1995) recommends a two-tiered approach to the serologic diagnosis of Lyme disease in people, consisting of use of an enzyme-linked immunosorbent assay [**ELISA**] or immunofluorescence assay [**IFA**] as a screening test, followed by Western blot [**WB**] analysis in patients with positive or equivocal ELISA or IFA results. This approach has also been adopted in the veterinary community and has been the gold standard for many years. An ELISA test was developed in 1989 to detect IgM antibodies against *B. burgdorferi* in equine serum to aide in surveillance of this emerging condition in the horse population (Magnarelli, 1989). With this tool, a serological survey was performed on horses from the states of NY, MD, DE, NJ, and PA. In 1990, 13% of the horses surveyed were seropositive for *B. burgdorferi* by ELISA (Bernard, 1990). A similar study was performed in 1992 at Texas A&M University, but only 1 individual from the 469 tested (0.2%) was positive, indicating that infection with *B. burgdorferi* was uncommon in horses in central Texas (Cohen, 1992). Over time, a pattern began to emerge. In 1998, an estimated 40% of horses in the Mid-Atlantic region showed serological evidence of *B. burgdorferi* exposure (Marion 1998). Currently, a large percentage of adult horses in the more eastern parts of the Northeast and Mid-Atlantic United States are or have been infected with *B. burgdorferi*. Most recently, a serological survey revealed that as many as 75% of adult horses in some of these areas are seropositive. Seroprevalence in other parts of the United States has not been studied or reported, but would be expected to fluctuate in a manner similar to that seen with the human form of the disease due to all of the factors outlined above (Diver, 2009).

It has been a topic of hot debate whether Lyme disease poses any real threat to horses. An early study on Equine Lyme Disease was conducted by Divers in 2003 on ponies bitten by adult *Ixodes scapularis* ticks infected with *B. burgdorferi*. The ticks were placed on the ponies for 7 days and all became infected; control ponies that had laboratory raised *I. scapularis* without *B. burgdorferi* infection attached in a similar manner for 7 days remained uninfected (Chang 2000). All ponies exposed to *B. burgdorferi* infected ticks developed detectable antibodies at 5-6 weeks. At 3-4 months after tick infection, the **ELISA** measured 200-300 units and remained that way until euthanasia 9 months after tick exposure. **Western blot** became positive at 10-12 weeks. *B. burgdorferi* was isolated from the skin biopsies taken near the sites of tick attachment throughout the duration of the study. This study revealed a possible preferred migration of the organism through connective, peri-neural, and peri-vascular tissue in the skin, fascia, muscle, and synovial membranes and proved that *B.burgdorferi* infection caused consistent and predictable microscopic disease. Pathology in these areas could certainly cause hyperesthesia and lameness, two of the most commonly reported clinical signs in humans and dogs, yet these ponies were not noted to be exhibiting any signs of illness. This conclusion supports the observation that, in some regions where many horses are exposed and infected by the organism, it is estimated that just 9% of horses that are seropositive actually develop clinical disease (Mangnarelli 1998).



So what about the horses that do become ill? From Divers work, it is known that when a tick carrying *B. burgdorferi* takes a meal from a horse, the bacteria travel into the skin. The first response is usually inflammation – this is the characteristic '**bulls-eye'** rash surrounding the tick bite (rarely seen on horses due to their hair). The bacteria multiply and migrate through the body. They travel and reside in the skin, fascia (bands of fibrous connective tissue that bind muscles and organs), and peri-neural tissues (those surrounding nerves), and often wind up in the synovial membranes of joints where they hide from the host's immune system. Because *B. burgdorferi* can affect almost any tissue, the signs of illness can vary from case to case, mimicking osteoarthritis and other inflammatory conditions that are far more common in horses. **Clinical signs** most commonly attributed to Lyme disease in horses include generalized stiffness, swollen joints, chronic weight loss, eye inflammation, mild fever, hepatitis, laminitis, muscle tenderness, head tilt, difficulty in swallowing, facial paralysis, sensitive skin (hyperesthesia), lethargy, and hyper-reactivity. Lyme disease can affect any body system, but in horses the musculoskeletal system and the nervous system appear to be favorite target sites of *B. burgdorferi*, making shifting lameness (75%) and behavior changes (50%) the most common clinical signs. *Anaplasma phagocytophilum* infection can occur simultaneously. The clinical signs of *Anaplasma* infection are fever, low blood platelet counts, leg edema, lethargy, jaundice, and sometimes muscle wasting or incoordination.

However, remember that many horses show no obvious signs of infection, or signs are so subtle that attributing things such as mild changes in gait to Lyme disease can be extremely challenging (Divers, 2009). That makes the diagnosis of Equine Lyme disease difficult due to the vague, nonspecific, and variable clinical signs, and some of the limitations of available tests. The main tests available are an ELISA, a Western Blot, a polymerase chain reaction (PCR) for Borrelia DNA, a C-6 ELISA SNAP kit (SNAP 3DX) marketed for dogs, and most recently a sensitive Lyme Multiplex assay.

The **ELISA** was the first available test (see above, Magnarelli 1989). This highly sensitive, class-specific, and polyvalent ELISA was developed to detect IgM antibody to total immunoglobulin (IgG) for *B. burgdorferi* in equine serum. From Divers work (above), it was documented that antibodies are detectable at 5-6 weeks after tick attachment [range 3-10 weeks] and the ELISA rose to 200-

300 units after 3-4 months. A high ELISA (>300 units) is significant and may be consistent with the presence of clinical disease. But ELISA tests only provide information regarding antibody levels produced against either whole cell or specific antigens; they do not differentiate between active infection and previous exposure. The **Western Blot assay** tests for certain *B.burgdorferi* proteins on immunoblot. The Western blot is most useful in those cases with moderate ELISA titers (200-300) or in cases in which the horse may have received the commercially available canine vaccine. If the ELISA titer is greater than 300, the probability that the Western Blot will be positive is 99%. A positive Western Blot is correlated with the presence of specific proteins from the organism. Tests that detect the spirochete directly can be more conclusive of current infection in some situations. Detection of *B. burgdorferi* DNA in a synovial membrane of a painful joint via **PCR assay** indicates active infection. The sensitivity of PCR to a synovial membrane biopsy in the horse is currently unknown. More recently, an on-site **C-6 ELISA SNAP** test marketed for detection of *Borrelia* infection in dogs was evaluated for detection of *Borrelia* infection in horses. The test kit is an ELISA that uses a synthetic peptide (C6) derived from the IR6 region within the *Borrelia* membrane protein VisE. Studies with canine samples suggest that C-6 ELISA SNAP is particularly useful in Lyme-endemic areas because it can be conveniently and reliably used in the animal clinic to determine the infection status of a dog irrespective of its vaccine history. The advantages of this SNAP test are speed, convenience, and low costs. Compared to the ELISA alone, the Western Blot and C-6 ELISA SNAP test are capable of differentiating between antibodies produced in response to the vaccine versus antibodies produced after natural exposure to *B. burdgorferi*. Divers and colleagues reported in 2008 that this kit marketed for dogs is a reasonable stall-side test to screen horses for *Borrelia* antibodies.

In 2011, the Animal Health Diagnostic Center at Cornell University developed a new Multiplex assay for the diagnosis of Lyme disease in dogs and horses. Although antibody testing had generally worked well for serum samples, it often failed for cerebrospinal fluid (CSF) samples. The Multiplex was originally designed to aid in detecting relatively low antibody concentrations in cerebrospinal fluid to confirm cases of neuroborreliosis. The **Lyme Multiplex assay** uses different outer surface proteins (Osp) of *B. burgdorferi* as markers for early infection, chronic infection, or vaccination (Wagner 2011). The increased analytical sensitivity provides an improved diagnostic tool for the detection of antibodies to *B.burgdorferi* in biological samples, including CSF. The principle of this assay is based on the differential expression of these antigens on the surface of *B.burgdorferi*. The expression of antigens on the organism differs, depending on its environment. In the ticks gut, *B. burgdorferi* expresses **OspA** (Mygland 2010). The antigen **OspC** is expressed after the tick bites during transmission of the spirochete and initial infection of the host (Yang 2004). The **OspF** antigen becomes expressed in the host and antibodies remain detectable during chronic infection (Pal 2004). These differential expression patterns of the *B.burgdorferi* outer surface protein antigens result in a variation of the onset of antibody responses after infection of mammals. Antibodies to OspC develop early after infection and are known as early infection markers in human patients (Akin, 1999). Antibodies to OspF are induced later during infection and seem to be maintained throughout the chronic infection stage (Wieneke, 2000). Antibodies to OspA are markers for vaccination in dogs and horses.

Treatment for Lyme Infection

The two most commonly used drugs for treating Lyme disease in horses are tetracycline given intravenously and doxycycline given orally. **Tetracycline** has been used most commonly for horses in the acute phase of infection who exhibit stiffness, limb edema, and fever. It is possible that these clinical signs are a result of *A. phagocytophilum* infection from the tick-bite rather than infection with *B. burgdorferi*. Horses with what are often believed to be more typical signs of Lyme disease (chronic stiffness, lameness, and hyperesthesia) are most frequently treated with **doxycycline** (10 mg/kg po q 12h). Duration of treatment is often 1 month, but this duration of treatment is only empirical. Horses treated with doxycycline should be observed for a change in stool consistency, as diarrhea 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 non-specific anti-inflammatory response because doxycycline inhibits metalloproteinase activity. Tetracycline (6.6 mg/kg IV q 24h) given for 1 week before beginning doxycycline treatment may yield a more rapid clinical response. **Ceftiofur** (2-4 mg/kg IV or IM q 12h) has also been used in treatment of horses with Lyme disease.

Treatment studies (Divers, 2003) suggest that tetracycline given intavenous is superior to orally administered doxycycline or intramuscular ceftiofur. The superiority of tetracycline over doxycycline might be related to expected higher tissue concentrations with tetracycline, because doxycycline has been shown to have low bioavailability when given orally to horses (Bryat, 2000). Some of the ponies treated with doxycycline or ceftiofur had a significant decline in antibody level during treatment, but antibody level increased after treatment was discontinued. This suggests that the drugs may inhibit proliferation of *B. burgdorferi* but may not eradicate the organism.

Although antibody titers decreased in experimentally infected ponies given the preceding treatments, similar declines in titer in association with those treatments have been rare in naturally infected horses. The reasons for this may be 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), re-infection, or antibody mimicry. Because erythema migrans (the rash associated with early Lyme infection) goes unrecognized in horses, detecting early infection is nearly impossible. In the experimentally infected ponies, the organism was eliminated from the body only in ponies that maintained ELISA titers below 110 units for 2 months after treatment was completed. Other treatments can be considered supportive include chondroprotective agents, non-steroidal anti-inflammatory agents, metronidazole as a potential treatment for the poorly documented cyst state, and acupuncture. Acupuncture may be especially valuable for management of hyper-esthesia syndromes that are often poorly responsive to treatment with non-steroidal anti-inflammatory drugs.

In short, the diagnosis of Lyme disease in horses is based on being housed in an endemic area, compatible clinical signs, ruling out of other causes for these signs, and supportive laboratory data to confirm the diagnosis. Researchers estimate that only about 10% of horses exposed to *B. burgdorferi* develop illness, and when they do the signs can be subtle and easily mistaken for other problems. Treating Lyme disease using antibiotics is advocated and theoretically curative; however, it is not as easy as it sounds. Early treatment seems critical. If the infection is not cleared within the first 5 months, it can become chronic and much more difficult to eradicate. It is possible that some infected horses may be infected for a prolonged duration, even life. Long term administration of medication may be required to clear the infection, and this can become expensive. And even following a prolonged course of antibiotic therapy, infection can persist or re-infection can occur. This possibility is supported by the fact that horses with high ELISA values rarely have any decline in titer when monitored for months or even years. Appropriate antimicrobial treatment in human patients with borreliosis remains equally controversial. Several studies (Klempner, 2001) have failed to show a benefit of prolonged antimicrobial treatment, particularly in those human patients with chronic infections.

Prevention and Vaccination: Prevention of Lyme disease in endemic areas could involve preventing tick exposure or prolonged attachment, early antimicrobial treatment after *Ixodes* exposure, or vaccination. Aggressive tick avoidance is one of the best ways to protect horses from Lyme disease. Ticks typically crawl around on the body looking for a feeding site for hours or days before biting. Simply **brushing** a horse each day, especially after riding in the woods, may be enough to remove ticks that have not yet attached themselves to the skin. Studies have shown that once a tick begins feeding, it does not transmit *B. burgdorferi* into the host's skin for another 36-48 hours, so removing attached ticks daily will also go a long way toward preventing Lyme disease. Look under the throatlatch, around the ears, neck, and belly. They aren't that hard to find if you are looking for them. If ticks are found on the horse, they should be identified to determine whether they are of *Ixodes* sp which is the only species of tick in North America known to transmit *B. burgdorferi*. Keep in mind that the larger brown ticks that are easier to spot are not the ones that carry Lyme disease. The deer ticks (*Ixodes scapularis*) that carry the spirochete are much smaller, about the size of a pinhead.

Look for **products** that are labeled for use against ticks and apply them as instructed. Efficacy in the horse is unfortunately variable. Use repellant sprays or spot-on permethrin products when adult ticks are most prevalent in late summer, fall, and the early part of winter even after fly populations dwindle. Adult ticks continue to be active until temperatures drop below 35^{o}F. Bear in mind the possibility of infection with larval or nymphal stages earlier in the year also means using repellant then – in other words nearly year-round tick repellant use.

Ticks find hosts to feed on by climbing up onto tall grasses and brushy undergrowth, then catching a lift when large animals brush past them. If pasture grasses are high enough, the ticks are likely to get onto a horse's legs or face during grazing. Keeping turnout areas **mowed** to less than 4-5 inches will limit ticks opportunities to get onto horses. Try to avoid allowing horses to graze in wooded regions, as this is prime habitat for *Ixodes* ticks (leaf litter).

Try to **reduce habitat** for the wild animals that host ticks. Once *B. burgdorferi* get established in the local wildlife, the bacteria are there to stay, and surveys done in endemic areas have found the spirochete in dozens of species, from small rodents, to foxes, opossums, raccoons, and feral cats, as well as horses. The white-footed mouse is an important reservoir for *B. burgdorferi*. Taking steps to limit mouse populations around your barn and pastures can help limit your horse's exposure to the bacteria. Keep weedy areas mowed down and remove woodpiles that give them cover. In the barn, keep grains and feed stored in sealed, rodent-proof containers, and clean up any spills immediately. Also, keep the ground clean under any bird feeders. Natural predators such as blacksnakes will help keep the mouse populations down. People often have strong emotions about the presence or absence of deer on their properties. Again, deer play a major role in the life-cycle of the *Ixodes* ticks that transmit the disease.

Currently there is no vaccine approved for use against Lyme disease in the horse, but there is one for dogs. The canine vaccine has an unusual mechanism for preventing infection. The vaccine stimulates the production of antibodies that travel from the host into the body of the tick when it starts sucking blood, attacking and disabling the bacteria before they enter the host's body. Cornell University researchers have investigated the use of a similar vaccine in horses, and the evidence suggests that it may work the same way. In the study, the researchers did not see any rise in titers after ticks fed on vaccinated horses, so they speculate that the vaccine actually killed the bacteria before they left the tick. This indicates that it might be a very effective vaccine. As of this writing more research is underway and may be published by next year.

We will administer the vaccine at the owner's request, but it should be noted that this vaccine is not licensed for use in horses. The study protocol is most closely imitated by the administration of three - 1 ml doses of dog vaccine. The vaccine is administered alone (without other vaccines against other diseases at the same time), the second dose three weeks after the first, and the third dose three months after the first. In other words, the first year three doses are required, followed by boosters every six months. Recent studies have shown that antibody titers from vaccination begin to decrease after six months, so boosters every six months are needed to maintain adequate levels of protection.

12/12 sam

Bibiography

Akin E, 1999: The immunoglobulin (IgG) antibody response to OspA and OspB correlates with severe and prolonged Lyme arthritis and the IgG response to P35 correlates with mild and brief arthritis. *Infect Immunol* 67:173-181.

Anderson JF, 1980: Vertebrate host relationships and distribution of *Ixodid* ticks in Connecticut, USA. *Journal of Medical Entomology* 17:314-323.

Anderson JF, 1989: Epizootiology of Borrelia in *Ixodes* tick vectors and reservoir hosts. *Rev Infect Dis* 11 [suppl 6}:S1451-S1459.

Anderson JF, 1990: *Borrelia burgdorferi* and *Ixodes scapularis* prevalence in the greater Philadelphia area. *Journal of Infectious Disease* 161:811-812.

Berger B, 1989: Dermatological manifestation of Lyme disease. *Review Infectious Disease* 11:S1475-S1481.

Bernard WV, 1990: Serologic survey for *Borrelia burgdorferi* antibody in horse referred to a Mid-Atlantic Veterinary Teaching Hospital. *Jour Amer Vet Med A* 196: 8;415-417.

Bertrand MR, 1996: Microclimate-dependent survival of unfed adult *Ixodes scapularis* in nature: life cycle and study design implications. *Journal of Medical Entomology* 33:619-627.

Bryat JE, 2000: Study of intra-gastric administration of doxycycline: pharmacokinetics including body fluid, endometrial and minimum inhibitory concentrations. *Equine Vet J* 32:233-238.

Burgdorfer W, 1982: Lyme disease – a tick borne spirochetosis? *Science* 216:1317-1319.

CDC, 1995: Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 44:590-591.

Chang YF, 2000: Experimental infection of ponies with *B. burgdorferi* by exposure to *Ixodid* ticks. *Vet Pathol* 37:68-76.

Cohen ND, 1992: Seroprevalence of antibodies to *Borrelia burgdorferi* in a population of horses in central Texas. Jour Amer Vet Med A 201: 7; 111-112.

Divers, T 2009: Lyme Disease, p 57-61. In Robinson NE [ed.], Current Therapy in Equine Medicine. WB Saunders Publ, St Louis, Missouri.

Duffy DC, 1994: *Ixodes scapularis* deer tick mesocoale populations in natural areas: effects of deer, area and location. *Journal of Medical Entomology* 31:152-158.

Eng TR, 1988: Greater risk of *Borrelia burgdorferi* infection in dogs than in people. *J Infect Dis* 158:1410-1411.

Klempner MS, 2001: Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *N Engl J Med* 345:85-92.

Lane RS, 1989: Lyme disease in California: interrelationship of *Ixodes pacificus*, the western fence lizard and *Borrelia burgdorferi*. *J Med Entomol* 26:272-278.

Magnarelli LA, 1986: Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State and North Carolina. *Journal of Wildlife Disease* 22:178-188.

Magnarelli LA, 1989: Class-specific and polyvalent enzyme-linked immunosorbent assays for detection of antibodies to *Borrelia burgdorferi* in equids. *Jour Amer Vet Med A* 195:10; 1115-1119.
Magnarelli LA , 1998: Borreliosis in equids in the Northeastern United States. *Am H Vet Res* 49:359-362.
Marion T, 1998: Lyme disease in horses: serological and antibody testing differences. *Amer A Equ Pract* 50: 144.
Myland A, 2010: European Federation of Neurological Societies: EFNS guidelines on the diagnosis of and management of European Lyme neuroborreliosis. *Eur J Neurol* 17:8-16.
Miller GL, 1990: The epidemiology of Lyme disease in the United States, 1987-1988. *Laboratory Medicine* 21:285-289.
Pal U, 2004: OspC facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. *J Clin Invest* 113:220-230.
Piesman J, 1987: Duration of tick attachment and *Borrelia burgdorferi* transmission. *J Clin Microbiol* 25:557-558.
Schmid GP, 1985: The global distribution of Lyme disease. *Rev Infect Dis* 7:41-49.
Wagner B, 2011: A Fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *B. burgdorferi* outer surface proteins in canine serum. *Vet Immunol Immunopathol* 140:190-198.
Wieneke CA, 2000: Evaluation of whole-cell and OspC enzyme-linked immunosorbent assays for discrimination of early Lyme borreliosis from OspA vaccination. *J Clin Microbiol* 38:313-317.
Wilson ML, 1985: Seasonal activity of immature *Ixodes scapularis*. *Journal of Medical Entomology* 22:408-414.
Yang XF, 2004: Essential role for OspA/B in the life cycle of the Lyme disease spirochete. *J Exp Med* 199:641-648.