Department of Microbiology and Infectious Diseases

University of Veterinary Medicine

Borrelia burgdorferi infection in horses.

Literature review 1980-2019

By Linda Werme

Supervisor:

Dr. Fodor László

Budapest, Hungary

2019

Table of contents

Introduction

The aim is to make a thorough but easily understood literature review regarding *Borrelia burgdorferi* infection in horses. The review will focus on epidemiological measures, seroprevalence, methods of detecting an infection and will put special emphasis on the several challenges present when trying to finalize a definite diagnosis of Lyme disease in horses.

The following questions will be addressed;

What is the level of seropositivity of Borrelia burgdorferi in horses?

Which measures are used to confirm an infection of Borrelia burgdorferi?

Would seropositivity of Borrelia burgdorferi be directly related to the clinical signs presented in horses?

What are the main challenges in confirming Borrelia burgdorferi as a causative agent of the development of clinical signs in horses?

Material and methods

Literature search

An initial search using the databases accessed from the University library as well as google scholar gave access to several journals, articles and case studies. The initial months were spent reading material and sorting out which material that were out of interest in the making of this thesis. The reference lists of the found material were in some cases further studied to be able to read the text in its original context.

The University library has access to well established databases such as MEDLINE, Science direct and several others. The following research criteria were used:

(Horses) AND (Borrelia)

(Horses) AND (Lyme)

(Ixodes) AND (Lyme)

(Horses) AND (Vaccine)

Other sources

For information regarding the different laboratory methods used to confirm *B. burgdorferi* infection in horses the developing companies own homepages were used in some cases, for example the homepage of Idexx (idexx.com). Some information was also retrieved from official websites such as the website of European Centre for Disease Prevention and Control (ecdc.europa.eu). In these cases, the material was originally obtained with the help of Google's search tool.

All sources used in this thesis can be found in the reference list and are referred to with numbers in the text.

Result of literature review

History

The disease was first recognized 1975 in the city of Old Lyme in Connecticut, USA where children presented a juvenile form of rheumatoid arthritis[1]. The aetiological agent of Lyme disease was identified and named *Borrelia burgdorferi* after its discoverer Burgdorferer et al. in 1982 and later in 1985 antibodies where confirmed through serology in horses of New England, USA[1].

The breakthrough of in vitro cultivation of borreliae occurred in 1971 when Kelly described a liquid medium which successfully maintained the spirochete *Borrelia hermsii* in 80 continuous subpages where after the spirochete was still proven to be infective[2]. This medium was to be named after Kelly and after some adjustments it was later used for the isolation of Lyme disease spirochetes from the mid-gut of an infected *Ixodes dammini* tick[2]. After further update, this medium is known today as `BSK medium´; Barbour- Stoenner- Kelly medium, who all took part in its development.

Aetiology

Borrelia burgdorferi is a gram negative, vector borne spirochete bacteria[3]. The bacteria have 7-11 bipolar flagella in the periplasmic space which allows it to stay motile in viscous media[1]. *Borrelia burgdorferi* has the morphological structure of a spiral and belong to the phylum of spirochetes together with *Leptospira* and *Treponema*[1 3]. The B. burgdorferi sensu latu species complex consist of several genospecies, for example: *Borrelia burgdorferi sensu stricto, Borrelia afzelii* and *Borrelia garinii*[1]. The exact genospecies of the infectious agent might vary in different geographical areas[1].

The general transmission of the bacteria is via infected ticks of the *Ixodes* complex[1]. The bacteria itself seems to be less metabolically active in an empty gut of a tick and seems to increase its metabolic activity during a blood meal[1]. Both its motility and morphological structure seems to change as well following this event which is thought to be essential for its transmission and adaption to the new vertebral host[1]. *B. burgdorferi* has an outer cell membrane that consist of Outer surface proteins (Osp´s) which can be expressed differently depending on the surrounding environment of the bacteria; when the spirochete is located in an empty tick gut, mainly Osp A is expressed on the cell membrane; when the bacteria is present in an engorged tick, or is located inside the new vertebral host, mainly Osp C is expressed[1]. In later chapters we will investigate

the Osp´s roles as antigens during the vertebral hosts immune response and consequently how the study of the various antibodies produced might be of benefit when trying to confirm the actual stage of infection.

Epidemiology

Vectors

Vectors of the *Ixodes* complex get infected with the spirochete bacteria during a blood meal from a potential reservoir host and may inoculate the spirochete into a new vertebral host during its next blood meal[1]. Ticks generally have three life stages; larva, nymph and adult. Ticks of the Ixodes complex requires one blood meal for every life stage for its proper development[4]. After each life stage they will be dropped to the ground following a blood meal and will undergo metamorphosis until the next life stage is reached[4]. The spirochete bacteria have the potential to stay infective during the metamorphosis and the infection can be acquired in any stage of the lifecycle[4]. The larvae of *Ixodes ricinus* might also acquire the infection from a transovarial route but this route is not suggested to be efficient enough for any further infection to the vertebral host[1]. Adult female ticks will drop to the ground following a blood meal and will then find a protected area where she will deposit her eggs. The female dies after the egg laying[4]. The hatched larvae are six-legged and are in the need of humidity for its survival[4]. Males will mate several times with numerous females[4]. Adult ticks and nymphs that are yet to have a blood meal might overwinter if the climate allows it, this will create two breeding seasons of that area[4].

Figure 1. Tick attachment on a horse's skin when visiting summer pastures

While *Ixodes ricinus* is considered the main vector in Europe, *Ixodes scapularis* and *Ixodes persulcatus* are considered the main vectors of North America and Asia respectively[1]. *Borrelia burgdorferi* has also been isolated from *Ixodes uriae* ticks retrieved from sea birds which could potentially spread the pathogen further across continents via their migratory routes[1]. *Borrelia burgdorferi* has been found in the digestive tracts of some mosquitoes and haematophagous flies but this route is not suggested to be the main contributory route regarding the dissemination of the pathogen[1].

Reservoir hosts

A reservoir host carries the pathogen for a significant amount of time and function as possible source of infection for the vector in question. Birds, medium- sized and small mammals are considered the main reservoir hosts for *B. burgdorferi*[1]. The exact type of reservoir host may vary in different geographical areas. Research suggests that the rate of survival of the spirochete in the midgut of the tick could be influenced by the species and type of blood consumed by the tick[1]. While larvae and nymphs usually infest smaller sized animals, larger animals, such as horses, are considered the main targets of adult ticks and their role as natural reservoir hosts are therefore questionable[1].

Infection route

Tick larvae or nymphs acquire the infection during their blood meals from an infected reservoir host, most often a small- or medium-sized wild mammal, the infection remains transstadial and the next developmental stage of the tick will be able to disseminate and inoculate the pathogen further[1]. A transovarian route exists but is not considered to be efficient enough for a further spread into the vertebral host[1]. Research suggests that female ticks are more often infected than male ticks[1]. It is worth to mention that all developmental stages could potentially be infected while co-feeding in close relationship on the same host[1].

After acquiring the infection, the spirochete bacteria will be present primarily in the lumen of the tick's digestive tract[1]. After a sufficient blood meal and following the engorgement of the tick, the pathogen will disseminate further through haemolymph to reach the salivary glands. The pathogen is likely to get inoculated into the new host via the saliva or by regurgitation[1 4]. After being inoculated, the bacteria tend to stay near the attachment site of the tick for some time before it starts to migrate through connective tissue and other tissues of preference in the new host[1]. The spirochete bacteria would not survive outside a host, a tick bite is therefore essential for further transmission[5].

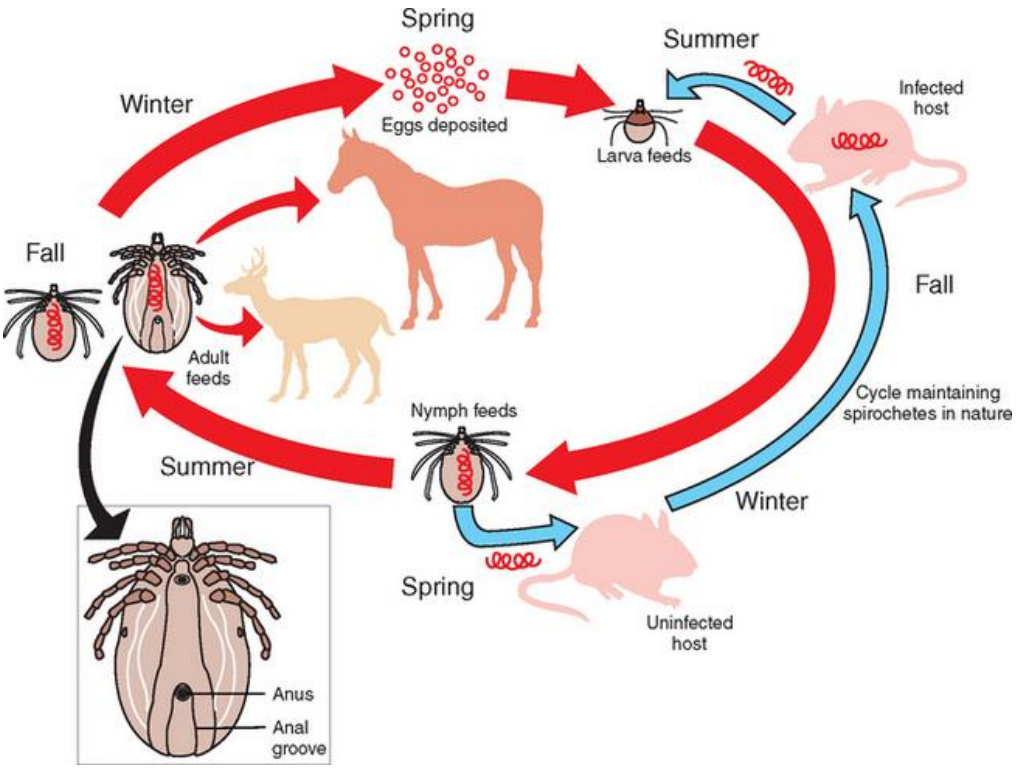

Figure 2. Two-year cycle of Borrelia burgdorferi in Horses.

The question has been raised whether direct contact of urine could serve as an additional route of infection, this after being able to observe viable *Borrelia burgdorferi* spirochetes in urine of clinically healthy horses in an endemic area[1]. Horses, as well as humans, are considered accidental and dead-end hosts for *B. burgdorferi*[6].

Prevalence of Borrelia burgdorferi in horses

The prevalence of *Borrelia burgdorferi* seropositive horses is suspected to be correlated to the prevalence of infected ticks in the area. Literature suggests that the seroprevalence of infected horses could be of higher incidence due to the possible increased time of exposure of the attaching tick on horses in comparison to humans that might observe it and remove it earlier[1]. An overall 30-40% of seropositivity is to be expected in horses of endemic areas, 5-10% are expected to develop clinical signs and the rest to remain asymptomatic[7]. A study performed in 2001 revealed an increase in number of infected ticks in Germany over a 10-year period[8]. Climate change or changes in wildlife management where mentioned as possible causes for such a change[8]. Similarly, the overall density of ticks in Denmark where estimated to have risen between the years of 1984 and 1998, warmer winters where mentioned as a possible attribute to this increase[7]. If assuming an increasing prevalence of infected ticks as well as a rise of the tick population itself, it is advisable to investigate the actual date of the different investigations when comparing the different estimations of seroprevalence of horses from different studies, the laboratory methods used must also be kept in mind when comparing results of different studies[7].

Even the time of the year for when the horses in different studies were tested might interfere with test results, it is suggested that the incidence of infection would be lower in horses during spring time than in late summer or autumn considering the fact that they then would have a longer exposure time to ticks and thereby risk of being infected[7].

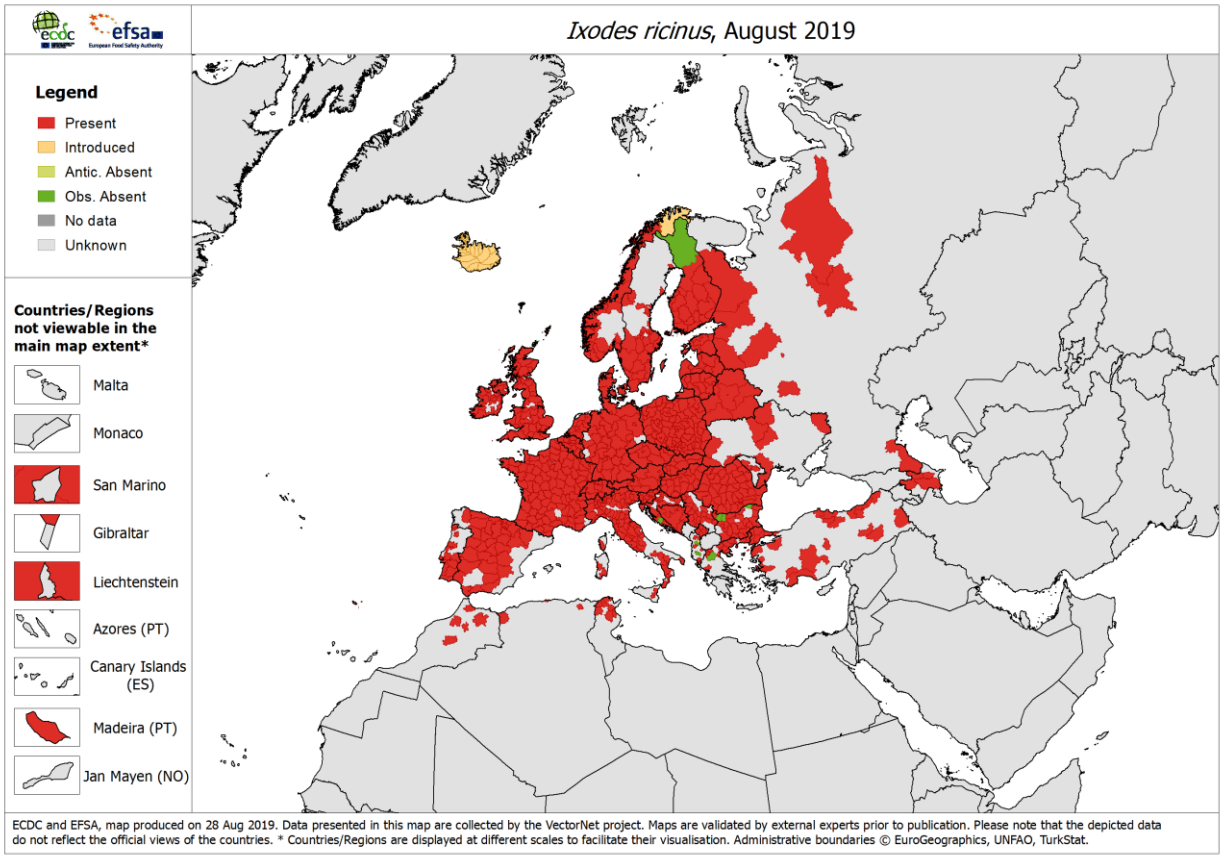

Figure 3. Presence of Ixodes ricinus, a vector of B. burgdorferi, Europe August 2019.

Out of 390 blood samples collected from Danish horses 29% where seropositive to *Borrelia burgdorferi.* The study covered several geographical areas in Denmark as well as horses of different ages, breeds and categories of use[7].

Estimated seroprevalence of *Borrelia sensu latu* in Horses in Europe[7]

Slovakia	47,8%	Germany	16,1%
Poland	25,6%	Denmark	29,0%
Sweden	16,8%		

Estimated seroprevalence of *Borrelia sensu latu* in the U.S[3]

North Eastern U. S	40 %

Pathogenesis

After the spirochete has been inoculated by the tick through the horse's skin, it has the ability to migrate and disseminate further through various tissues[3]. There seems to be certain predilection sites during the migration throughout the body such as: skin, fascia, perineural tissue and synovial membranes[1]. The bacteria tend to reside in places that are immunologically privileged sites, away from the hosts´ immune system, such as the eyes and nervous system[3]. When present in connective tissue, the bacteria could also be somewhat protected from being recognized by the immune system[1]. *Borrelia burgdorferi* contain a protein with haemolytic activity and a pore forming effect. This protein is suggested to be of benefit during the bacterial migration throughout various tissues in the body and could possibly help the bacteria to escape any macrophage lysosomal activity[1].

There are several genospecies of *Borrelia burgdorferi*. According to investigations in human medicine, these genospecies tend to have their own individual tissue tropism and manifest of clinical signs. It is suspected that this variation of genospecies and trophism is involved in the various clinical signs expressed in horses as well[1].

There is some indication that the bacteria might be involved in the development of Vitamin A deficiency. Vitamin A downregulates the synthesis of some cytokines that are important signal molecules and activators of the immune system. Any deficiency in Vitamin A and its downregulation would hence predispose the animal to a more extensive inflammatory response which could contribute to the development of an acute arthritic form of the disease[1].

Clinical signs

The following signs have been suggestive clinical signs of *Borrelia burgdorferi* in horses according to the literature: arthritis, lameness, muscle tenderness, anterior uveitis, encephalitis, abortion, foal mortality and low grade fever[1]. Additionally, pseudolymphoma has been describe at the site of the tick attachment[5]. More specific signs of neuroborreliosis could be: atrophy of spinal muscles, facial paresis, dysphagia, laryngeal dysfunction, tactile hyperesthesia, ataxia and behavioural changes[6 5]. The lameness is described to be episodic in most cases but could also be of chronic nature[4]. The clinical presentation of Lyme disease could possibly range from asymptomatic to a more systemic type of disease in horses and is often described as being a multi-systemic disorder[1]. Some ponies that were experimentally infected, presented skin lesions near the attachment site as their only obvious clinical sign[1]. Erythema migrans is assumed to be easier to identify in humans due to the morphological differences of skin and fur[1]. In human

medicine, disease of internal organs such as the heart, liver and kidney has been reported; this has not yet been reported in horses[4].

Figure 4. Recurrent fibrinous anterior uveitis.

Differential diagnosis

It is advisable that the clinician investigate if the patient is in an endemic area or an area with the presence of possible vectors before suspecting *B. burgdorferi* as a causative agent. The suggested clinical presentation of *Borrelia burgdorferi* in horses might mimic the clinical signs of several other diseases for example *Analplasma phagocytophilum*, influenza, infectious mononucleosis, chronic fatigue syndrome, multiple sclerosis, arthritis and other musculoskeletal problems[4]. Leptospira is more commonly associated with uveitis in horses but have slightly different appearance on smears and stains poorly with Wright´s stain in comparison to Borrelia[3]. According to the literature, there is a risk of serological cross reactivity between *Borrelia parkeri* and *Borrelia burgdorferi*[1].

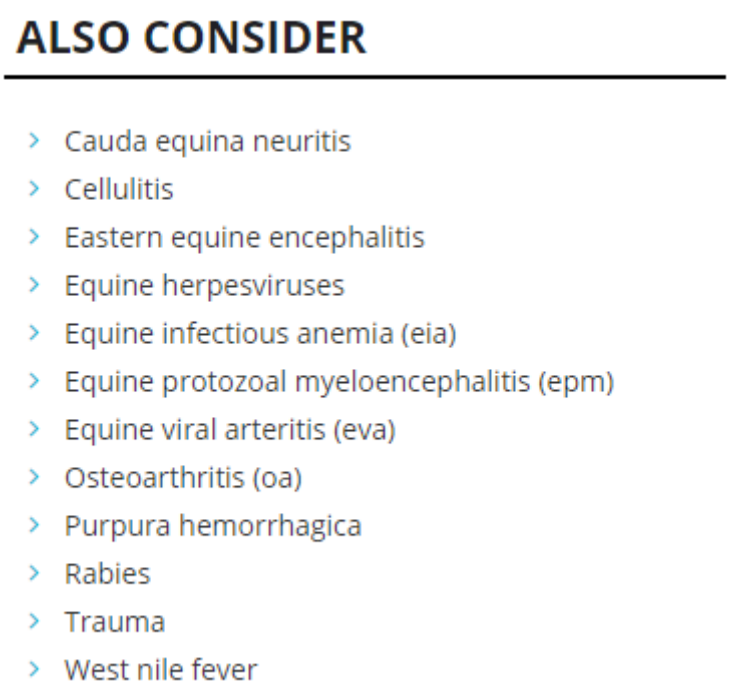

Figure 5. Potential differential diagnosis

Laboratory measures

The Multiplex assay

The Multiplex assay is able to detect the antibodies produced from various stages of infection which may give an indication of whether the horse is subject to a recent or more chronic type of infection[5]. Up to recently an ELISA test, followed by Western blot or Immunofluorescence assay (IFA) for confirmation, has been common methods for confirming *B. burgdorferi* infection in horses. The Multiplex assay, developed by Cornell University, is more specific and is also able to detect antibodies earlier than some other tests[6]. Some previously used methods used one single antigen, such as C6, in their detection of antibodies, while the Multiplex assay uses three *B. burgdorferi* antigens, namely Outer surface proteins A, C and F[6].

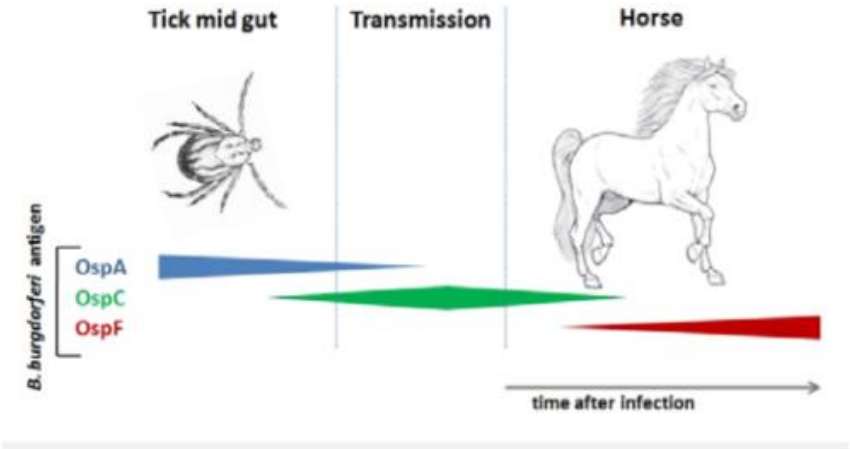

Figure 6. B. burgdorferi antigen expression

The type of outer surface protein expressed on the bacterial cell membrane is influenced by the surrounding environment[6]. When the bacteria are present in the tick's midgut the Osp A antigen is expressed[6]. During the blood meal and when leaving the ticks midgut, Osp C is expressed[6]. Osp C will also be maintained during early infection[6]. After being present in the horse's body for a while, Osp C disappears, and Osp F is expressed during the more chronic form of the infection[6]. The horse's immune system will produce different antibodies depending on which of the Osp antigens that are expressed at that time. The Multiplex assay is able to detect the type of antibodies produced and can thereby create a timeframe of the infection.

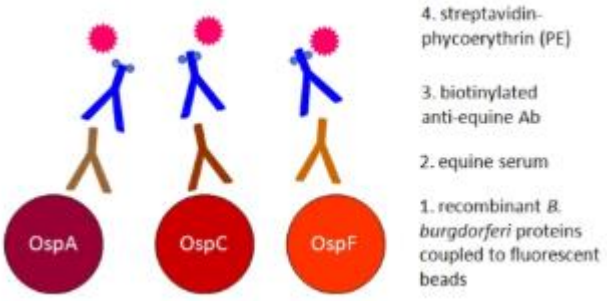

Figure 7. Multiplex assay

When performing the Multiplex assay, the Outer surface protein (Osp) specific antibodies of the horse sera will be bound to OspA, OspC or OspF multiplex beads and will be detected with the help of a fluorescent conjugate. The value of the Multiplex assay will be expressed as median fluorescent intensities (MFI)[6]. The assay is quantitative and provide information regarding all three antibody types from one single blood sample. The type of antibodies present may give an indication of the stage of infection. Osp A is generally not detected in horses that were naturally infected but may be present in horses with previous vaccination history[6]. As previously mentioned, Osp C indicates an early infection and Osp F a more chronic infection[6]. Osp C can be

detected around three weeks post infection and is undetectable by fourth to fifth months post infection[6]. Osp F is detectable from week five to eight post infection and will then stay elevated. If only Osp F antibodies were detected and not Osp C, the horse is considered to have been infected for at least five months[6].

The Multiplex assay ability to establish a time frame of the ongoing infection might be of interest in practice, since treatment of an early infection is said to be more successful than treatment of a chronic infection[6]. A rapid decline of antibodies is considered a more successful treatment, since antibacterial treatment will decrease the presence of bacteria and consequently the antibody production, which means that the quantitative properties of the assay can be used for post treatment evaluation[6]. In addition, the method may be used for evaluation of vaccination status post vaccination via its ability to differentiate between a vaccination and an actual infection with the help of an Osp A antigen marker.

Table 1 (*continued*)

Test	Laboratory	Antibody Targets	Interpretation	Pros	Cons
Equine multiplex assay (Serum and CSF)— not synovial fluid	AHDC, Cornell	3 recombinant antigens: OspA, OspC, and OspF	• Quantitative; results expressed as MFIs • Anti-OspA antibodies–vaccination and/or infection; correlate to antibodies detecting the 31-kDa band on WB • Anti-OspC antibodies–early infection; correlate to antibodies detecting approximate 22-kDa band on WB • Anti-OspF antibodies–chronic infection; correlate to antibodies detecting 29-kDa band on WB	• Detection of low-level antibody (pg/mL) • Potentially elucidates infection stage and vaccination status • Quantitative • Increasing levels may indicate active infection	• False-negative results might occur due to genetic variation in OspC • Experimental infection studies in horses confirming antibody kinetics have not been published • Dilutional linearity not reported
SNAP 4Dx (Serum, plasma, or anticoagulated whole blood)	IDEXX	• Synthetic peptide (C_6) that mimics specific Bb antigen (IR6, a highly conserved protein of VlsE)	• Qualitative; color development visually (subjectively) interpreted • Positive results indicate natural exposure, not vaccination • Anti-C_6 antibodies correlate to antibodies that detect the 39-kDa band on WB	• Inexpensive, easy to perform in clinic • Rapid results • Good agreement with multiplex OspF and WB • Vaccination status unlikely to affect results	• Subjective interpretation • Nonquantitative results

Figure 8. Description of the Equine multiplex assay and the SNAP4Dx methods for B. burgdorferi confirmation

ELISA SNAP®4DX

This ELISA test was initially developed for canine patients and is able to detect Heartworm, Lyme disease, Erlichia and Analplasma. More specifically it detects *B. burgdorferi* antibodies in serum using C6 peptide technology. The sensitivity of the test is according to Idexx laboratory 94,1 %[9]. Following a positive result of the ELISA snap test, it is advised to do a follow up test for its confirmation. Idexx laboratories suggest using the Lyme quant C6 quantitative test. This test can be used to confirm an active infection and if treatment might be indicated; the C6 antibody levels correlate to the organism load and viability in the body[10]. If the levels of

antibodies are high; the patient is likely to benefit from treatment[10]. It is advised to perform a second Lyme quant C6 test six months post antibiotic treatment to again assess the level of antibodies and response to treatment. As previously mentioned, the information provided by Idexx concerns canine patients.

Even though the ELISA SNAP®4DX was originally developed for canine patients, it has been proven to work in horses as well. Some research has shown that antibodies might be detectable for a shorter period post infection when using the ELISA snap in comparison to other ELISA methods[7].

Immunofluorescent antibody (IFA) testing

This method detects antibodies in serum and synovial fluid and was the first serological method used to detect Lyme disease[1 2]. The spirochetes are initially fixed to a microscope glass slide. The slide then gets incubated with antibodies from the serum as well as some other antibodies that are labelled with fluorescein isothiocyanate. The binding is observed with a fluorescence microscope which indirectly confirms the presence of *B. burgdorferi* antibodies. This test lacks the exact quantification of antibodies and is not highly specific when it comes to detecting only *B. burgdorferi* since the serum might consist of other adherent antibodies if the patient has been exposed to other bacteria that share the same antigen[2].

PCR Polymerase chain reaction

By means of PCR both live and dead pathogen, intact and fragmented spirochete DNA could be detected. The method is considered to have high sensitivity but provide no indication of whether the infection is active or passive[1]. PCR can according to the literature, use serum, synovial membrane, synovial fluid, cerebrospinal fluid and ocular fluid as test material[5 7]. It is possible to send in ticks to investigate if the tick is infected[4].

Western blot

The spirochetes are initially lysed using chemical methods and heat. This is followed by a step of separation of the various molecular components using sodium dodecyl sulphate-polyacrylamide gel electrophoresis[2]. The separated components function as antigens and get transferred onto a membrane where they bind to specific anti *B. burgdorferi* antibodies of the serum incubated with the membrane. These bindings are then observed using for example autoradiography. A negative sample is easy to analyze but the interpretation of an ensuring positive result is more difficult due to variations in reagents and methods used which creates interlaboratory variations[2].

Culturing

Many laboratories use BSK II medium to isolate and maintain *B. burgdorferi*, the spirochete is most successfully cultured in neutral pH, in a microaerophile environment with a temperature ranging between 30-37 degrees[2]. The spirochete is monitored using dark field light microscopy. Skin biopsies (from early erythema migrans lesions) and samples from various post-mortem lesions (for eg. blood, synovial fluid and eye fluid) are examples of test material that has been obtained during studies and investigations in the try of establishing cultures to identify the spirochete bacteria[1].

Direct methods

The spirochete bacteria have been observed in brain tissue using Direct immunofluorescence, in a histological section of the eye using Krajian silver staining and viable spirochetes has been observed in urine of clinically healthy horses in endemic areas[1].

Pathology and histopathology

Skin lesions, similar to erythema chronicum migrans in humans, have been observed in ponies near the attachment site of the tick; perineural and perivascular lymphohistiocytic aggregates were observed both subcutaneously and in deeper dermal layers[1]. Similar reactions were also observed in fascia and perisynovial membranes, the prescapular lymph nodes presented marked lymphoid hyperplasia[1]. The pathogen was also found in the heart, pericardium, bladder, kidney and in the meninges of the brain[1].

Results of laboratory investigations of interest

Case report of Encephalitis and its relation to Borreliosis

Following a case where an equine patient was presented with encephalitis, a brain sample confirmed the presence of spirochetes using direct immunofluorescence. Neither microscopy nor serology were used as additional resources in this case[1].

Case report of Arthritis and panuveitis and its relation to Borreliosis

A pony was presented with the clinical signs of arthritis and panuveitis. Microscopy confirmed the presence of a spirochete bacterium in a silver stained section of the eye and antibodies were

detected in serum and synovial fluid with immunofluorescence. Silver stains are generally difficult to interpret, and the culture performed was negative in this case[1].

Case report regarding uveitis and its relation to Borreliosis

A case report performed at Cornell university investigated two horses with the clinical sign of uveitis. The aim of the report was to isolate the spirochete bacteria within ocular fluid and to confirm the findings with the help of PCR. The first case involved a 16-year-old Icelandic horse that had uveitis along with alopecia around the eyes and mouth. The horse was hypersensitive and had a generalized pruritus. The uveitis was bilateral and the horse had turned blind. The clinical signs had been present for two months prior to this investigation. All values of the general clinical examination, such as heart rate and body temperature, where within normal range. Topical eye treatment and systemic NSAIDs had been previously administered but the ocular problems were described as reoccurring as soon as the treatment ended[3].

The patient had previously been tested negative for *leptospirosis.* Previous ELISA test were inconclusive and the Western blot negative when testing for *B. burgdorferi*. These tests were repeated with negative results at the University. Regular blood samples revealed an elevation of leukocytes, thrombocytes and fibrinogen. Several reflexes were absent during the ophthalmic examination and the eyes were subject to various pathological changes such as for example corneal wrinkling, yellow-green fibrinous aqueous humour and other changes related to chronic uveitis[3].

The horse was euthanized due to suffering from the pruritus, hypersensitivity and to some of the other clinical signs. Samples were taken from both eyes for cytology and a complete necropsy was performed. The aqueous and vitreous humour expressed high cellularity with an inflammatory infiltrate, additionally spirochete-like bacteria was found in the vitreous humour which were consistent with *B. burgdorferi* morphology. A primer set of the Osp A gene was used for PCR and confirmed *B. burgdorferi* in the vitreous humour of both eyes. Samples were negative when tested for *Leptospirosis.* The Multiplex assay was used as an additional resource to measure antibodies of the vitreous humour and confirmed the presence of Osp A and Osp F antibodies[3].

Even though *B. burgdorferi* was confirmed to be present in the vitreous humour of the eyes, other diseases and causes to the initiation of clinical signs could not be excluded. The presence of spirochete bacteria within the neutrophils of the cytospin smear, see below figure, though strongly suggests that the bacteria were either an initial cause or at least a contributing factor in the development of this ocular inflammation[3].

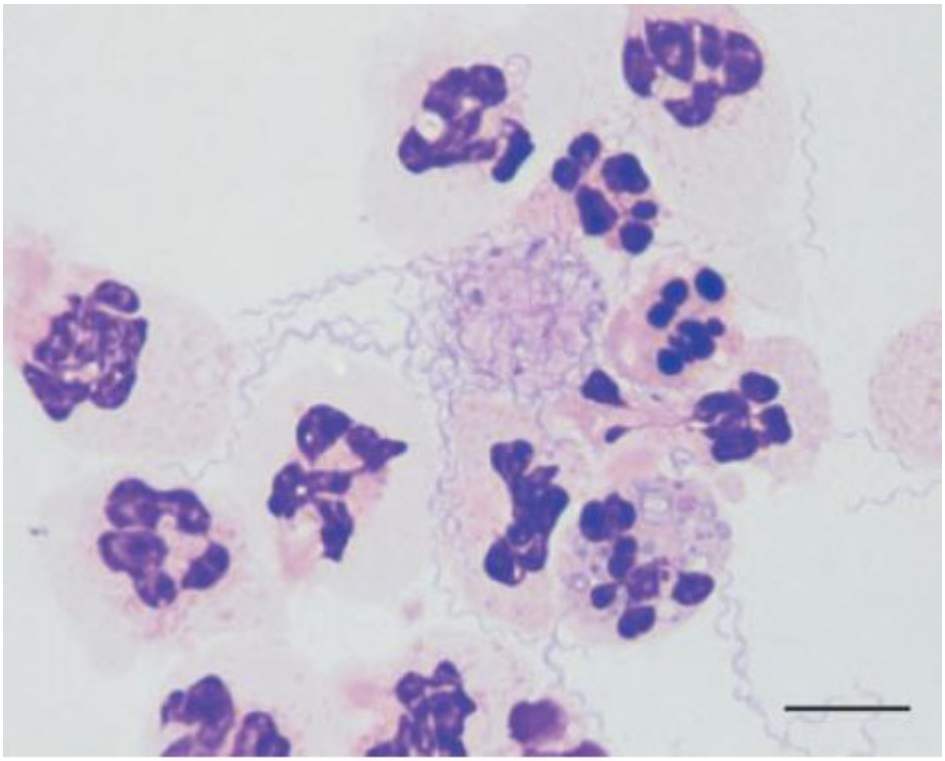

Figure 9. Vitreous humour. Neutrophils and spirochete bacteria. Cytospin smear. Wright´s x500. Bar = 10µm

The second case presented similar type of uveitis and involved a 13-year-old thoroughbred mare that appeared blind on clinical examination. PCR, smears of Modified silver stain and the Multiplex assay were used to identify the presence of B. burgdorferi of the different samples. The results were as follows[3]:

Left eye (**PCR**, OspA gene):	Right eye (**PCR**, OspA gene):
Aqueous humour: **positive**	Aqueous humor: negative
Vitreous humour: **positive**	Vitreous humor: no sample available
Serum: Negative	Serum: Negative

Left eye (Smear, **Modified Silver stain**)	Right eye (Smear, **Modified silver stain**)
Aqueous humour: negative	Aqueous humor: negative
Vitreous humour: **positive**	Vitreous humor: no sample available

Multiplex assay antibody detection

Osp A antibodies	Osp F	Osp C
Serum: **positive**	Serum: negative	Serum: Negative
Ocular fluids: **positive**	Ocular fluids: **positive**	Right aqueous humour: **positive**

The horse was euthanized due to it being unfit to participate in normal riding and training. On necropsy, an inflammatory response where present in multiple organ systems such as the skin, muscles, nervous tissue and other organs. Histological sections from samples originating from these sites revealed no presence of spirochete bacteria. High OspA antibodies were detected in all samples of ocular fluids, this could indicate the presence of an autoimmune response that possibly contributed to the pathogenesis. The possible relationship between Osp A antibodies and auto immunity has as to yet only been investigated in studies of humans with chronic Lyme disease. The presence of Osp A antibodies could also be related to a vaccination regime. The serum presented a lower titre of Osp A antibodies than the ocular fluids. This might support the idea of a more local production of antibodies in the eyes. The findings of Osp F antibodies usually indicate a chronic infection[3].

The bacteria were only identified in the vitreous humour of the eyes which further supports a more local inflammation of the eyes. Taking samples of vitreous humour with the help of needle aspiration is described to be more challenging than collecting aspirate from the aqueous humour. The sampling of vitreous humour often fails, even during post-mortem examinations. The samples of aqueous humour revealed no presence of the bacteria, the real use of this sampling method is therefore questionable[3].

Leptospirosis was part of the list of possible differential diagnosis of both horses but was ruled out due to the various tests performed. A previous European study did not find any significant correlation between clinical presentations of Equine recurrent uveitis and seropositivity to *B. burgdorferi* but there have been two documented cases prior to this investigation that suspected a possible relationship between uveitis and borreliosis in horses. This study is the only study known by the authors, that uses cytology to confirm *B. burgdorferi* in cases of recurrent equine uveitis. The authors find it challenging to finalize *B. burgdorferi* as a causative agent for uveitis in horses but still think that it should be part of a differential diagnosis, especially in endemic areas. Considering the risk of false negative serological results in horses with uveitis, the authors suggests that it could be worthwhile to test ocular fluids for the presence of antibodies as well as performing PCR if there is a suspicion of *B. burgdorferi* infection[3].

A Swedish case study of seropositivity in horses and the possible relationship to clinical signs

According to a Swedish study horses that lived in the southern and middle part of Sweden had higher incidence of *B. burgdorferi* infection than horses that lived in the colder northern part of Sweden. Mares were more often infected, as well as the age groups 11-15-year-old and 0-2-year-old horses respectively. Breeding mares had a higher incidence of being seropositive than for example racehorses. The seroprevalence was the highest during July- September and in horses

that had longer pasture exposure. The result suggests that the categories of horses that normally spend more time out on pastures also have a higher rate of infection. As previously mentioned, geographical location and climate seem to be of importance as well. Both horses with clinical signs and clinically healthy horses that were presented to veterinary clinics participated in the study. It could still be mentioned that some categories of horses might be overrepresented in this study; horses that compete might for example visit a veterinarian more often than for example a breeding stallion[11].

The main aim of the study was to investigate the possible relationship of seropositivity to some of the suggested clinical signs of *B. burgdorferi* such as lameness, fatigue, arthritis, hoof problems and unwillingness to be ridden. The study revealed that seropositivity to *B. burgdorferi* did not seem to be directly related to any of the clinical signs mentioned, apart from coffin-joint arthritis where a possible relation was found. The exact explanation and value of this result could not be fully clarified in this study. According to the authors, Borreliosis was often a presumed diagnose if the horse presented any of the clinical signs mentioned and in addition proved to be seropositive to *B. burgdorferi*. The authors hoped that this approach would change slightly after this study, since not many of the clinical signs could be directly related to the serological status of *B. burgdorferi*. It was found during other previous studies that some of the seropositive horses expressed clinical signs such as swollen joints, stiffness, laminitis, abortions and fever. At the same time, horses that did not express any clinical signs also proved to have antibody titres for *B. burgdorferi*. Another study concluded that early pregnancy failure was more common in horses with positive serology than in mares with negative serology[11].

Experimentally induced infection

Seven specific pathogen ponies were exposed to the pathogen via infected ticks, none of these ponies expressed any clinical signs other than mild skin lesions. The pathogen was detected with the help of PCR in some tissue samples and the ponies expressed high titres of *B. burgdorferi* antibodies in their blood. The bacteria were successfully cultured from skin biopsies that originated from the attachment site. They were also able to isolate the bacteria from post-mortem samples, which could indicate the possibility of *B. burgdorferi* to cause clinically healthy horses to be persistently infected[1].

Koch´s postulates

Koch´s postulates defines the relationship between successful isolation of bacteria and the presence of an actual disease. The following four criteria has to be met; the bacteria must be present in every case of the disease; the bacteria must be isolated from the diseased host and grow in pure culture; the specific disease must be reproduced when a pure culture of the bacteria

is inoculated into a healthy susceptible host and finally; the bacteria must be recoverable from an experimentally infected host. All criteria were met regarding *Borrelia burgdorferi* in a study performed by Chang et al, but the research lacked to prove any direct relationship to the clinical signs observed[5].

Borrelia burgdorferi as a conclusive diagnosis

In the attempt of finalizing *B. burgdorferi* as the actual cause of disease the following questioning scheme are often used when a patient is being presented to a clinic[6];

1. Does the horse live in an endemic area and could the Ixodes vector be present in the horses living environment?
2. Has there been any ticks found on the actual horse?
3. Could the clinical signs be compatible with signs of *B. burgdorferi*?
4. Have other causes of the clinical signs been ruled out?
5. Is the horse seropositive to *B. burgdorferi*?

If these criteria are interpreted as being part of a possible case of Borreliosis, the veterinarian might choose to go on with the recommended treatment.

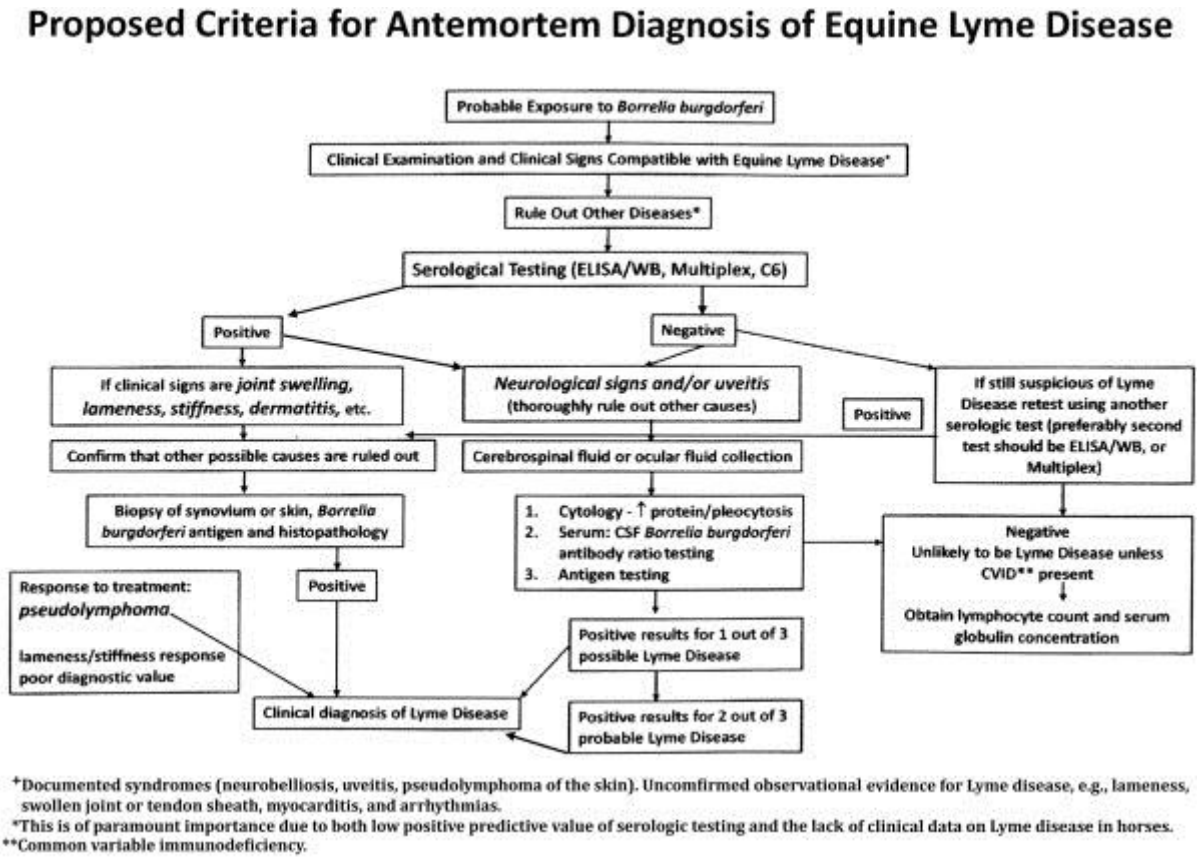

Figure 10. Guideline for antemortem diagnosis of Lyme disease in horses.

There is a wide controversy in the attempt of finalizing *Borrelia burgdorferi* in horses for many reasons; the wide list of possible differential diagnoses makes a final conclusive diagnosis more difficult as well as the fact that both clinically healthy and horses with clinical signs might be seropositive[1]. On the contrary, some infected patients might also be seronegative[2]. Possible co-infections with other pathogens, for example *Analplasma phagocytophylum*, that shares both vector and similarities in the clinical presentation, could either just contribute or in fact, be the whole reason to the clinical signs[1]. Studies revealed that 4,5% and 11% of the participating horses of a Swedish and Danish study respectively, where seropositive to both *B. burgdorferi* and *A. phagocytophylum* and that this relationship was of significance[7].

Bacterial culturing and isolation of the bacteria has been successful in both clinically healthy and clinically diseased horses and could not be relied upon for a definite diagnosis[1]. Studies have shown that horses with clinical signs might have higher antibody titre than subclinical horses. Even horses without clinical signs may present antibodies. A study showed that the antibodies rose for around 3-4 months, then became constant and detectable for about 9 months post infection[7].

Seropositivity is not always reliable, in some cases where the spirochete bacteria have been confirmed by the means of other methods, serology has still been negative. It takes some weeks before antibodies are expressed and detected in a serological sample and some testing has still proven to be negative even in prolonged cases[3]. Serological tests of horses with for example uveitis has proven to be negative while the spirochete has been confirmed using other laboratory methods[3]. The recently developed Multiplex assay is able to identify antibodies originating from various stages of the infection and could possible help in the clarification of the relationship between the possible various clinical signs and their relation to an active or chronic infection.

Treatment

A study proved tetracycline to be the most effective treatment providing 6,6 mg/kg IV q 12h for three weeks; this was the only treatment that successfully eliminated the pathogen of the infected host, using PCR and negative cultures as methods of reference[1]. Literature also suggests other options of treatment such as Doxycycline per os, Minocycline per os or intramuscular treatment with Ceftiofur[5]. It is important to realize that there might be differences in antimicrobial bioavailability in different species; Doxycycline for example, is considered to have low bioavailability when being administered to horses per os[1]. The use of antibiotics should only be administered to horses with clinical signs and that tested positive for *Borrelia burgdorferi*.

Additionally, antibiotics such as ceftiofur and metronidazole should not be part of the first line of defence considering the risk of development of drug resistance[5]. NSAIDs could be indicated if the horse experience pain or neurological problems[5].

Table 2
Antibiotic recommendations for *Borrelia burgdorferi* sensu lato infections

Drug	Dosage	Comments	Minimum Inhibitory Concentration for *Borrelia burgdorferi* sensu stricto or (*Borrelia burgdorferi* sensu lato Isolate)(Reference 8 Has All Authors Identified)	Quick Reference Disease Indications (Subjective)
Cefotaxime	25 mg/kg IV q6h Standard 500-kg horse: 12.5 g q6h NB 500-kg horse: 25 g q6h	High dosages (eg, 50 mg/kg IV q6h) for equine NB Excellent tissue penetration so efficacious in ocular and joint infections Suitable for distal limb perfusion (synovitis and osteoarthritis) Higher doses may lead to adverse effects (eg, colitis)	≤0.125 μg/mL 0.01–1 μg/mL	CNS Joint infections, intra-articular and distal limb perfusion Ocular infections
Ceftiofur	Ceftiofur sodium (Naxcel) 2.2 mg/kg IV q12h 500-kg horse: 1.1 g q12h Ceftiofur crystalline free acid (CFA, Exceede) 6.6 mg/kg IM days 1 and 4, then q7d 500-kg horse: 3.3 g per Rx	Research (REF 3a) indicates less "relapse" (increased Osp levels) post-treatment when compared with doxycycline. Excellent tissue penetration so efficacious in ocular and joint infections Long intervals between injections may increase patient comfort and compliance (Exceede) Sodium salt (Naxcel) suitable for distal limb perfusion (synovitis and osteoarthritis) Higher doses may lead to adverse effects (eg, colitis)	<0.04–0.08 μg/mL	CNS infections Ocular infections Joint infections, intra-articular and distal limb perfusion

(*continued on next page*)

Figure 11. Antibiotic recommendations.

Dexamethasone has been used with various outcomes in humans and should only be of consideration if the horse present uveitis or severe neurological signs[5]. Very few side effects have been reported following the usage of oral doxycycline, oral minocycline or tetracycline iv. for treatment; the most common side effect reported was soft manure[6]. The success of treatment can be evaluated by the means of a pre- and post- treatment blood sample where the presence of antibodies before and after the treatment. If the antibody value has decreased with at least 50 % between these two samples, during a set time frame, the treatment regime is considered successful[6]. The antibody titre will continue to decrease after a successful treatment and will become negative in the horse is not subject to any re-infection[6].

As previously mentioned, there is a risk of co-infections, where a different pathogen could serve as the possible causative agent of the observed clinical signs. It is possible that the treatment of choice would target this other pathogen as well and that any improvement would be due to the antibacterial effect acting on that pathogen instead.

Table 2
(*continued*)

Drug	Dosage	Comments	Minimum Inhibitory Concentration for *Borrelia burgdorferi* sensu stricto or (*Borrelia burgdorferi* sensu lato Isolate)(Reference 8 Has All Authors Identified)	Quick Reference Disease Indications (Subjective)
Doxycycline	10 mg/kg PO q12h 500-kg horse, 100-mg tablets: 50 tablets q12h	The most common antibiotic used for equine borreliosis Peak synovial fluid concentrations > than serum concentrations Use in suspected *B burgdorferi* synovitis is recommended Can be used in Borrelia-induced uveitis or NB, but mocular and CNS concentrations vs doxycycline @ 10 mg/kg ([see text]), has the additional benefit of intra-articular MMP inhibition/anti-inflammation	0.125–0.25 μg/mL ≤0.125–0.25 μg/mL	Joint and musculoskeletal disease
Metronidazole	15–25 mg/kg PO q6–8h 500-kg horse at 20 mg/kg = 10 g q6–q8h	Considered ineffective against motile spirochetes and effective vs resistant, round body ("stationary" or cystic) forms in vitro Used in concert with one of the other antibiotic choices on chart, which is effective for motile spirochetes. Oral administration may lead to patient inappetence IV formulation is expensive in adult horses	0.06–32 μg/mL 0.25–0.50 μg/mL	Chronic (resistant) Lyme disease in concert with an antibiotic effective vs motile spirochetes
Minocycline	4 mg/kg PO q12h 500-kg horse/ 100-mg tablets, 20 tablets q12h	Superior aqueous humor and CSF penetration (vs doxycycline) but tissue levels in vivo are questionable (may be below MIC for target organism) ±Recommended for ophthalmic and CNS cases of borreliosis Synovial concentrations are inferior to doxycycline, therefore a secondary choice for synovitis/ osteoarthritis	0.03–1 μg/mL 0.4–0.8 μg/mL	CNS and ocular infection

Figure 12. Antibiotic recommendations.

Prognosis

Early treatment with proper antibiotics is suggested but the prognosis itself is difficult to estimate due to the challenge of making a definite diagnosis. The prognosis depends on several factors such as; which organ system that is involved, the severity and duration of disease[1]. The prognosis is considered poor if the horse present signs of neuroborreliosis; there is only one documented case where the treatment was successful[5].

Prevention

The main method of prevention is to manually remove the tick; research of human medicine suggest that it takes several hours before the attached tick will inoculate and spread the bacteria into the new host. The recommendation is thereby to remove the tick as soon as possible and similar advice could be given to horse owners[1]. While grooming the horse, have a close look at the jaw area, ears, mane and inside of the legs. The tick should be removed with the help of tweezers, using the thumb and finger should be avoided because of the risk of squeezing tick material into the skin while trying to remove it[4]. An antiseptic ointment could be used at the site following tick removal.

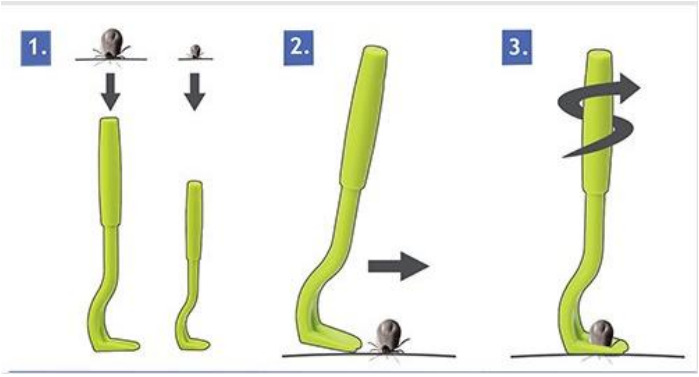

Figure 13. O´tom tick twister and suggested tick removal.

Topical treatments and tick repellents are other methods of prevention; the acaricide permethrin is suggested to have good effect but care must be taken when choosing topical treatment, not all are approved for horses[1.] Ticks are active during a longer period than many of the summer flies; depending on the local climate and weather conditions, tick repellents can be used for prevention until the outside temperature drops below freezing.

Tall grass is often a way for the ticks to reach bypassing potential hosts; avoiding these areas would be beneficial in prevention but is not always applicable to horses on pastures. Ticks usually lay their eggs in piles of leaves; these could potentially also be looked after and somewhat reduced. Rodents and pet animals on the farm could be infested by ticks; by keeping the rodent population under control and applying spot-on or similar on other pets, the overall tick population and risk of infection could be limited.

There is currently no approved vaccine for horses on the market but research suggest that vaccination may be beneficial for prevention in horses as well[12]. The effect of using commercial vaccines developed for dogs has not yet been proven[1.] A study performed by Cornell University revealed that antibodies were produced after administration of the canine vaccine but that the respond varied greatly between individuals and the effect seemed to be short-lived[13]. The low incidence of Lyme disease in horses together with the controversy in finalizing a definite diagnosis, are both probable contributing factors for making the development of vaccines less attractive[1]. The possibility of using vaccines for horses have been investigated in study performed by Chang et al. The study suggested that high concentrations of Osp A antibodies in the host plasma could possibly prevent the multiplication of the spirochete bacteria inside the tick gut[1].

Table 3
Canine Lyme (*Borrelia burgdorferi* s.s) vaccinations currently approved, for dogs, on the US market

Brand Name	Recombitek Lyme	Duramune Lyme	Novibac Lyme	Lyme Vax	VANGUARD crLyme
Manufacturer	Merial	Boehringer Ingelheim	Merck (Intervet, CN)	Zoetis	Zoetis
Vaccine antigen	Recombinant plasma-derived, subunit vaccine (OspA)	Killed whole-cell, bivalent bacterin (OspA, OspC)	Killed whole-cell, bivalent bacterin (OspA, OspC)	Killed whole-cell, bivalent bacterin (OspA, OspC)	Recombinant OspA and chimeric OspC
Adjuvant	None	Proprietary adjuvant	Proprietary non-Al adjuvant	Non-Al adjuvant	None
Dosing recommendations	1-mL sq vaccine; dogs should be administered 2 doses, 2–3 wk apart. Annual revaccination recommended		1-mL sq vaccine; dogs should be administered 2 doses, 2–4 wk apart. Annual revaccination recommended	1-mL sq vaccine; dogs should be administered 2 doses, 2–3 wk apart. Annual revaccination recommended	1-mL sq vaccine; dogs should be administered 2 doses, 3 wk apart. Annual revaccination recommended
		Do not administer sq to horses	Do not administer sq to horses	Do not administer sq to horses	

Dosing recommendations are for dogs. Horse dosages may vary and are off-label use only.

Figure 14. Canine vaccines against Lyme diseases currently approved on the US market.

Discussion

The general population of ticks seems to increase along with the prevalence of *B. burgdorferi* infected ticks[7 8]. Climate changes are mentioned as possible causes of this change[7 8]. Adult ticks and larvae that has not yet had their blood meal might overwinter if the climate allows it[4]. The prevalence of *B. burgdorferi* infected horses is proposed to be in direct relation to the prevalence of infected ticks in the area[1]. Some researchers are suggesting that the prevalence of infected horses could be higher than the infection rate of humans because of the possibility of longer exposure of the tick on the horse; humans have less fur and might observe and remove the tick easier than a horse is able to[1]. The risk of being infected increases if the horse is exposed to the attached tick during a longer period of time, it is therefore suggested to remove the tick as soon as possible[1]. Proper measures should be taken when removing the tick to prevent its body from being squeezed during the removal, something that also could increase the risk of infection[4].

An overall seropositivity of 30-40% is to be expected in horses in endemic areas[7]. Seropositivity in horses have been investigated during studies in several countries. The estimated seropositivity of different areas cannot be directly compared since several factors might have an impact on the results. The year when the study was performed is out of interest when comparing seropositivity in different geographical areas since the overall tick population is increasing, so might the seroprevalence of horses do. It is therefore not suggested to compare an older study in one country with a newer study in a neighbouring country, since a larger tick population and seroprevalence can be suspected in the newer study. The time of the year when the measurements where performed might also affect the result; if measuring the seroprevalence in late autumn, the horses might be exposed to two peaks of tick infestations in comparison to horses investigated during springtime[7]. Studies have shown that horses with a lifestyle that includes more pasture exposure generally also have a higher rate of infection, the type of horses participating in the studies also affects the results[11]. As with most test results, the sensitivity and specifity of the laboratory method used will have an influence on the test result when comparing different serological studies. In a try to estimate a mean value of the seroprevalence of *B.burgdorferi* infection in horses it is probably advisable to investigate the results of several studies where after a general estimate of the seroprevalence can be established.

The main methods of confirming an *B. burgdorferi* infection in horses has up to recently been by the use of ELISA, Western blot and Immunofluorescence assay (IFA)[6]. Usually a positive ELISA test was followed up with Western blot or an Immunofluorescence assay. The newly developed Multiplex assay was developed by Cornell university and uses three different antigens to detect the presence of three types of antibodies in the horse´s serum. The spirochete bacteria

will express different antigens depending on its surroundings for example when being present in the ticks ´midgut the antigen Osp A will be expressed and later when it has been present in the host for a while, Osp F will be expressed[6]. The horse's immune system will produce different type of antibodies in response to the type of antigen present at that stage. This will create an opportunity to use the Multiplex assay as an indication of how long the horse has been infected[6]. This information might help to clarify whether the different suspected clinical signs of *B. burgdorferi* is related to a more acute or chronic type of infection. Another method used for the confirmation of *B. burgdorferi* infection in horses is the ELISA SNAP®4DX that was originally developed for canine patients[7].

In some suspected cases of Borreliosis a spirochete bacteria itself was isolated and the horses presented clinical signs but the serology came out negative[3]. It is therefore suggested by some researchers to use alternative methods, such as for example PCR, and to use samples other than serum in addition to confirm the infection in cases of for example uveitis[3]. Serology may provide false negative results in cases where a more local inflammation is suspected as in the case above but even a positive serological result of *B. burgdorferi* is not reliable by itself when making a final diagnosis of the causative agent responsible for the observed clinical signs in horses. Clinically healthy horses have also been proven to be seropositive to B. burgdorferi and a positive serological result is therefore not directly related to clinical signs[11]. Investigations of horses with musculoskeletal or neurological signs revealed no higher incidence of seropositivity of these patients in comparison to the clinically healthy ones[1].

The wide list of possible differential diagnosis for *B. burgdorferi* make it more difficult to set a finial diagnosis. For example, there might be several reasons for a horse to be lame, both neurological, traumatic or structural anatomical conditions might present similar signs. The horse might be co infected with *Analplasma phagocytophilum* that shares both type of vector and possible clinical presentation with Borreliosis and it might be difficult to interpret the exact origin of the clinical signs in this case, providing antibiotics to such patient might erase the clinical signs but the exact origin of the clinical signs would remain unclear[1] . As previously mentioned, neither a positive serological result can confirm anything else than the presence of antibodies following an infection.

Summary

Both clinically healthy and horses with clinical signs can be seropositive to *Borrelia burgdorferi*[11]. The overall seroprevalence of *B. burgdorferi* in horses in endemic areas are estimated to be 30-40%[7]. There is still a controversy in the attempt to confirm *B. burgdorferi* as the causative agent for the development of clinical signs. Some studies found no relation between clinical signs and the presence of *B. burgdorferi* infection and some studies found a possible relationship with some of the clinical signs[1 11]. The spirochete bacteria can be present in samples other than serum even if the serology turned out to be negative[3]. *B. burgdorferi* has the ability to migrate through various tissues of the host and is considered a multisystemic disorder[3]. In immunologically favoured places such as the eye, there might be a more local antibody production that is not detected in the serum[3]. In horses that present signs of uveitis it is suggested to use additional laboratory measures and samples other than serum to confirm the presence of the spirochete[3].

The newly developed multiplex assay can be used to detect antibodies of the various stages of the infection[5]. By confirming the stage of infection and the time when the horse initially got infected, a comparison can be made between the actual stage of infection and the clinical signs thought to be caused by *B. burgdorferi* in a try to establish a clearer relationship between the two.

The time of attachment of the tick on the horse's body might be significant for the rate of infection; it is advisable to remove the tick as soon as possible without squeezing the tick's body, something that also could increase the risk of infection and inoculation of the bacteria into the new host[1 4].

The veterinarian must decide which treatment method that should be used based on the clinical signs, presence of vector and serological result. Broad spectrum antibiotics should not be part of the first line of defence and should not be administered without an initial positive serological result if *B. burgdorferi* is thought to be the reason for the clinical signs[5]. The success rate of treatment could be assessed with a pre- and post-blood sample where the post blood sample would show a marked decrease in the presence of antibodies after successful treatment[6].

As to yet, there is no vaccine on the market developed for equine patients but the vaccine aimed for canine patients has been tested on horses with various results[12].

Acknowledgements

I would like to thank my supervisor Dr. Fodor László and the Department of Microbiology and Infectious diseases at the University of Veterinary Medicine in Budapest for the guidance and assessment of this thesis.

References

1. Butler, C., Houwers, D., Jongejan, F. and van der Kolk, J. (2019). *Borrelia burgdorferi infections with special reference to horses. A review*. [online] Veterinary Quarterly, 27:4, 146-156 Taylor & Francis. Available at: https://doi.org/10.1080/01652176.2002.9695196 [Accessed 9 Sep. 2019].

2. Schwan, T., Simpson, W. and Rosa, P. (1991). *Laboratory confirmation of Lyme disease*. [online] PubMed Central (PMC). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3327992/ [Accessed 23 Oct. 2019].

3. Priest, H., Irby, N., Schlafer, D., Divers, T., Wagner, B., Glaser, A., Chang, Y. and Smith, M. (2012). Diagnosis of Borrelia-associated uveitis in two horses. *Veterinary Ophthalmology*, 15(6), pp.398-405.

4. Sellnow, L. (2019). *Lyme Disease in Horses – The Horse*. [online] The Horse. Available at: https://thehorse.com/14110/lyme-disease-in-horses/ [Accessed 31 Aug. 2019].

5. Prutton, J. (2019). *An update on Borrelia burgdorferi*. [online] Veterinary-practice.com. Available at: https://veterinary-practice.com/article/an-update-on-borrelia-burgdorferi [Accessed 31 Aug. 2019].

6. Cornell University College of Veterinary Medicine. (2019). *Lyme Disease Multiplex Testing for Horses*. [online] Available at: https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols/lyme-multiplex-horses [Accessed 20 Oct. 2019].

7. Hansen, M., Christoffersen, M., Thuesen, L., Petersen, M. and Bojesen, A. (2010). *Seroprevalence of Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in Danish horses*. [online] Acta veterinaria scandinavia. Available at: https://actavetscand.biomedcentral.com/articles/10.1186/1751-0147-52-3 [Accessed 29 Sep. 2019].

8. Kampen, H., Rotzel, D., Kurtenbach, K. and Maier, W. (2019). *Substantial Rise in the Prevalence of Lyme Borreliosis Spirochetes in a Region of Western Germany over a 10-Year Period*. [online] Europe PMC. Available at: https://europepmc.org/articles/PMC368359;jsessionid=86B68E6EE6EF3CC85C6598EDF0A11A93 [Accessed 9 Sep. 2019].

9. Idexx.com. (2019). *SNAP 4Dx Plus Test screens for 6 vector-borne diseases - IDEXX US*. [online] Available at: https://www.idexx.com/en/veterinary/snap-tests/snap-4dx-plus-test/ [Accessed 25 Oct. 2019].

10. Idexx.com. (2019). [online] Available at: https://www.idexx.com/files/lyme-disease-management-quant-c6.pdf [Accessed 23 Oct. 2019].

11. Egenvall, A., Franzén, P., Gunnarsson, A., Engvall, E., Vågsholm, I., Wikström, U. and Artursson, K. (2001). Cross-sectional study of the seroprevalence to Borrelia burgdorferi sensu lato and granulocytic Ehrlichia spp. and demographic, clinical and tick-exposure factors in Swedish horses. *Preventive Veterinary Medicine*, 49(3-4), pp.191-208.

12. Divers, T., Gardner, R., Madigan, J., Witonsky, S., Bertone, J., Swinebroad, E., Schutzer, S. and Johnson, A. (2018). *Borrelia burgdorferi Infection and Lyme Disease in North American Horses: A Consensus Statement*. [online] PMC Us National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5866975/ [Accessed 5 Oct. 2019].

13. Guarino, C., Asbie, S., Rohde, J., Glaser, A. and Wagner, B. (2017). *Vaccination of horses with Lyme vaccines for dogs induces short-lasting antibody responses*. [online] Sciencedirect.com. Available at: https://doi.org/10.1016/j.vaccine.2017.06.052 [Accessed 13 Nov. 2019].

Figure 1, Westgatelabs.co.uk. (2019). *Ticks*. [online] Available at: https://www.westgatelabs.co.uk/info-zone/parasites-affecting-horses/ticks/ [Accessed 25 Oct. 2019].
Figure 2, Divers, T. (2016). Lyme disease. *Veterinariankey.* Available at: https://veteriankey.com/wp-content/uploads/2016/06/B9781416024064500405_f35-01-9781416024064.jpg [Accessed 12 Oct. 2019]
Figure 3, Presence of Ixodes Ricinus. European Centre for Disease Prevention and Control. (2019). *Ixodes ricinus - current known distribution: July 2019*. [online] Available at: https://www.ecdc.europa.eu/en/publications-data/ixodes-ricinus-current-known-distribution-july-2019 [Accessed 22 Oct. 2019].
Figure 4, Swinebroad, E. (2019*). Borreliosis in Sport Horse Practice*. [online] Semanticscholar.org. Available at: https://www.semanticscholar.org/paper/Borreliosis-in-Sport-Horse-Practice.-Swinebroad/8ffbf8bbc112a9f44c79c419faf0eeac51ada857 [Accessed 22 Oct. 2019].
Figure 5, Horsedvm.co.uk. (2019). *Lyme Disease | HorseDVM Health A-Z.* [online] Available at: http://www.horsedvm.co.uk/disease/lyme-disease-in-horses/ [Accessed 22 Oct. 2019].
Figure 6, Figure 7, Cornell University College of Veterinary Medicine. (2019). *Lyme Disease Multiplex Testing for Horses*. [online] Available at: https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols/lyme-multiplex-horses [Accessed 20 Oct. 2019].
Figure 8, Swinebroad, E. (2019*). Borreliosis in Sport Horse Practice*. [online] Semanticscholar.org. Available at: https://www.semanticscholar.org/paper/Borreliosis-in-Sport-Horse-Practice.-Swinebroad/8ffbf8bbc112a9f44c79c419faf0eeac51ada857 [Accessed 22 Oct. 2019].
Figure 9, Vitreous humor. Priest, H., Irby, N., Schlafer, D., Divers, T., Wagner, B., Glaser, A., Chang, Y. and Smith, M. (2012). Diagnosis of Borrelia-associated uveitis in two horses. *Veterinary Ophthalmology*, 15(6), pp.398-405. Available at: http://eclinpath.com/june-2014-case-of-the-month/ [Accessed 11 Oct. 2019]
Figure 10, Divers, T., Gardner, R., Madigan, J., Witonsky, S., Bertone, J., Swinebroad, E., Schutzer, S. and Johnson, A. (2018). *Borrelia burgdorferi Infection and Lyme Disease in North American Horses: A Consensus Statement*. [online] PMC Us National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5866975/ [Accessed 5 Oct. 2019].
Figure 11, Figure 12, Swinebroad, E. (2019*). Borreliosis in Sport Horse Practice*. [online] Semanticscholar.org. Available at: https://www.semanticscholar.org/paper/Borreliosis-in-Sport-Horse-Practice.-Swinebroad/8ffbf8bbc112a9f44c79c419faf0eeac51ada857 [Accessed 22 Oct. 2019].
Figure 13, Westgatelabs.co.uk. (2019). *Ticks*. [online] Available at: https://www.westgatelabs.co.uk/info-zone/parasites-affecting-horses/ticks/ [Accessed 25 Oct. 2019].
Figure 14, Swinebroad, E. (2019*). Borreliosis in Sport Horse Practice*. [online] Semanticscholar.org. Available at: https://www.semanticscholar.org/paper/Borreliosis-in-Sport-Horse-Practice.-Swinebroad/8ffbf8bbc112a9f44c79c419faf0eeac51ada857 [Accessed 22 Oct. 2019].

Appendix 6. Electronic License Agreement and Copyright Declaration

HuVetA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Linda Werme

Contact information (e-mail): lwerme@hotmail.com

Title of document (to be uploaded):
Borrelia burgdorferi infection in horses.
Literature review 1980-2019.

Publication data of document: 2019

Number of files submitted: 1

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA may store more than one copy (accessible only to HuVetA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA as follows **(mark the appropriate box with an X)**:

- ☐ I grant unlimited online access,
- ☐ I grant access only through the intranet (IP range) of the University of Veterinary Medicine,
- ☒ I grant access only on one dedicated computer at the Ferenc Hutÿra Library,
- ☐ I grant unlimited online access only to the bibliographic data and abstract of the document.

Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

☒

I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetA do not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA in a way that is unlawful.

Date: Budapest, ...4....day ..11.....month..2019....year

Author/copyright owner
signature

HuVetA Magyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive *is an online veterinary repository operated by the Ferenc Hutÿra Library, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.*

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.
Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

Appendix 4.

I hereby confirm that I am familiar with the content of the thesis entitled "*Borrelia burgdorferi* infection in horses. Literature review 1980-2019" written by Linda Werme which I deem suitable for submission and defence.

Date: Budapest, 13th November, 2019

László Fodor
Department of Microbiology and Infectious Diseases