Polysynovitis in a horse due to Borrelia burgdorferi sensu lato infection – Case study

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Abstract

Lyme borreliosis (LB) is a multi-systemic tick-borne disease affecting both humans and animals, including horses, and is caused by a group of interrelated spirochetes classified within the Borrelia burgdorferi sensu lato (s.l.) complex. Despite the high reported seroprevalence in the European equine population for B. burgdorferi s.l., to-date no documented clinical cases have been described. A 6-year-old Paint gelding was referred with a history of three weeks of fever, intermittent lameness and digital flexor tendon sheath effusion of the right hind limb. Based on a strict diagnostic protocol, which included serological tests for infectious diseases and molecular investigations, a final diagnosis was made of polysynovitis due to B. burgdorferi s.l. infection. An unreported aspect observed in this case was the absence of the pathogen DNA in two of the affected joints. To the authors’ knowledge, the case described represents the first documented clinical case of equine LB in Italy. Moreover, the absence of pathogen DNA in two of the affected joints observed in this case revealed a possible similarity with the same condition described in humans, where an immunomediated pathogenesis for arthropathy due to B. burgdorferi s.l. infection is suspected. Since humans and horses share the same habitat, this report supports the role of the horse as potential sentinel for human biological risk.

Key words

Borrelia burgdorferi sensu lato, Borrelia afzelii, polysynovitis, equine

INTRODUCTION

Lyme borreliosis is a multi-systemic tick-borne disease affecting mainly humans, but also wild and domestic animals, including horses [1]. LB is caused by a group of interrelated spirochetes classified within the Borrelia burgdorferi s.l. complex, transmitted by ticks of the genus Ixodes. Equine B. burgdorferi infection appears to be present worldwide with the incidence mirroring, to a certain extent, that of humans, because the tick vector involved feeds on both species [2]. Furthermore, both humans and horses commonly share the same habitats. However, while human LB is considered the most common tick-borne disease in the northern hemisphere and has been widely investigated [2], equine borreliosis is still poorly documented. The lack of pathognomonic signs (e.g. erythema migrans and lymphocytoma) like those observed in human infection, and the broad spectrum of poor specific symptoms expressed in horses affected by LB, might explain the different awareness of the clinical importance of the disease in these two species [2].

Seropositivity for Borrelia burgdorferi s.l. has been reported among the equine population of several European countries [3, 4]. In Italy, Human LB was first described in 1985 [5]. Since then, it has been reported in a number of publications, and the disease is now thought to be endemic in several regions of Italy. Conversely, equine LB has been scarcely investigated throughout the country. However, recent reports suggest that the disease is endemic among horse populations in areas of central and southern Italy [1, 6, 7]. To-date, it appears that no clinical disease has been documented. In this report, a case of polysynovitis due to Borrelia afzelii infection is described, and, to the authors’ knowledge, it represents the first documented clinical case in Italy.

CASE STUDY

A 6-year-old Paint gelding was referred with a history of three weeks of fever, intermittent lameness and digital flexor tendon sheath effusion (DFTS) effusion of the right hind limb. In the four weeks prior to admission, the patient also presented weight loss, weakness, muscular atony, and clinical signs suggestive of laminitis. The referring vet treated the horse with ceftiofur (2.2 mg/kg IM twice daily) and marbofloxacin (2 mg/kg IV once a day) for two weeks with no improvement. On admission, the horse was lethargic with slightly elevated heart (50 BPM) and respiratory rates (24 BPM) and fever (39.3 °C). Examination of the musculoskeletal system revealed: generalized stiffness with gait abnormalities; thoraco-lumbar kyphosis; left mid-carpal joint, left medial femoro-tibial joint and right hind DFTS distension; non-painful distal limb oedema; reduced lateral flexion of the neck. Haematological findings included mild leukocytosis (WBC 10.6 × 106 u/L, reference 5.6–9.8 × 106 u/L) with mature neutrophilia (7.2 × 106 u/L, reference 3.0–6.0 × 106 u/L), normocytic normochromic anemia (RBC 5.26 × 106 u/L, reference 4.6–6.2 × 106 u/L), elevated heart (50 BPM) and respiratory rates (24 BPM). Serum biochemical parameters were unremarkable while serum protein electrophoresis revealed a low albumin to globulin ratio (0.55, reference 0.93–1.65), reduced albumin (2.55 g/dl, reference 3.1–4.7 g/dl) and α1-globulins (0.12 g/dl, reference 0.2–0.3 g/dl) and increased α2-globulins (1.23 g/dl, reference 0.5–0.9) and β-globulins (2.07 g/dl, reference 0.6–1.3 g/dl).
Evaluation of serum amyloid A (SAA) and fibrinogen, performed on a weekly basis starting from the day of admission, showed values ranging from 319.81–532.50 mg/L for SAA (mean 441.5 mg/L ± standard deviation (SD) 78.9 mg/L, reference 0.5–20 mL) and between 6.9–12.98 g/L for fibrinogen (mean 10.1 g/L ± SD 2 g/L, reference 2.0–4.5 g/L).

Radiographic examination excluded any bony abnormalities. Ultrasonographic examination of the swollen DFTS, of the left mid-carpal and of the medial femoro-tibial joints revealed effusion, possibly of fibrinous nature, and joint fluid aspirates revealed increasing white blood cell counts (1.300/μL; 4.600/μL and 5.800/μL, respectively; reference < 0.5 cells/μL) and of total protein concentrations (4.1 g/dl; 4.6 g/dl; 4.9 g/dl, respectively; reference 0.9–3.0 g/dL). The results of the cytological and bacteriological assessment of the joint fluid was unremarkable, whereas SAA concentrations were markedly increased in the left mid-carpal (211.83 mg/L) and medial femoro-tibial (187.25 mg/L) joints and the DFTS (410.68 mg/L) (Normally, levels appear to be very low or undetectable <0.48 mg/L) [8].

Blood culture was negative, as well as serological tests for the principal equine infectious diseases present in the region (Equine Herpesviruses, Equine Viral Arteritis, Equine Infectious Anemia, West Nile virus, Anaplasma phagocytophilum, Piroplasmosis). The serum tested positive for antibodies against B. burgdorferi using an in-clinic Enzyme Linked Immunosorbent Assay (ELISA) (Snap 4Dx, IDEXX Laboratories, Westbrook, ME, USA); therefore, an Immunofluorescence Antibody Testing (IFAT) was carried out. To obtain a quantitative assessment of the IgG and IgM antibodies against B. burgdorferi, commercial antigens (Fluoborrelia / Mega ScreenR Diagnostic Megacore, Horbranz, Austria) and fluorescein-conjugated anti-horse antibodies were used (rabbit-anti-horse-IgG-FITC/rabbit-anti-IgM-FITC, Sigma Immunochemicals, St Louis, MO, USA), diluted at 1:200 in Blue Evans solution. The sample was screened at an initial dilution of 1:80 (cut off) in phosphate buffered saline (pH 7.2), as described in the manufacturer’s protocol, and progressively diluted twofold in order to determine the end-point titer. The serum was found to be positive both for IgG and IgM antibodies with titers of 1:320 and 1:80, respectively.

For the purpose of screening for B. burgdorferi s.l. group DNA, a nested PCR assay was performed on DNA extracted from joint / tendon sheath fluid aspirates (QiAamp DNA Mini Kit Qiagen). The reaction was set up targeting the region containing the 23S-5S-23S intergenic spacer region (ISR) specific to the B. burgdorferi s.l. group, using the Borrelia afzelii genome as the reference sequence (GenBank: NC_008877). The primer combinations and PCR conditions are listed in Table 1.

All of the samples tested PCR-negative, with the exception of the right hind DFTS aspirate. This amplicon was sequenced and compared to publicly available data to confirm target specificity: Borrelia afzelii was the species with the highest number of hits.

Based on the clinical, immunological and biomolecular findings a diagnosis of LB was made and treatment with oxytetracycline (7.5 mg/kg, IV, SID) and corticosteroids (dexamethasone, 0.02 mg/kg, IV, tapering down the dose by 10% every three days) was initiated.

Despite the clinical improvement observed in the first 10 days of treatment, the horse continued to present weight loss and muscular atrophy. By the fourth week of treatment, the joints and digital sheats were no longer effused but the clinical condition continued to worsen.

After five weeks of treatment, the nested PCR protocol was repeated on blood and synovial fluid samples, with negative results. The antimicrobial therapy was therefore suspended, and treatment continued using a low-dose corticosteroid (dexamethasone 0.02 mg/Kg, IV), tapering down the dose by 10% every three days (phenylbutazone 2.2 mg/Kg, PO, SID). In spite of the long course of treatment, the horse did not improve and eventually became permanently recumbent and it was ultimately decided to administer euthanasia.

Based on the worsening of clinical signs, it was hypothesized that the bacteria were still present in the joint but not detectable with standard PCR. In order to confirm this suspicion and quantify Borrelia DNA levels in the joint and sheath fluid, a real-time quantitative PCR assay (qPCR) targeting the 23S-5S-23S ISR was formulated. Briefly, the 259 bp DNA fragment obtained with the nested PCR was cloned into a plasmid vector (pCR4-TOPO, Invitrogen) and used as the standard reference. A reference curve, relating the PCR cycle number as a function of the bacterial DNA concentration in the samples, was constructed using five serial ten-fold dilutions of the plasmid, starting from 21.65 × 10^5 bacterial genome equivalents diluted to 21.65 × 10. Genome equivalents (GE) were calculated using the single plasmid molecular weight. The efficiency of the standard curve was 81.6% (R^2=0.995).

The PCR positive DNA obtained from the right hind DFTS aspirate on admission, and the PCR negative sample collected from the same site five weeks following initiation of antibiotic treatment, tested positive at the qPCR assay, with GE numbers per μL of template well above detection limit. Interestingly, the Borrelia load in the treated sample was almost half of that in the sample obtained on admission (112.194 ± 14.425 GE/μL vs. 225.380 ± 43.218 GE/μL). The samples collected from the other affected joints proved negative.

On necropsy, lesions appeared to be confined to the joints, characterized by a moderate to marked swelling, irregular

### Table 1. Primer sequences and PCR conditions

| PRIMER | SEQUENCE 5’->3’ | AMPICRON LENGTH (bp) | PCR CYCLE |
|--------|----------------|----------------------|-----------|
| BorOutF | TGCCAGTTACAGGAATCTC | 569 | 94°C S’, 5x[94°C 20, 65°C->61°C 20, 72°C 20] |
| BorOutR | TCTGCGCAAGGTTAGTGCTGGG | | 35x[94°C 20, 61°C 20, 72°C 20] |
| BorInF | TGCCCTAAAATCCTAGGACATCCA | 259 | 94°C S’, 5x[94°C 20, 63°C->58°C 20, 72°C 20] |
| BorInR | ACTGCGAGTTCGGGGAAGAG | | 35x[94°C 20, 58°C 20, 72°C 20] |
| BorInF | TGCCCTAAAATCCTAGGACATCCA | 259 | qPCR 95°C S’, 50x[95°C 10, 61°C 20] |
hyperemic surface and abundant quantity of gelatinous synovial fluid in the joint cavity. On histological examination, the synovial membranes appeared to be affected by moderate to marked lymphomonocytic infiltration, associated with villus hyperplasia. Thickening due to hyalinization was occasionally detected in the arterioles of the synovial tissue. Moreover, scattered foci of calcification and neutrophilic infiltration were observed in the periarticular connective tissue, consistent with a chronic form of Lyme borreliosis [9].

**DISCUSSION**

The horse described in this report was reared and permanently housed in Tuscany, a region where a considerable seroprevalence for *B. burgdorferi* s.l. (24.3%) has been reported in the equine population [1]. Furthermore, several studies have demonstrated that *Ixodes ricinus* ticks in this region carry LB pathogen DNA and local cases of human neuroborreliosis have also been reported [10, 11, 12].

In Europe, LB is caused by the *B. burgdorferi* s.l. complex that comprises 18 known Borrelia genospecies. Among these, the most frequently reported genospecies in Italy include *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitaniae* and *B. spielmanii* that have all been documented in ticks collected in Tuscany [12, 13]. However, to-date, the genetic identity of the strains affecting horses in this region remains unknown, with the possible exception of *B. lusitaniae* reported to be present in the blood of naturally-infected horses in a recent survey carried out in Lazio, a neighbouring region of Tuscany [6].

While in humans different geno-species are associated with different clinical manifestations of the disease (e.g. *B. burgdorferi* sensu stricto is most often associated with arthritis, *B. garinii* with neuroborreliosis etc) [4], to-date, the variability in clinical presentations of equine LB cannot be attributed to the specific *Borrelia* geno-species implicated in the infection [10]. However, the majority of cases of disease are associated with *B. afzelii* [11], identified through the sequence analysis of the 23S–5S–23S rDNA, the same as in the presented case. This geno-species is usually associated with reservoir hosts represented mostly by rodents and with chronic skin condition (acrodermatitis chronica atrophicans) in humans.

From the clinical standpoint, equine LB is most commonly associated with musculoskeletal disorders, including lameness, laminitis, swollen joints and muscle tenderness, as result of the dissemination of the pathogen from the point of local infection, predominantly to connective tissues, signs that usually occur shortly after infection [3, 9, 15]. On the contrary, in humans, musculoskeletal disorders are generally considered as characteristic of the late stages of LB [16], often developing months after the onset of the disease.

In the case described, the horse presented polysynovitis and the diagnosis of LB was made basing on the following evidence 1) anamnestic data regarding housing in an endemic area, 2) clinical signs, 3) serological findings (both IgG and IgM class of antibodies) consistent with recent exposure to *B. burgdorferi* s.l., 4) detection of pathogen DNA in the fluid of affected synovial spaces, and 5) gross and histological joint changes.

Successful treatment of equine LB is generally accomplished using tetracyclines [17], but in the case described the clinical status did not improve despite the antimicrobial treatment. Moreover, in the presented case it was not possible to detect the pathogen from two of the affected joints. The same condition has been described in humans, where the pathogenesis of borreliosis arthropathy is not fully known [18]. The presence of Borrelia spirochetes in the connective tissues surrounding joints is thought to lead to the development of mild inflammation. Spirochetal lipoproteins act as potent inflammatory triggers for a variety of inflammatory cells [19], and the consequent post-inflammation tissue injury may set the stage for a subsequent, self-perpetuating, autoimmune reaction [20]. Human models of disease suggest that the progression towards chronic arthritis does not require the local presence of spirochetes. Instead, the chronic form of Lyme arthritis seems to be associated with the Th17 cell subset, a recently discovered type of helper T-cell. Pro-inflammatory cytokines (IL-6), produced initially by stimulated neutrophils, in combination with endogenous TGF-β, induce the development of Th17 cells from T-cell precursors. Th17 cells produce IL-17, which stimulates the production of other pro-inflammatory cytokines (IL-1β, TNF-α). IL-17 also induces the production of IL-6 by fibroblasts and synovial cells, thus creating a vicious inflammatory cycle [18]. This process has been proposed to explain the development of clinical signs in the absence of the microorganism in humans. Considering the absence of the pathogen DNA in two of the affected joints, a similar set of events may justify the progression of the disease in the horse of this report, although additional investigations are required to support this hypothesis. If confirmed, the possibility that horses can develop an autoimmune arthritis due to Borrelia infection would reveal a similarity between the disease in horses and human beings, supporting the role of horses as a potential model of human disease.

**CONCLUSION**

In the present case is reported *Borrelia afzelii* as causative agent of polysynovitis in a horse for the first time in Italy. From an epidemiological standpoint, since diffusion of Borrelia in the equine population is well documented, as the high reported seroprevalence demonstrates, the recognition of cases with evidence of clinical signs further supports the role of pasture horses as sentinel for human biological risk.

Many questions regarding equine LB still remain unanswered. Further studies appear essential to clarify the pathogenesis of this disease in horse, with special regards to musculoskeletal involvement. Moreover, the application of strict diagnostic protocols, including specific molecular investigation, as real time PCR, appear essential to achieve a correct final diagnosis.

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