

## 7. Summary

### Clinical evaluation of the direct and indirect detection of the causal agent of Lyme disease in the horse

The clinical diagnosis of Lyme disease (LD) in horses is often established by detection of *Borrelia (B.) burgdorferi* sensu lato (s.l.) antibodies in conjunction with clinical symptoms as specified in literature. Because of the lacking standardization of the tests it turned out that the detection of *Borrelia* in the specimens was very difficult. The aim of the study was to evaluate the common practice of serological testing for the establishment of the diagnosis LD in horses.

337 patients referred to the Clinic for Horses, Surgery and Radiology of the Freie Universität Berlin were examined clinically for a period of ten month and the collected samples were tested further in the laboratory of the BgVV<sup>1</sup> Berlin. The probands were classified in group A (horses independent of clinical symptoms, n=195) and group B (horses with clinical symptoms, n=142) after a full clinical examination.

In the study serum samples of horses (n=337) were tested using two commercial ELISA testkits (**A** and **B**), an ELISA (**C**) and IFT homemade at the BgVV for *B. burgdorferi* s.l. antibodies (immunglobulin G). In addition, specimens of skin, liquor, synovial fluid and blood of horses with clinical symptoms were analysed by PCR and cultivation (n=76).

In order to determine the sensitivity of the serological assays, both positive controls of the commercial ELISA testkits (**A** and **B**), 5 highly positive sera of each ELISA (**A**, **B** and **C**) and 3 sera tested negative in all of them were examined with Western blot (ALOMED<sup>2</sup>, n=20). The positive control of the ELISA **C** consisted of a sample that was high positive in all serological tests.

In a further study, 15 liquor samples were tested for *B. burgdorferi* s.l. antibodies (immunglobulin A, M and G) and the corresponding serum samples (n=15) for immunglobulin M and G using another ELISA (LGL<sup>3</sup>). In our investigations we examined Lyme disease in horses using direct methods for detection of *B. burgdorferi* s.l.: culture and Polymerase Chain Reaction (PCR). For the culture of *B. burgdorferi* skin (n=16), liquor (n=15) and synovial fluid (n=26) were inoculated into BSK- and MKP-medium native and with different inhibiting substances.

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Cultures were incubated for 3 months at 33°C, subcultivated and examined weekly by dark-field microscopy. Skin (n=16), liquor (n=15), synovial fluid (n=26) and blood (n=19) were tested for the presence of *B. burgdorferi* s.l. specific DNA by OspA-specific nested PCR.

In order to determine the specificity of the nested PCR the positive PCR-results were examined with Realtime PCR (ALOMED).

The results of the serologic testing revealed a high discrepancy within the tested probands. Following proportions of positive antibody titers were found (n=337): ELISA **A**: 61,1%, **B**: 9,8%, **C**: 3,3%. In the IFT 33,2% of samples were positive (cut off 1:128). Compatibility of results of serological tests existed in 22,0% of samples (74/337), only 1,8 % of samples were positive in all tests (6/337). There was no significant difference in serological results between horses with (group B) and without (group A) clinical symptoms of LD. A correlation between symptoms and seropositivity of serum samples could not be proved.

In the Western blot, positive (n=15) and negative in all ELISA (n=3) sera except the positive controls of the commercial ELISA testkits (**A** and **B**) were confirmed.

The LGL-ELISA detected incidences of seropositive samples (depending on dilution of serum) as follows: 53,3% (1:50), 26,7% (1:200) and 6,7% (1:400). These results are in correspondence with ELISA **A**.

The cultivation of *B. burgdorferi* was negative in all cases (n=57). In one of them we could find immobile spirochete-like forms. They are supposed to be "giant whips", flagellae of the contaminant flora, which are not involved in the development of Lyme disease.

In the OspA-specific nested PCR 3 skin (3/16), 2 liquor (2/15), 6 synovial fluid (6/26) and 1 blood (1/19) samples were positive for *B. burgdorferi* s.l.-DNA. In the Realtime PCR none of these 12 positive samples were positive for *B. burgdorferi* s.l.-DNA (0/12). A correlation between seropositivity of serum samples and the presence of specific DNA could not be proven.

These contradictory results revealed the necessity for evaluation of current direct and serological laboratory testing for *B. burgdorferi* s.l. Without standardised methods (concerning antigen coating, serum dilution, positive control) for the serodiagnostic tests the demands regarding specificity and sensitivity are not met. Reliable laboratory tools are a prerequisite under which a characteristic titer dynamic in conjunction with clinical signs can lead to the presumptive diagnosis LD. Due to the lack of standardization of PCR and the complex demands on cultivation direct detection of *B. burgdorferi* s.l. is not a suitable method for routine diagnostic at the moment.