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Short communication

Comparison of serum protein profiles of *Borrelia burgdorferi*-positive Bernese Mountain Dogs and dogs of other breeds using MALDI-TOF technique

M. Pisarek¹, M. Kalinowski¹, M. Skrzypczak², Ł. Mazurek¹, K. Michalak¹,
D. Pietras-Ożga¹, B. Dokuzeyli³, S. Winiarczyk¹, Ł. Adaszek¹

¹Department of Epizootology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine of the University of Life Sciences in Lublin, ul. Głęboka 30, 20-612 Lublin, Poland

²Second Department of Gynecology, Medical University of Lublin, 20-954 Lublin, Poland

³Department of Internal Medicine, Veterinary Faculty, Istanbul University-Cerrahpasa, 34320 Avcilar Campus, Avcilar, Istanbul, Turkey

Summary

The aim of the study was to compare the serum protein profile of Bernese Mountain Dogs (BMDs) reacting positive for Bb in snap testing with the serum protein profile of dogs of other breeds (healthy and with clinical borreliosis) using the MALDI time-of-flight (MALDI-TOF) technique. The observations included five groups of dogs. BMDs reacting positively to Bb in snap serological testing and showing symptoms of borreliosis (group 1), BMDs for which no borreliosis symptoms were determined but with seropositivity for Bb determined with snap serological tests (group 2), clinically healthy BMDs with no antibodies for Bb found in the serum (group 3), five dogs of different breeds, reacting positively in serological testing, in which borreliosis symptoms were observed (group 4), clinically healthy dogs of different breeds with negative reaction in tests towards Bb (group 5). A proteomic analysis demonstrated the presence of five identical protein fractions among all five groups. An additional two protein fractions of approximately 7.630 and 15.260 kDa were found in all the serum samples obtained from the dogs positive for borrelia in a snap test, both in those exhibiting symptoms of borreliosis, and seropositive BMDs not presenting symptoms of the disease. These two additional protein fractions may be used to differentiate between seropositive and seronegative *B. burgdorferi* dogs and may be considered a seropositivity marker, however, it cannot be used to differentiate between animals with the clinical form of the disease and those that are only seropositive.

Keywords: *Borrelia burgdorferi*, Bernese Mountain dogs, mass spectrometry

Introduction

Lyme disease is a multi-organ disease caused by *Borrelia burgdorferi* sensu lato (Bb) transmitted by *Ixodes* ticks (Zygner 2008, Tsao 2009, Zygner 2009, Stanek 2011, Dzięgiel 2014).

Bernese Mountain Dogs (BMDs) more often test positive for antibodies against Bb in rapid diagnostic tests without showing any clinical symptoms of the disease (Gerber 2007). Also, quantitative determination of the immunoglobulin level for spirochetes has indicated that BMDs may have an increased susceptibility to Bb of a hereditary nature. It is interesting that most of the studies showing higher Bb-sl-seroprevalence were performed in close proximity to each other in central Europe (Switzerland and southern Germany), the region where the highest prevalence of Bb-sl infested ticks was found (Gerber 2007). One might speculate about a regional effect, a close genetic relationship among the positive dogs (a genetic predisposition for infection) or a unique infectious species of Bb-sl in the area. More investigations are needed to evaluate the biological reasons and consequences of infections with Bb in BMDs.

A question arises whether the analysis of proteome of BMDs serum in healthy representatives of this breed is capable of demonstrating the specific proteins responsible for positive seroreactivity for Bb.

The aim of the study was to compare the serum protein profile of BMDs reacting positive for Bb in snap testing with the serum protein profile of dogs of other breeds (healthy and with clinical borreliosis) using the MALDI time-of-flight (MALDI-TOF) technique.

Materials and Methods

The current observations included five groups of dogs. The first group comprised 6 BMD individuals with positive reaction to Bb in snap serological testing (CaniV4 Vet Expert), for which clinical examination determined symptoms typical of borreliosis (joint swelling, fever, difficulty in moving), the second group included 10 BMD individuals in which no borreliosis symptoms were determined but in which snap serological tests demonstrated the presence of antibodies against Bb in the serum. The third group comprised 10 clinically healthy BMDs with no antibodies for Bb found in the blood serum via snap serological testing. The fourth group comprised five dogs of different breeds reacting positively in serological testing, in which borreliosis symptoms were observed. Group 5 included ten dogs of different breeds, clinically healthy, without positive reaction in testing for Bb.

From all dogs serum samples were collected for proteomic testing. Mass spectrometry was applied as a screening tool to determinate the presence and/or absence of some peaks between the groups with negative or positive *Borrelia* test results.

To generate high-quality, good-resolution mass spectra, a mixture of two matrices – DHB (2,5-Dihydroxybenzoic acid) (Bruker, Germany) and HCCA (α -cyano-4-hydroxycinnamic acid) (Bruker, Germany) – was adopted (Obama 2007, Signor 2013). Mass spectra were recorded in active positive reflector mode in the two mass ranges – 5,000-20,000 m/z and 20,000-10,000 m/z – using an Ultraflextreme MALDI TOF/TOF (Bruker, Germany) spectrometer and the flex Control 3.3 (Bruker, Germany) software.

Results and Discussion

Proteomic analysis demonstrated the presence of five identical protein fractions among all five groups. One, with m/z ~13,750 Da in the case of peptides and small proteins (spectra range: 5–20 kDa) and four for higher protein masses (spectra range: 20–100 kDa): ~21,900, ~27,350, ~32,850, ~65,440 m/z. Moreover, additional two protein fractions of approximately 7,630 and 15,260 kDa were found in all the serum samples obtained from the dogs positive for borrelia in snap testing, both in those showing symptoms of borreliosis, as well as seropositive BMDs not showing symptoms of the disease (groups 1, 2 and 4) (Fig. 1). These proteins were not found in the serum of any of the dogs negative in snap testing (groups 3 and 5). This indicates that the fraction discussed may be used to differentiate between seropositive and seronegative for *B. burgdorferi* in dogs and may be considered a seropositivity marker. The analysis of the mass spectrograms of the tested serum samples and the comparison of the control group and the test group spectra did not reveal any other significant differences (Fig. 1).

The assumption of the study was whether BMDs that do not exhibit clinical symptoms of borreliosis and with a positive reaction in snap testing for Bb possess a specific protein in their serum which can be used to identify such individuals – unfortunately this objective has not been met.

So far, the MALDI-TOF MS technique has been used to detect *Borrelia* bacteria in ticks (Fotso Fotso 2014), Calderaro 2014), and to perform microbiological identification of these microorganisms (Calderaro 2014, Neumann-Cip 2020).

In 2016, Dzięgiel et al. (Dzięgiel 2016) attempted to demonstrate specific proteins in the organisms of dogs infected with these bacteria that could be viewed as infection markers, to no avail, however.

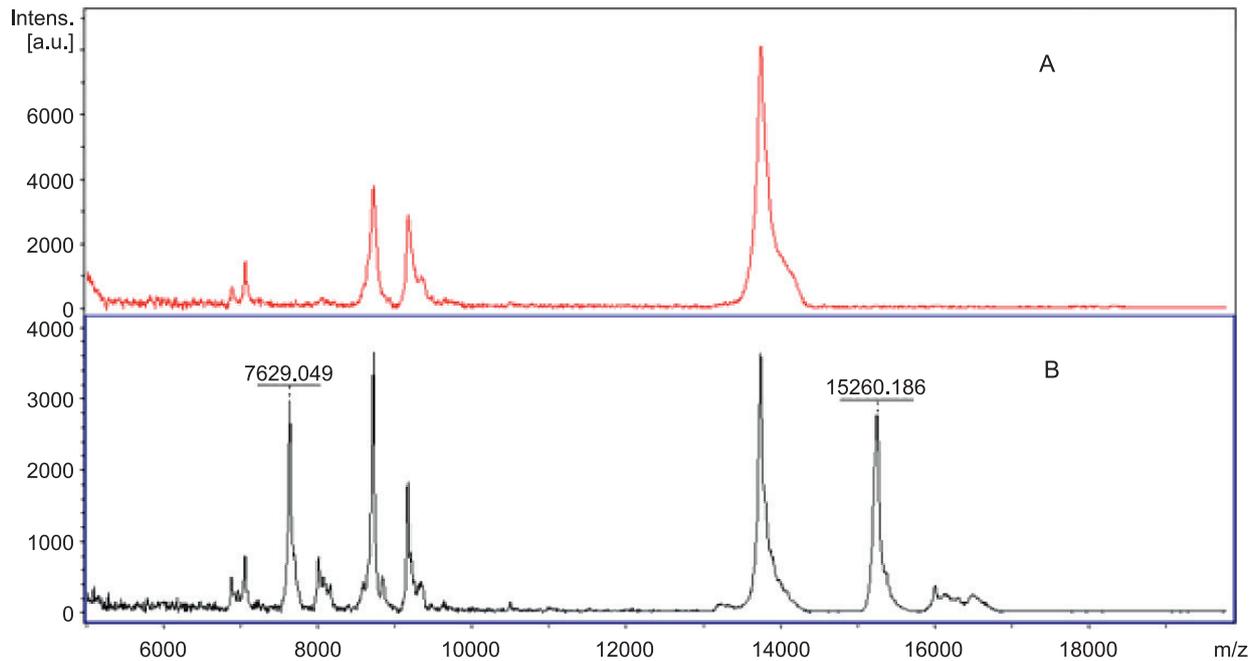


Fig. 1. MALDI-TOF mass spectrometry analysis of blood serum from dogs used in the study. a) results of serum analysis from dogs negative in snap testing (groups 3 and 5), b) results of results analysis of serum from dogs positive for borrelia in snap tests (groups 1, 2, 4).

In conclusion, BMDs more often had antibodies against Bb (Gerber 2009a, Gerber 2009b). It is interesting that most of the studies showing higher Bb-seroprevalence were performed in close proximity to each other in central Europe (Switzerland and southern Germany), the region reporting the highest prevalence of Bb-infested ticks (Gerber 2007). One might speculate about a regional effect, a close genetic relationship among the positive dogs, a genetic predisposition to infection, or a uniquely infectious species of Bb in the area. More investigations are needed to evaluate the biological reasons and consequences of infections with *B. burgdorferi* in BMDs.

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