Comparative Evaluation of a Lyme C6 multimer for the detection of *Borrelia burgdorferi*-specific antibodies in an endemic region in North America using Antech Diagnostics' Accuplex® BioCD assay.

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INTRODUCTION

Lyme disease is a zoonotic, vector-borne disease affecting humans, dogs, and other mammalian species. The disease is caused by infection with a diverse group of spirochetes of the *Borrelia burgdorferi* sensu lato complex. Lyme disease is considered one of the most common vector-borne diseases in North America with infection of hosts by tick (*Ixodes* spp.) transmission. Serological evaluation for antibodies directed against outer surface proteins of *B. burgdorferi* is the testing methodology of choice for Lyme disease surveillance in dogs.[1] The objective of this study is to determine the accuracy of a newly designed Lyme C6 multimer for detection of antibodies directed against Lyme C6 using using a silicon-wafer based automated fluorescence assay (Accuplex assay, BioCD, Antech Diagnostics).[2]

MATERIALS AND METHODS

Sample collection

All canine serum samples were submitted for serological testing for antibodies directed against *B. burgdorferi* to Antech Diagnostics' Lake Success, New York laboratory, from veterinary practices located in the Northeastern United States. A total of 415 samples were tested using the updated Accuplex Lyme C6 multimer and immunofluorescence (IFA) assays. All samples included in the study were negative for antibodies directed against the *B. burgdorferi* outer surface proteins, OspA and OspC, as determined by the Accuplex assay. Additionally, an investigation was conducted to obtain Lyme vaccination history, and only dogs that had no previous known history of Lyme vaccination were included for method comparison.

Borrelia burgdorferi targets

The *B. burgdorferi* peptides used in the study included OspA and OspC as previously described.[2] In addition, two 26-amino-acid C6 peptides, derived from two strains of *B. burgdorferi*, linked into a single multimer, were used as antigens for the Accuplex assay. The DNA coding for these peptides was commercially synthesized (GenScript, Piscataway, NJ) and cloned into pET-28a (+) vector. The protein was expressed in *E. coli* cells, purified using affinity chromatography, and tags removed according to the manufacturer's established protocols. Purified C6 protein was used for the Accuplex system applications.

Immunofluorescence assay for *Borrelia burgdorferi*

Sera from all dogs were tested blindly, and in parallel, for the presence of IgG antibodies to *B. burgdorferi* using indirect IFA. The protocol followed standard operating procedures established by Antech Diagnostics. IFA slides were obtained from Veterinary Medical Research & Development (VMRD, Pullman, WA). For the detection of IgG antibodies, a titer of 1:64 was considered equivocal and a titer of ≥ 1:128 was considered positive.

DATA AND STATISTICAL ANALYSIS

Statistical analysis was performed via MedCalc (MedCalc Software Ltd, Ostend, Belgium) and Microsoft Excel (Microsoft Excel 2018, Microsoft Corporation, Redmond, WA) statistical software. Standard formulas were used to calculate test performance of the Accuplex Lyme C6 multimer relative to the IFA reference method. A Chi-square test was used to determine sensitivity and specificity.

RESULTS

IFA-IgG antibodies directed against *B. burgdorferi* were detected in 61 of 415 serum samples resulting in a positivity rate of 14.7%. Antibodies directed against the Lyme C6 multimer were detected in 58 of the 415 serum samples resulting in a positivity rate of 14.0%. (Table 1). The sensitivity and specificity of the novel Accuplex Lyme C6 multimer assayed by BioCD are summarized in Table 2. The novel Accuplex C6 multimer has a sensitivity of 95.08% (86.29% to 98.97%) and specificity of 98.59% (96.73% to 99.54%). The percentage of samples that were identified as Lyme C6 positive is slightly higher than published data from the Companion Animal Parasite Council (CAPC) with regards to the seroprevalence of Lyme in the Northeastern United States. The slightly higher prevalence may reflect the exclusion of dogs with a known history of Lyme vaccination as well as samples positive for vaccine and/or acute exposure antibodies.[3]

Table 1

IFA VS. ACCUPLEX			
		IFA	
		Negative	Positive
ACCUPLEX	Negative	349	3
	Positive	5	58

Table 2

ACCUPLEX - C6 MULTIMER		
TEST	PERCENTAGE	95% CONFIDENCE INTERVAL
SENSITIVITY %	95.08%	86.29% to 98.97%
SPECIFICITY %	98.59%	96.73% to 99.54%

DISCUSSION

The prevalence of Lyme disease is increasing due to expansion in the geographic range of infected ticks, related to factors including climate change, bird migration, and wildlife movement. It is recommended that all dogs that live in, near, or travel to endemic areas be screened annually for antibodies directed against *B. burgdorferi*.[1] Although most dogs exposed to Lyme disease do not develop clinical signs, all dogs presenting with clinical signs potentially attributable to Lyme infection (Lyme arthritis and, less commonly, Lyme nephritis) should be screened as well. Benefits of identifying seropositive, clinically healthy animals are numerous including, follow-up screening for proteinuria, screening for other infections associated with tick exposure, identification of the need for tick prevention, and animal-human integrated zoonotic disease surveillance.[1] Antech Diagnostics newly designed Lyme C6 multimer simultaneously evaluates for antibodies to two C6 peptides found in the conserved invariable region 6 (IR6) within the borrelial surface protein VlsE. This region is highly immunogenic in the canine host and antibodies produced are indicative of natural infection or exposure. By excluding patients that were OspA or OspC positive or had a known history of Lyme vaccination, IFA positivity was considered indicative of natural exposure and was considered the best standard for this investigation. The Lyme C6 multimer showed excellent correlation to the Lyme IFA making Accuplex a reliable screening test for canine borreliosis.

CONCLUSION

The novel Accuplex Lyme C6 multimer demonstrated excellent sensitivity and specificity for the detection of antibodies directed against *B. burgdorferi* when compared to the IFA reference method.

REFERENCES

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