

Immune responses of 4321 European dogs to *Borrelia* antigens (2019 – 2021)

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Aim & Introduction

Our study aimed to evaluate the spectrum of immune responses (IgG) to a *Borrelia burgdorferi* line immunoassay (LIA) in the sera of European dogs, clinically suspected of *Borrelia burgdorferi* infection.

Among LIA antigens, C₆ peptide (C₆) represents the invariable sequence (IR₆) of the VlsE lipoprotein (VlsE/variable major protein-like sequence,

expressed. Both C₆ and VlsE antigens were described to be specific for active infection whereas they're absent in vaccinated dogs. OspC (outer-surface protein C) has been acknowledged as early marker of exposure/re-exposure in non-vaccinated dogs. OspC is detectable by 2 weeks after infection and wanes after 3-5 months without natural booster.

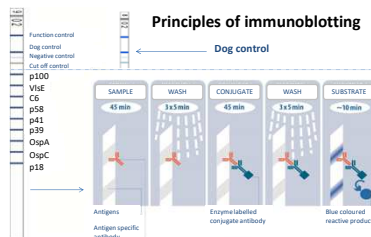
Antibodies against OspA (outer-surface protein A) are usually seen after vaccination.

Material & Methods

• Sera of 4321 dogs from 22 countries (80 % from Germany).

• **Borrelia burgdorferi** line immunoassay (Seramun, Germany) with 9 recombinant antigens: p18 (DbpA/ Decrin-binding protein A), p23 (OspC-mix), p31 (OspA-mix), p39 (BmpA/Borrelia membrane protein A), p41 (Flagellin), p58, C₆, VlsE (variable major protein-like sequence, expressed) and p83/100.

• **Evaluation criteria** according to directions supplied with the kit: **negative:** (≤ 1 band positive, except OspA, C₆/VlsE. **Positive:** (C₆+ and/or VlsE+ or > four other bands, OspA excluded. **Vaccinated:** OspA+ and/or together with ≤ 3 other bands, except VlsE/C₆. **Vaccination plus infection:** OspA+, OspC+ and/or VlsE+ or OspA+ plus ≥ four other bands. **Borderline:** 2-3 positive bands (excluding OspA, C₆/VlsE).



Results

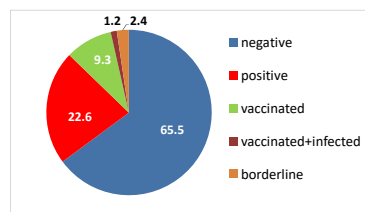
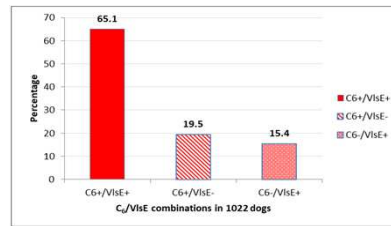


Fig. 1. Borrelia blot results (%) of 4321 dogs

Antibodies against	positive (n)	positive (%)	negative (n)	negative (%)
p18 (DbpA)*	582	13.5	3739	86.5
p23 (OspC-Mix)*	372	8.6	3949	91.4
p31 (OspA)**	458	10.6	3863	89.4
p39 (BmpA)**	551	12.8	3770	87.2
p41 (Flagellin)***	758	17.5	3563	82.5
P58****	620	14.3	3701	85.7
C ₆ peptide****	865	20	3456	80
VlsE**	822	19	3499	81
p83/100**	1380	31.9	2941	68.1

Origin of genospecies: *B. b. sensu stricto, B. garinii, B. afzelii, B. spielmanii; **B. afzelii; ***B. b. sensu stricto; ****B. garinii

Tab. 1. Frequencies of antibodies against individual proteins in 4321 dogs (n/%)



Consistent positive results to both infection indicating antigens (C₆+ / VlsE+) were 65.1 % (665/1022), while VlsE+ / C₆- was 15.4 % (157/1022) and C₆+ / VlsE- was 19.6 % (200/1022) (Fig. 2).

Fig. 2. Consistency of antibodies to both C₆ and VlsE proteins

Antibodies unique to OspC - an early marker of exposure - were found in 30 (0.4 %) dogs. 131 dogs (3 %) had antibodies to OspC but were negative for VlsE and C₆.

Antibodies to OspC combined with either VlsE+ or C₆+ were detected in 229 (5.3 %) dogs, indicating infection and/or re-exposure to the pathogen (Tab. 2).

OspC+/VlsE-/C ₆ - (n)	OspC+/VlsE+/C ₆ - (n)	OspC+/VlsE+/C ₆ + (n)	OspC+/VlsE-/C ₆ + (n)
131	12	217	12

Tab. 2. Positivities (number of dogs = n) of OspC in combination with VlsE and C₆

Dog breed	number (n)	VlsE+ (%)	C ₆ + (%)
Mix-breed	668	13.9	14.2
Labrador Retriever	323	13.9	16.1
Bernese Mountain dog	247	65.2	64
Golden Retriever	189	19	26.5
German Shepherd	144	13.9	10.4
Australian Shepherd	142	14.1	14.7
Border Collie	113	13.3	14.2

Tab. 3. Distribution of positivities (%) to VlsE or C₆ in breeds with > 100 individuals

Summary & Conclusions

- Immune responses to the infection-specific antigens VlsE and C₆ varied widely with inconsistent results in 35 % of the cases.
- Substantial, breed-specific differences were observed in anti-VlsE/C₆ responses, Bernese mountain dogs being the most highly represented breed in our sample population.
- Antibody responses uniquely to OspC were found in a small proportion of dogs, likely indicating very early infection in non-vaccinated dogs.
- Antibody responses to OspC in combination with VlsE/C₆ are likely to provide complementary information in assessing and staging (early) infection or re-infection by the pathogen.

Literature:

Gerber B, Eichenberger S, Wittenbrink M et al. Increased prevalence of *Borrelia burgdorferi* infections in Bernese Mountain Dogs: a possible breed predisposition. BMC Vet Res. 2007 Jul 12;3:15. doi: 10.1186/1746-6148-3-15. *** Littman NP, Gerber B, Goldstein RE et al. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med. 2018 May;32(3):887-903. doi: 10.1111/jvim.15085. *** Leschnik MW, Kirtz G, Khanakha G et al. Humoral immune response in dogs naturally infected with *Borrelia burgdorferi* sensu lato and in dogs after immunization with a *Borrelia* vaccine. Clin Vaccine Immunol. 2010 May;17(5):828-35. doi: 10.1128/01.00427.