Supplementary file 1: Table S1. Overview of total studies performed for completion of the prospective cohort study of 84 dogs in 4 age cohorts during 5 years and the study of 31 Bernese Mountain dogs presented for consultation in veterinary practice from 1990 to 2012.

Sequence Years	Part of study	Action	Workup	Results in brief	Reference
1 1990-1991	Enrollment of dogs in cohorts	Contact dog owners	Send letter of informed consent	4 age cohorts; 84 dogs in total	Hovius (1999); Hovius and Houwers (2007)
2 1990-1994	Quarterly clinical monitoring	Inspect dogs in veterinary cabinet	If appropriate, make diagnosis	Fever (n = 37) and lameness (n = 59)	Hovius and Houwers (2007)
3 1990-1994	Half-year serum sampling	Send letter or expect at veterinary cabinet	Check if the half-yearly visit is due	605 serum samples	Hovius (1999)
4 1990-1993	Evaluation tick infestation	Send yearly questionnaire	Collect ticks from skin of dogs	Score infestation intensity per year per dog	Hovius (1999)
5 1993	Evaluation tick infection	Collect ticks on mice and vegetation	Xenodiagnoses with PCR	High-density infected ticks on vegetation and mice	De Boer et al. (1993)
6 1993	Seasonal tick infestation	Collect ticks on dogs and vegetation	Collect ticks monthly for yearly distribution	90% of infestation in dogs during April-August	Hovius (1999)
7 1990-1994	Evaluate clinical monitoring	Make diagnosis for borreliosis or differential diagnosis.	Diagnose according to fever and lameness	14 dogs showing fever and lameness + 5 dogs showing malaise and lameness	Hovius and Houwers (2007); This study (Table 1)
8 1993, 1995	Perform IgG total whole cell ELISA	Deliver sera randomly, not in sequence	Sera, protocolized, performed at VMDC	High titers associated with fever and lameness	Hovius et al., (1999a); This study (Supplementary file 2)
9 1990-1998	*Borrelia* infection in ticks on dogs	Collect ticks feeding on the skin of dogs	PCR for *Borrelia* DNA in ticks	*B. burgdorfer*i (*s.s.*) most abundant	Hovius et al. (1998)
10 1995-1999	*Borrelia* infection in organs dog	Biopsies of skin and other organs of dogs	PCR for *Borrelia* of 4 clinical species	*B. burgdorfer*i (*s.s.*) mainly in skin and synovia	Hovius et al. (1999b)
11 1993-1996	Validation *Borrelia burgdorferi* (*s.s.*) whole cell ELISA	Compare with recomb Flagellin ELISA	Perform assay on neg. and other sera	*B. burgdorfer*i (*s.s.*) whole cell ELISA - no false positive results	Hovius et al. (1999a)
12 1998-2000	Different antibodies non-clinical dogs	SDS-PAGE blots with 4 *Borrelia* species	Assays with recombinant antibodies of 4 species	*B. burgdorfer*i (*s.s.*) specified antibodies in clinical not preclinical infection	Hovius et al. (2000)
13 1996-2000	Borreliacidal antibodyresponse	Test sera of clinical and healthy dogs	Immobilize different *Borrelia* with dog antibodies	3 *Borrelia* species-strains with different clinical dogs	Hovius et al. (2000)
14 1990-2012	Enrollment of 31 Bernese Mountain dogs (BMD)	Test sera for borreliacidal antibody response	Immobilize *B. afzelii* with BMD antibodies	60% of BMD persistent immobilization of *B. afzelii*	Hovius et al. (2000); This study
15 2004-2014	Retesting sera for C6 antibody	Employ IDEXX 4Dx in-house snap test	All sera cohort study and all BMD	60% of BMD persistent C6 antibody reaction	Hovius and Houwers (2007); This study
16 2011-2015	Test a subset for IgG1/G2 ELISA	Prepare antigen with RIVM VMDC AMC	Part sera of cohorts and of all BMD	Temporary positive in cohort, persistent in BMD	This study
17 2014	Test for *Borrelia* DNA in BMD	Biopsies sampled 1992–2012	Send 100 samples from 18 dogs	None of the samples (double tested) with *Borrelia* DNA	This study
18 2016	Time series of the 4 cohorts	Synchronize clinical and serology results per half-year	3.5 years (7 half-years) timeline	Alignment according to tick-seasons 1 to 12	This study (Supplementary file 2)

19 2018	Imputate missing values IgG1/IgG2	IgG1/IgG2 related to IgG T ELISA result	Place in timeline with clinical results	Alignment according to Low/High risk tick-season	This study (Supplementary file 1: Tables S2 and S3)
20 2019-2020	Explore and describe data	Calculate means per low risk-high-risk tick-season	Compare time trend IgG total with IgG1	Statistics (Fisher's exact test)	This study (Tables 2 and 3)
21 2020-2021	Evaluate IgG1 data in timeline	Calculate and graph low-risk and high-risk tick-season	Use only measured values (not imputed)	Statistics (Mixed Effect Analysis)	This study (Tables 4–6)
22 2020-2021	Compare clinical serology in time	Predict FL and MFL by serological variables	Use Supplementary files 2 and 3 including the imputation	Statistics (Generalized Estimating Equations)	This study (Table 7)
23 2020-2021	Explore BMD clinical serology	Predict FL by serological variables	Use Supplementary file 4 to compare serology	Statistics (Fisher's exact test)	This study (Supplementary file 4)
24 2020-2024	Write preliminary manuscript	Discuss hypothesis with co-authors	Experimental mice studies and IgG1	Total IgG and infection load; IgG1 indicates clearance	This study (Introduction and Discussion)
25 2022-2024	Write manuscript	Correct manuscript as indicated by authors	Make tables and graphs of results	Finalize manuscript with reference to earlier work	This study

Supplementary Tables S2 and S3

Calculation of imputed values for completing all half-year titers, shown in italics in Supplementary file 2. There were no missing data for clinical variables in the total of 588 consecutive half years; however approximately 25% of serological data were missing for total IgG ELISA and around 45% for IgG1 and IgG2 ELISA (mainly in cohorts 3 and 4). The frequency distribution and normality test of results of the three assays and their associations were used to determine the functions for imputing missing serological values (see *3.2. Serology: Total-IgG, IgG2 and IgG1 B. burgdorferi ss. whole cell ELISA titers*). The missing serological data were calculated separately for each cohort. Imputation of total-IgG ELISA values was done with the corresponding half-year mean, according to Supplementary Table S2 below.

Supplementary Table S2. Data used to calculate the imputed serological values. Imputation of total-IgG missing titer values derived from mean measured values per half-year. The mean value per sequential half-year for imputation was calculated together for the cohorts 1–3 (n = 67), and separately for cohort 4 (n = 17).

Half year	1	2	3	4	5	6	7
Total-IgG (Cohorts 1,2 & 3)	3	5	5	5	6	6	6
Total-IgG (Cohort 4)	5	6	6	7	6	7	7

IgG1 and IgG2, titers were imputed with the median of three clusters related to the total IgG titers 1 to 4; titers 5 and 6 and titers 7 and 9, derived from Fig 3B, according to the Supplementary Table S3 below.

Supplementary Table S3. Data used to calculate the imputed serological values of IgG1 and IgG2 missing titer values for each cohort separately. The median of the IgG1 and IgG2 titer values related to the three total-IgG titer clusters shown in Fig. 3B (see *Section 3.2*. Serology: Total IgG, IgG2 and IgG1 *B. burgdorferi* (*s.s.*) whole cell ELISA titers). Values of 1–4; 5 and 6; and 7–9 were used, separately per cohort. In Supplementary file 2, the results of imputed titer values are printed in italics-plain fonds in contrast with the measured titer values printed in straight-bold fonds.

IgG1/IgG2	Total-IgG 1–4	Total-IgG 5 and 6	Total-IgG 7–9
Cohort 1 IgG1/IgG2	<1 / 4	<1 / 5	1 / 6
Cohort 2 IgG1/IgG2	<1 / 4	<1 / 5	1 / 6
Cohort 3 IgG1/IgG2	<1 /4	1 / 5	2 / 6
Cohort 4 IgG1/IgG2	1 / 4	2 / 5	3 / 6

The directly measured and imputed serological variables were annotated for all studied 588 half years of the 84 dogs (see Supplementary file 2). Clinical signs were known for all quarter-years of the study period and had no missing values.

Supplementary Table S4. Extended Table 1 (columns 9 to 13): with detailed information on the diagnosis of the dogs from the prospective cohort study with a presumptive clinical diagnosis of Canine Lyme Disease (CLD), defined as fever and/or malaise followed by lameness.

1 2	3	4	5	6	7	8	9	10	11	12	13
Dog no. and breed	**Cohort no.**	**Tick-season (HR/LR) at clinic**	**Fever (°C)**	**Lameness**	**T-IgG and IgG1**		**Malaise and organ pathology**	**Possible differential diagnoses or concurrent morbidity**	**Workup FLb/MFLb**	**Discarding differential diagnosis**	**Confirmation of *Borrelia* infection**
					Clincal titers	**Max. titers**					
Fever and lameness in one quarter-year											
1 Alaskan Husky	1	4 LR	39.5	Stifle	6/<1	7/<1	Cough	Lumbar pain and strain	**FLb**	Yes	Exclusion dd
3 Labrador Retriever	1	3 LR	38.9	Stifle	5/<1	8/1	Urinary	Urinary gynecological	**FLb**	Yes	Exclusion dd
13 Labrador Retriever	1	5 LR	39.2	Stifle	7/3	7/3	Lameness	No strain or trauma	**FLb**	Yes	Exclusion dd
29 Golden Retriever	2	4 HR	40.0	Stifle	8/2	8/2	Skin, myocarditis	Gynecological and endocrine excluded	**FLb**	Yes	WB: Bb ss & Bg
40 Tatra Mountain Dog	2	2 LR	39.6	Elbow; stifle	7/2	8/2	Gastrointestinal; skin	Neck and back problems, prostatitis	**FLb**	Yes	Bactc.activ.: Bg /C6 WB: Bb ss & Bg
47 Scottish Terrier	3	5 HR	40.9	Stifle	7/<1	8/4	Gastrointestinal, urinary; skin	Stomatitis, helminth, nefritis, cystitis	**FLb**	Comorbidity	DNA Bb ss, Bb ss & Bg
48 Bernese Mountain Dog	3	4 LR	39.9	Stifle	8/7	8/8	Neuro; urinary	Anaplasmosis, prostatitis, liposarcoma	**FLb**	Yes	Bactc.activ.: Ba /C6 WB: Bb ss & Bg
58 Great Münsterlander	3	9 LR	40.6	Hind leg	7/<1	7/<1	Urinary	Malignant lymphoma liver	**FLb**	Comorbidity	DNA: Bb ss & Bg & Ba WB: Bb ss & Bg & Ba
63 Labrador Retriever	3	8 HR	39.6	Foot; stifle	8/1	8/1	Skin	Skin and nail problems	**FLb**	Yes	WB: Bb ss & Bg
72 Cross Labrador	4	9 LR	39.6	Stifle	8/4	8/4	Gastrointestinal; skin	HNP in youth, hepatitis, melanoma	**FLb**	Comorbidity	Bactc.activ.: Bg WB: Bb ss & Bg
75 Bouvier de Flanders	4	10 HR	39.5	Stifle	8/1	8/4	Urinary	Prostatitis and hip dysplasia	**FLb**	Yes	DNA: neg WB: Bb ss & Bg
78 Irish Terrier	4	13 HR	39.3	Stifle	8/2	8/2	Skin; neuro cardial	Stomatitis, nefritis, endocarditis, histiocytoma	**FLb**	Comorbidity	DNA: Bb ss WB: Bb ss
Fever and lameness in separate quarters											
77 Bernese Mountain Dog	4	12 LR	39.4	Stifle	8/3	8/3	Urinary	Stomatitis, hyperparathyroidism, nefritis	**MFLb**	Comorbidity	WB: Bb ss & Bg
83 Dachshund	4	13 LR	39.5	Stifle	5/2	8/3	Urinary; neuro cardial	Cushing dis., pyoderma, endocarditis	**MFLb**	Comorbidity	DNA: Bb ss & Bg & Ba; WB: Bg & Ba
Malaise and lameness in the same or different quarters											
14 Siberian Husky	1	3 LR	37.8	Elbow	8/<1	8/<1	Urinary; skin	Stomatitis, prediabetes, chronic cystitis	**MFLb**	Comorbidity	DNA: Bb ss & Bg/C6 WB: Bb ss & Bg Bactc.activ.: Bg
34 Bernese Mountain Dog	2	4 HR	38.5	Elbow	8/5	8/8	Gastrointestinal; skin	Stomatitis, prostatitis	MFLb	Yes	WB: Bb ss & Bg & Ba Bactc.activ.: Ba
51 Labrador Retriever	3	6 HR	38.6	Elbow	8/<1	8/4	Skin	Atopic dermatitis, hip dysplasia	**MFLb**	Comorbidity	Exclusion dd

74	Cross Labrador	4	11 HR	39.0	Hind leg	8/3	8/4	Hepatitis	Stomatitis, mastocytoma, hepatitis, hypothyroid	**MFLb**	Comorbidity	C6 antibody
79	Irish Terrier	4	12 HR	39.0	Stifle	8/1	8/1	Skin; urinary; cardial	Stomatitis, immune med. polyarthritis, endocarditis	**MFLb**	Comorbidity	DNA: Bb ss WB: Bb ss & Bg Bactc.activ.: Bg

Notes: Dogs from the prospective cohort study with a presumptive clinical diagnosis of Canine Lyme Disease (CLD), defined as fever or /and malaise followed by lameness. Column 1: dog number; Column 2: dog breed; Column 3: cohort number; Column 4: consecutive tick-seasons, in which the clinical signs, on which the presumptive clinical diagnoses, were made were observed. HR is High-risk tick-bite season (Q 2 and Q3), LR is low-risk tick-bite season (Q4 and the following Q1); Column 5: fever, defined as temperature ≥ 39.2 ˚C (dog 3 with a 0.3 °C lower being an exception); Column 6: affected joint or leg resulting in lameness; Column 7 shows the results of Total-IgG- and IgG1 whole cell ELISA titers observed concurrently with the clinical signs on which the clinical presumptive diagnoses was made; Column 8 shows the maximum observed Total-IgG- and IgG1 titers; Column 9: concurrent organ pathology's potentially also causing malaise; Column 10: concurrent morbidity and other diagnoses that in some cases may be the dominant clinical differential diagnoses that may compromise the immune system; Column 11: the differential diagnosis may not in all cases be excluded absolutely and therefore the presumptive diagnosis CLD is notated with different degrees of certainty: FLb (fever and lameness in the same quarter-year), MFLb (fever and lameness in separate quarter years, within one year or malaise and lameness in the same or separate quarter-years, within one year); Column 12: indicates three different states of the concurrent pathology, i.e. the differential diagnosis is completely discarded, the differential diagnosis is accepted, and the differential diagnosis is accepted as comorbidity supposedly immunocompromising the dog; Column 13: confirmation of *Borrelia* infection through other means than the high total IgG whole cell ELISA titer or IgG1 titer (columns 7 and 8): "DNA" indicates confirmation of infection by molecular detection of *Borrelia* species ("Bb ss" is *Borrelia burgdorferi* (*sensu stricto*), "Ba" is *Borrelia afzelii*, "Bg" is *Borrelia garinii*); "WB" signifies Western blots with mononuclear antibodies specifically detecting the indicated species; Bactc. active, signifies antibody mediated bactericidal activity of complement against the indicated species, abbreviated as for "DNA"; *Borrelia* specific antibody as determined with the in house IDEXX 4Dx Snap test.