Infections with *Babesia* spp. in dogs living in Germany (2007-2020)

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INTRODUCTION

Babesia (*B.*) spp. are vector-borne hematoprotozoal pathogens. Their distribution in Europe is linked to the occurrence of suitable vectors (Table 1).[1] Autochthonous infections with *B. canis* have been documented in dogs living in Germany[2,3,4,5,6,7], as have sporadic case reports of autochthonous *B. gibsoni* infections.[8] Infections with *B. vogeli* occur mainly in the Mediterranean area.[9]

Table 1: Occurrence of *Babesia* spp. in dogs in Europe

Pathogen	Vector	Distribution in Europe
Babesia canis	*Dermacentor reticulatus*	Western, Southern and Central Europe
Babesia vogeli	*Rhipicephalus sanguineus*	Southern Europe
Babesia (Theileria) annae	*Ixodes canisuga, (Ixodes hexagonus)*	Mainly Southern Europe
Babesia gibsoni, Babesia gibsoni-like	*Haemaphysalis* spp., *Dermacentor* spp.	Rare in Europe

AIMS OF THE STUDY

The aim of our study was to determine the number of dogs tested positive for *Babesia* spp. by PCR from 2007 to 2020 in a commercial laboratory. We further documented the presence or absence of any time spent abroad, blood work, tick infestation and ectoparasite prophylaxis.

MATERIAL AND METHODS

The database of the laboratory Laboklin (Bad Kissingen, Germany) was screened retrospectively for samples submitted by veterinarians in Germany for *Babesia* spp. PCR testing (18S rRNA-PCR with gel electrophoresis)[10] in dogs between January 2007 and December 2020. DNA isolated from samples with positive PCR results in this group from 2018-2019 was analyzed by 18S rRNA-[11] and Bc28.1-based PCR-RFLP[12] followed by cloning (Institute of Parasitology and Tropical Veterinary Medicine, FU Berlin, Germany) and sequencing (LGC Genomics GmbH, Berlin, Germany). Any available data on hematology and/or biochemistry results was gathered for this group. Information about time spent abroad, ectoparasite prophylaxis and tick infestation in the dogs tested from 2007-2020 was evaluated by means of written questionnaires completed by the treating veterinarians. All data presented in this poster were checked for normality by Kolmogorov-Smirnov-testing and suitable tests were chosen for calculation of statistical significance (Table 3). Odds ratios (OR) were calculated (Table 3). The statistical analysis was done via SPSS for Windows and $P < 0.05$ was stated as statistically significant using Fisher's exact test or Pearson's chi-squared test.

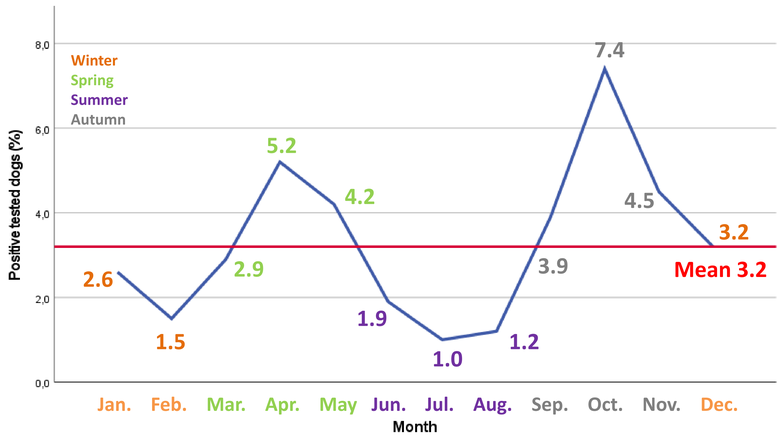

Figure 1: PCR-results of 20,914 dogs tested for *Babesia* spp. (659/20,914 [3.2%] tested positive) in the laboratory Laboklin (Bad Kissingen, Germany) from January 2007 to December 2020 ($P < 0.001$)

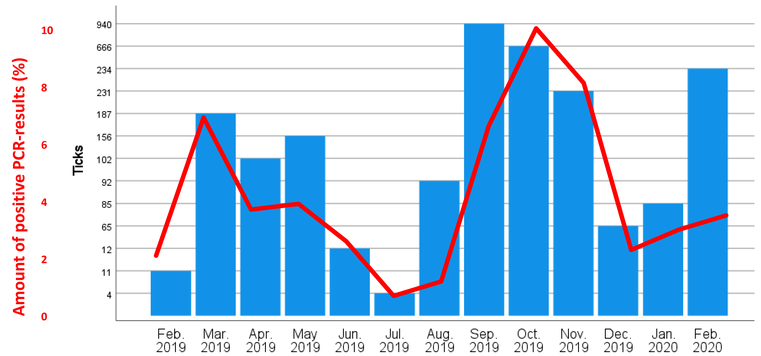

Figure 2: Number of collected *D. reticulatus* in Germany from 02/2019 to 02/2020 by Drehmann et al. (2020)[13] compared to amount of positive PCR-results in dogs tested for *Babesia* spp. (red line) in the same time-frame in the laboratory Laboklin (Bad Kissingen, Germany)

Table 2: Blood work in 80 dogs from Germany with complete blood count tested positive for *B. canis* by PCR, cloning and sequencing from 2018 to 2019 (Laboklin, Bad Kissingen, Germany) (CBC: *n* decreased [%] /*n* elevated [%])

	No stays abroad (*n*=23)	Travel (*n*=21)	Import (*n*=15)	Unknown (*n*=21)
WBC	12 (52) / 1 (4)	8 (38) / 5 (24)	3 (20) / 4 (27)	10 (48) / 4 (19)
HCT	20 (87) / 1 (4)	20 (95) / 0 (0)	13 (87) / 1 (7)	17 (81) / 1 (5)
THR	23 (100)/ 0 (0)	21 (100) / 0 (0)	10 (67) / 0(0)	18 (86) / 1 (5)
Bil ⇧	17/20 (85)	12/16 (75)	5/11 (45)	15/19 (79)
Crea ⇧	8/21 (38)	4/10 (40)	1/5 (20)	1/7 (14)
TP ⇩	12/21 (57)	9/16 (56)	6/11 (55)	4/19 (21)

WBC = White blood cell count, HCT = hematocrit, THR = platelet count, Bil = bilirubine, Crea = creatinine, TP = total protein

RESULTS

3.2% of 20,914 samples had positive PCR results for *Babesia* spp. Peaks were detected in April and October (Figure 1). DNA was available from 160 dogs, sequencing was possible in 152 dogs (95%). *B. canis* was identified by both PCRs in 141/152 dogs (92%). In 5 dogs, *B. canis* was detected by 18S rRNA only, as well as in 4 dogs *B. vogeli* (import Greece *n*=2, travel Austria/unknown *n*=1 each). In one dog each, *B. canis* (Bc28.1)/*B. vogeli* (18S rRNA) (unknown anamnesis) and *B. canis* (Bc28.1)/*B. gibsoni* (18S rRNA) were detected (import Romania). Questionnaires were available for 2,165 dogs (10%). In total, 905 dogs (48%) had never left Germany. PCR results were positive in dogs with (87/962, 9%) and without (62/905, 7%) stays abroad. Sex, seasonal distribution (comparing spring/autumn to summer/winter), tick infestation and ectoparasite prophylaxis had a statistically significant impact (Table 3). Pancytopenia occurred in 30/80 dogs tested positive for *B. canis* (38%; no stays abroad *n*=12 [52%], travel *n*=8 [38%], import *n*=2 [13%], unknown *n*=8 [38%], Table 2).

Table 3: Impact of selected conditions on *Babesia* spp. PCR test results

	N dogs	Odds ratio	*P*
Seasonal distribution	20,914	3.025	$P < 0.001$[1]
Stays abroad[A]	1,867	1.65 (stays abroad)	$P = 0.088$[1]
Sex	19,348	1.45 (male dogs)	$P < 0.001$[2]
Tick attachment[A]	868	7.62 (with ticks)	$P < 0.001$[1]
Ectoparasite prophylaxis[A]	770	3.02 (without prophylaxis)	$P = 0.001$[1]

[A]Dogs with unknown data were excluded from OR-calculation due to their high amount
[1]Fisher's exact test, [2]Pearson's chi-squared test

DISCUSSION AND CONCLUSIONS

In most dogs tested positive from 2018-2019 (95%), infections with *B. canis* were identified. In each of the dogs tested positive for *B. canis/B. vogeli* and *B. canis/B. gibsoni*, most likely coinfections were present. The high incidence of positive *Babesia* spp.-PCR testing correlates with high local activity of *D. reticulatus*[13] (Figure 2). Infections were more significantly observed in male dogs, dogs without ectoparasite prophylaxis, dogs with observed tick infestation and the known activity periods of *D. reticulatus*. Travel history and import are considered prominent sources of infection in Germany, but autochthonous infections with *B. canis* apparently occur in considerable numbers. The hematological and biochemical abnormalities in dogs tested positive for *B. canis* from 2018-2019 are consistent with literature data.[9]

Limitations of this study include lack of availability of clinical and background data, as only 10% of questionnaires were answered. The time between import or travel and PCR testing for *Babesia* spp. was not evaluated.

Possible links to changes in climate but also to changes in land use creating *D. reticulatus* habitats, increasing import of dogs from abroad and travel within Europe should be investigated further.

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