

## INTRODUCTION

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

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

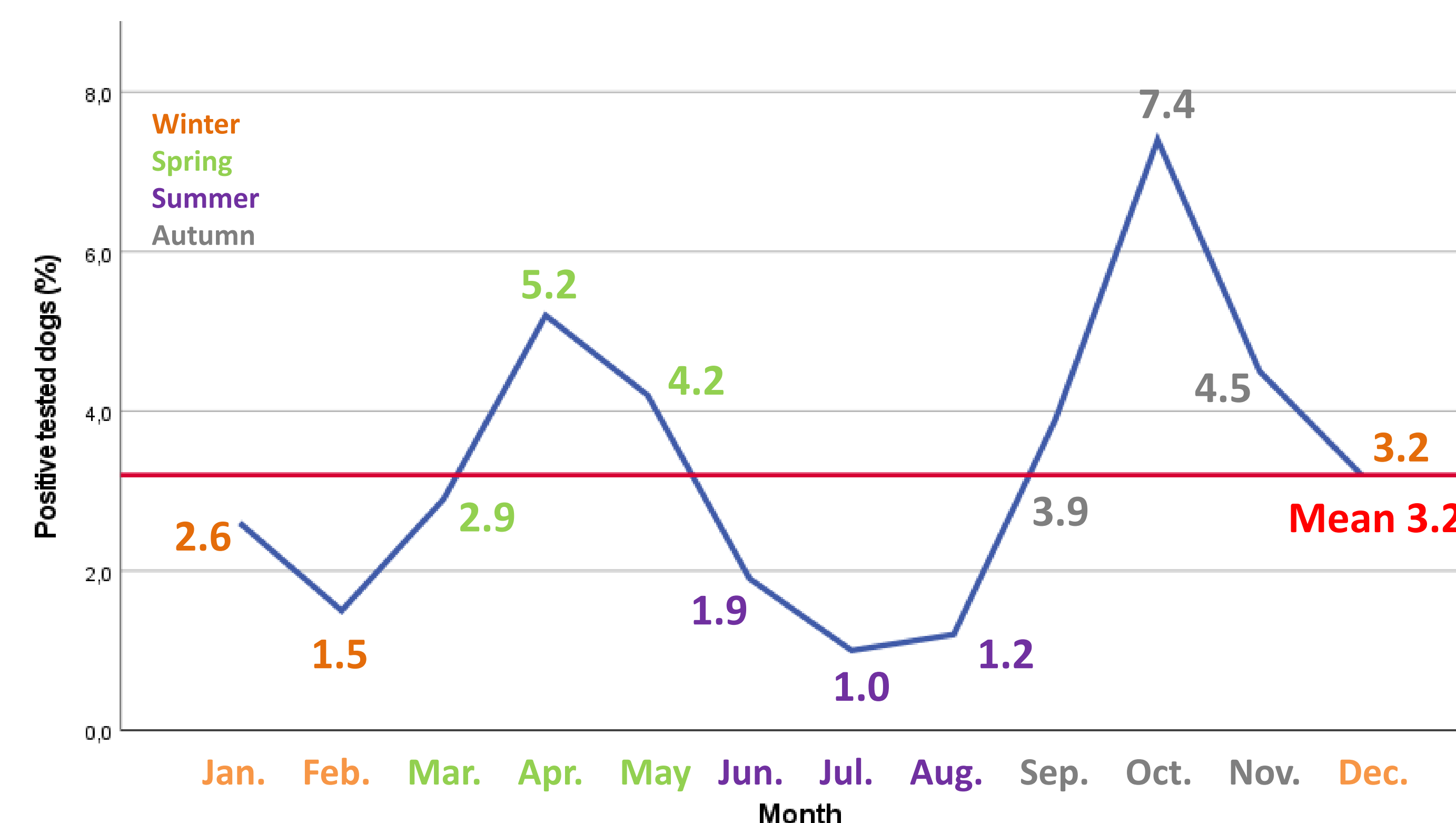
| Pathogen   | Vector  | Distribution in Europe               |
|--|---|--------------------------------------|
| <i>Babesia canis</i>                                     | <i>Dermacentor reticulatus</i>                          | Western, Southern and Central Europe |
| <i>Babesia vogeli</i>       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

## AIMS OF THE STUDY

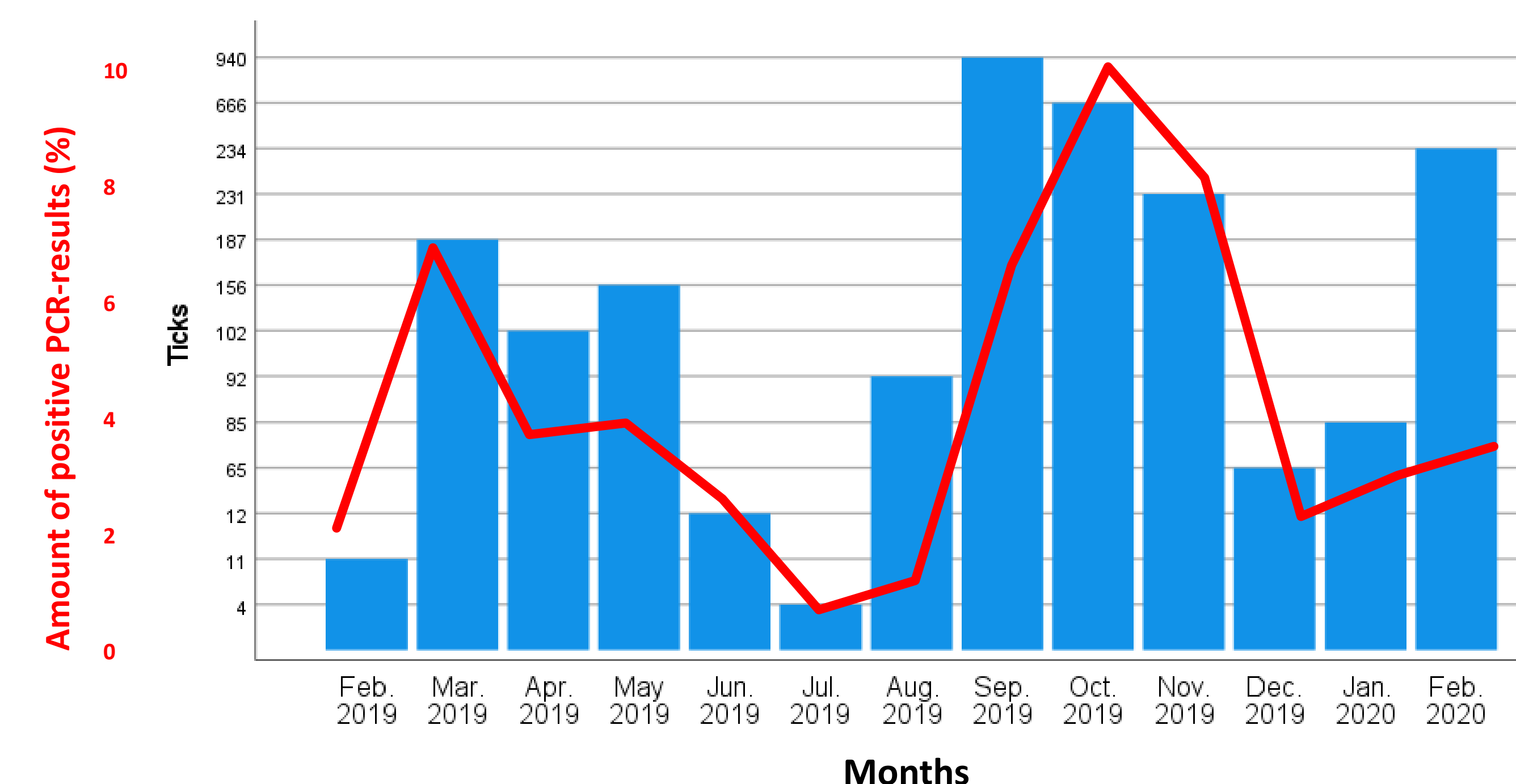
The aim of our study was to determine the number of dogs tested positive for *Babesia* spp. by PCR from 2007 to 2020 in a commercial laboratory. We further documented the presence or absence of any time spent abroad, blood work, tick infestation and ectoparasite prophylaxis.

## 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)<sup>10</sup> 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-<sup>11</sup> and Bc28.1-based PCR-RFLP<sup>12</sup> 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)<sup>13</sup> 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 <sup>A</sup>             | 1,867  | 1.65 (stays abroad)        | $P = 0.088^1$ |
| Sex                                   | 19,348 | 1.45 (male dogs)           | $P < 0.001^2$ |
| Tick attachment <sup>A</sup>          | 868    | 7.62 (with ticks)          | $P < 0.001^1$ |
| Ectoparasite prophylaxis <sup>A</sup> | 770    | 3.02 (without prophylaxis) | $P = 0.001^1$ |

<sup>A</sup>Dogs with unknown data were excluded from OR-calculation due to their high amount  
<sup>1</sup>Fisher's exact test, <sup>2</sup>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*<sup>13</sup> (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.<sup>9</sup> 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.

## REFERENCES

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