

Molecular detection of *Hepatozoon* species in domestic cats living in Germany

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INTRODUCTION

Hepatozoon (*H.*) species are protozoal, apicomplexan vector-borne infectious pathogens transmitted by blood-feeding arthropods to mammals, birds, reptiles and amphibians.¹⁻⁴ Three *Hepatozoon* species (*H. felis*, *H. canis* and *H. silvestris*) are known to infect cats in Europe. First reports of autochthonous infections in central Europe were recently published, including infections with *H. silvestris* in Switzerland⁵ and *H. felis* in Austria⁶.

AIMS OF THE STUDY

The objective of this study was to determine the prevalence of *Hepatozoon* species in samples from cats living in Germany.

MATERIAL AND METHODS

The study included cats tested for *Hepatozoon* species by PCR between 2007 and 2020 by the LABOKLIN laboratory. Coinfections with other vector-borne infectious agents were considered in cats tested by a "Feline Travel Profile" (Figure 3). From 2018 onwards, a partial 18S rRNA *Hepatozoon* gene fragment was sequenced from cats with positive PCR results. Data were checked for normal distribution by Kolmogorov-Smirnov test. Mann-Whitney-U-test was used to calculate statistical significance ($P < 0.05$) in non-normally distributed data. Veterinarians were contacted about the travel history of cats that tested positive.

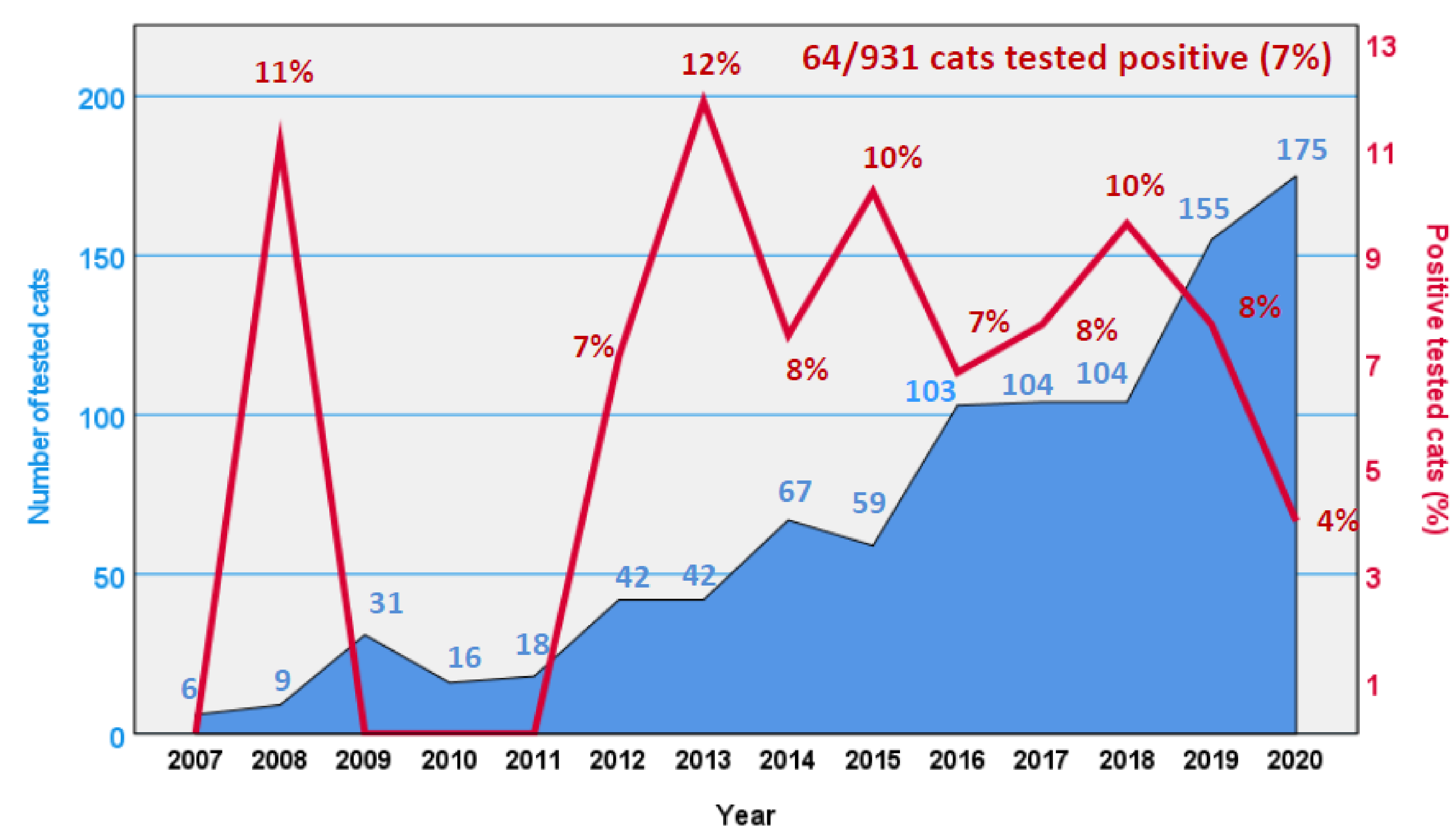


Figure 1: Results of the *Hepatozoon* species PCR in cats at Laboklin (Bad Kissingen, Germany) from 2007 to 2020

Table 1: Age distribution in cats tested by *Hepatozoon* species PCR at Laboklin from 2007 to 2020 [Data are n (%)]

Age	N tested cats	N negative tested cats	N positive tested cats
Kitten (≤ 6 months)	34 (4.3)	27 (79.4)	7 (20.6)
Junior (> 6 months to 3 years)	450 (57.3)	418 (92.9)	32 (7.1)
Adult ($> 3 - 7$ years)	140 (17.8)	128 (91.4)	12 (8.6)
Mature ($> 7 - 10$ years)	90 (11.5)	85 (94.4)	5 (5.6)
Senior ($> 11 - 14$ years)	54 (6.9)	52 (96.3)	2 (3.7)
Geriatric (≥ 15 years)	18 (2.3)	18 (100)	0 (0)
Unknown	145 (15.6)	139 (95.9)	6 (4.1)
Total	931 (100)	867 (93.1)	64 (6.9)

RESULTS

In total, 64 out of 931 cats (6.9%) tested positive for *Hepatozoon* species by PCR (Figure 1). There were no statistically significant differences regarding PCR test results and years ($U = 27018.00$, $Z = -0.353$, $P = 0.724$), sex ($U = 27501.00$, $Z = -0.123$, $P = 0.902$) or age group ($U = 18200.00$, $Z = -1.951$, $P = 0.051$). Cats aged < 3 years most frequently tested positive (Table 2). Forty-seven out of the 64 positive cats (73.4%) were imported from abroad (Figure 1), travel history was not available for the remainder of 17 cats (Figure 2). Fifty-two positive tested cats were screened by a "Feline Travel Profile", out of which 10 tested positive for additional vector-borne pathogens (Figure 3). The 18S rRNA gene of 16 samples was sequenced, showing 98.7-99.7% identity to *H. felis* from Spain (Accession Number AY620232).

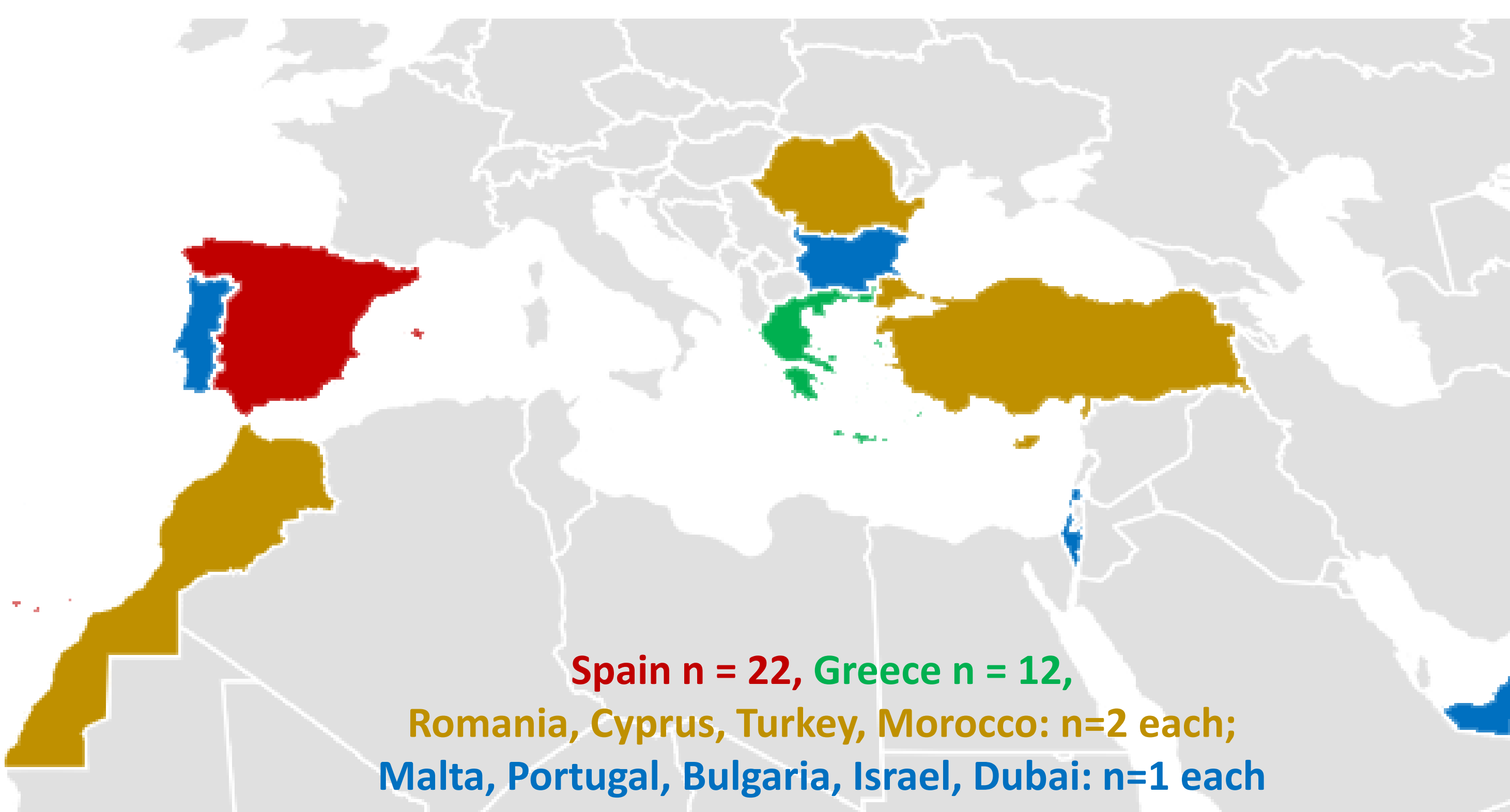


Figure 2: Travel history of 47/64 cats (73.4%) tested positive for *Hepatozoon* species by PCR

Discussion

Sixty-four out of 931 cats (6.9%) tested positive for *Hepatozoon* species. The incidence of infected cats appears to have increased since 2012, although no statistically significant differences were observed. This finding may be linked to the introduction of a so-called 'Feline travel profile' offered by LABOKLIN from April 2012 onwards. Other possibilities are a rising awareness of feline vector-borne pathogens as potential differential diagnoses or an increase in the number of imported cats. A relatively high number of positive tested cats were younger than 6 months of age (Table 1), which may suggest potential vertical transmission from queens to litters. The proof of autochthonous infection in Germany is missing, while still a possible cause for animals lacking complete history. All isolates were identified as *H. felis*. While there are reports that foxes in Germany tested positive for *H. canis* with genotypes typically found in dogs⁷, there do not seem to be records of infections in cats. Further investigation concerning possibility of autochthonous infections in cats with *H. felis* and *H. silvestris* respectively are needed.

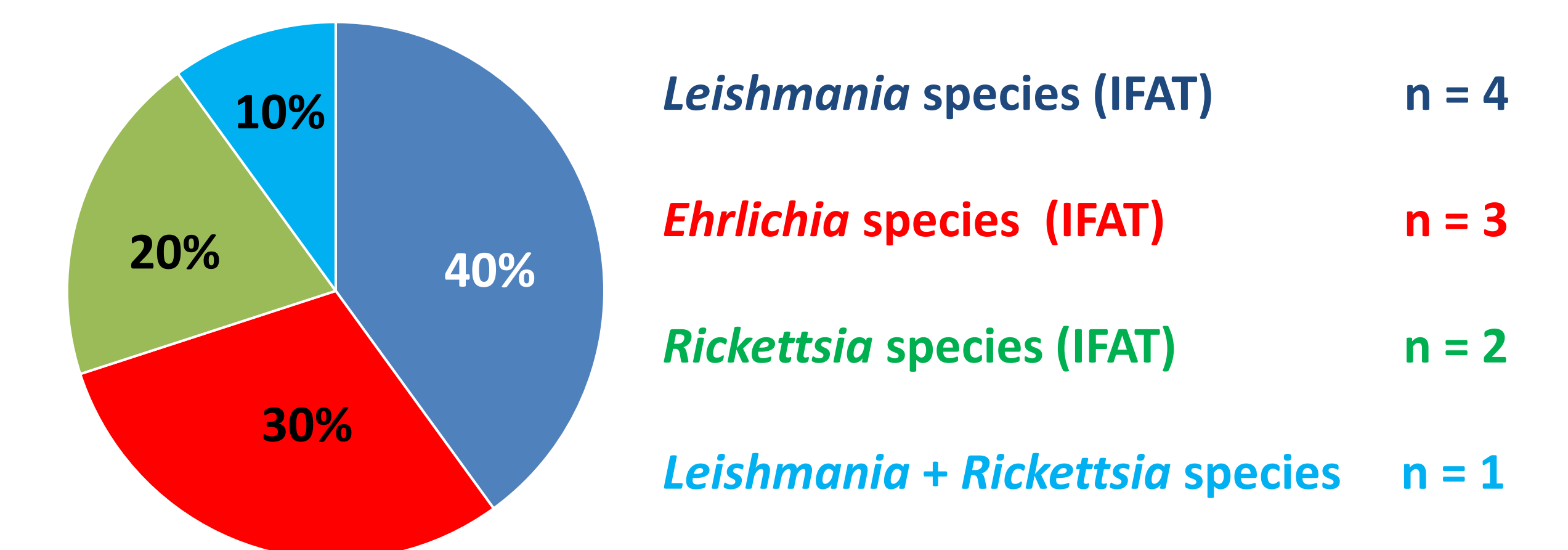


Figure 3: Serological coinfections in 10 cats tested positive by *Hepatozoon* species PCR

Limitations

The limitations of the study are associated with its retrospective study design. Owing to limited data availability, it was not possible to consider reasons for PCR testing (e. g. screening or clinical signs, travel or import history).

Conclusion

The pathogenesis of feline hepatozoonosis is not yet clear. Owing to a positive association between an outdoor lifestyle and *Hepatozoon* infection, blood-sucking arthropods are suspected vectors. In addition, transplacental infections may also play an important role. The epidemiology of *Hepatozoon* species infections in cats in central Europe requires further monitoring. Owing to the relatively high number of cats infected with *Hepatozoon* species in some regions, imported cats from endemic countries should be screened for vector-borne pathogens.

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