Retrospective evaluation of vector-borne pathogens in cats living in Germany (2012-2020)

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Research

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Abstract

Background: Blood-feeding arthropods can transmit parasitic, bacterial, or viral pathogens to domestic animals and wildlife. Vector-borne infections are gaining significance due to increasing travel and import of pets from abroad, as well as the changing climate in Europe. The main objective of this study was to assess the percentage of cats with positive test results for selected vector-borne pathogens in Germany and explore any possible association of such results with time spent abroad.

Methods: This retrospective study included test results from cats for which a 'Feline Travel Profile' established by the laboratory LABOKLIN had been requested by veterinarians in Germany between April 2012 and March 2020. This above-mentioned diagnostic panel includes the direct detection of *Hepatozoon* spp. and *Dirofilaria* spp. *via* PCR as well as indirect detection assays (IFAT) for *Ehrlichia* spp. and *Leishmania* spp. The panel was expanded to include an IFAT for *Rickettsia* spp. from July 2015 onwards.

Results: A total of 624 cats were tested using the "Feline Travel Profile". Serum for indirect detection assays was available for all 624 cats, EDTA-samples for direct detection methods were available from 618 cats. Positive test results were as follows: *Ehrlichia* spp. IFAT 73 out of 624 (12%), *Leishmania* spp. IFAT 22 out of 624 (4%), *Hepatozoon* spp. PCR 53 out of 618 (9%), *Dirofilaria* spp. PCR 1 out of 618 cats (0.2%) and, *Rickettsia* spp. IFAT 52 out of 467 cats (11%) tested from July 2015 onwards. Three cats had positive test results for more than one pathogen before 2015. After testing for *Rickettsia* spp. was included in 2015, 19 cats had positive test results for more than one pathogen (*Rickettsia* spp. were involved in 14 out of these 19 cats).

Conclusions: At least one pathogen could be detected in 175 out of 624 cats (28%) *via* indirect and/or direct detection methods. Four percent had positive test results for more than one pathogen. This data emphasizes the importance of considering the above-mentioned vector-borne infections as potential differential diagnoses in clinically symptomatic cats.

Introduction

Cats are at a high risk of coming in contact with blood-feeding arthropods such as fleas, ticks, or mosquitoes, especially outdoor or stray cats without ectoparasite prophylaxis [1, 2]. These vectors can transmit parasitic, bacterial, or viral pathogens, which may subsequently cause infection in competent hosts like cats. The patterns of occurrence of feline infectious agents are influenced largely by the distribution of transmitting vectors, e.g. the regional distribution of *Leishmania* spp. is associated with the occurrence of phlebotomine sand flies in the Mediterranean and South-Eastern Europe [3]. *Hepatozoon* spp. are transmitted by various blood-feeding arthropods worldwide, including ticks, mites, sandflies, tsetse flies, lice, kissing bugs, and leeches [4, 5]. Infections with *H. felis* or, less frequently, *H. canis* and *H. silvestris* have been detected in cats in the Mediterranean and South-Eastern Europe [4-8]. There are also single case reports of infections with *H. felis* in Austria [9] and *H. silvestris* in Switzerland [8]. *Dirofilaria* spp. are transmitted by mosquitoes. In cats, *D. immitis* is an established pathogenic species, whereas *D. repens* is known to be a cause of subclinical dirofilariasis [2, 10]. While these pathogens also generally occur within the Mediterranean and South-Eastern Europe, there has been one case report of a cat infected with *D. repens* in Poland [11]. *Rickettsia felis* has been detected in fleas in Germany [12], and as such autochthonous infections in cats in Germany are possible. Other documented vector-borne pathogens affecting cats in Europe include helminths (*Thelazia callipaeda*, *Dipylidium caninum*), bacteria (*Bartonella* spp., *Haemoplasma* spp., *Borrelia burgdorferi* complex, *Anaplasma (A.) phagocytophilum*, *A. platys*, *Coxiella burnetii*, *Francisella tularensis*), protozoa (*Babesia* spp., *Cytauxzoon* spp.) as well as viruses, namely *Flaviviridae* [2].

Among the pathogens examined in this study, *Rickettsia* spp., *Leishmania* spp., and *Dirofilaria* spp. have zoonotic potential and are consequently of importance for public health in Europe [2]. To the knowledge of the authors, there are presently no studies regarding the prevalence of antigens and/or antibodies to the vector-borne pathogens *Leishmania* spp., *Ehrlichia* spp, *Rickettsia* spp., *Dirofilaria* spp. and *Hepatozoon* spp. in cats in Germany. The aims of this study were to determine the percentage of positive test results for these vector-borne pathogens in cats for which samples were provided by veterinarians

in Germany to the veterinary laboratory (Bad Kissingen, Germany), and to determine if positive results were associated with a background history of time spent abroad.

Methods

This study included any “Feline Travel Profile” results of samples provided by veterinarians in Germany between April of 2012 and March of 2020. The panel includes a direct assay by polymerase chain reaction (PCR) of *Hepatozoon* spp. (TaqMan® realtime PCR, target: 18S rRNA) and *Dirofilaria* spp. (based on Rishniw et al., 2006 [13]). Furthermore, it includes immunofluorescence antibody testing (IFAT) for *Ehrlichia* spp. (MegaFLUO® EHRLICHIA canis, MegaCor Diagnostik GmbH, Hörbranz, Austria; ≥ 1:40 positive), and *Leishmania* spp. (MegaFLUO® LEISH, MegaCor Diagnostik GmbH, Hörbranz, Austria; > 1:64 positive), as well as *Rickettsia* spp. (RICKETTSIA CONORII IFA SLIDE, Viracell, Granada, Spain; > 1:128 positive) from July 2015 onwards (table 1). Where possible, information on time spent abroad was collected in questionnaires and telephone calls to the treating veterinarians. A descriptive statistical analysis of the data collected was made using SPSS for Windows (Version 27.0, SPSS Inc., Armonk, USA).

Table 1: Results of the diagnostic panel “Feline Travel Profile” as performed by the laboratory LABOKLIN (Bad Kissingen, Germany) in 624 cats from 04/2012 till 03/2020)

Time-Period	Total n/N (%)	Hepatozoon spp.[1,A] n/N (%)	Dirofilaria spp.[2,A] n/N (%)	Ehrlichia spp.[3] n/N (%)	Leishmania spp. [4] n/N (%)	Rickettsia spp.[5,B] n/N (%)
04/2012-03/2013	6/30 (20)	2/30 (7)	1/30 (3)	1/30 (3)	3/30 (10)	-
04/2013-03/2014	15/47 (31.9)	8/47 (17)	0/47 (0)	6/47 (13)	2/47 (4)	-
04/2014-03/2015	9/67 (13.4)	3/67 (5)	0/67 (0)	6/67 (9)	1/67 (2)	-
04/2015-03/2016	12/58 (20.7)	6/58 (10)	0/58 (0)	2/58 (3)	2/58 (3)	3/45 (7)
04/2016-03/2017	19/87 (21.8)	6/84 (7)	0/84 (0)	3/87 (3)	2/87 (2)	11/87 (13)
04/2017-03/2018	33/99 (33.3)	8/98 (8)	0/98 (0)	10/99 (10)	1/99 (1)	14/99 (14)
04/2018-03/2019	44/98 (44.9)	8/96 (8)	0/96 (0)	22/98 (22)	8/98 (8)	21/98 (21)
04/2019-03/2020	37/138 (26.8)	12/138 (9)	0/138	23/138 (17)	3/138 (2)	3/138 (2)
Total	**175/624 (28)**	**53/618 (9)**	**1/618 (0.2)**	**73/624 (12)**	**22/624 (4)**	**52/467 (11)**

[1] Polymerase Chain Reaction (PCR), TaqMan® realtime PCR, target: 18S rRNA

[2] PCR, based on Rishniw *et al.*, 2006

[3] Immunoflourescent antibody test (IFAT), MegaFLUO® EHRLICHIA canis (MegaCor Diagnostik GmbH, Hörbranz, Austria; ≥ 1:40 positive)

[4] IFAT, MegaFLUO® LEISH (MegaCor Diagnostik GmbH, Hörbranz, Austria; › 1:64 positive)

[5] IFAT, RICKETTSIA CONORII IFA SLIDE (Viracell, Granada, Spain; > 1:128 positive)

[A] EDTA blood for PCR was not provided for 6/624 cats

[B] Testing for *Rickettsia* spp. was performed from 07/2015 onwards

Results

Signalment and stays abroad

Six hundred and twenty-four cats were included in this study. Information on the breed was provided for 554/624 cats (89%). There were 20 different breeds of cats, predominantly European Shorthairs (423/554 cats, 76%) as well as mixed breeds (71/554 cats, 13%) and Siamese cats (17/554 cats, 3%). The sex of the animal was indicated for 573/624 cats (92%); of these, 308/573 cats (54%) were male, while 265/573 cats (46%) were female. The age of the animal was known in 536/624 cases (86%), of which the median age was 2 years (mean: 3.53 years; range: 0.2 – 18 years).

Information on time spent abroad was either unavailable or could not be requested retrospectively for 253/624 cats (41%). Eight out of 624 cats (1%) were born in Germany and never travelled. A travel history was available for 363/624 cats (58%). This included 29 countries, of which Spain (158/363 cats, 44%), Greece (53/363 cats, 15%), and Romania (33/363 cats, 9%) were most frequently named (table 2). Among this group of cats, 356/363 (98%) were imported to Germany from abroad, of which 38 cats were imported by animal rescue organisations and 15 cats were imported by private individuals after a holiday. One cat was imported from France and subsequently travelled to Turkey every year with its owner. Six of the 363 cats (2%) were born in Germany and accompanied their owners on vacation abroad, during which they would be allowed to roam freely in the respective foreign country (Spain, n=2; France/Italy/Romania/Bosnia each n=1).

Laboratory diagnostics

Results from 2951 direct and indirect detection assays on samples from 624 cats were evaluated. PCR testing was performed on samples from 618/624 cats (99.9%), for both *Hepatozoon* spp. and *Dirofilaria* spp. For 6/624 cats (0.1%), no EDTA blood was provided for analysis. Indirect testing *via* IFAT for *Ehrlichia* spp. and *Leishmania* spp. was performed for all 624 cats. After the addition of a *Rickettsia* spp. IFAT to the “Feline Travel Profile” in July 2015, 467/624 cats (75%) were also tested for this pathogen.

One hundred and seventy-five out of 624 cats (28%) had positive test results for at least one of the pathogens (table 1). PCR testing was reported as positive for *Hepatozoon* spp. in 53/618 cats (9%) and for *Dirofilaria* spp. in 1/618 cats (0.2%). IFAT testing showed the following: 73/624 cats (12%) had positive serology for *Ehrlichia* spp., 52/467 cats (11%) for *Rickettsia* spp., and 22/624 cats (4%) for *Leishmania* spp. For *Ehrlichia* spp. serology, titres of 1:40 (n=44), 1:320 (n=24) and 1:640 (n=5) were detected. Antibodies to *Rickettsia* spp. were found in 52 cats, with titres of 1:256 (n=33), 1:512 (n=14) and 1:1024 (n=5). The 22 cats with antibodies to *Leishmania* spp. had titres of 1:128 (n=14), 1:256 (n=3), 1:512 (n=4) and 1:1024 (n=1).

In 22/624 cats (4%), more than one pathogen was found by direct and/or indirect detection methods. This group includes three cats (14%) with positive test results prior to the addition of *Rickettsia* spp. IFAT to the “Feline Travel Profile” (*Leishmania* spp. IFAT/*Dirofilaria* spp. PCR, *Leishmania* spp. IFAT/*Hepatozoon* spp. PCR and *Leishmania*/*Ehrlichia* spp. IFAT) in July of 2015, and 19/22 cats (86%) after this addition. *Rickettsia* spp. were implicated in 14 of these 19 cats (74%). Overall, 19 cats had two concurrent positive test results for different pathogens (*Ehrlichia/Rickettsia* spp. IFAT [n=6]; *Leishmania/Rickettsia* spp. IFAT and *Leishmania* spp. IFAT*/Hepatozoon* spp. PCR [n=3, respectively]; *Rickettsia* spp. IFAT*/Hepatozoon* spp. PCR, *Ehrlichia* spp. IFAT/*Hepatozoon* spp. PCR, and *Ehrlichia/Leishmania* spp. IFAT [n=2, respectively], as well as *Leishmania* spp. IFAT*/Dirofilaria* spp. PCR [n=1]). Three cats had simultaneous positive test results for three pathogens (*Ehrlichia/Leishmania/Rickettsia* spp. IFAT [n=2], *Leishmania* spp. IFAT*/Rickettsia* spp. IFAT*/Hepatozoon* spp. PCR [n=1]).

Among the 363 cats with a history of time spent abroad, 110 (30%) had positive test results for at least one vector-borne pathogen. Three hundred and twenty of the 363 cats (88%) had been to a different country in the European Union and 44 (12%) had stayed in countries outside the European Union (primarily Turkey [n=12] and Dubai [n=5]) (table 2). One cat had been imported from France and subsequently accompanied its owner to Turkey every year, and it was thus included in both categories. Six cats were born in Germany and accompanied their owners on travels abroad, but all had entirely negative test results in this study. Test results were positive for more than 2-3 pathogens in 10/363 cats (3%), the majority of which had returned or come from Spain (n=5) and Greece (n=2).

There was a negative travel history in 8/624 cats (1%) tested by the “Feline Travel Profile”. Four of these eight cats had antibodies for *Rickettsia* spp.

Table 2: Positive test results in 363 cats with known stays abroad and introduction of the “Feline Travel Profile” diagnostic panel from 04/2012 up until (and including) 03/2020 in the laboratory LABOKLIN (Bad Kissingen, Germany)

Country	N	N tested positive /N total (%)	*Hepatozoon* spp.[1]	*Dirofilaria* spp.[2]	*Ehrlichia* spp.[3]	*Rickettsia* spp.[A, 4]	*Leishmania* spp.[5]	Stays abroad
Countries of the European Union								
Spain	158	51/158 (32)	19	-	21	11	5	131 imports, 20 animal welfare imports, 5 imports after holidays, 2 holidays
Greece	52	17/52 (33)	8	-	7	2	2	44 imports, 6 animal welfare imports, 2 imports after holidays
Romania	28	8/28 (29)	1	-	2	5	1	26 imports, 1 animal welfare imports, 1 holiday
Bulgaria	25	7/25 (28)	1	-	5	1	-	18 imports, 6 animal welfare imports, 1 import after holidays
Italy	23	3/23 (13)	-	-	-	3	-	20 imports, 1 import after holidays, 1 animal welfare import, 1 holiday
Croatia	15	3/15 (20)	-	-	2	1	-	11 imports, 4 imports after holidays
Portugal	9	2/9 (22)	1	-	1	-	-	8 imports, 1 animal welfare import
France	4[B]	0/4 (0)	-	-	-	-	-	3 imports[A]. 1 holiday
Cyprus	3	2/3 (67)	1	-	1	-	-	2 imports, 1 animal welfare imports
Malta	2	2/2 (100)	1	-	1	-	-	2 imports
Slovenia	1	0/1 (0)	-	-	-	-	-	1 import
Total EU	**320[B]**	**95/320 (30)**	**32**	**-**	**40**	**23**	**8**	**266 imports, 36**

								animal welfare imports, 13 imports after holidays, 5 holidays
Total Non-EU	44[A]	15/44 (34)	7	-	4	3	4	39 imports, 2 animal welfare imports, 2 imports after holidays, 1 holiday

[A] *Rickettsia* spp. IFAT has been added to the "Feline Travel Profil" from 07/2015 onwards.

[B] One cat which tested negative was imported from France and subsequently travelled to Turkey every year with its owner.

[1] Polymerase Chain Reaction (PCR), TaqMan® realtime PCR, target: 18S rRNA

[2] PCR, based on Rishniw et al., 2006

[3] Immunoflourescent antibody test (IFAT), MegaFLUO® EHRLICHIA canis (MegaCor Diagnostik GmbH, Hörbranz, Austria; ≥ 1:40 positive)

[4] IFAT, RICKETTSIA CONORII IFA SLIDE (Viracell, Granada, Spain; > 1:128 positive)

[5] IFAT, MegaFLUO® LEISH (MegaCor Diagnostik GmbH, Hör

Discussion

This study investigated 624 cats in Germany for the presence of *Hepatozoon* spp. and *Dirofilaria* spp. *via* direct detection methods as well as for the presence of antibodies against *Ehrlichia* spp., *Rickettsia* spp. and *Leishmania* spp. *via* indirect detection methods. A background history was available for 371 cats, the majority of which had either been imported or had spent time outside of Germany (363/371 cats, 98%). These numbers can be attributed to the fact that the testing panel used as the basis for this study to detect different vector-borne pathogens is offered as a "Feline Travel Profile" to veterinarians. The majority of the 363 cats with a known background history of time spent abroad had done so in other European countries (88%), but several non-European countries were also implicated (22%, table 2). Spain (n=158) and Greece (n=52) were most commonly involved, and many of the cats with a background history implicating either one of these countries had positive test results (Spain: 32%, Greece: 33%). Imports by animal welfare organizations may play a significant role for both these countries (Spain: 20 animal welfare imports, Greece 6 animal welfare imports). Similarly, 6 of 25 cats that had spent time in Bulgaria were imported to Germany by animal welfare organizations (table 2). The number of imported cats greatly outweighs that of cats accompanying their owners' travels, which contrasts with the findings of previous studies in dogs [14, 15]. The rising numbers of cats tested between 2012 and 2020 (table 1) may indicate that the import of cats is gaining importance in Germany. Together with the change in climate in many parts of Europe, this could contribute to an increase in the spread of pathogens and their potential vectors into previously non-endemic areas such as Germany, where they may spread further and form reservoirs for infection. Under suitable conditions, pathogens transmitted by imported vectors may cause infection in competent hosts endemic to Germany, of which cats are only one example. Moreover, endemic vectors which are potentially competent may be infected with previously non-endemic pathogens during a blood meal on infected cats and could proceed to contribute to the spread of these pathogens. One example of this phenomenon are presently isolated cases of autochthonous infections with *D. repens* [16-18] and *L. infantum* [19] in dogs in Germany.

To the knowledge of the authors, the prevalence of many vector-borne infectious pathogens in cats in Germany is still unknown, as for example for *Hepatozoon* spp. In this study, 9% of the cats tested for this pathogen had positive PCR results.

Direct detection methods demonstrate the presence of deoxyribonucleic acid or the antigen of a pathogen. Apart from infections with *H. canis* and *H. silvestris*, *H. felis* seems to be the primary infecting pathogen in cats [20-25]. Species differentiation showed the presence of *H. felis* in 7/53 cats infected with *Hepatozoon* spp. in this study. They had been imported from Spain (n=5), Greece, and Malta (n=1 respectively), which is consistent with the above cited literature. There is little knowledge about the pathogenesis, replication cycle, host range, and modes of transmission of *Hepatozoon* spp in cats. In addition to vector transmission, there are reports of transplacental transmission of *H. canis* and *H. felis* in cats [6]. Therefore, any female cat which was tested positive in this study and was not spayed (n=7) could transmit the pathogen in Germany to its kittens, regardless of any contact with a vector. Autochthonous infection with *H. felis* has been reported in a cat in Austria [9]. This may indicate the spread of the pathogen and/or vectors from historically endemic countries in the Mediterranean to more northern regions of Central Europe. In this study, 39/53 cats with positive test results had a history of travel/import to a known endemic area, and time spent abroad could not be excluded for any of the animals with positive test results. Consequently, this study provides no evidence of autochthonous infections in cats within Germany.

One cat in this study had positive PCR results for *Dirofilaria* spp., but further species differentiation was not done and a travel history/information on any time spent abroad was not available. This cat also had a positive IFAT for *Leishmania* spp., so contact with the pathogens in an endemic country in the Mediterranean is likely. Infections with *Dirofilaria* spp. in cats and dogs historically occurs in Mediterranean countries but have recently spread within these countries as for example Italy, Spain, France, Greece and Turkey [10]. *Dirofilaria repens* [26-28] has been the primary pathogen reported in Central and Eastern Europe, and it is currently considered an emerging zoonotic agent in all of Europe [29]. The prevalence of *Dirofilaria* spp. in cats varies from 0% to 33% across Europe [11, 30-40]. According to predictive models developed for dirofilariasis, temperatures during the summer may be suitable for the life cycle of larvae in mosquitoes even in colder regions like the United Kingdom, provided that reservoirs are present [10, 27, 41, 42]. It is to be noted that due to some diagnostic peculiarities, the true prevalence of *D. immitis* may be higher than indicated by the relatively low number of cats with positive test results in this study. Many of the immature pathogens are destroyed shortly after reaching the pulmonary arteries in cats, and the lifespan of the surviving pathogens is shorter in cats (2-4 years) than in most other species, such as dogs (5-7 years) [43]. Cats are rarely infected with more than five roundworms, which can be missed even in a post-mortem examination [44]. Microfilaremia is rare in cats, as less male worms are present [44]. Data on the prevalence of *Dirofilaria* spp. in cats in Germany is not yet available. A single case report from Central Europe describes a cat in Poland which was infected with *D. repens* and *Wolbachia* spp. [24].

Indirect detection methods were used to detect *Ehrlichia* spp., *Rickettsia* spp. and *Leishmania* spp. They demonstrate the presence of antibodies produced in response to the pathogen contact, but this does not necessarily correlate with the presence of disease. Seroconversion may not occur until two to three weeks after exposure and antibodies may be detectable for up to several years after disease resolution, depending on the pathogen. It is generally possible to distinguish more recent infections from those in the past by means of simultaneous Immunoglobulin M levels, or paired serum samples taken at intervals of two to four weeks. However, the former is unusual in routine diagnostics for the pathogens discussed and the latter is often not feasible in practice. The indirect IFAT utilised in this study detected Immunoglobulin G antibodies for all pathogens. Furthermore, the interpretation of IFAT can be subjective and so sensitivity can be low, especially where titres are low or borderline. Limitations may also include the possibility of cross reactivity with other pathogens, as well as false negative results in very young or immunosuppressed animals, or where investigations were done early in the natural history of the disease and therefore prior to seroconversion [45].

The IFAT used in this study detected antibodies to *Leishmania* spp. in 22/624 cats (4%). Cats in Mediterranean countries are generally infected by *L. infantum*. There is much variation in the reported prevalence of *Leishmania* spp. in cats tested by indirect assays not only between different European countries but also across different regions within one country, ranging from 0.1% to 60% [1, 30, 40, 46-68]. Dogs are currently the only known primary reservoir of infection [69]. It has been speculated that cats may be an additional reservoir, but this has not been confirmed [70]. Sandflies can be infected with *L. infantum* during a blood meal on an infected cat, and so cats could be instrumental in the spread of the pathogen in areas

with a high prevalence [71]. Consequently, cats with antibodies to the pathogen could be a reservoir for infection within Germany, provided they were also still infected. The presence of competent vectors like *P. perniciosus* has been reported in the South of Germany [72], as that of the potentially competent vector *P. mascitti* [72, 73]. There is little evidence on the susceptibility or resistance of cats to natural infection. Cats have a more efficient T-helper 1 cell immune response compared to dogs, which may be the cause of the lower prevalence of the pathogen in cats [46]. Twelve of the 22 cats with positive IFAT results (55%) in this study were imported to Germany from Mediterranean countries and Southeast Europe, where *L. infantum* is endemic. One of the 22 cats (5%) was imported from Brazil, where cats may be infected by not only *L. infantum* but also *L. amazonensis* or *L. braziliensis* [74-77].

Antibodies against *Ehrlichia* spp. were detected *via* IFAT in 12% of the tested cats. Previous studies involving indirect detection methods (IFAT) report a 1-18% prevalence of *Ehrlichia* spp. in cats in the Mediterranean [30, 50, 52, 54, 57, 78-82]. Data on the prevalence of antibodies against *Ehrlichia* spp. in cats in Germany is not currently available. IFAT may show some cross-reactivity with *E. chaffensis* (found in cats in the United States and Brazil) and *E. ewingii* (found in cats in the United States), as well as with *A. phagocytophilum* and *A. platys* at lower titres. Cross-reactivity due to contact with *A. phagocytophilum* in Germany cannot be excluded, especially in the 44 cats with a low titre of 1:40 in this study. Seropositivity in the remaining 29 animals with higher titres is most likely due to infection with *Ehrlichia*. A study in 479 cats in Southern Germany did not demonstrate any *Ehrlichia* spp. DNA [83]. Therefore, autochthonous natural infections in cats in Germany are unlikely and the infections most likely occurred abroad.

Eleven percent of 467 cats had positive IFAT results for *Rickettsia* spp. Seroprevalence in cats has been researched in Italy, Spain, and Portugal (IFAT/ELISA: 0-48.7%) [30, 46, 50, 52, 78, 84, 85]. Cats may be instrumental in the transmission cycle of some rickettsiae of the spotted fever group (SFG), especially *R. conorii* and *R. felis* [86, 87]. Antibodies for *R. conorii* have been detected in cats after infections with *Rhipicephalus sanguineus* [78, 84, 87]. *Rickettsia felis* is a well-established cause of the emerging flea-borne spotted fever, of which there have been several cases in humans worldwide [88, 89]. Cats will have antibodies for *Rickettsia* spp. after being infected (either natural or experimental) with fleas of the species *Ctenocephalides (C.) felis* [90]. The pathogen has also been detected *via* PCR in previously non-infected fleas after a blood meal on infected cats [91]. Consequently, *C. felis* can be considered a competent vector and autochthonous infections within Germany are possible. This study detected antibodies by means of IFAT, which is regarded as the gold standard for serological confirmation of pathogen contact in dogs and cats. There are, however, cross-reactions between any of the more than 20 species in the spotted fever group [87]. We detected antibodies to *Rickettsia* spp. in 52/467 cats (11%). There were 29 cats which were seropositive and had been imported from abroad, and it is unclear whether they were infected in Germany or in their country of origin. The four seropositive cats which had never left Germany were most likely infected with *R. felis.* The clinical importance of *Rickettsia* spp. infections in cats is still unknown. A study in clinically symptomatic cats found no association between positive antibody titres and fever, and no febrile cats in this study had positive PCR results for *R. felis* or *R. rickettsi* [92].

In this study, 22 of 624 cats (4%) had positive test results for more than one pathogen. It is known that co-infections may complicate diagnosis and treatment in dogs and may worsen their prognosis [2]. Coinfections with multiple vector-borne pathogens may occur in cats as well as dogs and humans, but their clinical consequences are still unknown and should be evaluated in further studies, especially in cats [93]. In this study, nine cats infected with *Hepatozoon* spp. also had antibodies against *Leishmania* spp. (n=4), *Rickettsia* spp. (n=3), and *Ehrlichia* spp. (n=2). Antibodies to *Leishmania* and *Ehrlichia* spp. were present in 12 cats infected with *Hepatozoon* spp., respectively. This indicates a pathogen contact with concurrent immunosuppression to be discussed in case of persistent infection, as it may result in increased susceptibility of infected animals to other pathogens [2].

Limitations of this study

Limitations of this study are mainly its retrospective design (e.g. no consistent histories) and the limited number of pathogens included. Certain vector-borne infectious pathogens such as *Cytauxzoon* spp. could not be included. Furthermore,

species differentiation for specific pathogens included in the study was not performed, except in the case of seven cats with positive test results for *H. felis*. There was also no information on the extent of ectoparasite prophylaxis in the cats, which may impact the prevalence of certain vector-borne pathogens. In the cats which had travelled with their owners to endemic countries, it was not possible to reliably document the duration or the time of the year of these travels. As many of the relevant vectors show pronounced seasonality, the time of year may significantly influence both incidence and prevalence of the pathogens they may transmit. The histories taken from the veterinarians only included the countries of stays abroad.

Conclusions

Of the cats included in this study, 28% had positive test results for at least one vector-borne pathogen. As vector-borne infections often remain undiagnosed, it is important to take thorough histories of any time spent abroad in all cats in which vector-transmitted infections are suspected. Owners of imported cats, or those who choose to take their cats with them on holidays abroad, should be given detailed information on any and all potential infections and resulting risks. Ectoparasite prophylaxis is advisable in all cats. The zoonotic potential of some pathogens such as *L. infantum*, *D. immitis*, and *D. repens* and their resulting importance in human medicine has to be noted [2].

Abbreviations

DAT: direct agglutination test; DNA: deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; FeLV: Feline leukemia virus; FIV: feline immunodeficiency virus; IFAT: Indirect immunofluorescence test; PCR: Polymerase chain reaction

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article. Parts of this study were presented as a poster at the DVG-Congress for Internal Medicine and Laboratory Diagnostics in Gießen, Germany (30 January–01 February 2020) and as an oral presentation at the International Research Conference on Veterinary Parasitology and Entomology in Copenhagen, Denmark (11-12 June 2020, Online Congress).

Authors' contributions

IS collected and evaluated the data and wrote the manuscript. BK and EM initiated and supervised the study and edited the manuscript. MV supported the statistical analyses and edited the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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