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# Retrospective Evaluation of Vector-borne Infections in Cats Living in Germany (2012-2020)

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**Keywords:** Arthropod-transmitted infections, Feline, Laboratory diagnostics

**Posted Date:** October 15th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-90832/v1

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**Version of Record:** A version of this preprint was published on February 25th, 2021. See the published version at https://doi.org/10.1186/s13071-021-04628-2.

## 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 the increase of travel, import of domestic animals from abroad, and due to the changing climate in Europe. The main objective of this retrospective study was to assess the prevalence of some vector-borne infections in cats in which a 'Feline Travel Profile' had been conducted.

**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 contains direct detection methods *via* PCR for *Hepatozoon* spp. and *Dirofilaria* spp. as well as indirect detection methods *via* IFAT for *Ehrlichia* spp. and *Leishmania* spp. The profile was expanded to include an IFAT for *Rickettsia* spp. from July 2015 onwards. The prevalence of the different vector-borne infectious agents was calculated.

**Results:** A total of 624 cats were tested using the 'Feline Travel Profile'. Serological samples for indirect detection methods were available for all 624 cats, EDTA-samples for direct detection methods for 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, tested from July 2015 onwards, *Rickettsia* spp. IFAT 52 out of 467 cats (11%). At least one infection was present in 175 out of 624 cats. Three coinfections were detected before 2015; after including the *Rickettsia* spp. test results there were 19 cats with confections (in 14 out of these 19 cats *Rickettsia* spp. were involved).

**Conclusions**: 175 out of 624 cats (28%) were tested positive for at least one vector-borne pathogen. Infections with multiple pathogens could be detected in 4% of the cats from 2012 to 2020. The data emphasizes the importance of considering the above-mentioned vector-borne infections as potential differential diagnoses in cats.


## Introduction

Cats are at a high risk of being in contact with blood-feeding arthropods such as fleas, ticks, or mosquitoes, especially outdoor or stray cats without any prophylaxis for ectoparasites [1, 2]. Such vectors can transmit parasitic, bacterial, or viral pathogens, which may subsequently cause infection in competent hosts like cats. This study includes infections with helminths (*Dirofilaria* (*D.*) spp.) as well as protozoa (*Leishmania* (*L.*) spp., *Hepatozoon* (*H.*) spp.) and bacteria (*Ehrlichia* (*E.*) spp., *Rickettsia (R.)* spp.).

Within Europe, infections with pathogens like *L. infantum*, *E. canis*, and *R. conorii* in cats are largely limited to the Mediterranean and Southeast Europe. This is due to the incidence of relevant vectors, namely *Rhipicephalus sanguineus* in the case of *E. canis*/ *R. conorii*, and most probably *Phlebotomus* (*P.*) spp. sandflies in the case of *L. infantum* [2]. *Hepatozoon* spp. are transmitted by various blood-feeding arthropods worldwide, including ticks, mites, sandflies, tsetse flies, lice, kissing bugs, and leeches [3, 4]. Mainly *H. felis* or, less frequently, *H. canis* and *H. silvestris* infections were detected in cats in the Mediterranean and Southeast Europe [3–7]. However, there are single case reports of *H. felis* in Austria [8] and *H. silvestris* in Switzerland [7]. *Dirofilaria* spp. are transmitted by mosquitoes. In cats, *D. immitis* is well described as a pathogenic species, whereas *D. repens* is known to be a cause of subclinical dirofilariasis [2, 9]. While these also generally occur within the Mediterranean and Southeast Europe, there has been one case report of a cat infected with *D. repens* in Poland [10]. Infection with *R. felis* may also occur in Germany [2] due to the local incidence of *Ctenocephalides felis* fleas as vectors [11]. Other documented vector-borne pathogens in cats within 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 viral infections with *Flaviviridae* [2].

Among the pathogens examined in this study, *Rickettsia* spp., *Leishmania* spp., and *Dirofilaria* spp. have zoonotic potential and consequently are of importance for public health in Europe [2]. The aim of this study was to determine the prevalence of the above named vector-borne pathogens in cats, by evaluating the results of the "Feline Travel Profile" panel performed on samples provided by veterinarians in Germany by the LABOKLIN (Bad Kissingen, Germany) veterinary laboratory. A secondary aim was to establish the travel history in the tested cats by telephone contact with the treating veterinarians.

## Methods

This study included any "Feline Travel Profile" panel results for samples from cats which were provided between April of 2012 and March of 2020 by veterinarians located in Germany. This panel includes a direct assay by polymerase chain reaction (PCR) of *Hepatozoon* spp. and *Dirofilaria* spp. Furthermore, it includes immunofluorescence antibody test (IFAT) as an indirect assay for *Ehrlichia* spp. and *Leishmania* spp., which was expanded to include testing for *Rickettsia* spp. from July 2015 onwards (Table 1). Wherever possible, information on any time spent abroad, as well as living conditions (ie. outdoor/indoor cat, other pets in the same household) and ectoparasite infection/prophylaxis was collected by means of questionnaires and telephone calls to the treating veterinarians. A descriptive statistical analysis of the data collected was performed with SPSS for Windows (Version 25.0, 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), own method (TaqMan® real time PCR)

[2] PCR, own method with special detection methodology (adapted from 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 stays abroad 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. For the remainder of this group, comprehensive information on the circumstances of the import was not available. 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). Eight cats out of 324 (1%) were born in Germany and never travelled. For 253/363 cats (41%) there was either no information on any time spent abroad, or this information could not be gained retrospectively. Information about living conditions and ectoparasite infections/prophylaxis was available for 18/624 cats (3%). Due to this small number, this information was not separately evaluated and will not be presented.

Table 2
Vector-borne infections in 624 cats with 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 (%) | Monoinfection *Hepatozoon* spp.[1] | Monoinfection *Dirofilaria* spp.[2] | Monoinfection *Ehrlichia* spp.[3] | Monoinfection *Rickettsia* spp.[A, 4] | Monoinfection *Leishmania* spp.[5] | Co-infections | Stays abraod |
|---|---|---|---|---|---|---|---|---|---|
| **Countries in the European Union (EU)** | | | | | | | | | |
| Spain | 158 | 51/158 (32) | 17 | - | 18 | 8 | 3 | 2 Ehrlichia/ Rickettsia; Rickettsia/ Hepatozoa; Leishmania/ Hepatozoa; Leishmania/ Ehrlichia | 131 imports, 20 animal welfare imports, 5 imports after holidays, 2 holidays |
| Greece | 52 | 17/52 (33) | 7 | - | 6 | 1 | 1 | Leishmania/ Hepatozoa; Ehrlichia/ Rickettsia | 44 imports, 6 animal welfare imports, 2 imports after holidays |
| Romania | 28 | 8/28 (29) | - | - | 2 | 5 | - | Leishmania/ Hepatozoa | 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 |

[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), own method (TaqMan® real time PCR)

[2] PCR, own method with special detection methodology (adapted from 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, Hörbranz, Austria; › 1:64 positive)

| Country | N | N tested positive /N total (%) | Monoinfection *Hepatozoon* spp.[1] | Monoinfection *Dirofilaria* spp.[2] | Monoinfection *Ehrlichia* spp.[3] | Monoinfection *Rickettsia* spp.[A, 4] | Monoinfection *Leishmania* spp.[5] | Co-infections | Stays abraod |
|---|---|---|---|---|---|---|---|---|---|
| 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)** | **28** | **-** | **36** | **19** | **4** | **8** | **266 imports, 36 animal welfare imports, 13 imports after holidays, 5 holidays** |
| | **Non-EU Countries** | | | | | | | | |
| Turkey | 12[B] | 3/12 (27) | 2 | - | - | 1 | - | - | 11 imports, 1 holiday[A] |
| Dubai | 5 | 1/5 (20) | - | - | - | - | - | Rickettsia/ Hepatozoa | 4 imports, 1 animal welfare import |
| Morocco | 3 | 3/3 (100) | 2 | - | 1 | - | - | - | 3 imports |
| Tunisia | 3 | 2/3 (67) | - | - | 1 | - | 1 | - | 2 imports, 1 animal welfare import |
| Bosnia | 3 | 1/3 (33) | - | - | - | - | 1 | - | 2 imports, 1 holiday |
| Ukraine | 3 | 1/3 (33) | - | - | 1 | - | - | - | 3 imports |
| Russia | 3 | 0/3 (0) | - | - | - | - | - | - | 3 imports |
| Brazil | 2 | 1/2 (50) | - | - | - | - | 1 | - | 1 import after holidays, 1 import |

[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), own method (TaqMan® real time PCR)

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| Country | N | N tested positive /N total (%) | Monoinfection *Hepatozoon* spp.[1] | Monoinfection *Dirofilaria* spp.[2] | Monoinfection *Ehrlichia* spp.[3] | Monoinfection *Rickettsia* spp.[A, 4] | Monoinfection *Leishmania* spp.[5] | Co-infections | Stays abraod |
|---|---|---|---|---|---|---|---|---|---|
| Egypt | 2 | 1/2 (50) | - | - | - | - | - | Ehrlichia/ Leishmania/ Rickettsia | 2 imports |
| Belarus | 1 | 1/1 (100) | 1 | - | - | - | - | - | 1 import |
| Israel | 1 | 1/1 (100) | 1 | - | - | - | - | - | 1 import after holidays |
| Lebanon | 1 | 0/1 (0) | | - | | | | | 1 import |
| Senegal/Togo | 1 | 0/1 (0) | - | - | - | - | - | - | 1 import |
| Tanzania | 1 | 0/1 (0) | | - | | | | | 1 import |
| Georgia | 1 | 0/1 (0) | - | - | - | - | - | - | 1 import |
| Montenegro | 1 | 0/1 (0) | - | - | - | - | - | - | 1 import |
| Serbia | 1 | 0/1 (0) | - | - | - | - | - | - | 1 import |
| **Total Non-EU** | **44[A]** | **15/44 (34)** | **6** | **-** | **3** | **1** | **3** | **2** | **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), own method (TaqMan® real time PCR)

[2] PCR, own method with special detection methodology (adapted from Rishniw et al., 2006)

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

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| Country | N | N tested positive /N total (%) | Monoinfection *Hepatozoon* spp.[1] | Monoinfection *Dirofilaria* spp.[2] | Monoinfection *Ehrlichia* spp.[3] | Monoinfection *Rickettsia* spp.[A, 4] | Monoinfection *Leishmania* spp.[5] | Co-infections | Stays abraod |
|---|---|---|---|---|---|---|---|---|---|
| **Total import/travel** | **363** | **110/363 (30)** | **34** | **-** | **39** | **20** | **7** | **10** | **303 imports, 38 animal welfare imports, 15 imports after holidays, 6 holidays, 1 import and holidays[A]** |
| Germany without stays abroad | 8 | 4/8 (50) | - | - | - | 4 | - | - | - |
| No history of stays abroad available | 253 | 61/253 (24) | 11 | - | 21 | 14 | 3 | 12 | No history available |
| **Total** | **624** | **175/624 (28)** | **45** | **-** | **60** | **38** | **10** | **22** | **-** |

[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), own method (TaqMan® real time PCR)

[2] PCR, own method with special detection methodology (adapted from 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, Hörbranz, Austria; › 1:64 positive)

## Laboratory diagnostics

Results from 2951 direct and indirect detection assays on samples from 624 cats were evaluated. PCR tests were performed on samples from 618/624 cats (99.9%) each for *Hepatozoon* spp. and *Dirofilaria* spp. In 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. Subsequent to 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%) tested positive 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 revealed the following: 73/624 cats (12%) tested positive 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. *Rickettsia* spp. antibodies were found in 52 cats with titres of 1:256 (n = 33), 1:512 (n = 14) and 1:1024 (n = 5). Of the 22 cats, which were tested positive for *Leishmania* spp., titres of 1:128 (n = 14), 1:256 (n = 3), 1:512 (n = 4) and 1:1024 (n = 1) were detected.

Evidence of co-infection with more than one pathogen could be found in 22/624 cats (4%). Three of 22 cats (14%) were tested positive before adding the *Rickettsia* spp. IFAT to the Feline Travel Profile (*Leishmania*/ *Dirofilaria* spp., *Leishmania*/ *Hepatozoon* spp. and *Leishmania*/ *Ehrlichia* spp.), 19/22 (86%) after adding detection of *Rickettsia* spp. antibodies in July 2015. *Rickettsia* spp. were involved in 14 out of these 19 cats (74%). In total, 19 cats tested positive for two pathogens simultaneously (*Ehrlichia/Rickettsia* spp. [n = 6]; *Leishmania/Rickettsia* spp. and *Leishmania/Hepatozoon* spp. [n = 3, respectively]; *Rickettsia/Hepatozoon* spp., *Ehrlichia/Hepatozoon* spp., and *Ehrlichia/Leishmania* spp. [n = 2, respectively], as well as *Leishmania/Dirofilaria* spp. [n = 1]). Three cats tested positive for co-infections with three pathogens simultaneously (*Ehrlichia/Leishmania/Rickettsia* spp. [n = 2], *Leishmania/Rickettsia/Hepatozoon* spp. [n = 1]).

Among the 363 cats with a history of any stays abroad, 110 (30%) tested positive for at least one vector-borne pathogen. The highest prevalence appeared to be in cats with a history of time spent in Spain (51/158 cats, 32%), Greece (17/52 cats 33%), and Romania (8/28 cats, 28%). Testing for infection with *Ehrlichia* spp. (39/363 cats, 11%), *Hepatozoon* spp. (34/363 cats, 9%), *Rickettsia* spp. (20/363 cats, 6%), and *Leishmania* spp. (7/363 cats, 2%) was reported as positive. There was evidence of co-infection with more than 2–3 pathogens in the cases of 10/363 cats (3%), most of which had returned or come from

Spain (n = 5) and Greece (n = 2) (Table 2). All of the six cats which were born in Germany but had accompanied their owners on travels abroad, were tested negative for any of the pathogens examined. Four of the eight cats (50%) which were reported not to have left Germany had antibodies for *Rickettsia* spp.

## Discussion

Overall, 175/624 cats (28%) were tested positive for at least one vector-borne pathogen. Within the group of cats with a history of any time spent abroad, this prevalence was 30% (110/363 cats). Previous studies in dogs living in Germany have found prevalences of 35% (imported dogs [12]), 13% (travelling dogs [13]), and 44% (any history of time spent abroad [14]). Any comparison of the prevalence of infection with vector-borne pathogens in dogs and cats is of limited value, for several reasons which include the following: different prevalence rates of some pathogens in dogs and in cats in endemic countries, variation in study design, difference in immune responses to infection in dogs and cats, different host preferences of specific pathogens, and inborn resistance mechanisms for some pathogens [15]. Moreover, cats exhibit a more thorough cleaning behaviour than dogs, which may cause them to remove a potential vector and therefore inhibit any possible disease transmission [16]. Cats far more rarely accompany their owners on travels abroad, and consequently there was a higher ratio of imported cats (98%, 356/363 cats) compared to cats which had been travelled with their owners (2%, 6/363 cats). Since all 6 cats had outdoor access, their risk of coming into contact with a relevant vector is similar to that of travel companion dogs. However, due to the small number of cats which had travelled, any attempts at interpretation of this data is not feasible. Beside that, the prevalence of vector-borne infections in imported cats and dogs are approximately the same.

The prevalence of vector-borne infections varies not only among countries but also within the countries themselves, as it is determined largely by geographical and climatic conditions, as well as the presence of suitable vectors and reservoirs for the pathogen [17, 18]. Not only the import of cats from abroad but also international travel and conveyance of goods is increasing in frequency. Coupled with the change in climate in many parts of Europe, this could contribute to an increased 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 *via* imported vectors may cause infection in competent hosts endemic to Germany, of which cats are one example. Moreover, endemic but potentially competent vectors may be infected with these previously non-endemic pathogens during a blood meal on infected cats, and proceed to contribute to the spread of these pathogens [2, 19, 20]. One example are isolated cases of autochthonous infections with *D. repens* [21–23] and *L. infantum* [24] in dogs in Germany, which has not been described in cats.

Direct detection methods demonstrate the presence of deoxyribonucleic acid or the antigen of a pathogen. PCR assays are used primarily in acute or peracute infections prior to seroconversion, or in the case of kittens due to the presence of maternal antibodies [2]. Despite the high sensitivity of the PCRs used in this study, false negatives are not uncommon in cats due to their propensity for having comparatively low pathogen concentrations in blood. This is suspected to be the case in *Rickettsia* spp., *A. phagocytophilum* or *Ehrlichia* spp. infections in cats [25, 26].

Indirect detection methods demonstrate the presence of antibodies after contact with the pathogen. This does not correlate with the presence of disease, as seroconversion may not occur until two to three weeks after exposure, and antibodies may be detectable for up to several years after disease resolution, both depending on the pathogen. Generally, it is possible to distinguish more recent infections from those which date further back by means of simultaneous Immunoglobulin M levels, or serum pairs taken at intervals of 2 to 4 weeks. However, the former is unusual in any routine diagnostics for the pathogens discussed, while the latter is often not feasible in practice. The indirect IFAT utilised in this study detected Immunoglobulin G antibodies for all pathogens. Additionally, due to the subjective microscopic assessment of samples, there is a possibility of human error negatively influencing the sensitivity in cases with low antibody titres. Further limitations may be due to cross reactivity with other pathogens, false negative results in very young animals or those which are immunosuppressed, as well as in those cases in which testing was done too early in the natural history of the disease and therefore prior to any seroconversion [27].

This study included detection assays for *Leishmania* spp., *Hepatozoon* spp., *Ehrlichia* spp., *Rickettsia* spp., and *Dirofilaria* spp. This selection was due to the framework of the corresponding testing panel offered by LABOKLIN, which facilitated the uniform testing of a population of cats by means of a set testing panel for a defined spectrum of pathogens. Due to the relatively late seroconversion of *Leishmania* spp. and the long prepatency of *Dirofilaria* spp., the prevalence of both pathogens may be higher than reported (*Leishmania* spp.: 4% (IFAT); *Dirofilaria* spp.: 0.2% (PCR)). In the following, every pathogen considered in this study will be discussed individually.

# Leishmania spp.

Cats in the Mediterranean countries are infected by the same *Leishmania* spp. as dogs in these regions, primarily *L. infantum.* There is much variation in the reported prevalence of *Leishmania* spp. in cats tested by indirect assays not only among different European countries but also across different regions within one country, ranging from 0.1–60% [1, 15, 28–52]. To the knowledge of the authors, there are no data on cats in Germany at this point in time. Utilising IFAT, this study found antibodies to *Leishmania* spp. in 22/624 cats (4%), and in 13 of the 363 cats (4%) with a history of any time spent abroad. Contrary to dogs or humans, in which horizontal or vertical transmission is possible, cats seem to be infected solely by vector transmission [53]. The prevalence of *Leishmania* spp. is lower in cats than in dogs, and cats are less likely to develop clinical signs if they are infected [1, 54]. Dogs are currently the only known primary reservoir of infection [55]. While cats are presumed to also be reservoir, this has not yet been proven [56]. There is little evidence on the susceptibility or resistance of cats to natural infection. Cats exhibit a more efficient T-helper cell-1 immune response than dogs, which may explain the lower prevalence of the pathogen in cats [15]. However, the pathogenesis of feline leishmaniasis remains unclear, as well as the role of cats in the life cycle of the pathogen. It has been shown that sandflies may become infected with *L. infantum* during a blood meal on an infected cat, and consequently cats may be instrumental in the spread of the pathogen in areas with a high prevalence [57]. It is therefore possible that the 22 cats in this study which were tested positive for antibodies

might transmit *L. infantum* further within Germany, provided they are still infected with the pathogen. Suitable competent vectors like *P. perniciosus* have been described in the South of Germany [58], as has *P. mascitti*, a potentially competent vector [59, 60].

Depending on the specific test utilised, it is usually recommended to use a titre cut-off of 1:80 when performing *Leishmania* spp. IFAT in cats [61]. In reference to this and according to manufacturer guidelines, this study used a cut-off of 1:64. Cross reactivity between different *Leishmania* spp. are probable in the 22 cats which tested positive in this study. Twelve of the 22 cats which tested positive (55%) were imported into Germany from Mediterranean countries and Southeast Europe, where *L. infantum* is endemic. One out of the 22 cats (5%) was imported from Brazil, where cats may be infected with not only *L. infantum* but also *L. amazonensis* or *L. braziliensis* [62–65]. In the remaining 9/22 cats (41%), it was not possible to obtain a travel or import history.

## Hepatozoon spp.

Infections with *H. felis*, *H. canis*, and *H. silvestris* have been described in cats. The prevalence of *Hepatozoon* spp. detected by PCR in Europe is between 0% and 38%, and all three *Hepatozoon* spp. which may infect cats in Europe have been previously described [1, 6, 28, 66–71]. To the knowledge of the authors, the prevalence of *Hepatozoon* spp. infections in cats in Germany is unknown. In this study, the pathogen was detected by PCR in 53/618 cats (9%). In 7 of these 53 cats (13%), which had been imported from Spain (n = 5), Greece, and Malta (n = 1, respectively), it was possible to detect *H. felis via* species differentiation. This result is in accordance with previous studies which have determined *H. felis* to be the primary infecting pathogen in cats [66–71]. In 39/53 cats which were tested positive for *Hepatozoon* spp. (74%), there was a history of travel/import consistent with an infection in an endemic area abroad. There is no evidence of autochthonous infections in cats within Germany, and therefore it is most likely, that the remaining 14/53 cats (26%) were also infected in an endemic region abroad. The only feline case report of an autochthonous infection with *H. felis* in Central Europe so far was from Austria [8].

There is little knowledge about the pathogenesis, replication cycle, host spectrum, and modes of transmission of *Hepatozoon* spp. in cats. In addition to vector transmission, there are reports of transplacental transmission in cats in the case of *H. canis* and *H. felis* [5, 72]. Therefore, any female cat which was tested positive in this study and had not been spayed (n = 7) might transmit the pathogen in Germany to their kittens, regardless of any contact with a vector. In the *Hepatozoon* spp. infected cats of this study, we detected coinfections with *Leishmania* spp. (n = 4), *Rickettsia* spp. (n = 3), and *Ehrlichia* spp. (n = 2).

## Ehrlichia spp.

*E. canis* or *E. canis*-like pathogens can infect cats [73, 74]. The prevalence of *Ehrlichia* spp. in the Mediterranean as tested by indirect detection methods (IFAT) in cats ranged between 1% and 18% [31, 32, 34, 36, 39, 75–79]. There does not seem to be any data on the prevalence of antibody testing in cats in Germany to this date. A study in 479 cats in South Germany did not demonstrate any *Ehrlichia* spp. DNA [26]. *Rhipicephalus sanguineus*, which is a potential vector for *E. canis*, is only found in Germany for short durations in specific temperatures, or as populations in constantly heated buildings [80]. Therefore, autochthonous natural infections in cats in Germany are unlikely.

Cross reactivity in indirect detection methods may occur 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 group of 44 cats with a titre of 1:40.

## Rickettsia spp.

Cats may be instrumental in the transmission cycle of some rickettsia of the spotted fever group (SFG), especially of *R. conorii* and *R. felis* [81, 82]. Dogs are a known reservoir for *R. conorii* and have been demonstrated to exhibit a clinical infection [83, 84]. This pathogen also has zoonotic potential. In cats, antibody titres to *R. conorii* have been shown after infections with *Rhipicephalus sanguineus* [75, 82, 85]. Seroprevalence has been examined in cats in Italy, Spain, and Portugal (IFAT/ELISA: 0-48.7%) [15, 31, 32, 34, 75, 85, 86]. *Rickettsia felis* is an established cause of the emerging flea-borne spotted fever, of which there have been several cases described in humans worldwide [87, 88]. Cats will have antibodies for *Rickettsia* spp. after infection (either natural or within the framework of an experiment) with fleas of the species *Ctenocephalides felis* [11]. The pathogen has also been detected by PCR in previously non-infected fleas after a blood meal on infected cats [89]. Consequently, *Ctenocephalides felis* is a competent vector and therefore autochthonous infections within Germany are possible.

This study utilised IFAT to detect antibodies, 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 [82]. We detected antibodies to *Rickettsia* spp. in 52/467 cats (11%). In those 29 cats which were seropositive and had reportedly been imported from abroad, there is a possibility of infection with rickettsia in either Germany or their home country. In the four cats which had never left Germany, an infection with *R. felis* is most likely. Species differentiation by PCR was not performed.

Furthermore, the clinical importance of *Rickettsia* spp. infections in cats is still unknown. For example, one study evaluated clinically ill cats for evidence of rickettsial infections, but no association between positive antibody titres and fever could be shown and no febrile cat had a positive PCR result for *R. felis* or *R. rickettsii* [90].

## Dirofilaria spp.

Infection with *Dirofilaria* spp. occurs primarily in dogs but has also been described in cats [9]. The prevalence of *Dirofilaria* spp. in cats varies between 0% and 33% across Europe [10, 31, 52, 91–99]. There does not seem to be any data on prevalence in Germany, specifically. A first case report in Europe describes a cat in Poland which was infected with *D. repens* and *Wolbachia* spp. [10]. Only one of the 618 cats tested for microfilaria by means of PCR (0.2%) was tested positive, and species differentiation was not performed. A travel history was not available for this cat. It seems most likely that it was infected abroad in an endemic country, especially considering the existing coinfection with *L. infantum*. Cats are in general more resistant to *Dirofilaria* spp. infections compared to dogs [100]. Additionally, some mosquito species which could function as vectors seem to prefer dogs to cats for their blood meals [101], which may explain the lower prevalence in cats. However due to some diagnostic peculiarities, the true prevalence in cats may be higher than that found in this study. A large fraction of the not yet mature pathogens is destroyed shortly after reaching the pulmonary arteries in cats, and consequently the duration of life of these pathogens is far shorter in cats (2–4 years) than it is in dogs (5–7 years) [102]. Cats are rarely infected with more than five roundworms, which may be overlooked even in a post-mortem examination [103]. Additionally, female roundworms are seen more in cats. Therefore microfilaraemia is rare in cats, as no male worms are available [103]. Antigen testing is prone to false negative results due to the low level of pathogens in cats, and therefore direct detection methods should only be utilised coupled with at least one specific antibody test as well as imaging modalities [9, 104]. Another possibility to increase the sensitivity is the heat pre-treatment of feline serum and/or plasma samples before analysis [105], which was not carried out through.

# Coinfections

In total, coinfections were detected in 22 out of 624 cats (4%) in this study. As it is known in dogs, coinfections may complicate the diagnoses and treatment in infected animals and may worsen the prognosis [2]. Coinfections with multiple vector-borne pathogens occur in cats as well as in dogs and humans, but the clinical consequences are still unknown and have to be evaluated in further studies, especially in cats [106].

*Leishmania* and *Ehrlichia* spp. infections, which were present in 12 positive tested cats each, may cause an immunosuppression, possibly making infected animals more susceptible for infections with other pathogens [2]. In 5 *Ehrlichia* spp. positive tested cats with low titres of 1:40, possible cross-reactions with *A. phagocytophilum* in Germany have to be taken in consideration.

# Limitations of this study

Limitations of this study are mainly its retrospective design (e.g. no consistent histories) and the limit of pathogens included. Moreover, certain vector-borne infections as e.g. *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 which were tested positive for *H. felis*. There was also no information on the presence or absence of ectoparasite prophylaxis in the cats, which may impact the prevalence of certain vector-borne pathogens. In the cats which had been travelled with their owners it was not possible to reliably document the duration of time or the time of the year spent in endemic countries. 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% were tested positive for at least one vector transmitted pathogen. As vector-borne infections often remain undiagnosed, it is important to take thorough histories of stays abroad in all cats in which vector-transmitted infections are at all suspected. Owners of such imported cats, or those who choose to take their cats with them on holiday abroad, should be diligently informed about 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

# Acknowledgements

Not applicable.

# Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

# 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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