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Prevalence of selected rickettsial infections in cats in Southern Germany

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ABSTRACT

Prevalence of *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia* DNA in blood of 479 cats collected in different veterinary clinics in Southern Germany was determined using a previously published conventional PCR using 16S-23S intergenic spacer primers (5′ CTG GGG ACT ACG GTC GCA AGA C 3′ – forward; 5′ CTC CAG TTT ATC ACT GGA AGT T 3′ – reverse). Purified amplicons were sequenced to confirm genus and species. Associations between rickettsial infections, and feline immunodeficiency virus (FIV), as well as feline leukemia virus (FeLV) status were evaluated. Rickettsial prevalence was 0.4% (2/479; CI: 0.01–1.62%). In the two infected cats, *Anaplasma phagocytophilum* DNA was amplified. These cats came from different environment and had outdoor access. Both were ill with many of their problems likely related to other diseases. However, one cat had neutrophilia with left shift and the other thrombocytopenia potentially caused by their *A. phagocytophilum* infection. There was no significant difference in the FIV and FeLV status between *A. phagocytophilum*-negative and -positive cats. *A. phagocytophilum* can cause infection in cats in Southern Germany, and appropriate tick control is recommended.

1. Introduction

Several rickettsial species have recently been recognized as pathogens in people and animals. The agents are obligatory gram negative intracellular bacteria and transmitted by arthropods [1]. Fleas and ticks are vectors for several rickettsial pathogens [2,3].

The order Rickettsiales harbors two families capable of infecting cats. The family Anaplasmatacea includes the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia.* These agents replicate intracellularly in white blood cells, red blood cells, platelets, or endothelial cells [1]. Although common in dogs, data on naturally occurring infections of cats are limited.

Among *Anaplasma* spp., *A. phagocytophilum* and *Anaplasma platys* have been described in cats. Confirmed *A. phagocytophilum* field infection in cats, based on PCR testing and gene sequencing, has been published as case reports in the United States [4,5] and in Europe, *e.g.*, Finland [6], Sweden [7], Switzerland [8], the United Kingdom [9], Italy [10], and Germany [11,12]. *A. phagocytophilum* is vectored by *Ixodes* spp. and occurs most commonly in cats living in regions endemic for this tick. Cats naturally infected with *A. phagocytophilum* showed anemia, anorexia, dehydration, fever, lethargy, tachypnea, and thrombocytopenia [4–11]. The vector for *A. platys* in cats is unknown, but is assumed to be *Rhipicephalus sanguineus*. Confirmed natural *A. platys* infection was reported in a cat in Brazil [13] and in a cat in the United States [14] that had thrombocytopenia and additional hematological and serum biochemical changes.

Molecular detection of *Ehrlichia* spp. in field cats has been reported in the United States [15], Brazil [16], and France [17]. *Ehrlichia* spp. are commonly transmitted by *R. sanguineus*. Clinical signs and laboratory changes described in infected cats include anemia, thrombocytopenia, fever, lethargy, anorexia, and dehydration [15–17].

Of the *Neorickettsia* spp., only *N. risticii* is suspected to infect cats based on the presence of antibodies in some naturally exposed cats in the United States [18] and Spain [19]. *N. risticii* is transmitted *via* trematodes that use snails as intermediate hosts [20]. When experimentally infected with *N. risticii*, cats can become clinically ill and show depression, mild fever, anorexia, diarrhea, and lymphadenomegalie [21].

The genus *Wolbachia* contains a single species, *W. pipientis*, a symbiont harbored in nematodes, such as *Dirofilaria immitis*.

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W. pipientis DNA commonly can be amplified from the blood of cats infected with *D. immitis*, which is not endemic in Germany but can occur in cats with a travel history [22].

Of the Anaplasmataceae, only the prevalence of *A. phagocytophilum* has been investigated in Germany so far in two studies, one from Northeastern Germany, the other from Southern Germany [11,12]. To the authors' knowledge, no study has investigated whether other Anaplasmataceae can be detected in cats in Germany. Thus, the aim of this study was to determine the prevalence of several important rickettsial infections, including *Anaplasma* spp., *Ehrlichia* spp., *Neorickettsia* spp., and *Wolbachia* spp., in cats in Southern Germany. Associations between rickettsial infections, feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) infection status were also evaluated.

2. Materials and methods

2.1. Animals

Blood samples of 479 cats were tested for *Anaplasma* spp., *Ehrlicha* spp., *Neorickettsia* spp., and *Wolbachia* spp. DNA. Cats were presented to different veterinary clinics in Southern Germany for various reasons.

In each cat, the health status was evaluated, a complete blood cell count (CBC) was performed, and FIV- and FeLV-status was examined. FIV antibodies were detected using a commercial ELISA (SNAP Kombi Plus FeLV/FIV antibody test®, IDEXX GmbH, Ludwigsburg, Germany). Status of FeLV infection in cats was investigated in all cats by performing tests for free FeLV p27 antigen in serum using a commercial ELISA (SNAP Kombi Plus FeLV/FIV antibody test; Idexx GmbH, Ludwigsburg, Germany), FeLV provirus using a PCR [23], as well as anti-FeLV-p45 antibodies using an indirect ELISA [23].

2.2. Assays

A total of 479 DNA samples that were previously extracted from whole blood using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics AG) had been stored at −80 °C until assayed in this study. The samples were thawed at room temperature and evaluated for *Anaplasma* spp., *Ehrlichia* spp., *Neorickettsia* spp., and *Wolbachia* spp. in a single PCR assay using 16S-23S intergenic spacer primers (5′ CTG GGG ACT ACG GTC GCA AGA C 3′ – forward; 5′ CTC CAG TTT ATC ACT GGA AGT T 3′ – reverse) in a 25 μl reaction. The primers amplified a product of 300 bp [4].

PCR products were visualized on a 1.5% agarose gel using 6X EZVision One DNA Dye (Amersco; Solon, OH) according to manufacturer specifications. Appropriate negative and positive controls were run with each sample, along with extract negative controls provided. The positive PCR controls were obtained from diagnostic whole blood samples received by the Center for Companion Animal Studies (Colorado State University Veterinary Teaching Hospital; Fort Collins, Colorado), positive controls were sequenced and confirmed by use of the same forward and reverse primers used for the PCR. Negative PCR control consisted of no DNA template added, but PCR (molecular grade) water being added in lieu of DNA template.

All positive samples were purified (QIAquick Gel Extraction Kit; Qiagen Germantown, MD) and sequenced to confirm genus and species (Colorado State University Proteomics and Metabolomics Facility; Fort Collins Colorado). Positive samples were sequenced using the forward primer only, from the PCR used to analyze the samples. From a 300 bp amplicon, >260 bp were usable for comparison to the GenBank BLAST database.

2.3. Statistical analysis

Statistical analysis was performed with Graph PadPrism 6.0. For determination of confidence intervals an exact binomial test was used [24]. The exact binomial test was one-tailed and was done to prove the alternative hypotheses that the prevalence of rickettsial infections are within the 95% confidence interval (CI). A significance level of <0.05 was chosen. Fisher's exact test was used to assess the associations between rickettsial infections, as well as feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) status.

3. Results

3.1. Animals

A total of 298 cats were male (62.2%) and 181 cats were female (37.8%). The cats ages ranged from three months to 19 years. Median age was 7.4 years (age of 40 cats was unknown). Of the 468 cats, 106 (22.6%) were purebred cats and 362 (77.4%) cats were Domestic Shorthair (DSH). Breed was unknown in 11 (2.3%) cats.

Both, free FeLV p27 antigen and FeLV provirus was detected in 9 of 479 cats, that therefore were classified as having FeLV (1.9%) progressive infection. Regressive FeLV infection was detected in the 7 of 479 cats (1.5%) that were antigen-negative and provirus-positive. Twenty-two cats (4.5%) were FeLV antigen- and provirus-negative but anti-p-45 antibody-positive and were therefore suspected of having abortive FeLV infections. A total of 7 cats were FIV antibody-positive (1.5%); two of these cats were also progressively infected with FeLV.

3.2. Prevalence of rickettsial infections

All PCR-negative and extract-negatives controls were negative on all PCR assays. Of the 479 cats, two were positive for rickettsial DNA giving a prevalence rate of 0.4%. On the basis of these data, prevalence of rickettsial infections was determined to be between 0.01% and 1.6% (95% CI: 0.01–1.611) [4]. The BLAST results for both positive amplicons showed that one cat had 99% homology to accession # CP006618.1 *Anaplasma phagocytophilum str. Dog2 genome* and the second cat had 99% homology to accession # KC470064.1 *Anaplasma phagocytophilum strain HN 16S ribosomal RNA gene*, complete sequence.

3.3. Association to FIV and FeLV infection

There was no statistically significant difference in the FIV ($p = 1.000$) and FeLV ($p = 1.000$) status between *A. phagocytophilum*-negative and -positive cats (Table 1).

3.4. A. phagocytophilum-*infected cats*

The first *A. phagocytophilum*-infected cat was a female, neutered DSH of unknown age and origin. The cat lived within a multi-cat household and had access to outdoors. The cat was presented with a history of illness for five days. On physical examination, the cat was dehydrated (5%), hypothermic (37.4 °C), had a reduced general condition, heart murmur (2/6), and corneal lesions on the right eye. The cat showed a non-regenerative anemia (Ht: 25%; reference range: 30–44%; MCV: 37.9 fl; reference range: 40–55 fl; reticulocytes: 0.0%), neutrophilia with a left shift (neutrophils: 18.27×10^9/l; reference range: $3–11 \times 10^9$/l; banded neutrophils: 3.09×10^9/l; reference range: $0–0.6 \times 10^9$/l), monocytosis (monocytes: 1.97×10^9/l; reference range: $0.04–0.5 \times 10^9$/l), and mild lymphocytosis (lymphocytes: 4.50×10^9/l; reference range: $1–4 \times 10^9$/l). In addition, the cat had renal azotemia and

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