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Exposure of client-owned cats to zoonotic vector-borne pathogens: Clinic-pathological alterations and infection risk analysis

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Abbreviations:
VBD
vector-borne disease
CSD
cat-scratch disease
EDTA
ethylenediaminetetraacetic acid
IFAT
immunofluorescence antibody test
CBC
complete blood count
ALT
alanine aminotransferase
AST
aspartate aminotransferase
SAP
serum alkaline phosphatase
GGT
gamma glutamyl transferase
CI
confidence interval
OR
odds ratio

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ABSTRACT

Zoonotic Vector-Borne Diseases (VBDs) represent a relevant health issue for pets and humans. Italy is a major epidemiological hub for feline VBDs, because of suitable conditions for vector biology and disease transmission patterns. The present study investigated the exposure to major zoonotic arthropod-borne pathogens of cats in Italy, along with the evaluation of clinic-pathological features and a risk factor analysis. Out of 167 examined cats, 52 (31.1%) were seropositive for at least one vector-borne pathogen, being positivity for *Bartonella henselae* the most recorded (18%). Also, various cats seroreacted for *Rickettsia felis* (10.8%) and *Rickettisa typhi* (4.2%), *Leishmania infantum* (3%), *Anaplasma phagocytophilum* (2.4%) and *Ehrlichia canis* (2.4%). Forty-six cats were tested also for antibodies against *D. immitis* and two (4.3%) scored positive. The statistical analysis showed a positive association between flea infestation and seropositivity to *B. henselae*, other than an association between the administration of monthly ectoparasiticide treatments and seronegativity for *Rickettsia* spp.; seropositive cats were older than negative animals and the lifestyle (i.e. indoor vs outdoor) was not correlated with exposure to vector-borne pathogens. The majority of seropositive cats appeared clinically healthy or showed aspecific clinical signs. Around 80% of seropositive cats had one or more biochemical and/or complete blood count abnormalities. The present data confirm the endemicity of zoonotic feline VBDs in Italy and indicate that awareness on arthropod infections and transmitted pathogens should be kept high and possible implemented, towards the protection of animal and human health with adequate surveillance plans.

1. Introduction

Vector-Borne Diseases are caused by different pathogens transmitted to vertebrate hosts by invertebrate vectors [1]. These diseases are of growing concern in veterinary medicine and public health, due to their spreading and relevant pathogenic role [2–5].

Especially in Southern Europe, different factors have led to a geographic spread of VBDs of pets. For instance, a general decrease of veterinary cares has increased the number of free-roaming and stray animals [6]. Climate changes favor reproduction rates of arthropods and survival and development of transmitted pathogens [7,8]. Increased movements of animals foster the introduction of new diseases in previously free areas, where host populations are not immunized [9]. As occurred for the mosquitoes *Aedes albopictus* and *Aedes koreicus* [10,11], the intensification of the international trade of goods is primarily involved in the spreading of new vectors and VBDs [12].

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Cats are considered less frequently affected by VBDs then dogs and there is a paucity of data on feline VBDs if compared with studies carried out for dogs in the past decade [6].

This could also be due to a possible natural and genetically controlled immunological resistance of cats to arthropods and the pathogens they transmit [13]. Thus, lack of detailed information, underestimation of disease prevalence and underdiagnosed infections may impair our knowledge on feline VBDs.

Under a practical standpoint, clinic-pathological information on zoonotic feline VBDs is still scant, due to the sub-clinical and aspecific nature of clinical signs, the frequent occurrence of comorbidities concurring to a misrepresentation of the clinical pictures, and to the scarce perception of practitioners [4–6,14].

Cats with undiagnosed VBDs can play an important epidemiological role and concur to the spread of pathogens, as they act as source of infection for invertebrate hosts [15] or transmit directly pathogens to humans, as for instance in the case of *Bartonella henselae* [16]. Indeed, the epidemiological role of cats as potential sentinel of zoonotic diseases, under a "One Health" point of view, should be improved and reconsidered [17]. The implementation of epidemiological (i.e. risk factors analysis) and clinic-pathologic features is pivotal to improve diagnostic approaches and veterinary and sanitary awareness on feline VBDs. Thus, the present study has been carried out for evaluating the exposure of cats living in selected areas of Italy to major zoonotic VBDs, along with an evaluation of clinic-pathological alterations and analysis of potential risk factors.

2. Materials and methods

2.1. Animals and study areas

Overall 167 client-owned cats referred to the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, University of Teramo – Abruzzo, Central Italy (Site A, n = 122) and at different veterinary practices located in Apulia, Southern Italy (Site B, n = 45), were selected for the study. Detailed information on age, sex, lifestyle, habitat, presence/absence of clinical signs were recorded. An owner consent has been signed for sampled cats.

2.2. Clinical examination and sampling

All cats were subjected to a complete physical examination for the presence of clinical signs potentially related to VBDs, such as fever, lethargy, anorexia, depression, dermatological alterations, lymphadenopathy, ocular manifestations, mucosal abnormalities. After a thorough clinic examination, all cats were bled (3 ml) and each blood sample was divided in two aliquots, i.e. with and without EDTA, and processed immediately after the collection as follows.

2.3. Serology

Indirect immunofluorescence antibody assays were performed on cat sera with the procedures recently described [15,18–20] using the following materials:

- Anti-*Bartonella henselae*-IgG-antibodies: IFAT "Mega FLUO BARTONELLA *henselae*" (Megacor Diagnostik GmbH) kit (cut-off 1:64);
- Antibodies against *Rickettsia typhi* and *Rickettsia felis*: commercial antigen (slides coated with purified individual substrate antigens of *R. typhi* and *R. felis* - Fuller Laboratories) and anti-cat IgG (FLUO FITC anti-cat IgG conjugate);
- Antibodies against *Ehrlichia canis*: commercial antigen (slides coated with *E. canis* antigens - MegaScreen FLUO *E. canis*) and anti-cat IgG (FLUO FITC anti-cat IgG conjugate–Fuller Laboratories);
- Antibodies against *Leishmania infantum*: commercial slides coated with promastigotes (MegaFLUO Leish - Megacor Diagnostik GmbH) and anti-cat IgG (FLUO FITC anti-cat IgG conjugate).
- Antibodies against *Anaplasma phagocytophilum*: commercial antigen (slides coated with *A. phagocytophilum* antigens - MegaScreen FLUO *A. phagocytophilum*) and anti-cat IgG (FLUO FITC anti-cat IgG conjugate - Fuller Laboratories).

The amount of serum of 46 cats was sufficient also for testing the presence of antibodies against *Dirofilaria immitis* with the feline Heartworm Antibody Test Kit "Solostep® FH Cassette" (HESKA).

2.4. Complete blood count, biochemical profile and blood smears

Complete blood count, biochemical profile and blood smear evaluation were performed for cats seropositive to at least one VBD agent. Giemsa stained blood smears were performed as previously described [15] for the detection of inclusions suggestive of target pathogens.

2.5. Statistical analysis

A statistical analysis has been performed to evaluate the association of possible risk factors (i.e. age, sex, lifestyle, travel history, multi-cat households, ectoparasiticide treatments, presence or history of ectoparasite infestations) with seropositivity to VBD agents using Mann-Whitney or Fisher's exact test. The statistical analysis has been carried out considering either the seropositivity to at least one pathogen or to the different pathogens investigated in the present study. Furthermore, the determined odds ratio was used to measure the strength of association between the values of each factor and the presence of infections. Clinical laboratory analyses of cats seropositive to at least one VBD agent have been here presented as frequency of observation. The statistical analysis has been performed using GraphPad Prism version 6.0.1 (GraphPad Software, La Jolla, California, USA).

3. Results

3.1. Serology

Overall, 52/167 cats (31.1%) scored positive for at least one VBD, specifically 35 (28.7%) from site A and 17 (37.8%) from site B. Thirty cats (18%) seroreacted for *B. henselae*, while antibodies against *R. felis* and *R. typhi* were detected, respectively, in 18 (10.8%) and 7 (4.2%) cats. Five (3%) cats seroreacted against *L. infantum* while seroreaction against *A. phagocytophilum* and *E. canis* was detected in 4 and 4 cats each (2.4%). No cats scored positive for *A. platys*. Out of the 46 cats examined for *D. immitis*, 2 (4.3%) tested positive for antibodies (Table 1).

Thirty-one cats (including those tested also for *D. immitis*) had monospecific infections while 15 were seropositive for different VBDs in different combinations, being the combinations *R. felis* and *R. typhi* the most recorded (See Discussions, Section 4) (Table 2). Among cats that were not tested for *D. immitis* monospecific and polispecific exposure was recorded in 4 and 2 cats respectively (Table 2).

Table 1
Overall prevalence of the different Vector-Borne Pathogens investigated in the present survey. P = positive; T = total of sampled animals; NF = not found.

Pathogen	Site A P/T (%)	Site B P/T (%)	Total
Bartonella henselae	22/122 (18.0)	8/45 (17.8)	30/167 (18)
Leishmania infantum	4/122 (3.3)	1/45 (2.2)	5/167 (3)
Rickettsia felis	10/122 (8.2)	8/45 (17.8)	18/167 (10.8)
Rickettsia typhi	1/122 (0.8)	6/45 (13.3)	7/167 (4.2)
Ehrlichia canis	3/122 (2.4)	1/45 (2.2)	4/167 (2.4)
Anaplasma phagocytophilum	2/122 (1.6)	2/45 (4.4)	4/167 (2.4)
Dirofilaria immitis[*]	2/46 (1.6)	NF	2/46 (1.6)

[*] Forty-six cats positive to at least one Vector-Borne Pathogen were tested also for *Dirofilaria immitis*.

Table 2
Number and percentage of infections with Vector-Borne Pathogens either in cats tested also for *Dirofilaria immitis* ($^{D.i.}$) or not (*) in the present study. P = positive; T = total of sampled animals.

Monospecific infections	P/T (%)*D.i.*	P/T (%)*
Bartonella henselae	21/46 (46.6)	3/6 (50)
Leishmania infantum	1/46 (2.2)	–
Rickettsia felis	3/46 (6.5)	1/6 (16.7)
Ehrlichia canis	3/46 (6.5)	–
Anaplasma phagocytophilum	3/46 (6.5)	–
Total	31/46 (67.4)	4/6 (66.6)
Mixed infections		
Rickettsia felis + Rickettsia typhi	6/46 (13.0)	–
Rickettsia felis + Bartonella henselae	3/46 (6.5)	1/6 (16.7)
Rickettsia felis + Ehrlichia canis	1/46 (2.2)	–
Rickettsia felis + Leishmania infantum	2/46 (4.3)	–
Rickettsia felis + Rickettsia typhi + Bartonella henselae	1/46 (2.2)	–
Anaplasma phagocytophilum + Leishmania infantum	–	1/6 (16.7)
Dirofilaria immitis + Bartonella henselae	1/46 (2.2)	–
Dirofilaria immitis + Leishmania infantum	1/46 (2.2)	–
Total	15/46 (32.6)	2/6 (33.3)

3.2. Clinical findings at physical evaluation

Among positive cats, 9/52 (17.3%) showed at least one clinical sign. Four of them had aspecific signs, such lethargy, anorexia, dehydration, diarrhea and fever. Upper and lower respiratory signs (i.e. oculo-nasal discharge and cough) were also recorded in 6 cats.

3.3. Complete blood count, biochemical profile and blood smears

CBC and serum chemistry were performed for 42 and 44 of the 52 seropositive cats respectively. CBC and biochemical profile were not obtained for 10 and 8 cats respectively, due to difficulties in using adequate quantities of blood from these animals. Laboratory abnormalities (Tables 3 and 4) were present in 33/42 (78.6%) cats with CBC and in 35/44 (79.5%) cats with serum chemistry analysis. No pathogens were detected at blood smear evaluation.

Table 3
Serum chemistry abnormalities of 44 cats of the present study infected with at least one Vector-Borne Pathogen. AST (Reference Interval: 10–40 U/l), ALT (Reference Interval: 10–50 U/l), CK (Reference Interval: 90–320 U/l), SAP (Reference Interval: 0–47 U/l), Creatinine (Reference Interval: 0.7–1.8 mg/dl), Urea (Reference Interval: 20–65 mg/dl), Glucose (Reference Interval: 75–160 mg/dl), Bilirubin (Reference Interval: 0-0.7 mg/dl), Triglycerides (Reference Interval: 40–150 mg/dl), Total cholesterol (Reference Interval: 60–200 mg/dl), GGT (Reference Interval: 0–5 U/l). AST = aspartate-aminotransferase; ALT = alanine-aminotransferase; CK = creatine kinase; GGT = gamma-glutamil-transferase.

Parameter	Low n/%	High n/%
AST	–	5/11.3
ALT	–	15/34.1
CK	–	5/11.4
SAP	–	4/9.1
Creatinine	–	6/13.6
Urea	–	6/13.6
Glucose	–	12/27.3
Bilirubin	–	1/2.3
Triglycerides	12/27.3	9/20.4
Total cholesterol	1/2.3	18/40.9
GGT	–	5/11.4
Total protein	–	6/13.6
Albumin	5/11.4	–
Globulin	–	3/6.8

Table 4
Complete blood count abnormalities of 42 cats of the present study infected with at least one Vector-Borne Pathogen. Hematocrit (Reference Interval: 24–45%), Leucocytes (Reference Interval: 5000–19000/mm^3), Reticulocytes (Reference Interval: 0–80000/mm^3), Lymphocytes (Reference Interval: 1500–7500/mm^3), Monocytes (Reference Interval: 100–850/mm^3), Neutrophils (Reference Interval: 2000–12500/mm^3), Eosinophils (Reference Interval: 0–750/mm^3), Basophils (Reference Interval: 0–90/mm^3).

Parameter	Absence or Low n/%	Presence or High n/%
Hematocrit	3/7.1	–
Leucocytes	1/2.4	8/19
Reticulocytes	–	1/2.4
Lymphocytes	5/11.9	4/9.5
Monocytes	–	2/4.8
Neutrophils	5/11.9	7/16.7
Eosinophils	–	3/7.1
Basophils	–	17/40.5
Platelet estimates	3/7.1	1/2.4
Band neutrophils	–	2/4.8
Platelet aggregates	–	20/47.6
Rouleaux	–	1/2.4
Hypochromasia	–	1/2.4
Activated lymphocytes	–	1/2.4

3.4. Risk analysis

The Mann-Whitney test showed that the median age of positive cats was 48 months, while negative cats had median age of 36 months ($p = 0.02$). The Fisher's exact test revealed statistically relevant associations between the presence or history of flea infestation and *B. henselae* seropositivity, being 7/18 cats infested with fleas (38.9%; $p = 0.02$; 95% CI: 1.22–9.93; OR = 3.48) seropositive at the IFAT. The same test showed that seronegativity to *Rickettsia* spp. was more frequent in cats that were subjected to monthly ectoparasiticide treatments (n. 1/53, 1.9%) ($p = 0.01$; 95% CI = 1.14–68.32; OR = 8.84). No associations were observed between lifestyle (i.e. indoor vs outdoor) and the exposition to at least one pathogen investigated in the present study; indeed, outdoor seropositive cats were 27/92 (29.3%) while seropositive cats without access to the external environment were 25/75 (33.3%; $p = 0.62$). No any other statistically significant associations were detected.

4. Discussion

The present study showed that privately owned cats of Central and Southern Italy, including those mainly living indoor, are exposed to different zoonotic VBDs.

Bartonella henselae is worldwide distributed [14] and, accordingly, seropositivity to this pathogen has been the most recorded in the present study. These findings are similar to those observed in other regions of Southern Italy [21,22] and in outdoor cats from the North of the Country [23]. Also, these data are consistent with the results of two studies recently carried out in other countries of the Mediterranean Basin, that demonstrated high values of seropositivity to *B. henselae*, i.e. 50% and 58.8% in Spain [24] and Greece [15] respectively. If compared to these latter data, the lower prevalence rate here found is probably due to the fact that study cats were privately owned, including also indoor animals (n. 75/167 cats). This is of importance given that the aforementioned studies evaluated the presence of the infection only in cats with flea infestation [24] and in stray or free-roaming cats [15], thus supporting the evidence that outdoor or free-ranging animals are more prone to be infected by ectoparasites and transmitted pathogens [14]. However, 25/52 (48.1%) of cats seropositive to at least one pathogen examined in the present study were housed indoor and, of them, 10/25 (40%) seroreacted for *B. henselae*. Furthermore, 8/25 (32%) indoor cats that scored seropositive for at least one agent of VBD were

seropositive for *Rickettsia* spp. Although the seropositivity indicates the exposure to pathogens, and an evidence of pathogen DNA in the blood of the animals was not obtained, seropositive animals could potentially contribute to the circulation of flea-borne diseases in domestic environment, increasing the risk of infection for humans and animals sharing the same habitat.

Despite its presence has not been evaluated in the present study, *Bartonella clarridgeiae* is suspected to be another flea-borne agent of CSD [16]. Indeed, recent studies documented the occurrence of *B. clarridgeiae* in outdoor cats from Southern Italy [21,22] and the presence of this latter pathogen should be considered in designing further surveys investigating feline VBDs.

With regard to rickettsial infections, the fact that all study cats seropositive for *R. typhi* were also positive to *R. felis* could be due either to a multiple infection with these two pathogens or to cross-reactions. In fact, it is known that serological cross-reaction between *R. felis* and other rickettsiae of the typhus group, such as *R. typhi*, occurs [25,26]. This is of particular relevance given the different pathogenic potential of these two bacteria in humans, as murine typhus caused by *R. typhi* is more severe than flea-borne spotted fever by *R. felis* [27]. Furthermore, despite the presence of feline rickettsial infections has been documented in recent studies in Europe, including Italy [15,21,24], data on prevalence and circulation of these bacteria in cat populations are scanty and their presence is probably still underestimated. Hence, new studies aimed either at expanding epidemiologic and clinical knowledge on feline rickettsiosis are necessary.

Leishmaniosis is now considered an emerging disease of cats and it has been documented in different European countries (e.g. Italy, Spain, Greece, Portugal, France) with various seroprevalence rates, similar or higher to those here recorded [5,20]. Outdoor and stray cats from other studies carried out in Italy showed a higher seroprevalence for *L. infantum* than those here found, likely for the higher number of cats with more chances of ectoparasite infestation, contrarily to privately owned animals here examined [22,23,28]. Human visceral leishmaniosis is a major zoonotic disease and the role of cats in the urban cycle of *L. infantum* as an additional source of infection should be further investigated [29]. Privately owned cats can also be potentially involved in the *L. infantum* life cycle, as suggested by the present results. In fact, four out the five *L. infantum* seropositive cats of the present study lived indoor.

Feline infections with Anaplasmataceae have been described in different European countries (e.g. Spain, Portugal, Germany, Greece, Italy) [15,17,21,22,30,31]. The seroprevalence for *A. phagocytophilum* and *E. canis* is lower if compared to values obtained in previous surveys carried out in Southern Italy [21,22]. However, these data confirm that the exposure to these tick-borne pathogens should be not erroneously neglected even in animals with limited outdoor access. In particular, 4/4 and 2/4 of cats seropositive to *A. phagocytophilum* and *E. canis* respectively, lived indoors, suggesting that also these animals may favor the circulation of zoonotic tick-borne diseases.

The presence of *D. immitis* seroreactivity in cats here recorded is not exceptional, being exposure of cats already documented in cats from Central and Southern Italy [32,33]. Nonetheless, the prevalence rate of *D. immitis* in cats is significantly lower if compared to dogs (approximately the 10% of the known prevalence of canine infections) [34] and, given the documented southward expansion of canine *D. immitis* infections in Italy [32,33,35,36], there is the need to improve awareness on this disease regardless animal habitat even in areas that are currently considered non-endemic. For this reasons, *D. immitis* exposure in cat populations should encourage further surveys providing new data on the prevalence of this filariid in dogs from the same areas, which could be higher than expected. This is of importance given that microfilariaemic dogs act as source of infection for competent mosquito vectors, spurring the spread of heartworm disease and increasing the risk of infection for both animals and humans.

Cats displaying seroreaction for various VBDs can either be asymptomatic or show aspecific clinical signs with a wide variety of laboratory abnormalities [4,5,37–41]. Accordingly, the vast majority of seropositive cats of this study were apparently healthy (82.7%) while some (17.3%) had non-specific clinical alterations. However, these signs are only suggestive of a non-specific malaise and not necessarily related to an active vector-borne infection [15]. Various seropositive cats of the present study showed one or more laboratory abnormalities even in absence of overt clinical alterations, although it is unclear if directly imputable to a real infection or to other concurrent diseases. However, the alterations herein found are consistent with findings previously documented; for instance, three seropositive cats (i.e. two to *B. henselae* and one to *E. canis*) showed moderate thrombocytopenia as already observed in a previous study [22]. The half (21/42; 50%) of seropositive cats had a CBC compatible with an inflammatory status or an antigenic stimulation (i.e. presence of immature band neutrophils, activated lymphocytes, basophilia and eosinophilia). Also, 25% seropositive cats showed hyperproteinemia, hypoalbuminemia and/or hyperglobulinemia secondary to the immunity response. Accordingly, these alterations have been already observed in cats exposed to various VBDs, e.g. *B. henselae*, *E. canis* and *L. infantum* infections [5,40,42]. Basophilia is a rare finding in small animals [43] and, interestingly, it has been observed in 12/23 (52%) of the cats seropositive for *B. henselae*. Basophils are an integral component of hypersensitivity reactions and usually, basophilia is accompanied by eosinophilia [43]. However, eight *B. henselae* seropositive cats showed mild to moderate basophilia without eosinophilia. Therefore, diagnostic tests for *B. henselae* infection should be taken into account in cats with basophilia, even if eosinophilia is absent.

Several positive cats to at least one agent of VBD had mild increased liver enzymes and the half of these animals (11/22) seroreacted for *B. henselae*. These data fit with a previous study, where the 45% of cats with increased liver enzymes were seropositive for *B. henselae* [44]. A liver involvement has also been suspected in previously described cases of feline and canine anaplasmosis [45,46], canine ehrlichiosis [47] and canine leishmaniosis [48] and in humans with murine typhus [49]. These data suggest that the presence of VBDs, and especially of *B. henselae,* should be investigated in cats with unexplained increased liver enzyme, although further studies are warranted to ultimately confirm the association between VBDs and liver disorders.

An accurate evaluation of the drivers and risk factors potentially involved in the epidemiology of zoonotic VBDs is of primary importance. As expected, the higher median age of seropositive cats observed in this study population is related to a longer period of time of exposure to ectoparasites and related VBDs. Exposure to ectoparasites and outdoor access are key factor in the epidemiology of feline VBDs. However, no statistically significant difference in terms of seroprevalence between indoor and outdoor housed animals has emerged from this study. Even though unexpected, these data are similar to those observed in others epidemiological surveys in Southern European Countries, i.e. Cyprus, Spain and Portugal, being the exposure to *Bartonella* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Rickettsia* spp. similar in cats housed strictly indoor vs cats allowed to roam outdoor [17,24,50].

These results support the hypothesis that privately owned cats, including those conducing a mainly indoor lifestyle, could act as epidemiological sentinels and their role should be further evaluated, given their close contact to humans.

With the exception of a significant association between the use of ectoparasiticides and *Rickettsia* spp. seronegativity, no other association between ectoparasiticide treatments and positivity/negativity to the different pathogens emerged from the statistical analysis, as in a previous study [22]. Nevertheless, chemoprevention against ectoparasites is the most effective control measure against feline VBDs and the present data show that ectoparasiticide treatments should be applied also in indoor cats with no or limited outdoor access.

In conclusion, feline zoonotic VBDs are endemic in Italy, and these

data show that cats can be exposed to vector-borne pathogens with relatively high prevalence regardless of the lifestyle. Thus, preventative control measures are advisable even in animals living indoor, in order to protect both animal and human health. Given that some risk factors are still not completely understood and that the clinical features are not well defined, these data could spur further studies focused on these features of feline VBDs.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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