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Serological and Molecular Investigation of *Rickettsia* spp. and *Anaplasma* spp. in Domestic Cats from Sicily, Italy

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INTRODUCTION & AIM

Vector-borne pathogens such as *Rickettsia* spp. and *Anaplasma* spp. are increasingly recognized as emerging threats to both human and animal health. In the Mediterranean region, climatic and ecological conditions favor the proliferation of arthropod vectors, particularly ticks and fleas, enhancing the risk of pathogen circulation among domestic and wild hosts. Domestic cats (*Felis catus*), due to their close association with humans and frequent infestation by ectoparasites, may act as sentinels, incidental hosts, or potential reservoirs for these pathogens. Understanding their role is essential to evaluate the risk of zoonotic transmission and to improve surveillance within a One Health framework (Figure 1).
The aim of this study was to investigate exposure and infection rates of *Rickettsia* spp. and *Anaplasma* spp. in domestic cats from Sicily (Italy) (Figure 2). The study also included molecular screening of ectoparasites collected from these cats to detect the presence of vector-borne pathogens and assess their possible role in transmission cycles.

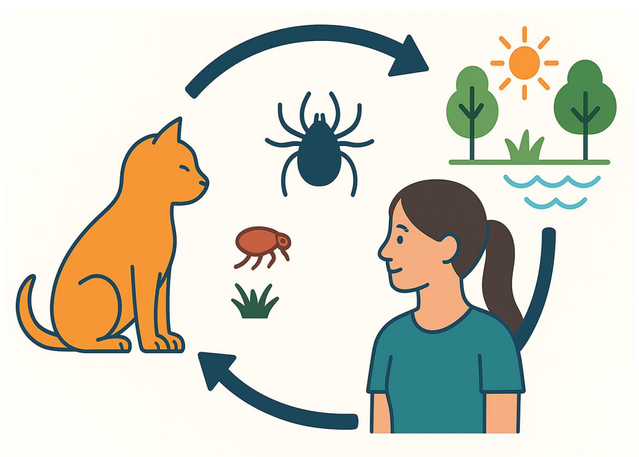

Figure 1. Tick-borne pathogens: a One Health perspective

Figure 2. Map of Sicily (Italy) showing the sampling area.

METHODS

Between 2024 and 2025, blood and serum samples were collected from domestic cats across different areas of Sicily. Serum samples were tested for antibodies against *Rickettsia* spp. and *Anaplasma* spp. using an indirect immunofluorescence assay (IFA) with commercial kits (Fuller Laboratories, USA), following the manufacturer's instructions.
Ectoparasites (ticks and fleas) collected from the examined cats were morphologically identified using standard taxonomic keys.
Genomic DNA was extracted individually from each sample (blood, ticks and fleas) using the DNeasy Blood and Tissue Kit (Qiagen, Germany). Ticks and fleas were first mechanically homogenized in 200 µL of tissue lysis buffer, followed by digestion with proteinase K at 56°C overnight to ensure complete lysis. Subsequent purification steps were performed according to the manufacturer's instructions. The extracted DNA was eluted and stored at −20°C until further molecular analyses. To confirm morphological identification, a fragment of the *mitochondrial cytochrome c oxidase subunit I* (*COI*) gene (~710 bp) was amplified by polymerase chain reaction (PCR) using universal invertebrate primers [1]. PCR reactions were performed under standard conditions, and the amplified products were verified by agarose gel electrophoresis and sequenced for species confirmation. The obtained sequences were compared with reference data in GenBank using the BLAST algorithm to confirm the taxonomic identity of the ectoparasites.
Blood, tick, and flea DNA samples were tested for the presence of *Rickettsia* and *Anaplasma* DNA by PCR, targeting the *ompA*, *ompB*, and *gltA* genes for *Rickettsia* spp., and the 16S rRNA gene for *Anaplasma* spp. (Table 1). PCR assays were carried out using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA) in a final volume of 50 µL, containing GoTaq G2 DNA Polymerase (Promega Italia s.r.l., Milan, Italy) and 5 µL of DNA template. Positive and negative controls were included in each run. Amplicons were separated by electrophoresis on 2% agarose gels stained with SYBR Safe DNA gel stain (Thermo Fisher Scientific) and visualized under UV illumination. PCR products of the expected size were purified, quantified, and submitted for sequencing to Macrogen Europe (Amsterdam, The Netherlands). Raw chromatograms were examined and edited using Chromas software (Technelysium Pty Ltd., Tewantin, Australia). Consensus sequences were generated in FASTA format and analyzed using the Basic Local Alignment Search Tool (BLAST, version 2.13.0; National Center for Biotechnology Information, USA) to determine the closest matching species based on sequence similarity.

Pathogen	Gene Target	PCR Assay	Primer/Probe Sequences	Reference
Rickettsia spp.	*ompA*	Nested PCR	Rr190.70p 5′-ATGGCGAATATTTCTCCAAAA-3′- Rr190.701n 5′-GTTCCGTTAATGGCAGCATCT--3′ Rr190.602n 5′-AGTGCAGCATTCGCTCCCCCT-3′	[2]
Rickettsia spp.	*ompB*	Nested PCR	rompB OF 5′-GTAACCGGAAGTAATCGTTTCGTAA-3′ rompB OR 5′-GCTTTATAACCAGCTAAACCACC-3′ rompB SFG IF 5′-GTTTAATACGTGCTGCTAACCAA- 3′ rompB SFG IR 5′-GGTTTGGCCCATATACCATAAG-3′	[3]
Rickettsia spp.	*gltA*	PCR	409D 5′-CCTATGGCTATTATGCTTGC-3′ 1258N ATTCCAAAAAGTACAGTGAACA-3′	[4]
Anaplasma spp.	*16S-rRNA*	Nested PCR	EE1 5′-TCCTGGCTCAGAACGAACGCTGGCGGC-3′ EE2 5′-AGTCACTGACCCAACCTTAAATGGCTG-3′ EE3 5′-GTCGAACGGATTATTCTTTATAGCTTGC-3′ EE4 5′-CCCTTCCGTTAAGAAGGATCTAATCTCC-3′	[5]

Table 1. Primers and PCR assays used for the molecular detection of *Rickettsia* spp. and *Anaplasma* spp. in feline blood and ectoparasite samples. The table summarizes the target genes, type of PCR assay, primer sequences, and corresponding literature references.

RESULTS & DISCUSSION

A total of 80 blood and serum samples were collected from domestic cats. Serological analysis showed high levels of exposure to vector-borne pathogens: antibodies against *Rickettsia* spp. were detected in 56.3% (45/80) of cats, while antibodies against *Anaplasma* spp. were found in 12.6% (10/80).
Rickettsia felis DNA was identified in 3.8% (3/80) of feline blood samples, whereas no *Anaplasma* spp. DNA was detected. Among the ectoparasites analyzed, *R. felis* DNA was detected in one of two fleas (*Ctenocephalides felis*), and *Anaplasma phagocytophilum* DNA was found in one *Ixodes ricinus* tick out of eight examined (Table 2).
The marked difference between seroprevalence and molecular findings indicates that cats are frequently exposed to *Rickettsia* spp. and *Anaplasma* spp., but active infections are uncommon or transient. This discrepancy likely reflects a short bacteremic phase that reduces the likelihood of molecular detection in blood samples. The detection of pathogen DNA in fleas and ticks confirms environmental circulation of these microorganisms and supports their epidemiological relevance in Sicily. However, the presence of pathogen DNA in ectoparasites collected from cats does not necessarily imply that cats serve as reservoirs; they may instead represent incidental hosts exposed to infected vectors.
These findings align with previous studies conducted in Mediterranean regions, where *R.felis* and *A. phagocytophilum* are endemic but show variable prevalence depending on environmental and ecological conditions. The results emphasize the importance of continuous monitoring of companion animals and their ectoparasites to better understand vector-borne disease dynamics and potential zoonotic risks.

Pathogen	Test	Sample Type	Number of Samples	Positive	Prevalence (%)	Detected Species
Rickettsia spp.	IFA	feline serum	80	45	**56.3%**	Antibodies against *Rickettsia* spp.
Anaplasma spp.	IFA	feline serum	80	10	**12.6%**	Antibodies against *Anaplasma* spp.
Rickettsia spp.	PCR	feline whole blood (EDTA)	80	3	**3.8%**	*Rickettsia felis* DNA
Anaplasma spp.	PCR	feline whole blood (EDTA)	80	0	**0%**	No DNA detected
Rickettsia spp.	PCR	Fleas (*Ctenocephalides felis*)	2	1	**50%**	*R. felis* DNA
Anaplasma spp.	PCR	Ticks (*Ixodes ricinus*)	8	1	**12.5%**	*Anaplasma phagocytophilum* DNA

Table 2. Serological and molecular detection of *Rickettsia* spp. and *Anaplasma* spp. in domestic cats (*Felis catus*) and their ectoparasites from Sicily. The table summarizes the prevalence of antibodies detected by IFA and the PCR-positive samples from feline blood, fleas and ticks.

CONCLUSION

This study showed that domestic cats in Sicily are widely exposed to *Rickettsia* spp. and *Anaplasma* spp., although molecular evidence of active infection is limited. The detection of pathogen DNA in ectoparasites highlights the circulation of these agents in the environment and their potential role in transmission.
The results reinforce the need for integrated surveillance programs involving companion animals, vectors, and humans within a One Health approach. Such interdisciplinary monitoring is crucial to better assess the epidemiological significance of feline hosts and to mitigate the public health risks posed by emerging vector-borne pathogens in Mediterranean areas.

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FUTURE WORK / REFERENCES

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