P-39

A PCR prevalence study of vector-borne pathogens of cats in Greece - the major challenges

Panagiota Ligda^{1*}, Anastasios Ligdas¹, Smaragda Sotiraki¹

1. Laboratory for Parasitology and Parasitic Diseases, Veterinary Research Institute of Thessaloniki, Hellenic Agricultural Organisation (ELGO-DIMITRA), Thessaloniki, Greece. *giota.lig@hotmail.com

Background

Vector-borne pathogens (VBPs) represent a growing global threat, posing unprecedented challenges to veterinarians, especially in endemic areas. Greece is in the Mediterranean basin with favorable environmental conditions for pathogen transmission and poorly managed stray dog and cat populations. Numerous studies and publications have reported the country to be a highly endemic area for various VBPs, most of them focusing on dogs [1] and only rarely on cats [2]. The aim of this study was to investigate the presence of VBPs in cats in Greece.

Materials and methods

Blood and conjunctival swab samples were collected from 207 adult household cats and molecularly analyzed to identify the presence of *Leishmania infantum* (blood and conjunctival swab samples), *Ehrlichia* spp, *Anaplasma* spp, *Bartonella henselae* and *Hepatozoon* spp (blood samples). Precisely, for the detection of *L. infantum* a TaqMan real-time qPCR assay targeting a 120 bp fragment of the kinetoplast minicircle DNA, was performed as previously reported [3]. For the presence of *Ehrlichia* spp, a PCR targeting a 345 bp fragment of the 16S rRNA gene [4] of various species including *E. canis, E. chaffeensis, E. muris, E. ruminantium, A. phagocytophilum, A. platys, A. marginale, A. centrale, Wolbachia pipientis, Neorickettsia sennetsu, N. risticii and N. helminthoeca, was performed according to previously described thermal-cycling conditions [5]. For the presence of <i>Anaplasma* DNA, species-specific nested PCRs for *A. platys* and *A. phagocytophilum* targeting a 678 bp and a 546 bp fragment of the 16S rRNA gene, respectively, were performed according to previously described protocols [6]. For the detection of *B. henselae* a PCR targeting the citrate synthase gene was performed [7], while for *Hepatozoon* a partial sequence of 18S rRNA gene was amplified according to previously described protocols [8].

Results

L. infantum was detected in 6.3% (n=13/207) of the cats, either in blood and/or in conjunctival swabs (Table 1). Parasitic load in blood samples was up to 2,509 genome copies/ml, while it was as high as 7,678 copies in conjunctival swabs. For *Ehrlichia* spp the infection level was 9%, for *Anaplasma* spp 8.4%, for *B. henselae* 14.2%, while for *Hepatozoon* spp was 40.6%. Sequencing results confirmed the presence of *B. henselae* and *H. cati*

Pathogen	Infection level
Leishmania infantum	6.30%
<i>Ehrlichia</i> spp	9%
Anaplasma spp	8.40%
Bartonella henselae	14.20%
Hepatozoon spp	40.60%

Table 1. Infection level of cats with the different pathogens analysed.

Conclusions

As shown, parasitic infections in cats in Greece are present and cannot be neglected, raising issues regarding the animals' health/welfare status and the spread of zoonotic pathogens. The domestic cat is an extremely flexible, adaptable species and a successful predator, in most cases capable of surviving without human support. This holds particularly true for the Greek cat population, where a significant

proportion of cats reside outdoors. Managing such infestations poses a considerable challenge, necessitating a shift in owners' perspectives towards preventive measures and therapies.

Funding: Self-funded

Conflict of interest: None declared.

References

- Kostopoulou D, Gizzarelli M, Ligda P, Foglia Manzillo V, Saratsi K, Montagnaro S, et al. Mapping the canine vector-borne disease risk in a Mediterranean area. Parasit Vectors 2020;13:282. <u>https://doi.org/10.1186/s13071-020-04153-8</u> Kokkinaki, K.C.G, Saridomichelakis, M.N., Skampardonis, V., Mataragka, A., Ikonomopoulos, J.; Leontides, L., Mylonakis, M.E.,
- 2. Steiner, J.M., Suchodolski, J.S., Xenoulis, P.G. Prevalence and Risk Factors for *Bartonella* spp. and *Haemoplasma* Infections in Cats from Greece. Vet. Sci. 2022, 9, 337. https://doi.org/10.3390/vetsci9070337
- 3. Francino O, Altet L, Sánchez-Robert E, Rodriguez A, Solano-Gallego L, Alberola J, et al. Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. Vet Parasitol. 2006;137:214–221
- 4. Inokuma H, Raoult D, Brouqui P. Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. J Clin Microbiol. 2000;38:4219–4221.
- 5. Brown GK, Martin AR, Roberts TK, Aitken RJ. Detection of *Ehrlichia platys* in dogs in Australia. Aust Vet J. 2001;79:554–558
- 6. Springer A, Montenegro VM, Schicht S, Globokar Vrohvec M, Pantchev N, Balzer J, et al. Seroprevalence and current infections of Canine Vector-Borne Diseases in Costa Rica. Front Vet Sci. 2019;6:164
- 7. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;3:1797-1803
- 8. Inokuma H, Okuda M, Ohno K, Shimoda K, Onishi T. Analysis of the 18S rRNA gene sequence of a *Hepatozoon* detected in two Japanese dogs. Vet Parasitol. 2002;106:265-271