

SUMMARY

of the doctoral thesis entitled:

EPIDEMIOLOGY AND DIAGNOSIS OF SOME DIGESTIVE PROTOZOA IN CARNIVORES, IN THE SOUTHERN PART OF ROMANIA

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KEYWORDS: endoparasitofauna in carnivores; digestive protozoa; giardiasis and cystoisosporosis in dogs and cats; diagnostic; epidemiology; zoonotic risk

Parasitic infestations, especially those with zoonotic potential, are important public health problems (Mitrea, 2011; Mitrea et al., 2012). Carnivores are hosts for more than 60 potentially zoonotic parasites (Sarvi et al., 2018). The presence of these animals in close contact with humans constitutes a potentially high risk of infestation, especially for children due to less frequent hygiene practices than those of adults, as well as greater exposure to contact with soil contaminated with parasite eggs or cysts. Gastrointestinal parasites of dogs such as *Toxocara canis*, *Canine hookworm*, *Echinococcus* spp., *Canine dipylidium*, *Giardia duodenalis*, *Cryptosporidium* spp. is of considerable importance in the field of public health through its zoonotic potential (Youn, 2009).

Parasitic infestations have certain common features such as more frequent damage to young animals, while adult animals have subclinical infestations, representing sources of infestation; the variability of pathogenicity, infestations being often asymptomatic and self-limiting, under certain conditions; the lack of specificity of the signs of the disease, and their expression is frequently associated with the intestinal phase; the difficulty of establishing a definite diagnosis, in certain situations, since the negative result of analyzing a sample of feces does not exclude the disease (Uiterwijk et al., 2018).

The early diagnosis of parasitosis is very important, both for the well-being and health of pets, and for reducing the risks of transmission to humans, in the case of zoonotic species.

The management of digestive endoparasitoses, as in the case of other parasitoses, requires a definite diagnosis. Establishing a definite diagnosis, through sensitive and specific methods, is the basis of any therapeutic and preventive approach. Laboratory tests are based on the detection, by microscopic examination of specific parasitic elements in faecal samples, as well as on immunological tests (copro-antigens in the case of certain species) or molecular biology, procedures and techniques characterized by variable sensitivities, which influence the methodology of investigation, depending on the purpose pursued.

Improving knowledge on the prevalence of endo-parasitoses in general and those with zoonotic risk, such as giardiasis in particular, in animals in different areas and different communities contributes to the improvement of animal health and the development of control measures, including for public health risk assessment.

In this context, the present work addresses, through integrated studies, the problem of the epidemiology of digestive protozoa, mainly giardiasis and cystisporosis, in pets, dogs and cats from the South of Romania, in order to assess the potential risks both for animal health and for the human one. Thus, the main objectives of the studies were:

- **investigating the structure of the intestinal parasitofauna in dogs and cats, in South-Eastern Romania; epidemiology of some protozoa: cystoisosporosis and giardiasis:**

- epidemiological aspects of endoparasitic infestations in dogs and cats, both in terms of their unique presence and polyparasitism;
- identifying the risk factors associated with parasitism in these animals, with an emphasis on the presence of giardiasis, in light of the risks associated with both animal and human health.
- **coproparasitological investigations, including rapid tests, regarding infestations with *Giardia duodenalis* in dogs and cats;**
- **molecular identification of infestation with *Giardia duodenalis* the carnivore.**

The thesis is structured in 6 chapters integrated into the two parts of the thesis: Section I – bibliographic study, with two chapters (32 pages, representing approx. 1/3 of the total thesis) and Section II – propre research with 4 chapters related to the studies carried out. The thesis is completed by 29 tables and 43 figures (graphics / images / original photos) and a bibliographic list of 175 titles.

The first part of the thesis – the bibliographic study, presents updated, synthetic information regarding the best-known digestive protozoa in carnivores, giardiasis and cystoisosporosis.

Thus, chapter I - Giardiasis in carnivores, begins with information on etiology, history, and taxonomy *Giardia*, morphology and biology of species of this genus, elements of epidemiology and ways of disease transmission. In the second part of the chapter, the risk factors for public health presented by their presence are presented *Giardia* in animals, the prevalence of infestation with this parasite as described in the specialized literature, elements of pathogenesis and diagnostic techniques.

Similarly, the second chapter is dedicated to bibliographic studies on cystisporosis, problematizing the etiology, history, taxonomy, morphology and biology of the species of the genus *Cystoisospora* and continuing through elements of epidemiology, pathogenesis, and diagnosis.

Section II of the thesis is structured in 4 chapters that describe the studies integrated to achieve the objectives targeted in the research plan.

The third chapter, presents an epidemiological study on the structure of the gastrointestinal parasitofauna in dogs and cats from the south-eastern region of Romania, and in this context the problem of the epidemiology of some digestive protozoa (giardiasis, mainly), with the assessment of potential risks both for animal health and for the human one. Coproparasitological investigations were carried out in the period 2013-2019 on a total number of 1459 dogs and 253 cats from Brăila, Bucharest, Călărași, Ialomița, Ilfov, Prahova and Vâlcea counties. Animals with owners, from public or private shelters, kennels, and community animals were included.

The coproparasitological examination was carried out by the zinc sulfate flotation method. The results indicated a prevalence of parasitism of 44.83% in dogs versus 23.32% in cats, significantly correlated with species. Also, polyparasitism prevailed in dogs compared to cats. No correlations with clinical signs were identified. In dogs, helminths predominated (35.3%), and in cats protozoa, *Giardia* having the highest prevalence (11.86%). Among the predictors of parasitism were the age, breed, origin of the animals. Age under 1 year correlated with the presence of *Giardia* and *Cystoisospora*. The study dedicated to giardiasis in 107 symptomatic animals indicated prevalences of 21.4% in dogs versus 5.6% in cats (rapid test). Bacteriological examination confirmed mixed intestinal infections. The results highlight the need for consolidated measures to prevent and control digestive parasites in animals, including to reduce zoonotic risks.

The fourth chapter details the results of investigations into infestations with *Giardia duodenalis* to carnivores and the identification of potential associated risk factors, carried out on a number of 107 animals with digestive clinical signs (89 dogs, 18 cats) originating from Bucharest and the Bucharest metropolitan area. The investigations were carried out by complementary methods, including the immunochromatographic test *Giardia* copro-antigen, the coproparasitological examination by the zinc sulfate flotation method and the bacteriological examination. The results of the study indicated a prevalence of infestation with *G. duodenalis* of

21.4% in tested dogs and 5.6% in cats. The prevalence rate was higher in younger dogs (≤ 1 year) at 30.8% compared to adults (17.2%) and those over 8 years (20%). Microscopic examination also confirmed the presence of associated intestinal parasites. Thus, besides *G. duodenalis* (20.45%) the following species were identified: *Toxocara canis* (11.4%), *Isospora* spp. (6.8%), *Canine hookworm* (4.5%) and *Trichuris fox* (2.3%). At the same time, mixed infestations were found in dogs positive for *Giardia*. The data obtained highlight the relevant public health risks associated with this protozoan.

In the fifth chapter the optimization and application of a modern molecular biology technique, namely Real-Time PCR, for the detection of specific *Giardia* parasite DNA in fecal samples from dogs and cats is presented. The results obtained by this molecular method confirm the sensitivity and specificity of the technique, feasible both in routine diagnosis and in carrying out epidemiological studies on *Giardia* infestations in domestic carnivores.

The conclusions of the integrated studies of this thesis highlight that *G. duodenalis*, in single or mixed parasitic infestations, was identified as the main causative agent of digestive disorders and respectively present in the endo-parasitofauna of the carnivores included in the study, with a higher prevalence rate in young animals.

At the same time, it was confirmed that infections with *G. duodenalis* they can be associated with multiple parasitic infestations and bacterial infections. They emphasize the need to carry out complete, complementary investigations, especially in the case of clinical and epidemiological suspicion, as a basis for applying an appropriate management and therapy protocol to companion animals. In addition, these results confirm the need to apply parasitological control measures, including to reduce public health risks.