

ABSTRACT

Vector borne diseases have become a focal point of present day research, with many posing a significant threat to both human and veterinary patients. While the most clinically impactful vector-borne parasites tend to belong to the Protozoa reign, nematodes have begun to garner some much-deserved attention over the past century (ANDERSON, 2000). Outside of the already established *Dirofilaria* spp. and *Onchocerca* spp., modern medicine has been facing a reemerging and expanding threat posed by *Thelazia* spp., *Setaria* spp. and *Parafilaria* spp. (PANAITESCU et al., 1999; ȚĂLU et al., 2012; MATHER & TREUTING, 2012; BRADBURY et al., 2018A; MARTINEZ-SANCHEZ et al., 2021; DOLFF et al., 2020). As one of the unique taxa of parasitic nematodes, *Thelazia* spp. represents a co-evolutionary model for both insects and nematodes (OTRANTO & TRAVERSA, 2005). These eyeworms inhabit the segments preceding the eye, namely the conjunctiva and the tear ducts. Along with other members of the Spirurina suborder, they hold the distinction of being the only nematodes for which the secretophagous flies act as vectors (STOFFOLANO 1970).

While the overall distribution of the disease appears ubiquitous, eluding the polar regions (SKRJABIN et al., 1971), and the reservoir role of domestic and wild carnivores for *T. callipaeda* being clearly established (OTRANTO & TRAVERSA, 2005; DO VALE et al., 2019), additional insight is required to ascertain the current distribution and host range of *Thelazia* spp. in herbivores. Despite humans not considered as playing an important role in its life cycle, aberrant transmission has been reported (WEI et al., 2020; BRADBURY et al., 2018A; SINGH & KHINDRIA, 2018). With most cases linked to mild symptoms, the vast majority of cases go unnoticed (DJUNGU et al., 2014), facilitating both transmission and evolution of disease severity (NAEM, 2007). Scarce reports originating from Romania have continuously underlined the neglected nature of this disease, which correlated with its high prevalence in cattle (~20%) (DULCEANU 1971) and overall increasing diversity of its hosts (MIHALCA et al., 2016) create a conundrum for clinicians. Moreover, thelaziosis was described as a potential threat for reintroduction efforts of the European bison (DEMIANSK & KACZOR, 2014).

The first part (I) of the current thesis is comprised of three chapters, providing an up to date summary of the information available in scientific publications on the taxonomy, distribution, pathogenesis and diagnosis of thelaziosis, as well as its historical development in Romania. Chapter I.1. focuses on the taxonomic journey of genus *Thelazia*, introducing its current standing within modern nomenclature, along with its tumultuous historical development. Chapter I.2. builds upon its predecessor by providing insight into the main species of *Thelazia* of clinical importance, their life cycle, distribution, pathogenicity and diagnostic methods. Chapter I.3. creates the required premise for the current study, by providing the historical distribution of the disease within Romania.

The second part (II) of the thesis establishes the aims of the study, contingent on four original studies focused on the epidemiology, morphology and diagnosis of *Thelazia* spp. in domestic and wild herbivores from Romania. These are then followed by the conclusions and recommendations sections, along with the originality of the study itself. The cited references conclude the thesis, with 182 titles.

Through the study presented in Chapter II.1. we established the presence of *T. lacrymalis* in horses from Romania, further building on the very few existent reports on the distribution of this nematode. The aim of this study was to assess the presence, prevalence and distribution of *T. lacrymalis* in horses from Romania. The eyes of 273 horses, slaughtered at two abattoirs from the Northwestern and Western regions of Romania, were examined for the presence of *T. lacrymalis* between March and November 2021. Of the 273 animals sampled, 12 (4.39%) were positive for *Thelazia* spp. Eighty-seven nematodes were recovered, all morphologically identified as *T. lacrymalis*. The intensity of infestation varied between one and 33 nematodes/animal while five animals presented a bilateral infestation and seven a unilateral one. The highest prevalence was encountered in Pannonian ecoregion (12.12%) while the lowest was in the Alpine ecoregion (0%). Seventy-five intact specimens underwent detailed morphometric analysis, of the 18-20 parameters, resulting in notable differences in striation lengths compared to the data available in other reports. BLAST analysis identified a 96.46-98.60% similarity to the only other COI gene sequence available for *T. lacrymalis*. Lastly, through phylogenetic analysis, it separated *T. lacrymalis* into a different clade within the genus.

Chapter II.2. details a study focused on the presence of *T. callipaeda* in the European hare in Romania. The aim of this study was to evaluate the status of the Romanian populations of European brown hares, *Lepus europaeus* as reservoir hosts for *T. callipaeda*. Between November 2019 and November 2021, the eyes of 326 *L. europaeus* carcasses were examined for the presence of ocular parasites. Four (1.23%) hares harbored *T. callipaeda* infection, with a total of 84 nematodes collected (mean intensity 21 nematodes/host), with 45 males, 39 females (two sexually immature, seven with only eggs, and 30 with eggs and larvae). One specimen from each host was successfully sequenced resulting in a 100% similarity with several other sequences of *T. callipaeda* haplotype 1. Despite the low prevalence, *Lepus europaeus* appears to be a reservoir host for the disease. The study also stands as the only quantitative analysis of the distribution of *T. callipaeda* in lagomorphs.

Building on the previous two studies, through Chapter II.3. we aimed to provide a detailed epidemiological, morphological and molecular description of *Thelazia rhodesi*, *T. gulosa* and *T. skrjabini*. The eyes of 705 slaughtered cattle were sampled and subjected to morphological identification, morphometric analysis, and molecular characterization. PCR amplification and sequence analysis were performed based on the cytochrome oxidase subunit 1 (*COI*) gene. A novel PCR method was developed based on the consensus sequence from each species. Specific forward primers were designed for each of the three species, and a reverse primer (COIntR) was used for all reactions. A consensus thermal profile was established by gradient PCR amplification of each species separately. A prevalence of 19.3% was observed, which varied significantly with ecogeographical factors. A total of 585 *Thelazia* nematodes were recovered, with *T. rhodesi* being the most abundant, followed by *T. skrjabini* and *T. gulosa*. Morphometric and molecular analyses supported the morphological identification, yielding unique sequences for each species. The study provides the most comprehensive phylogenetic analysis of the genus, which also serves as the basis of a novel multiplex PCR, which is able to successfully diagnose infections with one or more *Thelazia* species. Lastly, it underlines the severity of the knowledge gap within the framework of neglected diseases, by comparing the historical prevalence from Europe to that obtained in the current study, highlighting the similarity of values despite modern therapeutic options.

Concluding the thesis, the in-prep study detailed in Chapter II.4. focused on the presence of two of the three known *Thelazia* species in bovines, in wild European bison and farmed American bison. Between November 2021 and January 2023, both eyes of seven American bisons (5 slaughtered, 2 brought to the Pathology department following their death), from two different locations in Romania were collected and examined for the presence of eyeworms. Additionally, two European bisons, originating from reintroduction sites were also sampled, during routine necropsy. Three of the seven examined American bisons were infected by *Thelazia*, while both European bisons harbored an infection. Thirty-eight nematodes were recovered from the conjunctival sacks and lachrymal ducts of the animals. Two species were morphologically identified in both the American and European bison: *T. skrjabini* and *T. rhodesi*. The research manages to establish the presence of the disease in both species, adding several new sequences of *T. rhodesi* and *T. skrjabini* to Genbank. Moreover, building on the previous study, it underlines the importance of livestock as a potential reservoir for the disease, affecting reintroduction efforts of susceptible European bison.

References

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