

The diversity of parasitic fauna in the European brown hare (*Lepus europaeus* Pallas, 1778) from the southern region of the Republic of Moldova

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Abstract. The paper presents the results of research on the parasitic fauna of the European brown hare (*Lepus europaeus* Pallas, 1778) in the Southern region of the Republic of Moldova. This area hosts a diversity of 12 parasitic species, classified into 3 phyla: Nematoda, Platyhelminthes, and Apicomplexa, as well as in 4 taxonomic classes: Cestoda, Trematoda, Secernentea, and Gonoidasida. Within the class Cestoda, one species was identified, *Cysticercus pisiformis* (Zeder, 1803); in Trematoda, two species were described: *Fasciola hepatica* (Linnaeus, 1758) and *Dicrocoelium lanceolatum* (Rudolphi, 1919); in Secernentea, six species were found, namely: *Trichocephalus leporis* (Frölich, 1789), *Strongyloides papillosus* (Wedl, 1856), *Trichostrongylus retortaeformis* (Zeder, 1800), *Passalurus ambiguus* (Rudolphi, 1819), *Trichostrongylus probolurus* (Railliet, 1896), and *Trichuris leporis* (Frölich, 1789); and in the class Gonoidasida, 3 species of *Eimeria* were identified: *Eimeria leporis* (Nieschulz, 1923), *Eimeria stiedae* (Lindemann, 1865), and *Eimeria intestinalis* (Cheissin, 1948). Among the identified helminths, two species – *Fasciola hepatica* (Linnaeus, 1758) and *Dicrocoelium lanceolatum* (Rudolphi, 1919), are known to have the potential to parasitize humans as well. The research revealed that parasitic agents were present in 100% of the collected samples. Regarding the infestation typology, 45 samples (25.5%) were found to have infestations with a single parasitic species, while 165 samples (74.5%) exhibited polyparasitic infestations, with 2 to 6 coexisting species. These results provide a detailed overview of the parasitic fauna affecting the European brown hare in this region, highlighting the importance of continuing research on the impact of these parasites on wildlife health and their potential impact on human health.

Keywords: parasitic fauna, European brown hare, single infestations, polyparasitic infestations, parasitic species.

Diversitatea parazitofaunei la iepurele-de-câmp (*Lepus europaeus* Pallas, 1778) din zona de sud a Republicii Moldova

Rezumat. În lucrare sunt prezentate rezultatele cercetărilor realizate asupra faunei parazitare a iepurelui-de-câmp (*Lepus europaeus* Pallas, 1778), din Zona de Sud a Republicii Moldova. Această zonă adăpostește o diversitate de 12 specii parazitare, încadrate în 3 încrengături: *Nematoda*, *Platyhelminthes* și *Apicomplexa*, și în 4 clase taxonomice:

Cestoda, *Trematoda*, *Secernentea* și *Gonoidasida*. Astfel, în cadrul clasei *Cestoda* a fost identificată o specie, *Cysticercus pisiformis* (Zeder, 1803), în *Trematoda* au fost descrise două specii: *Fasciola hepatica* (Linnaeus, 1758) și *Dicrocoelium lanceolatum* (Rudolphi, 1919), în *Secernentea* au fost găsite șase specii, respectiv: *Trichocephalus leporis* (Frölich, 1789), *Strongyloides papillosus* (Wedl, 1856), *Trichostrongylus retortaeformis* (Zeder, 1800), *Passalurus ambiguus* (Rudolphi, 1819), *Trichostrongylus probolurus* (Raillet, 1896) și *Trichuris leporis* (Frölich, 1789), iar în cadrul clasei *Gonoidasida* au fost identificate 3 specii de *Eimeria*: *Eimeria leporis* (Nieschulz, 1923), *Eimeria stiedae* (Lindemann, 1865) și *Eimeria intestinalis* (Cheissin, 1948). Dintre helminții identificați, două specii, *Fasciola hepatica* (Linnaeus, 1758) și *Dicrocoelium lanceolatum* (Rudolphi, 1919), sunt cunoscute ca având și posibilitatea de a parazita omul. Cercetările au relevat faptul că, în eșantioanele prelevate, agenții parazitari au fost prezenți în 100% dintre cazuri. În ceea ce privește tipologia infestării, în 45 de probe (25,5%) au fost detectate infestări cu o singură specie de parazit, iar în 165 de probe (74,5%) au fost observate infestări poliparazitare, cu 2 până la 6 specii coexistente. Aceste rezultate oferă o imagine detaliată a diversității faunei parazitare care afectează iepurele-de-câmp din această regiune, subliniind importanța continuării cercetărilor asupra impactului acestor paraziți asupra sănătății faunei sălbatice și a potențialului lor impact asupra sănătății umane.

Cuvinte-cheie: parazitofauna, iepure-de-câmp, monoinvazii, poliinvazii, specii parazitare.

1. INTRODUCTION

The European brown hare (**Lepus europaeus** Pallas, 1778) represents an essential component of the wildlife in the Republic of Moldova, having significant ecological importance, especially in agroecosystem landscapes. It is a typical inhabitant of agricultural areas, preferring cultivated land, where hare populations are more abundant due to the available food resources. Among the factors that can negatively influence hare populations are, in particular, parasitic and infectious diseases [1].

Previous studies suggest that European brown hare populations (**Lepus europaeus**) in Europe have significantly decreased in recent decades due to multiple ecological and biological stress factors. Infectious and parasitic diseases are among the main causes negatively affecting the survival and reproductive rates of these populations [8]. In the Republic of Moldova, since 2012, there has been a significant increase in hare populations, with the autumn population rising from 62,000 to 145,000 individuals by spring 2017, with densities ranging from 140 to 200 hares per 1000 ha in the ecotone areas of agroecosystems. In the autumn of 2019, the hare population reached approximately 246,000 individuals [1, 7].

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At the same time, research has highlighted that the European rabbit (*Oryctolagus cuniculus**), an invasive species that has been successful worldwide, can serve as an important reservoir for numerous parasitic species. In Europe, this species is found across vast areas of the continent, and its expansion to European islands has been facilitated by human activities. The European rabbit can act as a host for a wide range of pathogens, including gastrointestinal parasites, which can affect both wildlife and domestic animals [4].

Changes brought about by the reorganization of livestock units and the increase in the number of small farms, along with the relocation of animals from complexes to private households, have led to significant transformations in the parasitic fauna. Thus, animals that were previously kept in stables, now grazing in various anthropogenic stations or natural reserves, can transmit pathogens to wildlife [3].

The evolution of zoonoses represents a major risk factor, causing significant damage through the loss of human and animal lives, as well as reduced working capacity of affected people. In the case of livestock, these diseases can lead to a decrease in productivity and confiscation of infected organs [2].

In Romania, in Arad County, studies on the European brown hare (*Lepus europaeus**) have shown a high prevalence of gastrointestinal parasitoses, estimated at around 87.5% (from 24 samples examined, 21 were positive). The most frequently identified parasite was *Eimeria* spp.*, present in 17 samples (80.95%), followed by *Cysticercus pisi-formis** in 2 samples (9.52%), *Trichocephalus leporis** in 13 samples (61.90%), and *Trichostrongylus* spp.* in 7 samples (33.33%). The presence of gastrointestinal parasites and larval cestodes in the European brown hare constitutes an important risk factor for their health and for domestic carnivores feeding on infected hares [5].

These observations highlight the need for further research and the implementation of monitoring measures for wildlife health to prevent the spread of parasitic diseases, which can affect both wild and domestic animal populations. The seasonal dynamics of helminth circulation in the external environment in intermediate and definitive hosts depend on both the biological and ecological characteristics of various species of trematodes, cestodes, nematodes, as well as climatic conditions. Abundance for several species of helminths occurs at the end of summer and is geographically dependent. Maximum indices of invasion extent occur at the end of summer and autumn [10].

At the same time, it has been established that wild animals in natural biocenoses are less infected with helminths than domestic animals. This is likely due to the development pattern of invasions in natural biocenoses, the greater resistance of wild animal organisms, lower population density per unit area, and seasonal non-periodic migrations.

Additionally, wild animals use plants containing alkaloids, resins, and other substances that stop or limit the development of helminths in the host organism, which ultimately determine the extent and intensity of the invasion as well as the overall structure of the parasitic population in animals [9].

In natural biocenoses, wild herbivores serve as reservoirs for the spread of helminths to domestic animals, while in agrobiocenoses (forest, steppe), the reverse circulation of parasitic agents is observed [11].

The infestation of wild mammals with various parasitic agents demonstrates that they play a role in maintaining the epizootic chain of these diseases and are important for the infestation of humans and domestic animals. It is essential that parasitic diseases in wildlife be monitored annually, allowing for the assessment of their evolution, the emergence of new parasitic agents, and the development of measures to combat them [6].

The epidemiological implications of parasitoses in wild hares, the increase of sanitary standards for hunting products, and the importance given to their health status motivate the goal of this study, namely, to determine the prevalence of gastrointestinal parasitic infestations in the European brown hares (*Lepus europaeus*) from hunting grounds in the Republic of Moldova.

The aim of the research was to assess the diversity of the most dangerous zoonotic parasitic invasions from the classes Cestoda, Trematoda, Secernentea, and Gonoidasida in the European brown hare from the Southern Region of the Republic of Moldova [12].

The diversity of the parasitic fauna in the European brown hare (*Lepus europaeus* Pallas, 1778) from the Southern Region of the Republic of Moldova is a topic of major interest for ecological and sanitary studies, with significant implications for wildlife health, as well as for the prevention of the risks of parasite transmission to other species, including domestic animals and humans. As an emblematic representative of the wildlife in this region, the European brown hare harbours a variety of parasitic species, which vary both in terms of taxonomic groups and in terms of infestation levels and their impact on its health [13].

Studies conducted in the Southern Region of the Republic of Moldova have identified a considerable diversity of parasitic fauna in the European brown hare. These parasitic species come from various groups, ranging from cestodes and trematodes to nematodes and protozoa, highlighting the complexity of the ecological relationships within the ecosystem in which the European brown hare lives. Thus, this diversity reflects not only the variety of parasites that colonize its organism but also the variability of ecological conditions in the study area, which favor the development and proliferation of parasites.

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The parasites affecting the European brown hare can have a significant impact on its health, negatively influencing its living conditions, reproductive capacity, and even the survival of populations. For example, parasites from the Trematoda and Secernentea classes can cause damage to internal organs, affecting digestion and nutrient absorption, which can lead to a general weakening of the body. In cases of severe infestations, these can contribute to the decline of hare populations, especially when parasitic diseases are not efficiently controlled or treated [14].

The parasitic diversity of the European brown hare does not only affect this species but can also have a cascading effect on other components of the ecosystem. For instance, hares are an important food source for wild carnivores, and the parasites affecting the hares can be transmitted to these predators through direct contact or by consuming infested meat. Moreover, some of the parasite species identified, such as **Fasciola hepatica** and **Dicrocoelium lanceolatum**, are known for their ability to parasitize other mammal species, including domestic animals and even humans. This can lead to the emergence of zoonoses, which increases the importance of monitoring the health of wildlife [15].

Another significant aspect of the parasitic fauna diversity in European brown hares is the risk of parasite transmission to humans, especially in the case of zoonotic parasites like **Fasciola hepatica** and **Dicrocoelium lanceolatum**. These species can affect not only farm animals but also human health, particularly in rural areas where direct contact with wildlife is more frequent. Therefore, studying the parasitic diversity in hares has direct implications for public health, highlighting the need for preventive measures and control of zoonotic diseases [16].

Considering this parasitic diversity, it is essential for authorities and researchers to develop strategies for monitoring and conserving European brown hare populations. These strategies should include periodic health assessments of wildlife, early detection of parasitic infestations, and the implementation of effective control measures, including habitat management and pathogen spread prevention. Additionally, educating rural populations about the risks associated with parasitic diseases and implementing good hygiene practices are important aspects of preventing disease transmission [17].

The parasitic fauna diversity of the European brown hare in the Southern Region of the Republic of Moldova highlights the ecological complexity of this ecosystem and emphasizes the importance of continuous wildlife health monitoring. By identifying and studying these parasites, effective measures can be adopted to protect hare populations and prevent risks associated with zoonotic diseases. This research is essential not only for the protection of wildlife but also for public health and the conservation of biodiversity in the studied regions [18].

2. MATERIALS AND METHODS

The parasitological research was conducted within the Parasitology and Helminthology Laboratory of the Institute of Zoology from the Academy of Sciences of Moldova (ASM). These investigations are essential for a better understanding of the distribution and impact of parasites on the health of the European brown hare populations in this region, and the data obtained will contribute to the development of effective strategies for the monitoring and control of parasitic diseases.

To establish the diversity of the most dangerous zoonotic parasites in European brown hares, 214 biological samples were collected in 2021 from the Southern region of the Republic of Moldova. These samples were subjected to detailed analysis for the identification of parasitic agents, using advanced and varied diagnostic methods. Techniques such as coprological methods (Fulleborn, Darling), coprolarvosopic methods (Popov, Baermann), partial parasitological investigations (according to K.I. Skriabin), and successive washing methods were applied, all aimed at providing a comprehensive evaluation of the parasitic fauna.

Parasitological evaluation was performed by determining the degree of parasite spread (indicated by the prevalence index – EI, %) and the intensity of invasion (measured in specimens – II), using a Novex Holland B microscope with 20–40x magnification and a 10x WF 20mm visual field. The results obtained were processed statistically using Excel software, ensuring a thorough analysis of the data.

3. RESULTS AND DISCUSSIONS

As a result of the evaluation of the diversity of the most dangerous zoonotic parasitic invasions in the European brown hare from the Northern region of the Republic of Moldova, biological samples were investigated, and it was established that they were infested with various parasitic agents from the following classes: Cestoda – 1 species, Trematoda – 2 species, Secernentea – 6 species, and Gonoidasida – 3 species (Table 1).

Parasitological examination conducted on 180 coprological samples collected from European brown hares in the Southern region of the Republic of Moldova revealed that parasitic agents were present in all 180 samples (100% of cases).

Invasions consisting of a single species of parasite were present in 74 samples (41.1%), while polyparasitism (involving multiple species) was identified in 106 samples (58.9%) (Figure 1).

From the total number of polyparasitism samples, the following associations were more frequently established: with 2 species of parasites – in 52 samples (49.0%), of which 18

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Table 1. Diversity of helminth fauna in *Lepus europaeus*, Pallas, 1778, from the Southern region of the Republic of Moldova.

Invasion		Level of infestation	
		EI (%)	II (ex.)
Class Cestoda			
1.	<i>Cysticercus pisiformis</i> (Zeder,1803)	25.0	19-21
Class Trematoda			
2.	<i>Fasciola hepatica</i> (Linnaeus, 1758)	3.5	2.8
3.	<i>Dicrocoelium lanceolatum</i> (Rudolphi,1919)	7.1	1.7
Class Secernentea			
4	<i>Trichocephalus leporis</i> (Frolich, 1789)	13.4	6.0
5.	<i>Strongyloides papillosus</i> (Wedl, 1856)	79.1	128
6.	<i>Trichostrongylus retortaeformis</i> (Zeder,1800)	4.1	2.0
7.	<i>Passalurus ambiguus</i> (Rudolphi, 1819)	14.6	3.0
8.	<i>Trichostrongylus probolurus</i> (Railliet,1896)	15.3	6.0
9.	<i>Trichuris leporis</i> (Frölich, 1789)	17.5	8.0
Class Gonoidasida			
12	<i>Eimeria leporis</i> (Nieschulz, 1923)	39.8	98
13	<i>Eimeria stiedae</i> (Lindemann, 1865)	57.1	64
14	<i>Eimeria intestinalis</i> (Cheissin, 1948)	14.3	17

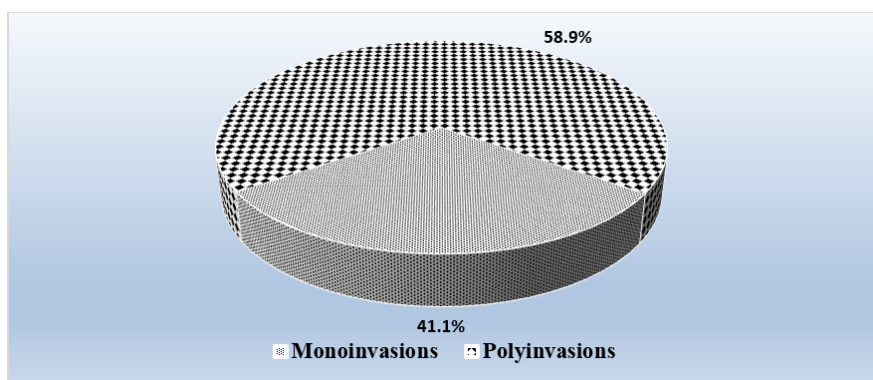


Figure 1. The level of infestation, in the form of mono- and polyinvasions, in European brown hare (*Lepus europaeus* Pallas, 1778) from the Southern region of the Republic of Moldova

samples formed associations of *Strongyloides papillosus* + *Fasciola hepatica* (34.6%), 13 samples formed by the species *Trichostrongylus retortaeformis* + *Eimeria leporis* (25.0%), 14 samples formed by *Strongyloides papillosus* + *Dicrocoelium lanceolatum* (27.0%), and 7 samples formed by the species *Eimeria intestinalis* + *Cysticercus pisiformis* (13.5%).

In 21 samples (19.8% of cases), polyparasitic associations formed by 3 species of parasites were found: *Strongyloides papillosus* + *Trichostrongylus probolurus* + *Graphidium strigosum* in 10 samples (47.6%); *Nematodirus abnormalis* + *Trichuris leporis* + *Cysticercus pisiformis* in 5 samples (23.8%); *Trichocephalus leporis* + *Trichostrongylus retortaeformis* + *Nematodirus abnormalis* in 3 samples (14.3%); *Passalurus ambiguus* + *Trichuris leporis* + *Graphidium strigosum* in 2 samples (9.5%); and *Strongyloides papillosus* + *Nematodirus abnormalis* + *Cysticercus pisiformis* in one sample (4.8%).

Polyparasitic associations consisting of 4 species of parasites were identified in 17 samples (16.0%) and included: *Strongyloides papillosus* + *Fasciola hepatica* + *Trichostrongylus probolurus* + *Trichuris leporis* in 8 samples (47.0%); *Nematodirus abnormalis* + *Dicrocoelium lanceolatum* + *Trichocephalus leporis* + *Eimeria leporis* in 5 samples (29.5%); *Strongyloides papillosus* + *Trichuris leporis* + *Passalurus ambiguus* + *Trichostrongylus probolurus* in 3 samples (17.6%); and *Cysticercus pisiformis* + *Strongyloides papillosus* + *Trichuris leporis* + *Eimeria stiedae* in one sample (5.9%).

As a result of the laboratory parasitological examination, 11 samples (10.5%) showed polyparasitic associations consisting of 5 species: *Strongyloides papillosus* + *Trichuris leporis* + *Trichocephalus leporis* + *Fasciola hepatica* + *Nematodirus abnormalis* in 5 samples (45.5%); *Strongyloides papillosus* + *Dicrocoelium lanceolatum* + *Eimeria intestinalis* + *Passalurus ambiguus* + *Trichuris leporis* in 3 samples (27.3%); *Cysticercus pisiformis* + *Nematodirus abnormalis* + *Trichocephalus leporis* + *Passalurus ambiguus* + *Eimeria stiedae* in 2 samples (18.2%); and *Eimeria exigua* + *Trichuris leporis* + *Passalurus ambiguus* + *Cysticercus pisiformis* + *Trichocephalus leporis* in one sample (9.0%).

The research results revealed the presence of mixed infestations, consisting of complex combinations of parasites, which indicate the diversity and complexity of parasitic infestations in the field rabbit population from the Southern region of the Republic of Moldova. Thus, mixed infestations consisting of six species of parasites were identified in 5 samples (4.7%). These infestations consist of combinations of parasites that vary depending on the type and degree of infestation, as well as the type of ecological relationship they have with their host. In detail, two important parasite combinations were detected: the mixed infestation with species of parasites – *Strongyloides papillosus* + *Trichostrongylus retortaeformis* + *Trichuris leporis* + *Trichocephalus leporis* + *Eimeria*

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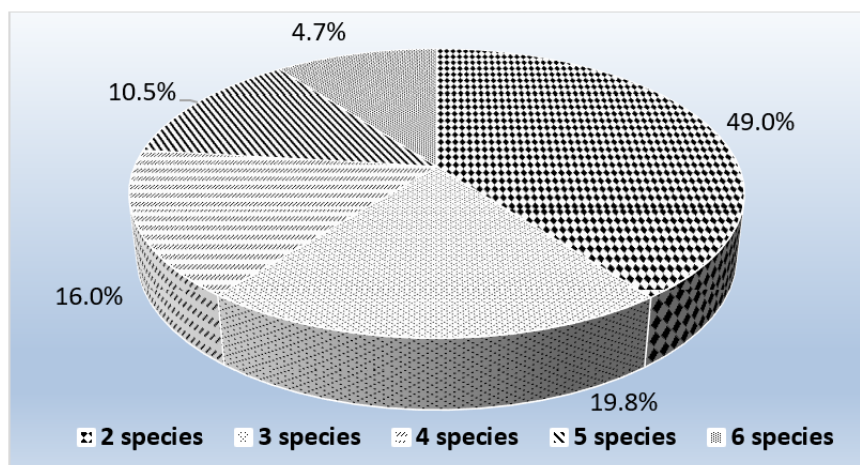


Figure 2. Associations with parasitic agents identified in European brown hare (*Lepus europaeus* Pallas, 1778) from the Southern region of the Republic of Moldova

exigua + *Nematodirus abnormalis* – identified in 3 samples (60.0%); the mixed infestation with species of parasites – *Strongyloides papillosus* + *Trichostrongylus retortaeformis* + *Nematodirus abnormalis* + *Cysticercus pisiformis* + *Eimeria stiedae* + *Graphidium strigosum* – detected in 2 samples (40.0%).

These combinations reflect the complexity of infestations in field rabbits and suggest a diverse interaction between parasites from different groups, which can contribute to a significant health impact on the host.

Additionally, the parasitological analysis highlighted a division of parasite species based on their development mode, into two main categories: Biohelminths (15.0%) and Geohelminths (85.0%).

Biohelminths are species that require intermediate hosts to complete their life cycle. These species are generally more complex in their biology, requiring another animal (usually a different species) for the larval stage to develop before infecting the final host. Geohelminths are parasite species that develop without the need for intermediate hosts. They are more commonly found in external environments, such as soil or vegetation, where larvae can survive for a period and infect the host directly through contact with the contaminated environment (Figure 3).

This distinction between biohelminths and geohelminths is important for understanding how these parasites spread and infect field rabbit populations. Geohelminths, for example, may pose a higher risk for rapid infestation spread since they develop in the external

environment and do not depend on other animal species to complete their evolutionary cycle. On the other hand, biohelminths, which require intermediate hosts, can be more easily controlled through the management of these intermediate species, but they require careful monitoring of complex ecological flows.

These results emphasize the complexity of parasitic diseases in field rabbits in this region and the need for integrated strategies for parasite management, which should include measures to limit the spread of geohelminths and monitor biohelminthic species.

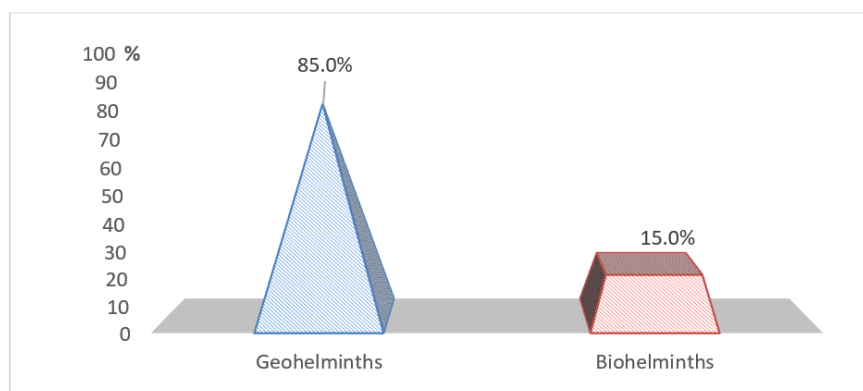


Figure 3. Division of parasite species identified in European brown hare (*Lepus europaeus* Pallas, 1778) from the Southern region of the Republic of Moldova, according to the mode of development of their evolutionary cycle

The results of the parasitological research conducted on European brown hare (*Lepus europaeus*) in the Southern region of the Republic of Moldova highlight a high level of infestation with various parasitic agents, which poses a significant risk not only to the health of the rabbits but also to domestic animals and humans, especially in the case of zoonotic species.

The studies identified a total of 12 species of parasites, which are divided into two main categories: parasites specific to wild rabbits and those common to other animal species, both wild and domestic. Of the 12 species identified, 4 species (33.3%) are exclusive to European brown hare, namely: *Cysticercus pisiformis*, *Trichuris leporis*, *Trichocephalus leporis*, and *Passalurus ambiguus*. These species are specially adapted to their host, and their presence in the rabbit's body can lead to various health disorders, such as affecting the digestive system or other internal organs.

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On the other hand, 8 species (66.7%) are common to other wild and domestic animal species, including *Strongyloides papillosus*, *Trichostrongylus probolurus*, *Trichostrongylus retortaeformis*, *Fasciola hepatica*, *Dicrocoelium lanceolatum*, *Eimeria stiedae*, *Eimeria leporis*, and *Eimeria intestinalis*. These species are widespread in various ecosystems and can affect a wide range of animals, including cattle, sheep, or even carnivores. Additionally, two of these species (*Fasciola hepatica* and *Dicrocoelium lanceolatum*) present a particular risk, as they are capable of parasitizing humans as well, making them important from a public health perspective (Figure 4).

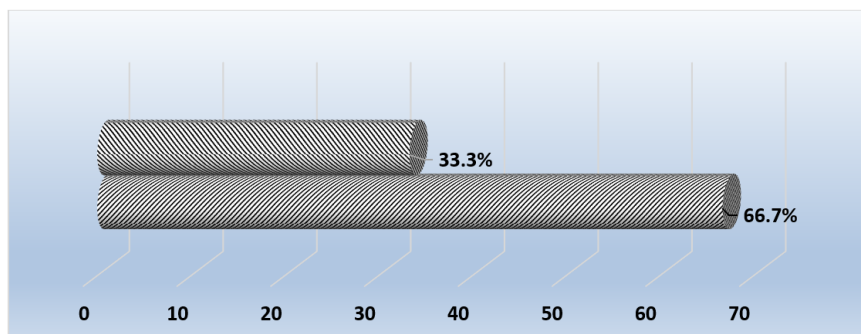


Figure 4. Host specificity of parasite species identified in European brown hare (*Lepus europaeus* Pallas, 1778) from the Southern region of the Republic of Moldova

4. CONCLUSIONS

- (1) For the European brown hare (*Lepus europaeus* Pallas, 1778) population in the Southern region of the Republic of Moldova, a high level of infestation with dangerous parasitic agents was observed. Taxonomically, 12 species of parasites were identified, distributed across the classes Cestoda (1 species), Trematoda (2 species), Secernentea (6 species), and Gonoidasida (3 species).
- (2) It was found that in all samples collected (100%), parasitic agents were identified. Single-species infestations were found in 74 samples (41.1%), while polyparasitic infestations (comprising 2–6 different species) were present in 106 samples (58.9%).
- (3) Of the 12 species identified, 4 species (33.3%) are exclusively specific to European brown hare, including *Cysticercus pisiformis*, *Trichuris leporis*, *Trichocephalus leporis*, and *Passalurus ambiguus*. In contrast, 8 species (66.7%) are common to other wild and domestic animal species, including *Strongyloides papillosus*,

Trichostrongylus probolurus, *Trichostrongylus retortaeformis*, *Fasciola hepatica*, *Dicrocoelium lanceolatum*, *Eimeria stiedae*, *Eimeria leporis*, and *Eimeria intestinalis*. It is important to note that two of these species (*Fasciola hepatica* and *Dicrocoelium lanceolatum*) are capable of parasitizing humans, posing a potential risk to human health.

- (4) These observations highlight both the diversity of the parasitic fauna in wild rabbits in this region and the risks associated with certain species that can affect other animals, including humans. The results emphasize the need for continuous monitoring of rabbit populations and their parasites to prevent the spread of zoonotic diseases.

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