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Prevalence of gastrointestinal parasitic infections in cattle and sheep in two regions of Romania

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Abstract: Amongst ruminant livestock diseases, the gastrointestinal (GIT) helminths and protozoan parasite infections result in a significant socio-economic concern worldwide. During the period between October 2017 and November 2018, a total of 788 fresh fecal specimens from cattle (n = 303) and sheep (n = 485) were screened for the presence of gastrointestinal helminths and protozoan parasites in two historical regions (Crisana and Banat) of western Romania. Laboratory processing of fecal samples with the simple salt flotation (Willis) and sedimentation techniques, followed by microscopic analysis revealed that 86.1% of cattle and 92.6% of sheep were found to be positive for at least one parasitic stage of GIT helminths and/or protozoa. Five endoparasites were identified in both cattle (C) and sheep (S), and their prevalence was as follows: *Eimeria* spp. 24.1% (C), 43.7% (S); *Balantidium* spp. 10.2% (C), 1% (S); *Fasciola/Paramphistomum* spp. 55.8% (C), 75% (S); *Dicrocoelium* spp. 14.2% (C), 3.2% (S); and *Strongylid* eggs 46.9% (C), 71.3% (S). In addition, *Toxocara* spp. (0.3%) genera were found in cattle, while *Nematodirus* spp. (24.5%) and *Moniezia* spp. (3.5%) were observed only in sheep. The strongylid infections were significantly more prevalent ($P < 0.05$) in cattle older than 8 years, while the coccidial ones were positively associated ($P < 0.05$) with sheep younger than 2 years. The study provides useful information for veterinary practitioners in order to develop effective prevention and control strategies against gastrointestinal parasitic infections in the studied regions.

Key words: Gastrointestinal parasites, sheep, cattle, Romania, prevalence

1. Introduction

Amongst ruminant livestock diseases, the gastrointestinal (GIT) helminths and protozoan parasite infections result in significant economic losses worldwide. Out of them, the decrease of the feed intake and efficiency of its utilization resulting in the growth rate, lower outputs of animal products (e.g., carcass quality weight, wool growth), infertility or reduced milk production are the most frequently encountered adverse effects. In addition, GIT parasite infections can increase the susceptibility of host to other several bacterial and viral diseases, resulting in carcasses and organs condemnation within the slaughtering process [1,2].

Results of several investigations conducted at worldwide level highlighted that despite all financial efforts during prophylactic or therapeutic campaigns, based on appropriate integrated parasite management programs, the extensivity of GIT parasitic infections, both in ruminant livestock extensive and intensive production systems, reaches important values [3,4]. In this regard, according to statistical data, the most significant damages are due to the occurrence of GIT helminths [5].

In Romania, the breeding of the ruminant livestock, especially in rural communities within backyard and/or small scale integrated farms with agro-pastoral feeding, is considered an important economic sector of the food industry. Results of previously conducted studies targeting the assessment of GIT parasite prevalence in cattle and sheep, from various areas of the country, highlighted different prevalence values [6–9]. However, most of the studies conducted on sheep were focused on the screening of a limited number of parasites. In addition, their availability is mostly confined to local literature and language, with poor international visibility.

The current study was undertaken to assess the occurrence and epidemiological significance of GIT helminths and protozoan parasites in cattle and sheep populations in two regions (Crisana and Banat) of western Romania.

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2. Materials and methods

The study was performed in two historical neighbor western Romanian regions, named Banat and Crisana (between 46° 07′ N, 20° 15′ E and 45° 06′ N, 22° 40′ E). The screened area has a temperate-continental climate with vegetation rich in floristic composition, covering a surface of about 46.266 km² with a total of 285,882 cattle and 2,808,138 sheep population. During the period between October 2017 and November 2018, a total of 788 fresh fecal specimens (~50 g) from cattle (n = 303) and sheep (n = 485) were collected directly from the rectum and transferred to sterile individually labeled plastic containers. The probability sampling procedure was used, consisting of the random selection from each herd of the tested animals. Clinically healthy cattle from five small-scale integrated livestock farms (up to 100 heads) and 75 private households (with 1–3 heads), and sheep from eight flocks were investigated. In all situations, no history of any routine deworming activity of the last month before sampling data has been recorded for the sampled animals. The sampled cattle were raised under semiintensive (housed with grazing) farming system. In this system the animals graze during the day on native grasses and shrubs, especially in the summer season under a herder supervision, sharing the same grazing field with other livestock (e.g., horse, goat) or wildlife, with free access of different parasitic stages. The grazed animals drank from ponds sourced from rainwater, irrigation channels or from brooks crossing the grazing fields. The sheep flocks graze free-range during the day and are housed at the night on the ground fenced on a narrow area.

Data regarding the origin and age of the animals were provided by the breeders. All of the investigated cattle were female and crossbred of different combination forms between Holstein–Friesian, Simmental, Limousin, Belgian Blue, and Bălţată Românească pure breeds. The investigated sheep were autochthonous Ţurcană breed.

All the samples were preserved in adequate conditions and transported under refrigeration to the Faculty of Veterinary Medicine Timisoara at the Parasitology and Parasitic Diseases clinic in order to be subjected to processing and microscopic examinations within 48 h.

Standard parasitical screening methods, namely the Willis technique followed by sedimentation, were used as previously described [10–12] in order to detect GIT parasites; approximately 2 g of fecal samples which were taken directly from the rectum was processed. The presence of parasite oocysts, eggs, larvae, and cysts were recorded in fecal pellet suspensions within examination under a light microscope at 10× and 40× objectives magnification. The observed parasitic stages were identified at genera level using morphological identification keys described in [13] and [11,14]. Each examined animal was counted as positive when one or more parasitic stage was recorded in its fecal samples.

The Minitab 16 Statistical Software (Minitab, Inc., State College, Pennsylvania) was used in order to evaluate the possible statistically significant differences between the gathered data and the infection prevalence. The differences were considered to be statistically significant when the P value was lower than 0.05.

3. Results

Out of 303 examined cattle, 261 (86.1%, 95% CI = 81.6–89.7) were found to be positive for at least one parasitic stage of GIT parasites. The light microscope appearances of all the identified parasitic stages are shown in Figure. In this host species a variety of parasites were found including oocysts of protozoans (*Eimeria* spp. coccidians: 24.1%, 95% CI = 19.5–29.4; *Balantidium coli*: 10.2%, 95% CI = 7.2–14.3), digenetic trematodes (*Fasciola*/*Paramphistomum* spp.: 55.8%, 95% CI = 50–61.4; and *Dicrocoelium lanceolatum*: 14.2%, 95% CI = 10.6–18.8), and different nematodes (*Toxocara* spp.: 0.3%, 95% CI = 0.2–2.1; Strongylid eggs: 46.9%, 95% CI = 81.6–89.7).

A total of 449 (92.6%, 95% CI = 89.8–94.7) sheep from the 485 examined were infected with different GIT helminths and/or protozoa and the identified parasites were digenetic trematodes (*Fasciola*/*Paramphistomum* spp.: 75%, 95% CI = 71–78.8 and *D. lanceolatum*: 32%, 95% CI = 27.9–36.3), different nematodes (*Nematodirus* spp.: 24.5%, 95% CI = 20.8–28.7; other Strongylus spp.: 71.3%, 95% CI = 67.1–75.3), protozoan oocysts of (*Eimeria* spp. coccidians: 43.7%, 95% CI = 39.3–48.3; *B. coli*: 1%, 95% CI = 0.4 – 2.5), and cestodes (*Moniezia* spp.: 3.5%, 95% CI = 2.1–3.7).

The summary of the study results according to the recorded epidemiological data are presented in Table. Statistical analysis showed significant differences (P = 0.0004) of *Fasciola*/*Paramphistomum* spp. infection between cattle living in Banat (72.1%) and Crişana (49.3%) regions. Instead, the prevalence of the digestive strongyles was significantly higher (P = 0.0182) in Crisana comparing with Banat region. The digestive strongyles were significantly more prevalent in cattle older than 8 years comparing with the ≤ 2 year (P = 0.0457) and 2 to 8 years (P = 0.4632) age groups.

In sheep, a significantly higher prevalence values were registered for *Eimeria* spp. (P = 0.0002), *D. lanceolatum* (P = 0.0001), *Nematodirus* spp. (P = 0.0001), and other Strongylus spp. (P = 0.0001) in Banat comparing with Crisana region. Contrarily, the prevalence of *Fasciola*/*Paramhistomum* spp. was positively associated with Crisana region.

The distribution of GIT parasites amongst different age groups of sheep showed that *Eimeria* spp. prevalence was significantly higher in ≤ 2 year age group, compared to 2 to 8 year (P = 0.0501) and >8 year (P = 0.0320) age groups,

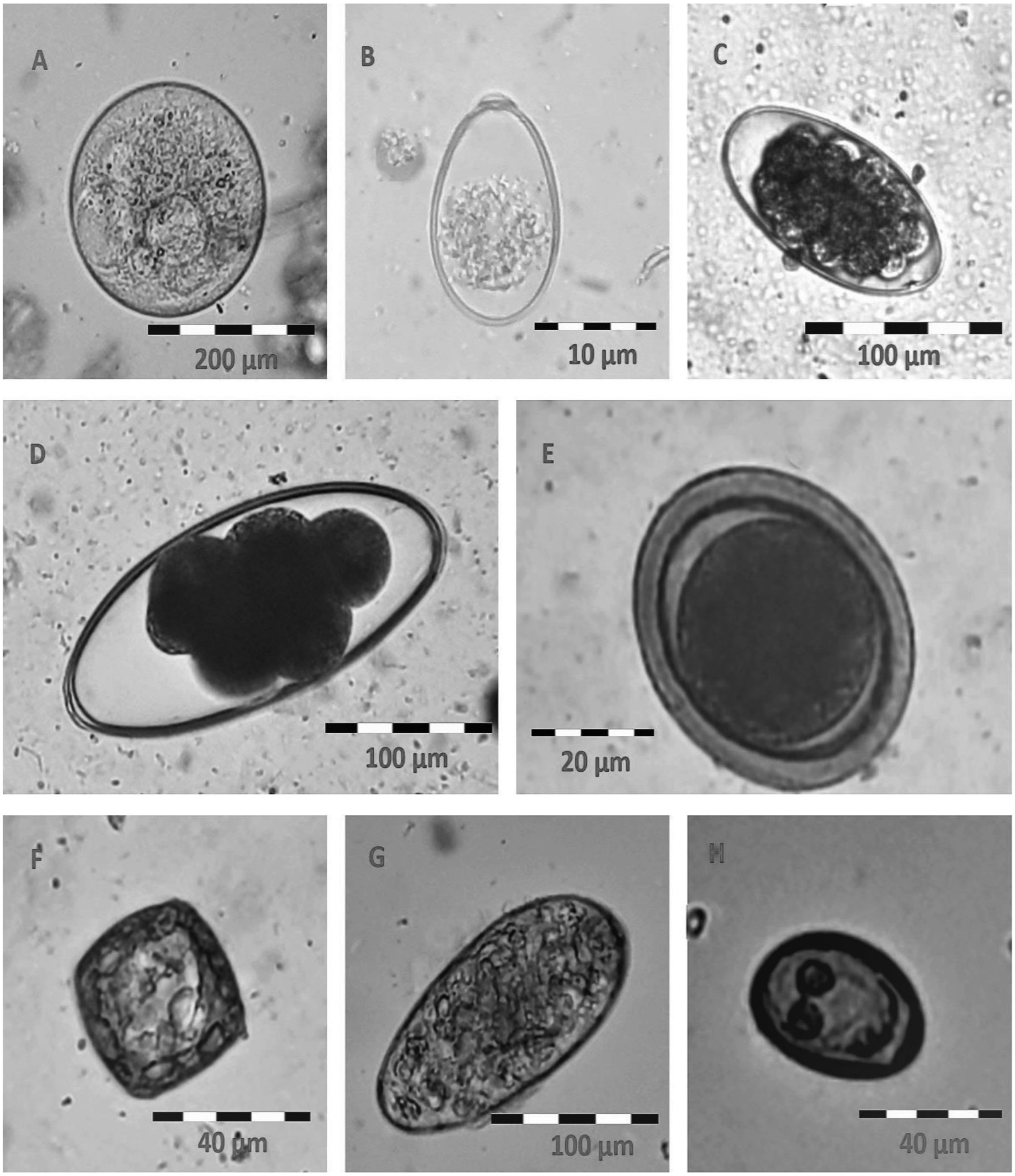

Figure. Parasitic stages observed under the light microscope in the cattle and sheep fecal samples. **A.** *Balantidium coli* **B.** *Eimeria* spp. **C.** *Strongylid* spp. **D.** *Nematodirus* spp. **E.** *Toxocara vitulorum* **F.** *Moniezia* spp. **G.** *Fasciola/Paramphistomum* spp. **H.** *Dicrocoelium lanceolatum*.

respectively. Moreover, the percentage of 2 to 8 year aged sheep infected with digestive strongyles was significantly higher compared to ≤ 2 year (P = 0.0007), as well as >8 year (P = 0.0141) age groups.

The registered overall GIT parasite prevalence in sheep (92.6; 449/485) was significantly higher (P = 0.0051) compared with cattle (86.1%; 261/303).

4. Discussion

The results of the current study demonstrated that the presence of GIT parasites in cattle and sheep is a common finding in the screened two historical regions (Crisana and Banat) of western Romania. In cattle, different parasitic stages of the six different parasites (*Eimeria* spp., *B. coli*, *Fasciola/Paramphistomum* spp., *D. lanceolatum*, *Toxocara*

Table. Distribution of the identified gastrointestinal parasites in the screened ovine and bovine population according to the recorded data.

Identified parasites	No. of infected/(%) [95% CI]									
	Sheep					Cattle				
	Region		Age			Region		Age		
	Banat (*n*=378)	Crisana (*n*=98)	≤2 years (*n*=107)	2 to 8 years (*n*=370)	>8 years (*n*=8)	Banat (*n*=86)	Crișana (*n*=217)	≤2 years (*n*=90)	2 to 8 years (*n*=169)	>8 years (*n*=44)
Balantidium coli	-	5 (5.1) [1.9–12.1]	-	5 (1.3) [0.5–3.3]	-	31 (36.1) [26.2–47.2]	-	13 (14.4) [8.2–23.8]	18 (10.7) [0.1–16.6]	-
Eimeria spp.	185 (47.8)* [73.2–81.8]	26 (26.5) [18.4–36.6]	56 (52.3)* [42.5–62]	154 (41.6) [36.6–46.8]	1 (12.5) [0.7–53.3]	21 (24.4) [16.1–35.1]	51 (23.5) [18.1–29.8]	21 (23.3) [15.3–33.7]	41 (24.3) [18.2–31.6]	10 (22.7) [12–38.2]
Fasciola/ Paramphistomum spp.	281 (72.6) [67.8–76.9]	83 (84.7)* [75.7–91]	37 (34.6) [25.8–44.5]	319 (86.2) [82.2–89.5]	8 (100) [59.8–1]	62 (72.1)* [61.2–81]	107 (49.3) [42.5–56.1]	32 (35,6) [26–46.4]	109 (63.9) [56.1–71]	28 (63.6) [47.7–77.1]
Dicrocoelium lanceolatum	140 (36.2)* [31.4–41.2]	15 (15.3) [9.1–24.3]	3 (7.5) [3.5–14.7]	129 (38.4) [33.4–43.6]	5 (62.5) [25.9–89.8]	43 (50) [39.1–61)	-	18 (20) [12.6–30]	23 (13.6) [0.1–19.9]	2 (4.5) [0.8–16.7]
Moniezia spp.	17 (4.4) [2.7–7.1]	-	-	17 (4.6) [2.8–7.4]	-	-	-	-	-	-
Toxocara spp.	-	-	-	-	-	-	1 (0.5) [0.2–2.9]	-	-	1 (2.3) [0.1–13.5]
Nematodirus spp.	114 (29.5)* [25–34.3]	5 (5.1) [1.9–12.1]	24 (22.4) [15.2–31.7]	95 (25.7) [21.4–30.5]	-	-	-	-	-	-
Strongylid spp.	301 (77.8)* [73.2–81.8]	45 (45.9) [35.9–56.2]	63 (58.9) [53.6–72.5]	280 (75.7)* [70.9–79.9)	3 (37.5) [10.2–74.1]	31 (36.1) [26.2–47.2]	111 (51.2)* [44.3–57.9]	16 (52.2) [41.5–62.8]	95 (64.6) [48.4–63.8]	31 (70.5)* [54.6–82.8]
Negative	36 (9.3) [6.7–12.7]	-	18 (16.8)* [10.5–25.6]	18 (4.9) [3–7.7]	-	3 (3.5) [0.9–10.6]	61 (28.1)* [22.3–34.7]	35 (38.9)* [29–49.8]	22 (13) [0.1–19.3]	7 (15.9) [7.2 – 30.7]

*: $P < 0.05$

spp. and Strongylid nematodes) with different prevalence values (Table) were identified. Comparing with the results of several other studies conducted in different countries, our overall GIT parasites prevalence value (86.1%) was higher than that recorded in Sri Lanka (11.6% [3]), Ethiopia (61%; [15]), India (67.2%; [16]), and Colombia (50.5%, [17]); close to the one found in Nigeria (82.5%); [18]), but lower than that obtained in Ghana (93.8%); [2]). In Romania, a small-scale integrated study in the eastern part of the country highlighted that 79% of the tested intensively and traditionally reared cattle were found to be positive at least one parasitic stage of GIT parasites [9]. Moreover, in another investigation carried out in the central region of Romania, processing fecal samples from intensively reared Angus breed, an overall prevalence of 90.3% was obtained for different GIT parasites [7]. The fact that the tested bovine population shares communal grazing with several other livestock herds, which can increase the degree of pasture contamination and diversity of parasitic elements, can support the recorded relatively high prevalence value in our study. It is important to mention that caution should be taken when comparing the results of different epidemiological studies because the registered overall prevalence values can be significantly influenced by several factors including the design of the study (e.g., period of study, number of the processed fecal samples, methodologies used for parasite identification, and farm management including feeding and deworming practices) or climatic (e.g., rainfall, humidity, temperature) and environmental (e.g., vegetation, soil, presence/absence of intermediate hosts) parameters of the screened region, which can favor the survival of the infective stages of the parasites and their intermediate hosts.

A significantly higher proportion of cattle were infected with *Fasciola*/*Paramphistomum* spp. in Banat region, and with Strongylid nematodes in Crisana region. Similar to our results a positive territorially association with GIT parasites infection in cattle has been reported in other studies [2]. Being two neighbor regions of the country with very similar geo-ecological conditions, the registered differences cannot be explained exactly. However, the possible variations in husbandry practices, soil and vegetation type, or the abundance of surface waters that can maintain favorable habitats to the developments of the infective stages of the parasitic can greatly contribute to the observed differences. In this regard, further studies are necessary including the possible influence of climatic conditions and herd management factors in order to draw a causal territory association of the infection.

It is not surprising that the prevalence of Strongylid infection was significantly higher in cattle older than 8 years comparing with the other two tested age groups. In this regard, the repetitive and longer exposure of older cattle to the infective stages of the Strongylid nematodes, beside the different immunological status of the tested age groups can support these findings [3]. In agreement with our findings, a significantly higher ($P < 0.05$) value of Strongylid infections were reported in the Ethiopian cattle older than 7 years by Telila et al. [19], comparing with cattle younger than 2 years. Contrarily, in the same country, Regassa et al. [20] observed that younger animals were more susceptible to GIT parasites infections.

Fecal examination of sheep revealed that the overall prevalence rate of GIT parasites was 92.6%. In comparison, coprological surveys with similar design conducted in Turkey [21], Spain [22], Bulgaria [23], Republic of Moldova [24], Kenya [25], Brazil [26], Ethiopia [20], Serbia [27], Papua New Guinea [28], India [29], Serbia [30], Nigeria [31], Colombia [17], and Pakistan [32] the prevalence of GIT parasites was 100%, 100%, 98.5%, 94%, 80%, 76.6%, 75.3%, 74.5%, 72%, 68.7%, 64.1% 63.2%, 63.0%, and 53.3%, respectively. In Romania, the study conducted by Indre et al. [33] in several flocks from the western part of the country highlighted an overall GIT parasites prevalence value of 75%, but in this study the investigations were focused only on the survey of the occurrence of GIT nematodes and trematodes. The registered high overall parasite prevalence in the current study may be attributed to several factors including the lack of rotational grazing and anthelmintic treatments, management failures, poor nutrition, or the grazing of mixed-species flocks in the same field.

Like in the case of cattle, significant associations were found between the occurrence of some parasites and the screened regions (*Eimeria* spp., *D. lanceolatum*, *Nematodirus* spp. and Strongylid spp. with Banat region; and *Fasciola*/*Paramhistomum* spp. with Crişana region). In the lack of any investigation, aiming to evaluate the influence of climatic and environmental conditions as possible risk factors on the acquiring of parasite infections, it is very difficult to assess the mechanisms behind these differences.

In the present study, a significantly higher prevalence of *Eimeria* spp. in sheep younger than 2 years was obtained. This finding complies with those reported in previous works [34,35] and can be strongly linked by the age-related development of immunity against this parasite. The possible acquired immunity against parasites by older animals has been experimentally demonstrated [36].

The observed relatively high parasitic diversity, present in the recording of six parasites in cattle (*B. coli*, *D. lanceolatum, Eimeria* spp., *Fasciola/Paramphistomum* spp., *Toxocara* spp., and Strongylid spp.) and seven parasites in sheep (*B. coli*, *D. lanceolatum, Eimeria* spp., *Fasciola/ Paramphistomum* spp., *Nematodirus* spp. *Moniezia* spp., and Strongylid spp.), can be considered common findings, considering that the semiintensive farming system with communal grazing field offers favorable conditions for the development of parasite vectors (e.g., aquatic and dry snails as intermediate hosts for *Fasciola/Paramphistomum* and *D. lanceolatum,* respectively; or oribatid mites for *Moniezia* spp.) which can largely enhance the parasitic fauna heterogeneity.

Even if both species were raised under the same systems grazing on pastures, the obtained results showed that sheep were more susceptible to internal parasites. Similar findings were published by León et al. [17] in Brazil and by Khan et al. [37] in Pakistan. This observation can be sustained by several predisposing factors including different defoliation regimes of plants by the tested species or the possibility of grazing by sheep the hardly accessible unclean and unsafe areas due to their greater mobility.

In conclusion, the results obtained in the current study suggest that GIT parasites are widespread in the tested cattle and sheep populations, in two historical regions (Crisana and Banat) of western Romania. Many parasite infections in both species were positively associated with the age and screened spatial regions, but the influence of the climatic conditions and herd management practices, as possible risk factors, remains entirely unexplored. In this regards, further studies are recommended, beside the evaluation of the economic impact of GI parasites on large and small ruminant livestock. Moreover, the study provides useful information for veterinary practitioners in order to develop effective prevention and control strategies against GIT parasitic infections.

Contribution of authors

CBS and KI have contributed equally to this article.

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