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PREVALENCE OF GASTROINTESTINAL PARASITES IN BACKYARD DOMESTIC RABBITS IN THE CENTRAL AND NORTH-WEST ROMANIA

PREVALENȚA PARAZIȚILOR GASTROINTESTINALI LA IEPURI DOMESTICI DIN GOSPODĂRII PRIVATE DIN CENTRUL ȘI NORD-VESTUL ROMÂNIEI

C. MAGDAȘ[1)], Mirabela Oana DUMITRACHE[1),*)], G. D'AMICO[1)], R. PINTEA[1)], Viorica MIRCEAN[1)], Adriana GYÖRKE[1)]

ABSTRACT | REZUMAT

This study aimed to assess the prevalence of gastrointestinal parasites occurring in rabbits farmed in the backyard production system. The study was conducted between January 2017 and May 2017 in the central and North-West Romania (Satu-Mare, Cluj, Bistrița, Bihor, and Harghita counties). One-hundred-fifty-four fecal samples were collected from rabbits aged between 2 and 12-months old. Flotation and McMaster methods were used to analyze the feces sample. A prevalence of 62.3% of gastrointestinal parasites was recorded; a single infection was detected in 48.1% of samples and mixed infection in 14.3%. *Eimeria* spp. (57.8%) was the most prevalent parasite identified in domestic rabbits, with an average intensity of 6571 oocysts/g feces. *P. ambiguus, S. papillosus,* and digestive strongyles were identified in 14.3%, 3.2%, and 5.2% of samples, respectively.

Keywords: Eimeria spp., Passalurus, Graphidium, Obeliscoides, rabbits

Scopul acestui studiu a fost evaluarea prevalenței paraziților gastrointestinali la iepuri domestici crescuți în sistem extensiv. Studiul a fost realizat în perioada ianuarie 2017-mai 2017, animalele luate în studiu provenind din centrul și nord-vestul României (județele Satu-Mare, Cluj, Bistrița, Bihor și Harghita). O sută cincizeci și patru probe de fecale au fost recoltate de la iepuri de diferite vârste (între 2 și 12 luni). Probele de fecale au fost analizate folosind metode de flotație și McMaster. Prevalența paraziților gastro-intestinali a fost de 62,3%. Infestație unică a fost detectată la 48,1% probe și infestație mixtă la 14,3% probe. La iepurii luați în studiu, cel mai frecvent identificat parazit a fost *Eimeria* spp. (57,8%), cu o intensitate medie de 6571 oochisturi/g fecale. *P. ambiguus*, *S. papillosus* și strongilii digestivi au fost identificați în 14,3%, 3,2% și respectiv 5,2% din cazuri.

Cuvinte cheie: *Eimeria spp., Passalurus, Graphidium, Obeliscoides,* iepuri

Domestic rabbits are raised as farm animals for meat production as well as pet animals. The largest worldwide meat-rabbit producer is China, followed by the European Union. In Europe, rabbit farming is most frequent in France, Hungary, Italy, Portugal, and Spain. Sixty-six percentages of the rabbits are found in conventional farms and slaughtered in approved slaughterhouses, while 34% are reared and processed in the backyard system (8). In Romania, the rabbit farming followed a decline from around 5,000,000 rabbits in 1989 to 85,000 in 2018 (12); nowadays, most of the rabbits are reared in the backyard system. In 2020, even if the consumption of rabbit meat registered a global increasing trend, in Europe, including Romania, the consumption of rabbit meat decreased (8, 12). Rabbit meat is considered a functional food due to its high digestibility, low level of cholesterol, high level of proteins with essential amino acids, high levels of PUFA and n-3 FA, and because it is a significant source of vitamin B and phosphorus (6).

Gastroenteric disorders are the main cause of morbidity and mortality (5-15%) in the growing rabbits reared in conventional rabbit farming (8). The main causes are parasites and bacteria (2). *Eimeria* spp. and *Passalurus ambiguus* are the most commonly identified gastrointestinal parasites (26). Coccidiosis is a protozoan disease caused by 14 *Eimeria* species in rabbits; it is characterized by diarrhea and high morbidity and mortality in farmed animals. Young rabbits are highly susceptible to the infection, particularly after weaning, while adult rabbits are asymptomatic carriers of *Eimeria* spp. (22). *P. ambiguus* is considered non-pathogenic for adult rabbits, but in youngsters can occur a heavy infection leading to the enteritis complex around weaning (26).

Given that in the backyard system, the prophylactic treatments for gastrointestinal parasites are rarely performed, we assessed the prevalence and intensity of gastrointestinal parasites in domestic rabbits reared in backyard system in different Romania's counties.

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
*) *Corresponding author: mirabela.dumitrache@usamvcluj.ro*

MATERIALS AND METHODS

Animals and samples

We conducted a prospective epidemiological study to assess the prevalence of gastrointestinal parasites in rabbits raised in backyard farming system. The study was carried out between January and May 2017 in the following counties: Satu Mare (localities: Păuleşti, Ambud, Peten, Hrip and Amati), Bihor (localities: Suplacu), Cluj (localities: Cluj-Napoca, Vâlcele, and Ceanu Mare), Bistriţa-Năsăud (localities: Năsaud), and Harghita (localities: Corund, Praid, and Sovata) (Fig. 1). One fecal specimen was sampled per age category from each household. The collected samples were common or individual, depending on the accommodation of the animals.

Sample analysis

Fecal samples were analyzed using coproparasitological techniques such as flotation with sodium chloride (specific gravity 1.28) and McMaster. The oocysts and eggs were identified according to their morphological characters (18, using an optical microscope Olympus BX61, Japan). Positive samples were further tested using the McMaster technique to assess the number of oocysts/eggs per gram (OPG/EPG) of fecal sample.

Fig. 1. Area of sampling

Data analysis

The frequency and prevalence with a 95% confidence interval (CI) were calculated for each identified parasite. Results were analyzed considering overall data, the different age categories, and size of the groups. Three age groups were defined: >2 up to 4 months (kits), 4-6 months (young rabbits), and >12 months (adult rabbits), according to the EFSA recommendations for age groups (2019). Based on the household size, the following size groups were considered: 1-9 rabbits/household, 10-15 rabbits/household, and 20-50 rabbits/household. A Chi-square test was used to statistically analyze the differences in prevalence among the variables. The average and standard error were calculated for OPG and EPG. Before further processing, the distribution data was assessed by D'Agostino-Pearson's normality test. If not normally distributed, the data undergo logarithmic transformation (base 10). The ANO VA repeated measures analysis of variance test was used to identify statistically significant differences among age groups. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed with EpiInfo 3.5.1 (CDC, USA) and MedCalc Statistical Software 19.0.4. (MedCalc Software Ltd, Ostend, Belgium).

RESULTS AND DISCUSSIONS

One-hundred-fifty-four fecal samples were collected from 1628 rabbits. The group size varied from 1 to 50 rabbits/household with an average of 117 rabbits/household. The age of rabbits was between 2 and 12 months (average 8±4.4 months) (Fig. 2). The animals were raised in wooden cages (Fig. 3), being separated based on the age category and gender in adults.

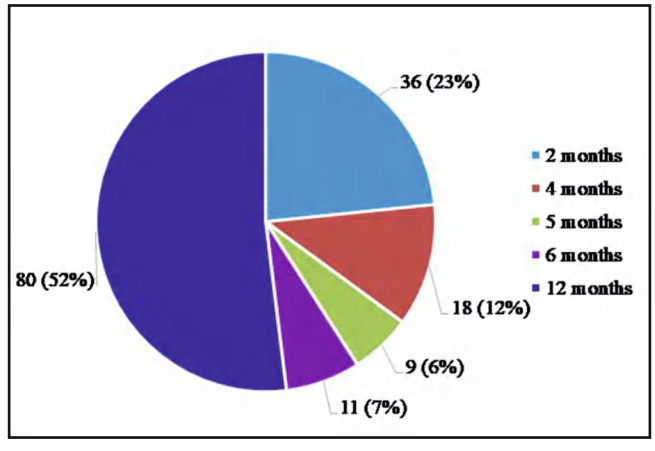

Fig. 2. Number of samples by age of the rabbits

Fig. 3. Accommodation of rabbits

Overall, 96 (62.3%) out of 154 examined samples were positive for at least one gastrointestinal GIT parasite; the number of single infections (74/154; 48.1

Table 1

Frequency and prevalence [*n*(%)] of gastrointestinal parasites detected in domestic rabbits from backyard system overall and by age category

	Total (*n* = 154)	Kits (*n*=36)	Young rabbits (*n*=38)	Adult rabbits (*n*=80)	*p*
Eimeria spp.	89 (57.8)***	15 (41.7)***	30 (79.0)***	44 (55.0)***	0.004
P. ambiguus	22 (14.3)	6 (16.7)	9 (23.7)	7 (8.8)	0.09
S. papillosus	5 (3.2)	0	2 (5.3)	3 (3.8)	0.41
Digestive strongyles	8 (5.2)	3 (8.3)	2 (5.3)	3 (3.8)	0.59
Single infection	74 (48.1)***	14 (38.9)***	24 (63.2)***	36 (45.0)***	0.08
Mixed infection	22 (14.3)	5 (13.9)	8 (21.1)	9 (11.3)	0.36
Total	96 (62.3)	19 (52.8)	32 (84.2)	45 (56.3)	0.006

Table 2

Frequency and prevalence [*n*(%)] of gastrointestinal parasites detected in domestic rabbits from backyard system overall and by age category

	1-9 rabbits/household (*n*=64)	10-15 rabbits/household (*n*=62)	20-50 rabbits/household (*n*=28)	*p*
Eimeria spp.	35 (54.7)	43 (69.4)	11 (39.3)	0.02
P. ambiguus	13 (20.3)	5 (8.1)	4 (14.3)	0.15
S. papillosus	1 (1.6)	3 (4.8)	1 (3.6)	0.58
Digestive strongyles	4 (6.3)	1 (1.6)	3 (10.7)	0.17
Single infection	25 (39.1)	37 (59.7)	12 (42.9)	0.06
Mixed infection	12 (18.8)	7 (11.3)	3 (10.7)	0.41
Total	37 (57.8)	44 (71.0)	15 (53.6)	0.18

Table 3

The OPG/EPG values (mean±std. err.) for the gastrointestinal parasites detected in domestic rabbits from the backyard system

	Total	Kits	Young rabbits	Adult rabbits
Eimeria spp.	6571 (±1568)	3088 (±656)	4027 (±709)	9516 (±3115)
P. ambiguus	927 (±640)	717 (±154)	1089 (±260)	900 (±648)
S. papillosus	500 (±207)	0	100 (±0)	767 (±233)
Digestive strongyles	588 (±667)	733 (±318)	567 (±120)	400 (±300)

%) was statistically higher [$X^2(1,308)=40.921$, $p< 0.00001$] compared to mixed infections (22/154; 14.3%), regardless of age categories (Table 1).

Overall, the following parasites were identified in rabbits: *Eimeria* spp. (89/154; 57.8%), *P. ambiguus* (22/154; 14.3%),digestive strongyles (8/154; 5.2%), and *S. papillosus* (5/154; 3.2%) (Table 1, Fig. 3). Young rabbits (4-6 months old) were the most infected with the identified GIT parasites, less with digestive strongyles (Table 1). The prevalence of each GIT parasite increased until the age of 5 months and then decreased (Fig. 5). GIT parasites were detected at a higher rate in households with 10-15 rabbits (71.0%) compared to the ones with smaller (1-9 rabbits: 57.8 %) or larger groups (20-50 rabbits: 53.6%) (Table 2).

Eimeria spp. prevalence was significantly higher [$X^2(3, N=616) = 187.81$, $p<0.00001$] compared to the other detected infections. The highest prevalence of *Eimeria* spp. was observed in young rabbits (79.0%) [$X^2(2, N=154) = 11.07$, $p<0.004$] and in households with 10-15 rabbits (69.4%) (Table 1, 2). However, the highest count was registered in adult rabbits (9516±3115) (Table 3). The intensity of parasitism for each species is presented in Table 3 (overall and by age groups). High counts (1000 OPG/EPG) were registered in the case of *Eimeria* spp. (400-123,400), regardless of the age group, and for *P. ambiguus* in young rabbits (200-2,800) (Table 3).

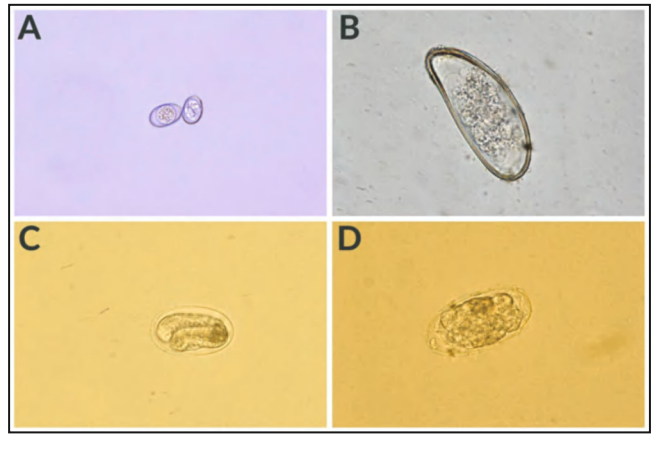

Fig. 4. *Eimeria* spp. oocysts: unsporulated and sporulated oocysts (400x) [A]; *P. ambiguus* egg (400x) [B]; *S. papillosus* egg (400x) [C]; strongyle egg (400x) [D]

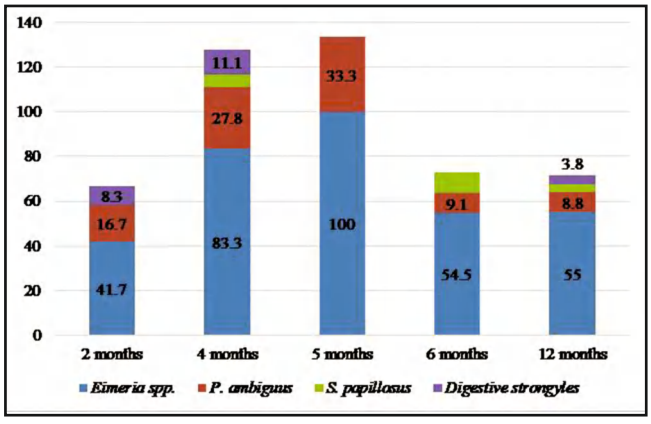

Fig. 5. Prevalence (%) of GIT parasites in household rabbits according to the age

The European rabbit (*Oryctolagus cuniculus*) is one of the 40 species in the family Leporidae and the only extensively domesticated rabbit species. All the rabbits included in our study came from household growth systems, raised for meat production. The rabbits were accommodated in wooden cages, with wooden plank floors. In this type of cage, the feces eliminated by the rabbits remain on the floor. Based on personal observation, in such growth systems in Romania, the owner cleans the cages every 1-2 weeks. Under these conditions, the parasitic elements eliminated through the faeces have a high chance of evolving to the infective stage and to be a source of (re) contamination for rabbits. This growth system reduces the risk of infection with parasites having a heteroxenous life cycle and increases the risk of infection for parasites having monoxenous life cycle due to the possibility of the host to host transmission or reinfection. The cecotrophy is favored in this case by the presence of the complete floor that does not allow the elimination of feces, having a great significance for *Passalurus* infection.

In our study, the infection with *Eimeria* spp. (57.8 %) was the most prevalent. In rabbits, two forms of coccidiosis can occur: intestinal and hepatic. Coccidiosis and nutritional errors are the most common causes of diarrhea and losses in rabbits worldwide (22). Young rabbits are the most affected, especially after weaning, while adults are asymptomatic carriers. We found a prevalence of 41.7% in kits, 79.0% in young rabbits, and 55.0% in adult rabbits. Similar data were reported in Poland: 84.9% in 0-6 months old rabbits, 54.6% in 6-12 months old rabbits, and 77.8% in rabbits older than 12 months (22). In a study performed on dead postweaning kits, 86.50% of them were diarrheic rabbits due to *Eimeria* infection (9). Interestingly, the OPG value increased with the age. Pilarczyk et al. (2020) found a decreasing trend based on age, from 11541 oocysts/g feces in 0-6 months old rabbits to 8863 oocysts/g feces in rabbits older than 12 months (22). However, there is no correlation between oocyst excretion and the severity of the disease (21). Globally, the infection rate with *Eimeria* spp. in rabbits varies between 20% and 80%, and frequently, mixed infection with more than 2 species is reported (21). *E. intestinalis, E. flavescens*, and *E. stiedae* are the most pathogenic species in rabbits. We have not performed identification of the *Eimeria* spp. in our study. In the present study, the total prevalence for helminth infection was 18.2%. We have found three types of nematodes, respectively *P. ambiguus*, *S. papillosus,* and digestive strongyles.

P. ambiguus (Rudolphi, 1819), known as lagomorphs pinworm, is an oxyurid found in the caecum and colon of domestic and wild rabbits. Contamination of the rabbits occurs after the ingestion of the eggs containing the third stage larvae, so the larvae remains protected inside the egg, making the infective element very resistant in the environment. Autoinfections by coprophagy play an important role in breeding systems with poor hygienic conditions (7). *P. ambiguus* infections in rabbits are not generally very pathogenic even if high infections occur, the reason why the infection often remains unknown by breeders (20). A study performed by Rinaldi et al. (2007) regarding the circadian rhythm of *P. ambiguus* egg excretion revealed that the highest number of eggs was found in the samples collected during the afternoon and night hours of the day (23). In the present study, *P. ambiguus* was the nematode species with the highest prevalence found (14.3%), being more prevalent in young rabbits (23.7%) than in kits (16.7%) and adults (8.8%). Infections with *P. ambiguus* have been reported in domestic rabbits in different studies reporting coproscopic examination and/or necropsy outcomes. The reported prevalences have a very wide range, from 3% to 75%, suggesting that *P. ambiguus* is the most prevalent helminth in rabbits (1, 10, 13, 19, 24). In our study, we have found prevalences what fit between these findings, with EPG values slightly higher in young rabbits (1089±260) compared with kits (717 ± 154) and adults (900±648).

Graphidium strigosum, Obeliscoides cuniculi, and *Trichostrongylus retortaeformis* are the digestive strongyles which have been found more often in natural infections in domesticated rabbits. *G. strigosum* and *O. cuniculi* inhabit the mucosa of the stomach and *T. retortaeformis* is a parasite of the small intestine (7). The digestive strongyles of rabbits have a direct life cycle; contamination occurs by ingestion of free-living third-stage infective larvae.

The *Graphidium* genus (Haemonchidae) includes a single species, *G. strigosum* (Dujardin, 1845), which is an exclusive parasite found in European lagomorphs (17). *G. strigosum* is one of the species where the infective stages have a higher sensitivity to high and low

temperatures (14). According to Manssoni et al. (2011), at 24°C hatched L1 larvae can reach the infective L3 stage in 5 days and the patent period can last until 13 months (17). Different studies have shown a prevalence between 1.09-16.5% in domestic rabbits (16, 19, 24). In wild rabbits, *G. strigosum* is considered as a nematode causing high mortality, with prevalences that can reach 78% (3).

Reports of *O. cunniculi* (Trichostrongylidae, Measures and Anderson, 1983) infection in *Oryctolagus cuniculus* in Europe are rare, but it is a common parasite in American lagomorphs. This parasite has a low host specificity, being occasionally reported also in rodents and ruminants (11). A study made in Poland on 274 slaughtered rabbits, shown a 0.36% prevalence of infection with *O. cuniculi* (24).

The members of *Trichostrongylus* genus are mainly parasites of ruminants. Their presence in rabbits and ruminants suggests the possible transfer between the two hosts (4). The life cycle of *T. retortaeformis* (Trichostrongylidae, Zeder, 1800) has been reproduced by Audebert et al. (2002), at 24°C L3 larvae can form in 5 days, the prepatent period was 12 days and the patent period lasted five and a half months. The authors found no significant differences among the life cycle development of the different *Trichostrongylus* species of lagomorphs and ruminants (4). The prevalence of infection with *T. retortaeformis* in domestic rabbits may vary from 5.47 to 42% according to different authors (13, 19, 24). In the present study, the total prevalence of infection with digestive strongyles was 5.2%, respectively 8.3% in kits, 5.3% in young rabbits, and 3.8% in adult rabbits. EPG values were higher in kits (733±318) compared to young (567± 120) and adult (400±300) rabbits.

S. papillosus (Strongyloididae, Wedl, 1856) is known as a common intestinal nematode of ruminants, but infection can also occur in rabbits, which can develop in severe infection with anorexia, bodyweight loss, anemia, gastrointestinal motor disturbances, and subsequent death (15, 25). Severe forms are most likely in the young animals, being associated with poor hygiene conditions. *Strongyloides* is a geohelminth, which has an unusual life cycle, with two types of development: homogonic (direct) and heterogonic (indirect), depending on environmental temperatures. In case of the direct life cycle, a rhabditiform larva emerges from the egg, molts and after 2–3 days turns into an infective larva (5). Skin penetration is the main way of infection and only the female worms are parasitic. Data regarding the prevalence of *S. papillosus* in rabbits are scarce. Prevalences between 0.9-13.3% are mentioned for *S. papillosus* infection in domestic rabbits by different authors (10). In our study, we have found a slightly higher prevalence in young rabbits (5.3%) compared with adult rabbits (3.8%), while the EPG values were higher in adult (767±233) rabbits compared with the young (100±0) ones, respectively.

CONCLUSIONS

In the present study performed on 154 feces samples collected from rabbits raised in the backyard farming system, with *Eimeria* spp. (57.8%) and *P. ambiguus* (14.3%) were the most prevalent parasitic agents, followed by *S. papillosus* (3.2%) and digestive strongyles (5.2%). The highest prevalences were found in young rabbits with ages between 4 and 6 months. The category of rabbits having the highest risk of infection with GIT parasites was found in the rabbits raised in households with 10-15 individuals. The obtained results suggest that more rigorous coproparasitological surveillance and implementation of appropriate preventive strategies is necessary to avoid the parasitic infections in rabbits from the backyard system. The focus should be directed to the awareness of these parasitic diseases, prevention measures necessary to assure basic zoohygenic conditions, and the education of breeders.

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