



ORIGINAL ARTICLE

PREVALENCE AND SPECIES COMPOSITION OF ENDOPARASITES IN HOBBY AND MEAT RABBITS IN EASTERN SLOVAKIA WITH SPECIAL FOCUS ON *EIMERIA* SPP.

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Endoparasitosis in rabbits often represents a hidden problem and causes serious financial loss to breeders as it occurs without any evident clinical symptoms. The subject of the present investigation was to identify the rate of prevalence of endoparasites, with special attention being paid to coccidiosis. The investigation was carried out at selected rabbit farms in five villages in the Gelnica District in Eastern Slovakia in the period from September 2019 to August 2020. A total of 87 faecal samples were examined using the flotation method and the McMaster technique. Of these, 83 samples tested positive for intestinal parasites, representing a positivity rate of 95.4%. Coccidian oocysts of *Eimeria* spp. were present in all positive samples, and *Passalurus ambiguus* eggs were found in six cases (7.2%). The overall oocyst counts for *Eimeria* spp. ranged from 50 to 27,000 oocysts per gram (OPG), while the egg counts of *P. ambiguus* ranged from 50 to 250 eggs per gram (EPG). To determine the species composition of the genus *Eimeria*, molecular analysis of the first internal transcribed spacer (ITS-1) region of ribosomal DNA was performed on selected samples with the highest concentrations of oocysts. The analysis revealed the presence of five coccidian species: *Eimeria magna*, *Eimeria flavescens*, *Eimeria piriiformis*, *Eimeria irresidua*, and one unidentified *Eimeria* sp.

Keywords: coccidiosis; coprological methods; *Eimeria* spp.; PCR; rabbit

INTRODUCTION

Coccidiosis is a gastrointestinal parasitic infection that affects rabbits and hares, and it has a notably negative economic impact, especially in farmed rabbit populations (*Oryctolagus cuniculus*) worldwide [1, 2]. Coccidiosis

in rabbits is caused by one or more species of unicellular parasites belonging to the genus *Eimeria*, subphylum Apicomplexa. Globally, a large number of *Eimeria* species have been described; however, most commonly, 15 species of the genus *Eimeria* are believed to be responsible for rabbit coccidiosis, including: *E. stiedae*, *E. magna*, *E. matsubayashi*,

E. neoleporis, *E. nagpurensis*, *E. irresidua*, *E. flavescens*, *E. piriformis*, *E. intestinalis*, *E. exigua*, *E. roobroucki*, *E. perforans*, *E. vej dovskyi*, *E. coecicola*, and *E. media*. The only exception is *E. stiedae*, which parasitises the bile ducts of the liver and is considered one of the most pathogenic species, while *E. magna*, *E. irresidua*, *E. perforans*, and *E. media* belong to the most frequently diagnosed species in domestic rabbits [3, 4, 5, 6, 7, 8, 9, 10, 11, 12].

Young rabbits, especially after weaning and between the ages of 6 weeks and 6 months, represent the most vulnerable group [13]. Another group of susceptible individuals includes older and immunocompromised ones, while adult rabbits are often asymptomatic carriers and rarely present with clinical symptoms of coccidiosis. No gender- and breed-dependent predisposition to that disease has been observed [13, 14].

The life cycle of that parasite is direct and comprises the exogenous phase (sporogony) and the endogenous phases (schizogony and gametogony). They are transmitted through the faecal-oral route, and the severity of infection depends on the number of ingested oocysts [15]. The intake of sporulated oocysts, containing 8 sporozoites, is followed by the schizogony phase [16]. The sporozoites are released from sporocysts and invade the epithelial cells of the small intestine and appendix of rabbits. The exception is the *Eimeria stiedae* species, which colonises the cells of the liver and bile ducts. During the gametogony phase (sexual reproduction), a new generation of oocysts is produced at the parasite's predilection sites. Upon completion of the cycle, these oocysts are excreted in the faeces into the external environment. The exogenous phase (sporogony) takes place in the external environment and leads to the creation of a new generation of infectious oocysts [17, 18]. Clinical symptoms of intestinal and hepatic coccidiosis are very similar. Generally, the clinical condition of affected rabbits is characterised by apathy, anorexia, and weight loss, primarily caused by a reduced intake of food during the first four weeks. In untreated cases, the condition may progress to a chronic stage [19].

Post-mortem observations in cases of infection caused by *E. stiedae* reveal hepatomegaly accompanied by typical multifocal necrotic lesions on the hepatic parenchyma. The lesions are 1 – 2 mm large, of white or cream colour, filled with an exudate dispersed across the entire parenchyma [14, 17, 20].

Diagnosis of coccidial infection is primarily based on the detection of oocysts in examined faecal samples. Spe-

cies identification within the genus *Eimeria* is traditionally performed through morphological and morphometric evaluation of oocysts, sporocysts, and sporozoites [21]. However, microscopic identification requires considerable expertise and is often time-consuming, particularly when distinguishing closely related species. For this reason, molecular techniques such as polymerase chain reaction (PCR) have increasingly been employed to improve the accuracy and reliability of species identification [18].

In addition to protozoa, rabbits also present with helminths. *Passalurus ambiguus* (an oxyurid worm) is the most frequently detected intestinal nematode in rabbits and hares and the second most common intestinal parasite after *Eimeria* spp. [22]. *P. ambiguus* parasitises the appendix and large intestine of infected individuals, completing a direct life cycle. Infection occurs when rabbits ingest these eggs from the environment, commonly through contaminated feed, water, or materials, or via caecotrophy, resulting in autoinfection. Adult females deposit sticky, embryonated eggs – typically oval, asymmetrical, light brown in colour, and featuring a polar plug – around the perineal region [14, 17, 23, 24, 25]. In most cases, rabbits infected with *P. ambiguus* are asymptomatic, even when the intensity of infection is high. However, in young and weakened rabbits, the risk of clinical symptoms is higher. Those may include intensive itching around the rectum, digestive disorders, diarrhoea, weight loss, and even rectal prolapse [17, 26, 27].

The aim of the present study was to determine the prevalence and species composition of endoparasites in hobby and meat rabbits in selected localities of Eastern Slovakia, with particular emphasis on coccidial infections caused by *Eimeria* spp., using coprological and molecular diagnostic methods, in order to contribute to a better understanding of the epidemiological situation of these infections in rabbit breeding. In addition, this study provides the first PCR-based molecular confirmation of *Eimeria* species infecting rabbits in Slovakia.

MATERIAL AND METHODS

Sample collection

The investigation was carried out from September 2019 to August 2020. A total of 87 faecal samples were analysed, including 76 samples collected from individu-

al rabbits and 11 pooled samples obtained from groups of breeding females with young. The study focused on selected farms in the town of Gelnica ($n = 43$), followed by the villages of Mníšek nad Hnilcom ($n = 19$), Prakovce ($n = 15$), Jaklovce ($n = 5$), and Kojšov ($n = 5$), all located within a 20-kilometer radius in the Gelnica District of Eastern Slovakia. The majority of the samples originated from hobby rabbits, particularly those involved in rabbit hopping, a popular regional activity, and from rabbits raised for meat production. A detailed overview of the distribution of rabbits by location and category is presented in Table 1. The examined faecal samples were mostly collected from the Czech Spotted Rabbit ($n = 18/87$), New Zealand White Rabbit ($n = 13/87$), and Rhön Rabbit ($n = 18/87$) breeds. All hobby and meat rabbits included in the study were in the range of 6 months to 3 years.

Coprological examination

After collection, the faecal samples were stored at refrigerator temperature (4 °C) and subsequently transported to the laboratory for coprological examination. The samples were examined using a qualitative flotation method to confirm the presence of protozoan oocysts and helminth eggs. To assess the intensity of infection, the quantitative McMaster technique was applied according to the method described by Taylor (2007) [14].

Molecular identification of *Eimeria* spp.

The examined faecal samples with the highest concentrations of *Eimeria* spp. oocysts (ranging from 10,000 to 27,000 OPG) were subjected to molecular analysis in order to identify the *Eimeria* species infecting rabbits in the studied region. The total genomic DNA extraction was carried out using a ZR Faecal DNA MiniPrep™ commercial kit (ZymoResearch, USA) following the manufacturer's instructions and subsequently stored in a freezer at -20

°C and later used for the PCR analysis. DNA samples were used for amplification of the internal transcribed spacer 1 (ITS-1) region of ribosomal DNA according to the procedure published by Oliveira (2011) [18] using universal primers for the *Eimeria* species: forward ITS1-F (GG-GAAGTTGCGTAAATAGA) and reverse ITS1-R (CTG-CGTCCTTCATCGAT) with resulting length fragments of about 400 bp. The total PCR reaction mixture, with a volume of 50 µl, consisted of 25 µL of OneTaq® 2X Master Mix with Standard Buffer (New England Biolabs), 21 µL of nuclease-free water, 1 µL of each of the two primers (Sigma, GB), and 2 µL of DNA template. The amplification program consisted of the initial denaturation step at 94 °C lasting for 2 min, with 30 cycles repeated at 94 °C for 1 min, at 58 °C for 1 min, at 72 °C for 1 min, and a final extension phase at 72 °C for 7 min [18] in a programmable Mastercycler Nexus X2 (Eppendorf, Germany).

The amplified PCR products were visualized by electrophoresis on a 1% agarose gel and observed under a UV transilluminator. All positive products were sent to the commercial laboratory Microsynth SeqLab (Vienna, Austria) for purification and bidirectional sequencing using the same primers as in the PCR. Sequencing was performed using the Sanger method. The obtained sequences were edited using MEGA X software [28] and assembled using GeneTool Lite 1.0 (BioTools Inc., USA). The resulting sequences were compared with reference sequences in the GenBank database (NCBI) using the BLAST algorithm.

RESULTS

Coprological analysis

Flotation analysis revealed the overall presence of *Eimeria* spp. oocysts in 83 out of 87 (95.4%) (Table 2).

Table 1. Distribution of rabbits by category and location

Location	Hobby rabbits	Meat rabbits	Breeding females with young	Total
Gelnica	24	16	3	43
Mníšek nad Hnilcom	5	9	5	19
Prakovce	15	-	-	15
Jaklovce	-	3	2	5
Kojšov	-	4	1	5
Total	44	32	11	87

Note: Dash (–) indicates that no animals of the given category were reported in the respective location

Table 2. Positivity for *Eimeria* spp. in the Gelnica District

Location	Positive Hobby Rabbits / Total Examined	Positive Meat Rabbits / Total Examined	Positive Breeding Females with Young / Total Exam- ined	Overall positivity (%)
Gelnica	23/24	14/16	3/3	93.0
Mníšek nad Hnilcom	5/5	8/9	5/5	94.7
Prakovce	15/15	-	-	100
Jaklovce	-	3/3	2/2	100
Kojšov	-	4/4	1/1	100

Eggs of *P. ambiguus* were confirmed in 6 samples (7.2%) originating from both hobby and meat rabbits of three breeds (Czech Spotted Rabbit, New Zealand White Rabbit, and Rhön Rabbit) kept in Gelnica, Mníšek nad Hnilcom, and Prakovce. In contrast, no *P. ambiguus* eggs were detected in any of the individual or pooled samples collected from breeding females with young in Jaklovce and Kojšov.

The McMaster quantitative technique was employed to determine the intensity of infection caused by *Eimeria* spp. oocysts (OPG – oocysts per gram of faeces) and to detect the presence of *P. ambiguus* eggs (EPG – eggs per gram). Across all examined samples, the intensity of coccidian infection ranged from 50 to 27,000 OPG (Table 3).

Molecular analysis of *Eimeria* spp.

Molecular analysis of the ITS-1 ribosomal DNA region was performed on 15 samples with a high concentration of oocysts in rabbit faeces in order to detect the presence of *Eimeria* spp. and determine their species composition. Sequencing and subsequent sequence editing yielded eight high-quality sequences, which showed 96.45–100% identity to *Eimeria* species in the GenBank database. The sequences obtained in this study were deposited in GenBank under accession numbers PZ160625–PZ160632. In these eight isolates, five *Eimeria* species were identified: *E. piriformis*, *E. magna*, *E. irresidua*, *E. flavescens*, and one unidentified *Eimeria* sp. (Table 4). The first sample (E1) was collected from breeding females with young of the Czech Spotted Rabbit breed from Gelnica, which ex-

hibited a high intensity of infection caused by *Eimeria* spp. oocysts (16,400 OPG). Two isolates from this sample were identified: E1a as *E. piriformis* and E1b as *E. magna*. The second positive sample (E2) originated from a female of the diminutive Lionhead breed from Gelnica. Sample 3 (E3) was collected from a diminutive male Hermelin rabbit from Gelnica. In both cases (E2 and E3), *Eimeria* sp. were detected; however, species-level identification was not achieved due to low sequence similarity (96.45% and 96.63%) with reference sequences available in the GenBank database. The fourth sample (E4) originated from a male Rhön Rabbit from Prakovce, which was used for breeding and kept under extensive rearing conditions. The fifth sample (E5) was collected from a female Czech Spotted Rabbit from Mníšek nad Hnilcom, housed together with her kits, and showing the highest intensity of coccidian infection (27,000 OPG). The E6 sample was obtained from a male Hermelin rabbit from Gelnica and the E7 sample from a pregnant female Czech Spotted rabbit from the Mníšek nad Hnilcom area (Table 4).

DISCUSSION

Coccidiosis in rabbits is a major parasitic disease that significantly increases breeding costs. In the present study, 87 faecal samples from rabbits were examined microscopically using the flotation method. The samples showed a high positivity rate of 95.4% for *Eimeria* spp. oocysts. This is notably higher compared to a 2015 study that in-

Table 3. Intensity of infection with *Eimeria* spp. and *Passalurus ambiguus* in examined rabbit breeds

Rabbit breed	Locality	<i>Eimeria</i> spp. (OPG range)	<i>Passalurus ambiguus</i> (EPG range)
Czech Spotted Rabbit	Gelnica, Mníšek nad Hnilcom	250 – 16,400 (max. 27,000 in pooled sample)	50 – 150
New Zealand Rabbit	Jaklovce, Kojšov	50 – 4,500	not detected
Rhön Rabbit	Prakovce	50 – 4,400	200 – 250

Table 4. Identification of *Eimeria* spp. based on ITS-1 rDNA and GenBank accession numbers

Sample code	<i>Eimeria</i> spp.	GenBank accession no.	Highest identity with GenBank	
			Percent identity	Accession number
E1a	<i>E. piriformis</i>	PZ160625	100%	HM768889.1 host: Rabbit Czech Republic [18]
E1b	<i>E. magna</i>	PZ160626	98.60%	MK590197.1 host: Rabbit China
E2	<i>Eimeria</i> sp.	PZ160627	<i>Eimeria perforans</i> 96.45%	HM768888.1 host: Rabbit France [18]
E3	<i>Eimeria</i> sp.	PZ160628	<i>Eimeria perforans</i> 96.63%	HM768888.1 host: Rabbit France [18]
E4	<i>E. irresidua</i>	PZ160629	100%	HM768885.1 host: Rabbit Czech Republic [18]
E5	<i>E. irresidua</i>	PZ160630	100%	HM768885.1 host: Rabbit Czech Republic [18]
E6	<i>E. magna</i>	PZ160631	98.60%	MK590197.1 host: Rabbit China
E7	<i>E. flavescens</i>	PZ160632	99.18%	MN535227.1 host: Rabbit Mexico

vestigated endoparasite prevalence in rabbits from 23 breeder farms in Eastern Slovakia, which reported a 72.5% infection rate with *Eimeria* spp. [29].

In Romania, 236 rabbit faecal samples were examined, revealing a 77.56% infection rate with *Eimeria* spp. [12]. In wild European brown hares from Austria and the Czech Republic, Chroust et al. (2012) reported very high prevalence in young animals (97.9% in Austria and 95.5% in the Czech Republic), while adults showed lower rates (60.0% and 64.6%, respectively) [30]. In Poland, a survey of 14 small rabbit farms identified ten *Eimeria* species, with prevalence ranging from 8.0% to 98.5% depending on the species, and the highest mean oocyst count reached 21,100 OPG in mid-May [31].

Research conducted in Romania showed that domestic rabbits exhibited a prevalence of *Eimeria* spp. infection of 52.3% [32]. In three Algerian regions, Maziz-Bettahar et al. (2018) reported a post-weaning coccidian prevalence of 90%. Across the examined farms, two to six *Eimeria* species were detected per location, with eight species identified in total. The most prevalent were *E. magna* (42.5%), *E. media* (17.6%), and *E. irresidua* (14.9%). High oocyst excretion was significantly associated with limited anti-coccidial use (25.0% of farms) and poor hygienic condi-

tions (65.0% of farms) [33]. In the Sichuan Province of southwestern China, an investigation based on 110 samples collected from 11 farms reported an overall *Eimeria* prevalence of 56.4%, with the highest infection rate occurring in weaned rabbits (74.0%). These results indicate that kits and recently weaned rabbits constitute the most vulnerable age category, representing the period of greatest susceptibility to eimeriosis [34].

Using a specifically designed pair of primers for ITS-1 region amplification and applying the PCR analysis proposed by Oliveira, eight samples from rabbits reared in the Czech Republic and France were examined, revealing the highest presence of oocysts of *E. magna*, *E. flavescens*, *E. piriformis*, *E. perforans*, and *E. irresidua*. The scientific study conducted by Oliveira (2011) [18] was used as the basis for subsequent published studies. In China, Brazil and Mexico, the species that were most frequently detected included *E. flavescens* (61.82%), *E. magna* (42.5%), *E. intestinalis* (11.3%), and *E. perforans* (35.2%) [1, 35, 36].

In Germany, *E. media*, *E. magna*, and *E. perforans* were the most commonly detected species, whereas *E. exigua* was observed relatively infrequently [37]. These findings demonstrate the widespread occurrence of *Eimeria*

infections in European rabbit and hare populations and underscore the importance of ongoing monitoring and control measures. Although numerous studies have identified *Eimeria* spp. in domestic rabbits solely on the basis of oocyst morphology [8, 21, 38], accurate species differentiation remains challenging due to the considerable morphological overlap among the many *Eimeria* species capable of infecting rabbits [39].

Infections caused by *P. ambigua* may affect the production performance, especially in young and weaned kits. The intensity of infection may also vary depending on the season and the age of the rabbits [22]. Among all examined samples collected in the Gelnica District, the prevalence of *Passalurus* eggs was 7.2%, with egg counts ranging from 50 to 200 EPG. This represents a 3.0% lower prevalence compared to a 2015 study conducted in Eastern Slovakia, which reported a prevalence of 10.2% [29]. In a study from Poland, Szkucik et al. (2014) detected *P. ambigua* eggs with a prevalence of 5.83% [40]. In Southern Italy, infection intensity was high, with 82.3% of examined rabbits testing positive using the FLOTAC technique [41]. The most recent study from Ukraine reported morbidity rates of passalurosis ranging from 37.70% to 41.67% in domestic rabbits, with the highest infection intensity (82.76%) observed in animals aged 1–2 years [42]. A study conducted in south-western Romania reported a 4.4% prevalence of *P. ambigua* eggs in domestic rabbits, based on coproscopic examination of 111 individuals [32]. In contrast, research performed in Hungary on rabbit farms revealed a different pattern, showing a significantly lower infection rate in the growing stage (5–11 weeks old); the highest infection rate was detected in samples collected from young rabbits during the lactation period [43].

It is important to highlight that the post-weaning period represents the highest risk phase for digestive diseases in rabbits, where endoparasitism and bacterial infections are among the most common health issues [44].

CONCLUSION

The present study aimed to investigate the occurrence of intestinal parasites in rabbits from selected rabbit farms in the Gelnica District, focusing on hobby and meat rabbits raised on these farms. A total of 87 faecal samples were

collected from individual rabbits as well as from groups of breeding females with their young. Coprological examination revealed a high overall prevalence of oocysts of *Eimeria* spp. (95.4%), despite the absence of overt clinical signs in most animals. The intensity of infection varied across breeds and locations, with the highest oocyst counts observed in breeding females and their kits. Eggs of the *P. ambigua* were also detected, although at a lower prevalence (7.2%), mainly in hobby and meat rabbits from specific breeds and sites. Molecular analysis using PCR allowed species-level identification of *Eimeria* spp., providing new insights into the genetic diversity of these parasites in Slovakian rabbit populations. This represents the first molecular characterization of *Eimeria* spp. in rabbits in Slovakia. Given the high prevalence and potential impact on rabbit health and breeder economy, routine parasitological monitoring and early intervention strategies are strongly recommended. Regular screening for intestinal parasites, particularly *Eimeria* spp. and *P. ambigua* can help mitigate financial losses and improve animal welfare in both hobby and commercial rabbit production systems. Effective prevention of coccidiosis in commercial rabbit production requires strict hygiene management, including regular removal of faeces, sanitation of cages and equipment, and environmental conditions. In addition, regular parasitological monitoring and the appropriate use of anticoccidial drugs can help reduce the spread of infection, minimize economic losses, and improve animal welfare.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

No ethical approval was necessary for this study.

Conflict of Interest

The authors declare no conflicts of interest.

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Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

Authors' Contributions

Conceptualization: A. S., G. Š.; methodology: A. S., G. Š., N. J.; sample collection: N. J.; data curation: A. S., G. Š.; manuscript writing: A. S., G. Š., N. J.; molecular identification: A. S.; funding acquisition: G. Š. All authors have read and agreed to the published version of the manuscript.

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