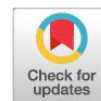


Research Article



Eimeria Oocysts and *Passalurus ambiguus* Infection of Farmed Rabbits Depending on the Age

CSONGOR DEMETER¹, ZOLTÁN NÉMET², ZSOLT GERENCSÉR¹, RÓBERT JURÁSKÓ¹, ANDRÁS MAYER¹, BORÓKA BÁRDOS^{1*}, ANETT DEMETER-JEREMIÁS³, ERIKA LENCSES-VARGA⁴, ZSOLT MATICS⁴

¹Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Institute of Animal Sciences, H-7400 Kaposvár, Guba S. u. 40, Hungary; ²University of Veterinary Medicine, Department of Pathology, H-2225 Üllő, Dóra major, Hungary; ³S and K-Lap Ltd., Kartal, Császár út 135, Hungary; ⁴Széchenyi István University, Department of Animal Science, H-9200 Mosonmagyaróvár, Vár tér 2, Hungary.

Abstract | The aim of the study was to investigate the *Eimeria* spp. and *Passalurus ambiguus* infections of rabbits by day of life. From 2018 to 2024, pooled faecal samples were collected from 29 Hungarian and 2 Slovakian rabbit farms. Low level of *Eimeria* oocysts infection was observed during lactation. In the week following the weaning the proportion of positive samples increased. Between day 43 and slaughter age, the proportion of positive samples was consistently high. The average OPG values (number of *Eimeria* oocysts per gram faeces), never reached 5,000 during the lactation period, but a critical period started at 42 days of age, with average OPG values above 10,000 in several cases. High number of oocysts were observed until the end of fattening period. The exponential smoothing model estimated the onset of *Eimeria* infection at 38–40 days of age ($p=0.023$). The proportion of *P. ambiguus* eggs positive samples reached 50% already in the lactation period. From day 29 to slaughter age, a relatively low rate of infected samples was detected. For *P. ambiguus*, the model estimated 7 days of age as the increase ($p=0.001$) of infection. It can be concluded that *P. ambiguus* eggs and *Eimeria* oocysts can be detected in the faeces of rabbits during their whole life cycle. During the fattening period (5–11 weeks of age), the *Eimeria* infection is on high level while the *P. ambiguus* infection is in low level.

Keywords | Rabbit farming, *Eimeria* infection, *Passalurus ambiguus* infection, Coccidiosis, Pinworm, Age-dependent

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***Correspondence** | Boróka Bárdos, Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Institute of Animal Sciences, H-7400 Kaposvár, Guba S. u. 40, Hungary; **Email:** borokabardos@gmail.com

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INTRODUCTION

Even in modern husbandry, with adequate feeding and hygiene conditions, digestive diseases of rabbits can still hinder production. The animal health risk factors for rabbits kept on industrial farms have not changed over the years. Viruses, bacteria, fungi and parasites that cause respiratory and digestive problems are still present on farms.

Most common health problems in rabbits were parasitic infections (24.3%), bacterial diseases (21.0%), feed-borne and metabolic diseases (18.0%) and viral infections (11.6%).

Besides bacteria, parasites are responsible for a large proportion of gastrointestinal diseases, the most common endoparasitic infection in large-scale farmed rabbits is

coccidiosis, in which several *Eimeria* species play a role (Vetési, 1990). In addition to *Eimeria* species, an oxyurid nematode, *Passalurus ambiguus* is a common parasite in the intestinal tract of rabbits (Licois, 2004; Peeters *et al.*, 1981). Parasites in the digestive tract of rabbits can cause diarrhoea and, in severe cases, mortality and thus significant economic losses (Peeters *et al.*, 1981; Licois, 2004; Sioutas *et al.*, 2021).

Twelve different *Eimeria* species are known to occur in rabbits (Demeter *et al.* 2023), eleven of which infect the intestinal tract, while one (*Eimeria stiedai*) reproduces in the bile ducts.

Balicka-Ramisz *et al.* (2020) identified ten *Eimeria* species in their study on 14 farms in Poland, with *E. media*, *E. magna*, *E. perforans* and *E. irresidua* species being the most abundant. Yin *et al.* (2016) collected faecal samples from 11 farms in Sichuan province, southwest China, and reported identifying 9 *Eimeria* species. In their cases, the species detected were *E. perforans*, *E. media*, *E. irresidua*, *E. magna* and *E. intestinalis* in the highest proportions. El-Shahawi *et al.* (2012) in Egypt identified eight *Eimeria* species and *E. intestinalis* and *E. coecicola* were the most dominant species. In Saudi Arabia, Abdel-Baki and Al-Quraishy (2013), identified ten *Eimeria* species, including *E. coecicola*, *E. magna*, *E. perforans* and *E. media* as the most common. In the subtropical mountainous region of India, Laha *et al.* (2015) identified six *Eimeria* species (*E. magna*, *E. exigua*, *E. piriformis*, *E. coecicola*, *E. intestinalis* and *E. media*) and attributed them to mortality from coccidiosis. In our previous study Demeter *et al.* (2023), oocysts of eight *Eimeria* species were identified from faecal samples from large-scale rabbit farms in Hungary: *E. media*, *E. magna*, *E. coecicola*, *E. irresidua*, *E. flavescens*, *E. piriformis*, *E. perforans* and *E. intestinalis*.

Individual species of rabbit coccidia differ in pathogenicity. Intestinal coccidia causes more or less severe disease in rabbits, depending mainly on the infection dose, the *Eimeria* species, the immune status and the age of the animals (Pakandl, 2009). Adult animals are mostly asymptomatic to coccidiosis caused by *Eimeria* spp. and generally rabbits younger than 20 days of age are not infected, however Dürr and Pellérdy, (1969) were able to infect suckling rabbits between the first and ninth day of life, but using huge numbers of liver coccidia, despite which oocyst excretion was very low. Literature data and practical experience suggest that young animals are particularly susceptible to coccidiosis in the post-weaning phase (Drouet-Viard *et al.*, 1997; Pakandl and Hlášková, 2007).

In our previous study (Demeter *et al.*, 2023), 71% of the oocysts identified were represented by species of low pathogenicity, while 25% were from moderate and 4% from

highly pathogenic species. The proportions varied significantly by life stage. The lactation period (weeks 1-5 of life) was dominated by weakly pathogenic species, with moderate and highly pathogenic species present in less than 10%. The post-weaning period (weeks 6-9 of life) showed an increase in the proportion of oocysts of moderately pathogenic species, but it was interesting that highly pathogenic species were not detected in this period. During the final phase of fattening (weeks 10-13 of life), the proportion of mildly pathogenic species was again higher, the proportion of moderately pathogenic species decreased and a highly pathogenic *Eimeria* species (*E. flavescens*) was detected in this life stage.

In addition to *Eimeria* oocysts, domestic and wild living rabbits are also commonly infected with *Passalurus ambiguus* worldwide which is one of the most common oxyurid nematodes found in rabbits. *P. ambiguus* is species- or host-specific, i.e. it only lives in rabbits (<https://vcahospitals.com>).

There are usually no clinical signs of the presence of *Passalurus sp.* parasites, even in cases of severe infection (a rabbit can be infected with up to 1000 parasites). Eggs and live adult worms can only be detected in the faeces, not around the anus (<http://www.medirabbit.com>).

Based on the literature, it is not possible to clearly determine the severity of effect of *P. ambiguus* on the health of rabbits. In most cases, it can live undetected in the digestive tract of rabbits, however some researchers, such as Mykhailiutenko *et al.* (2019) have found that *P. ambiguus* infection can manifest clinically as intense itching around the anus, various digestive disorders and weight loss. In rabbits, the chronic presence of *P. ambiguus* in the large intestine has resulted in pathomorphological lesions in the colon, with localised damage to the upper epithelium of the colon. *P. ambiguus* is an aetiological factor in the occurrence of chronic hurriculitis colitis. According to Sioutas *et al.* (2021) and Hönich *et al.* (1978), heavy infestation with *P. ambiguus* can cause intestinal inflammation, and parasites in the gut have several adverse effects, both toxic and mechanical, as well as nutrient depletion, which negatively affect host health. Symptoms can include diarrhoea, weight loss, neurological symptoms and even death. At the location of the *Passalurus sp.* worms in the digestive tract of the rabbits, inflammatory and pathological changes can be observed (<http://www.medirabbit.com>).

According to Sioutas *et al.* (2021) *P. ambiguus* infection can affect the production of both mothers and young rabbits. During the lactation period, suckling rabbits can be infected with contaminated litter or other materials. Based on the length of prepatent period of *P. ambiguus* (55-60 days) (Mehlhorn, 2008), it is therefore recommended to examine

the faeces of rabbits around the age of 67 days.

According to Abdel-Gaber *et al.* (2019), early diagnosis and control of parasites help maintain a high level of production, reduce losses and it is also advantageous from animal welfare aspect.

The study aimed to examine the distribution of *Eimeria* and *P. ambiguus* infections of rabbits by day of life, their possible correlations and recommendations for the timing of anti-parasitic treatments to prevent a high infection rate.

MATERIALS AND METHODS

ANIMALS

From 2018 to 2022, faecal samples were collected from 29 Hungarian and 2 Slovakian rabbit farms, and from 2022 to 2024, sampling was limited to Hungarian farms. The sampling represented about half of the meat-producing rabbit population in Hungary and 80% in Slovakia. The survey covered farms with a herd size between 200 and 6000 rabbits. Sampling and analysis fully covered the different stages of large-scale production.

FEED AND HUSBANDRY

The flocks were fed with a complete granulated feed mixture appropriate to age and production level (Table 1). The chemical composition of the feeds used has not changed significantly over the years. The diets used at does and at the postweaning and finishing stages of fattening generally did not contain anti-parasitic supplements (medications). In cases of severe infection, anti-parasitic treatments were used on veterinary prescription and under supervision.

Table 1: Chemical composition of diets in each production cycle.

Item	Doe Diet	Weaning Diet	Finisher Diet	Diet for future breeding animals
Moisture, %	11-12	11-12	11-12	11-12
Crude protein, %	17.3-17.5	15.3-16.0	15.3-15.6	15.0-15.5
Crude fat, %	3.5-3.9	2.6-3.5	3.8-4.3	2.5-2.9
Crude fibre, %	14 - 15	16.3-16.8	15.5-16	19-20
Ash, %	7.3	6.6	6.6	6.8
ADF	17-17.5	19- 19.7	18.5-19.2	23-24
NDF	32- 32.6	37-37.5	36-36.8	38.5-39.1

ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber.

Faecal samples were obtained from rabbits kept in standard, welfare-improved cages and grid-floored box housing systems.

SAMPLE NUMBERS, SAMPLE COLLECTION AND PARASITOLOGICAL ANALYSIS

A total of 4060 samples for *Eimeria* oocysts and 5469 samples for *P. ambiguus* were evaluated between March 2018 and March 2024 with an unequal distribution per periods.

In all cases, the fresh faeces samples were taken from the manure channel after daily manure removal. According to our methodology, samples were taken from the first, middle and last thirds of the manure channels. A minimum of 2-5 g of faeces per row was collected and mixed, i.e. the sample was not an individual sample but a pooled sample of animals of the same age group in the barn.

Surface enrichment tests were performed in the laboratory of S&K-Lap Ltd. The samples were analysed within 48 hours after collection. The surface enrichment solution was an aqueous solution of magnesium sulphate (400 g MgSO₄ and 1 litre distilled water). Faecal samples were processed using McMaster method according to the recommendations of the Royal Veterinary College and FAO (<https://www.rvc.ac.uk>). During the surface enrichment study, the number of *Eimeria* oocysts and *Passalurus ambiguus* eggs was recorded (Figure 1A, 1B).

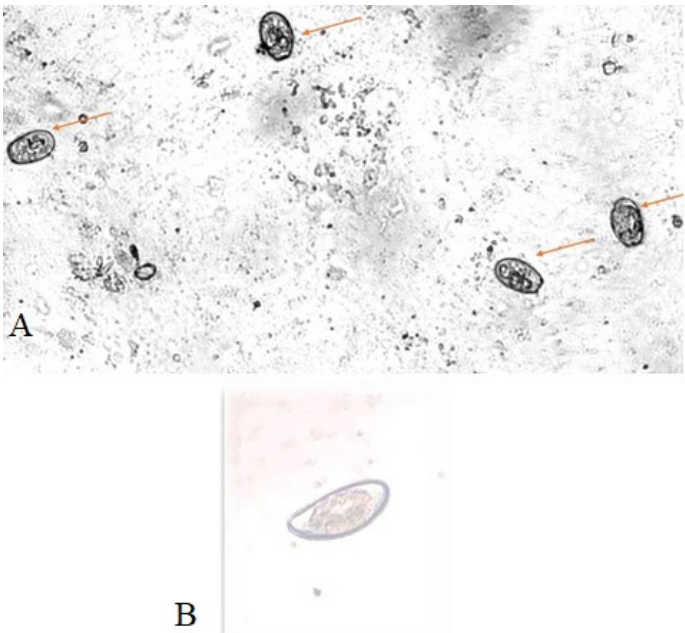


Figure 1: A: Sporulated *Eimeria* oocysts. B: *Passalurus ambiguus* egg.

The number of developmental stage individuals of *P. ambiguus* larvae present in the counting chamber during the same enrichment procedure was not recorded. The number of *Eimeria* oocysts was expressed as OPG value (oocysts per gram).

STATISTICAL ANALYSIS

The IBM SPSS 29.0 software package was used for statistical analysis. The data were analysed using a predic-

tive statistical model, including a so-called exponential smoothing model. Using the current observed value and the previous smoothing state, formulas are given to calculate the smoothing states of each observed point. The smoothing equations give the weighted average of the current value and the previous states of the time series. The weight of the current value or state is a model parameter with a value between 0 and 1, and the previous values are exponentially decreasing. The data are evaluated both as a fraction and as a percentage. The relationship between *Eimeria* oocyst and *P. ambiguus* infestation was analysed using Pearson correlation.

RESULTS AND DISCUSSION

EIMERIA OOCYST BURDEN RATE BY AGE

The proportion of positive samples for *Eimeria* oocysts was still low in the lactation period. In the first week (with one exception), the percentage of positive samples was between 2-8%. From days 9-10, the trend was increasing, and 12-15%, in two cases, even above 30%, were seen, as shown in Figure 2. Between days 20-30, values of 0-16% were observed. Between days 32-35, a significant change was observed, with oocysts found in all samples tested in 2 cases and positive samples above 10% on the other days. In the one week after the weaning (days 35-42), the percentage of positive samples varied between 10-60%. From day 42 onwards, the percentage of positive samples was consistently high, sometimes above 60%, until slaughter age (70-77 days).

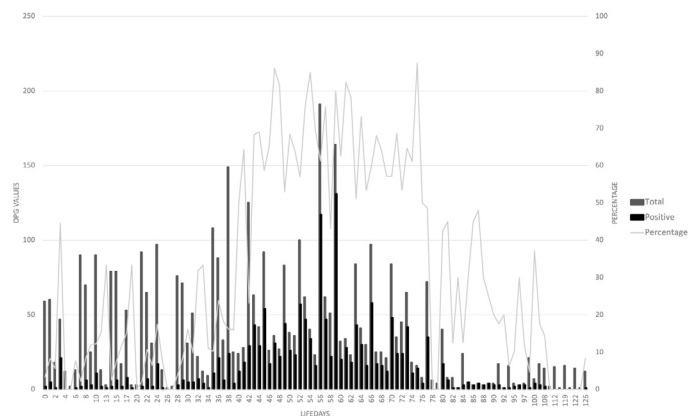


Figure 2: The total number of samples and the number and ratio of oocyst positive samples.

Based on the exponential smoothing model, a significant increase ($p=0.023$) in infection was observed on day 38, based on the OPG count in the faeces. Based on the percentage data, the model estimates a significant increase in infection on day 40.

The figure clearly shows the prediction based on the estimation model, where the peak of the infection can be

placed on days 38-40, since from there onwards a significantly higher OPG presence is seen compared to the sample numbers up to and including day 80.

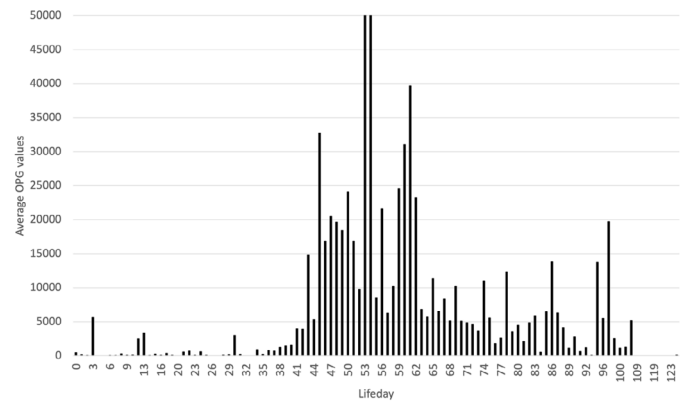


Figure 3: Average OPG values in faeces samples depending on the age of rabbits.

We also compared the average OPG values of the samples received per day of life. Figure 3 shows that, except for one case, there was no average OPG count above 5000 during the lactation period. In the post-weaning period, a sharp increase was observed, with the excretion period starting at 42 days of age, where in several cases the average OPG value could reach levels of 10,000. However, the oocyst release does not disappear at the end of fattening, and high average oocyst counts were often observed at over 80 days of age.

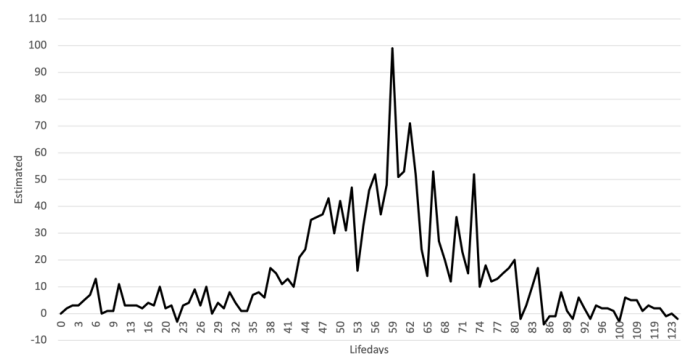


Figure 4: Evolution of model-estimated oocyst infection per day of life.

PASSALURUS AMBIGUUS RESULTS

The proportion of positive samples for the presence of *P. ambiguus* eggs was already high during the lactation period (Figure 4). Infestation rates of 50% were already observed during the first week of life, followed by relatively high peaks in the proportion of positive samples during the next 3 weeks of lactation (highest positive rates of 20%, 25% and 33% in the 2nd, 3rd and 4th week of life, respectively). From day 29 until slaughter age (up to 79 days of age), a relatively low level of infestation was detected, with no positive samples reaching the 10% level. However, from

day 80 to the end of the study period, high *P. ambigua* infection peaks were again observed, with positive samples reaching up to 66 %. For *P. ambigua*, both in number of specimens and percentages, the estimation model predicted day 7 as the peak of infection.

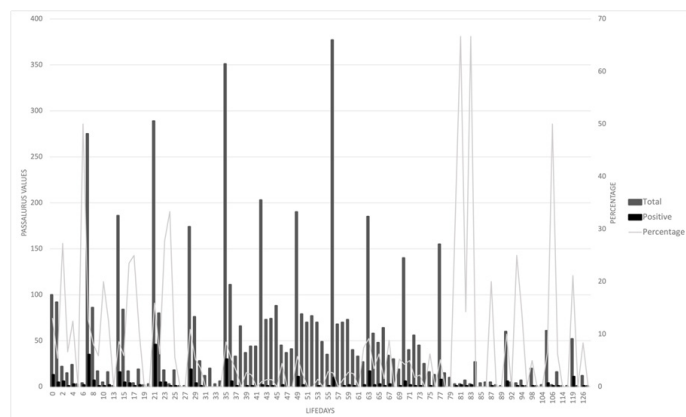


Figure 5: The total number of samples and the number and ratio of *Passalurus* positive samples.

The Figure 5. shows that even with a relatively large sample size, the number of positive samples is high until the weaning. The start of the infection upsurge is also clearly visible, which the model estimates ($p=0.001$) to be on day 7, as shown in Figure 6.

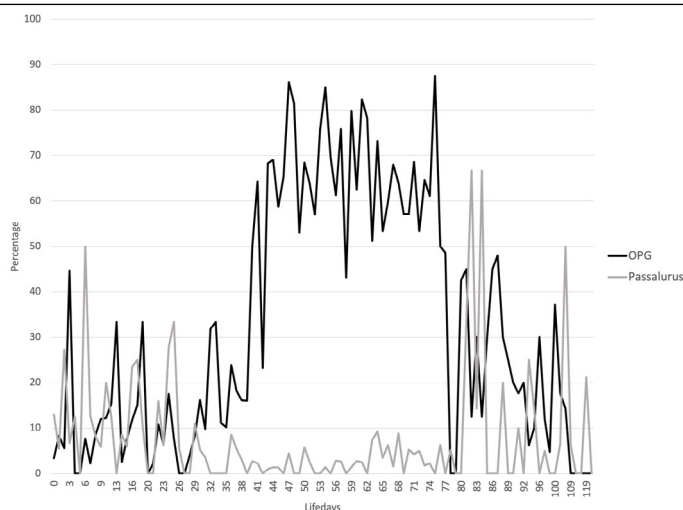


Figure 7: Percentage of positive samples of *Eimeria* oocysts and *Passalurus* eggs.

Pearson correlation shows a correlation between the percentage of oocyst and *P. ambigua* positive samples ($p=0.004$), with a weak negative correlation ($r=-0.278$).

There is a paucity of literature on the age-dependent infection of *Eimeria* oocysts and *Passalurus ambigua* of rabbits based on large scale monitoring data.

Our results suggest that *Eimeria* oocysts shedding of rabbits is continuous but strongly influenced by age. This statement is supported by [Pilarczyk et al. \(2020\)](#) as age is a determinant of parasite development, resulting in a more pronounced difference in zootechnical parameters in younger animals. Based on our results, the highest infection rates were found in rabbits younger than 6 months of age. However, our results are not in accordance with the observation of [Pakandl and Hlášková \(2007\)](#) that infections cannot be observed in suckling rabbits younger than 19 days of age. Our monitoring showed infection rates between 2-8% in the first week, 12-15% after days 9-10 and 0-16% between days 20-30. As our study is not based on faeces samples of individual rabbits but on pooled samples of rabbits at the same production stage, the results obtained during lactation could be due to oocyst shedding by the rabbit does.

Our results are in accordance with [Pakandl and Hlášková \(2007\)](#) that coccidiosis typically affects young rabbits at 5-6 weeks of age, immediately after weaning, when young rabbits have low resistance to infection. The weaning phase is, therefore, a critical period and can lead to significant losses. In line with the results of several research groups ([Maziz et al., 2018](#)), the prediction based on our estimation model suggests that the peak of infection is between days 38 and 40, as the prediction show significantly higher *Eimeria* oocyst presence from this age up to day 80. Sim-

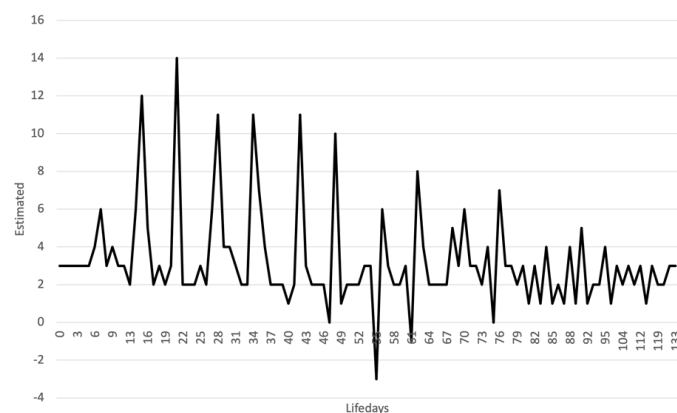


Figure 6: Evolution of model-estimated *Passalurus ambigua* infection per day of life.

The percentage of *Eimeria* oocyst and *P. ambigua* was assessed together. Based on this estimate, the onset of oocyst infection is estimated to be on day 40 in terms of percentages, while *P. ambigua* infection occurs as early as day 7. Figure 7 shows that similar infection rates are observed for *Eimeria* oocysts and *P. ambigua* eggs in the lactation period and in the post-fattening period, but that in the intermediate period, the oocyst presence increases significantly, while the proportion of *P. ambigua* positive samples remains low. When the oocyst infestation flares up, the *P. ambigua* infestation level drops.

ilarly to our results, Ilić *et al.* (2018) reported a higher rate of *Eimeria* oocyst infection in young rabbits (50.6%) than in older ones (37.6%).

Sultan *et al.* (2015) reported that *P. ambiguus* can be observed in rabbits worldwide, but still, there are few studies that specifically address this worm. In Hungarian large-scale rabbit farms, *P. ambiguus* infestation ratios are low (3–5%) and depending on the season (Demeter *et al.*, 2023) while in Egypt, *P. ambiguus* is one of the most prevalent worms in domestic rabbits, infecting up to 40% of samples (Sultan *et al.*, 2015).

According to Sioutas *et al.* (2021) *P. ambiguus* infection can affect both the production of rabbit does and young rabbits. Lactating does can infect their offspring, and the prepatency period of *P. ambiguus* is 55–60 days (Mehlhorn, 2008), therefore they recommend examination of rabbits' faeces around 67 days of age. Our results have not or only partially confirmed the need to test the rabbits at this age, as a relatively low level of infection was detected from day 29 to slaughter age (up to 79 days of age). In our examinations, occurrence of *P. ambiguus* egg in rabbits' faeces was relatively higher during the lactation period (up to the 29th day of life) than later on. Based on the prepatency period of *P. ambiguus*, eggs were presumably excreted by adults. Our study does not support the findings of Ashmawy *et al.* (2010) that younger animals are more frequently infected than adults, although the authors do not specify the age of the animals.

P. ambiguus and *Eimeria* spp. are frequently occurring parasites in the intestinal tract of rabbits, which, among other symptoms, can cause diarrhoea and, in severe cases, death. The strong infection of rabbits can contribute to the development of intestinal inflammatory disease complex that occurs around weaning (Sioutas *et al.*, 2021). To support this, we looked for a relationship between the interaction of the parasites in our study, in which there was a weak negative correlation between the presence of *Eimeria* oocyst and *P. ambiguus* infection.

CONCLUSIONS AND RECOMMENDATIONS

Passalurus eggs and *Eimeria* oocysts can be detected in the faeces of rabbits even during lactation. After weaning, oocyst shedding is very high and remains high until the end of fattening, while the frequency of *P. ambiguus* shedding drops to very low levels. In the "broodling" period, both *P. ambiguus* and *Eimeria* oocysts are sometimes shed with high frequency in the faeces of rabbits. Based on an estimation model, the peak of *P. ambiguus* infestation is around day 7, while the high peak of *Eimeria* oocyst shed-

ding is around day 38–40 of life. The frequent and high oocyst shedding during the fattening period highlights the need for monitoring studies and anti-parasite treatments.

In summary, we can say that the economic damage caused by these two parasites is significant in large-scale rabbit farms, so monitoring studies are essential. In contrast to other farm animals (e.g. Poultry), parasitological monitoring in rabbits is a less researched area, so in the light of our results, we consider it important to continuously check and control further herd-level tests. Furthermore, the physiological effects of other parasites, fungi and bacteria and their relationships could be researched.

NOVELTY STATEMENTS

Based on a large amount of monitoring data collected over several years from industrial rabbit farming, we determined with the help of the exponential smoothing model estimated the onset of *Eimeria* infection at 38–40 days of age. For *Passalurus ambiguus* the estimation model predicted day 7 as the peak of infection. A weak negative correlation was found between the presence of *Eimeria* oocyst and *Passalurus ambiguus* infection.

AUTHOR'S CONTRIBUTIONS

Csongor Demeter, Zoltán Németh, Zsolt Gerencsér and Zsolt Matics, conceived and designed the study. Anett Demeter-Jeremiás, Róbert Juráskó, András Mayer, conducted data gathering. Boróka Bárdos, performed statistical analyses. Csongor Demeter, Erika Lencsés-Varga and Zsolt Matics, wrote the article.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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