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SURVEY OF THE SEASONAL DEPENDENCY OF *EIMERIA* OOCYSTS AND *PASSALURUS AMBIGUUS* INFECTIONS IN INDUSTRIAL RABBIT FARMS

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Abstract: A major problem of large-scale rabbit farming is digestive diseases. Relatively few data on parasitic diseases of industrial rabbit populations are available. The aim of the study was to evaluate the seasonal-dependent incidence of *Eimeria* spp. and *Passalurus ambiguus* infections at large-scale rabbit farms. The survey was carried out between 2018 and 2022 at 29 Hungarian and 2 Slovakian rabbit farms. Altogether, 7612 faecal samples were examined. *Eimeria* oocysts and *P. ambiguus* eggs were detected on all rabbit farms examined, although only a relatively small portion of faecal samples was found positive. *Eimeria* oocysts and *P. ambiguus* eggs were found in 32 and 6% of the samples, respectively. Our findings for the seasonal-dependent evaluation of collected data showed that the highest proportion of *Eimeria* positive samples occurred during the summer (35.3%) and autumn (36.2%) periods. In winter, a significantly lower infection rate was found (29.5 %; $P<0.05$). The most favourable results were observed in spring (25.1%; $P<0.05$). As for *P. ambiguus*, the highest rate of infection was measured in spring (8.4%) and the lowest in summer (4.5%; $P<0.01$). The autumn and winter periods showed intermediate results (6.7 and 5.5 %, respectively).

Key Words: rabbits, pinworm, coccidiosis, parasitology, season.

INTRODUCTION

Approximately 4-4.5 million slaughter rabbits are produced in Hungary every year from 100-105 thousand rabbit does, mostly housed in industrial rabbit farms (Juráskó, 2022). Currently, one of the biggest challenges for industrial rabbit production in Hungary is the prevention and treatment of digestive disorders. Unfortunately, gastrointestinal problems are common in rabbits, especially diarrhoea, which most often occurs in young rabbits after weaning (Lafeber Vet, 2022).

Parasites can play a significant role in causing diseases of the digestive system. One of the main parasitic infections in rabbits is coccidiosis (Yan *et al.,* 2013). Coccidiosis is caused by a protozoa of the genus *Eimeria*, and according to the literature, the infection is always present in investigated rabbit farms, and to our current knowledge it is impossible to eliminate it (Vancraeynest *et al.,* 2008). Eleven *Eimeria* species have been identified in domestic rabbits (Pakandl, 2009), and the simultaneous presence of several species occurs very often (Catchpole and Norton, 1979). Based on their pathogenicity, the *Eimeria* species can be classified as follows: non-pathogenic to slightly pathogenic (*E. media, E. exigua, E. perforans, E. coecicola*), moderately pathogenic (*E. irresidua, E. magna, E. piriformis*), extremely

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pathogenic coccidia (*E. intestinalis, E. flavescens*) and *E. stiedae,* which causes hepatic coccidiosis (Coudert *et al.,* 1995). In addition to the different pathogenicity, the species have different sporulation times (between 21-72 h) and prepatent periods (4-17 d; Vetési, 1990).

Both clinical and subclinical coccidiosis infections can cause significant economic losses on rabbit farms, such as nutritional indigestion, poor absorption, dehydration, diarrhoea, body weight loss, increased susceptibility to bacterial and viral infections and a high level of mortality in highly infected stocks (Pakandl, 2009). In rabbit farming, effective drugs and hygiene measures are extremely important in the control of *Eimeria* infection (Vereecken *et al.,* 2012). A strong correlation was observed between *Eimeria* infection, hygiene status and preventive treatments (Vereecken *et al.,* 2012). In general, breeding rabbits (bucks and does) are resistant to diseases resulting from *Eimeria* infection. However, asymptomatic animals can also carry the pathogens and excrete them in moderate quantities. The highest morbidity and mortality rates were observed in weaned rabbits (El-Ashram *et al.,* 2020). By evaluating responses to different doses of experimental oocyst infections, it was found that clinical signs are only detectable with high doses (Pakandl, 2009).

The effect of the seasons on coccidiosis has already been investigated in livestock species kept on pasture (cattle: Lassen *et al.,* 2014; sheep, goats: Mohamaden *et al.,* 2018) and a generally higher number of oocysts was observed in the hot, rainy season than in the cold, dry season. Grès *et al.* (2003) examined the occurrence of oocysts in wild rabbits in France and found a higher oocyst excretion in spring and autumn than in summer.

Only a few data are available in the literature concerning oocyst occurrence in domestic rabbits. The effects of age, group size and season on the prevalence of oocysts were examined under very different backyard housing conditions in Kenya (Okumu *et al.,* 2014), in Mexico (de Guevara *et al.,* 2019), in Poland and Ukraine: (Basiaga *et al.,* 2020) and in Poland (Pilarczyk *et al.,* 2020). At a Polish rabbitry, Kornaś *et al.* (2015) studied the effect of age and season on the level of parasite infection during a three-year period on 22-70 samples per year.

Passalurus ambiguus is species or host specific and one of the most common oxyurid nematodes found in domestic and wild rabbits worldwide (Abdel-Gaber *et al.,* 2019). Based on the literature data, five different pinworm species were identified in rabbits, from which *P. ambiguus* was the most frequently reported in domestic rabbits (in Poland: Kornaś *et al.,* 2015; in Egypt: Hussein *et al.,* 2022). Self-infection with *P. ambiguus* can lead to permanent reintroduction of the parasite into the body if no measures are taken against it. Suckling kits can be infected by contaminated nest or other materials.

P. ambiguus often lives unnoticed in rabbits, but strong infection can cause intestinal inflammation (Hönich *et al.,* 1978), diarrhoea, weight loss, neurological symptoms and even death (Mykhailiutenko *et al.,* 2019; Sioutas *et al.,* 2021). *P. ambiguus* infection can affect the production of both does and young rabbits (Sioutas *et al.,* 2021).

Our aim was to assess the infection of *Eimeria* oocysts and *P. ambiguus* in large-scale rabbit farms based on the seasons.

MATERIALS AND METHODS

Manure samples were collected in 29 Hungarian and 2 Slovakian rabbit farms. This represented approximately half of Hungary's and 80% of Slovakia's meat-producing rabbit population. The study covered farms with herd sizes between 200 and 6000 rabbit does. Sampling was conducted in all stages of breeding. As for the housing technology, the pooled faecal samples came from rabbits kept in improved cages and slatted boxes according to standards and animal welfare aspects.

Feed and housing

All herds were fed with a complete pelleted feed mixture suitable for age and production level. Diets used for does and in the post-weaning and finishing stages of fattening typically did not contain anthelmintic supplements. In case of severe infection, ad hoc anti-parasitic treatments (Robenidin 50-66 ppm, Diclazuril 1 ppm) were used under veterinary prescription and supervision.

In the farms, rabbits were handled according to the principles stated in European Directive 2010/63/EU and according to the Hungarian legal requirements (32/1999. /III. 31./ and 178/2009. /XII. 29./).

Sample numbers, sample collection and parasitological analysis

Regarding *Eimeria* oocysts, 5,723 faecal samples were collected and examined in this study between March 2018 and February 2022, unevenly distributed. In the case of *P. ambiguus*, 7612 samples were evaluated in the time frame between March 2018 and April 2022, unevenly distributed by period.

The samples were collected according to a standardised method from the first, middle and last third of the manure channels of barns. We collected a minimum of 10-20 g fresh faeces per row and mixed them, i.e. the samples were not individual but a mixed pooled sample of the rabbits in the same age group in the barn.

The flotation enrichment tests were carried out in the laboratory of S&K-Lap Ltd (Kartal, Hungary). The samples were examined within 48-72 h after collection. For the flotation test, an aqueous solution of magnesium sulphate ($MgSO_4$) was used.

Faecal samples were processed exclusively according to the McMaster method based on the recommendation of the Royal Veterinary College and the FAO (The RVC/FAO Guide to Veterinary Diagnostic Parasitology). During the flotation test, the number of *Eimeria* oocysts (Figure 1) and *Passalurus* eggs (Figure 2) was recorded.

The results of *Eimeria* oocysts were expressed as OPG numbers (oocysts per gram). The numerical results were classified into categories to facilitate the analysis, so the test result could be considered as negative (OPG=0), low (1≤OPG≤358), high (359≤OPG≤5000) or very high (5000<OPG) oocyst count.

Statistical analyses

The occurrence rate of OPG negative and OPG positive faecal samples in each season, as well as the occurrence rate of *P. ambiguus* negative and *P. ambiguus* positive faecal samples in each season, were analysed by Chi-square test, using the SPSS 10.0 software package.

Figure 1: Microscopic image of an *Eimeria* oocyst (200× magnification).

Figure 2: Microscopic image of a *Passalurus ambiguus* egg (200× magnification).

RESULTS AND DISCUSSION

Eimeria oocyst monitoring results

Of the 5723 tested samples, 3889 samples showed negative results (68.0%), 404 samples showed low (7.1%), 775 samples showed high (13.5%) and 655 samples showed very high (11.4%) OPG levels. On av., 75% of the samples contained no oocysts or only low levels. Our results show lower prevalence of oocyst infection in industrial rabbit farms compared to the data in literature, originated mainly from small scale or backyard farms in Mexico (48%; de Guevara *et al.,* 2019), in Serbia (51%; Ilić *et al.,* 2018), in Saudi Arabia (75%; Abdel-Baki

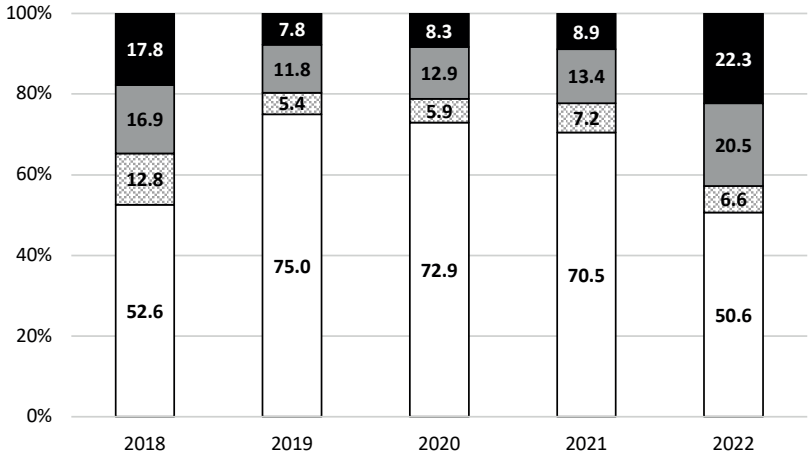

Figure 3: Distribution of samples showing different levels of oocyst excretion in different years of data collection. ☐ 0 oocyst per gram (OPG); ▨ 1-358 OPG; ▩ 359-5000 OPG; ■ 5000< OPG.

and Al-Quarishy, 2013) and in Poland (75%; Pilarczyk *et al.,* 2020; 79%; Szkucik *et al.,* 2014). However, Elshahawy and Elgoniemy (2018) observed prevalence data of *Eimeria* oocysts (34%) similar to ours in Egypt in rabbits housed in galvanised wire cages.

Eimeria infection levels according to the year

Figure 3 shows the distribution of samples by OPG categories processed per year of examination.

The results of the first year showed a relatively high proportion of positive samples, which was followed by the introduction of strict prevention measures. The *Eimeria* infection rate improved as a result of treatments carried out to control the disease, as new chemical agents and disinfection procedures were introduced to production. In the latter period, the trend worsened again and the proportion of samples showing high or very high OPG levels increased.

OPG ratio depending on the season

In the study of Kornaś *et al.* (2015) coccidiosis was a permanently present problem of the examined Polish rabbit farm throughout the year. Considering the data of our entire observation period, the highest proportion of OPG positive samples occurred in summer and autumn periods (Figure 4). In these two seasons, 35-36% of the samples contained oocysts. The winter period showed a more favourable picture, since we found a significantly higher proportion of OPG negative samples (71%; $P<0.05$). The most favourable results were observed in spring ($P<0.05$), when almost 75%

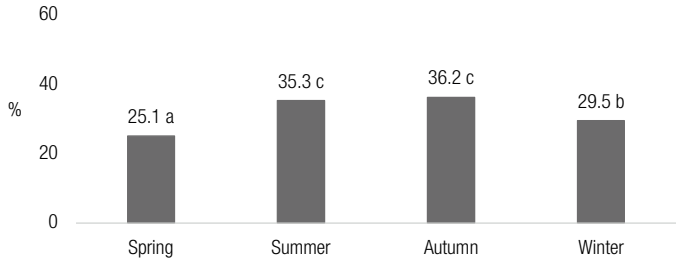

Figure 4: Ratios of *Eimeria* positive samples depending on the different seasons. [a,b,c]Different letters show significant differences ($P<0.05$).

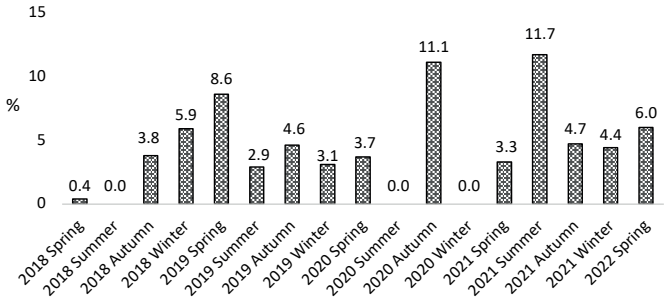

Figure 5: Ratios of *Passalurus ambiguus* positive samples per year and season.

of the samples were free of oocysts. Partly opposite results were observed by de Guevara *et al.* (2019) in Mexican backyard rabbit farms. The highest prevalence of *Eimeria* oocysts was found in the coldest and driest winter season (71.3%). The autumn and summer seasons showed slightly more favourable results (56.2 and 48.1 %, respectively), while the lowest level of oocyst infection was observed in spring (17.1%) which is the warmest season in Mexico.

Awais *et al.* (2012) investigated the seasonal incidence of coccidiosis in broiler chickens kept under large-scale conditions. According to their results, the prevalence of coccidiosis was significantly ($P<0.05$) higher in autumn (60.0%), followed by summer (47.4%), spring (36.9%) and winter (29.9%).

P. ambiguus monitoring results

From the 7612 samples tested, 335 samples showed positive for Oxyurida eggs (4.4%), so nearly 96% of the samples were free of *P. ambiguus*. Our results shows a much more favourable picture than the observations of Hussein *et al.* (2022), who detected *P. ambiguus* infection in 90 out of 200 rabbits (45%) in Egypt.

In 143 of the positive samples (1.9% of all tested samples), only the larval form of presumably *P. ambiguus* could be detected, 270 samples (3.5%) contained only *P. ambiguus* eggs (Figure 1), and in 65 samples both developmental forms were observed (0.9%).

Figure 5 shows the occurrence of *P. ambiguus* positive samples for each season in the ratio of tested samples. Regarding contamination of the samples, the results for each year (2018: 3.43%; 2019: 4.61%; 2020: 3.45%; 2021: 5.58%) and the trends within the year are different. Infection peaks occurred at different periods in each year.

Figure 6 shows the *P. ambiguus* infestation according to the seasons. Considering the data for the entire study period, the lowest proportion of *P. ambiguus* positive samples occurred in summer (3.0%; $P<0.05$). The infection rates measured in autumn and winter were identical and did not differ from the infection rate measured in spring (4.4, 4.4 and 5.7%, respectively; $P=0.06$). Partially similar results were reported by Le Normand *et al.* (2016), as they also recorded the lowest occurrence of *P. ambiguus* in summer, but found that the highest discharge occurred in autumn, followed by spring and winter.

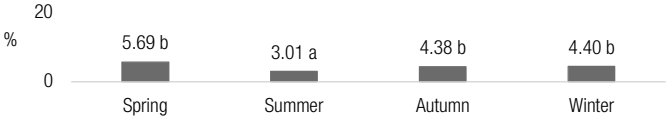

Figure 6: Ratios of *Passalurus ambiguus* infected samples in the different seasons. [a,b]Different letters show significant differences ($P<0.05$).

CONCLUSIONS

In general, lower *Eimeria* oocyst and *P. ambiguus* infections were observed in the large-scale rabbit farms examined compared to the results in the literature about small scale or backyard farms.

From the research into the effects of seasons on oocysts, we can conclude that the infection rate was the lowest in spring and the highest in summer and autumn, although the winter period is not to be neglected either. The excretion levels of *P. ambiguus* were different between the seasons, but unlike oocysts, the most favourable picture was shown in the summer period.

Based on our findings, regular parasitological monitoring of rabbit farms is highly recommended, since both the *Eimeria* oocyst and *P. ambiguus* are present in large-scale farms throughout the year.

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