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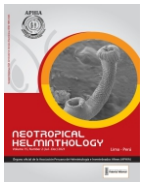

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

EIMERIA OOCYST EXCRETION IN NATURALLY INFECTED RABBIT IN A FARM FOR MEAT PRODUCTION

EXCRECIÓN DE OOQUISTES DE *EIMERIA* EN CONEJOS INFECTADOS NATURALMENTE EN UNA GRANJA DE PRODUCCIÓN DE CARNE

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ABSTRACT

Coccidiosis is cause of economic losses due to weight loss and growth retardation, decreased feeding efficiency, diarrhea and death of weaning rabbits. The main objective of the work was to evaluate the dynamic change of *Eimeria* oocyst shedding in the reproductive stage and in weaned litters during the fattening period, in a farm for meat production, with a history of coccidiosis infection. A faecal sample of the following physiological condition was obtained weekly: gestation (G1, G2, G3 and G4) and lactation (L1, L2, L3 and L4), not pregnant or not lactating were also included (V). In weaned rabbits, faecal sample / litters were obtained weekly, until rabbits reached the slaughter weight (2.5 kg). All samples were examined by flotation and the McMaster method. Co-infection with six species of the genus *Eimeria* could be observed in all positive animals. The infection rate in rabbits varied according to physiological state, being 22.7% (G3-L3), 13.4% (G4), 9.1% (G1-L4), 20% (GL), 4.6% (G2-L2). During the first week of lactation (L1), no positivity was observed. Parasitic loads ranged from 40 to 13820 OPG; the highest counts were observed in L2 and G3. In the second half of lactation, females have seven times the risk of excreting oocysts in the feces compared to the first half. Litter rabbits excreted oocyst during all fattening period; however, during weeks 3 to 7 the oocyst excretion was higher. This is the first report in which it is described the oocysts mixture of six *Eimeria* species (*E. vejdovskyi*, *E. coecicola*, *E. magna, E. exigua*, *E. media* and *E. stiedae)* from the rabbit for meat production in the region. These findings show that breeding rabbits are asymptomatic carriers of coccidian infections and can shed oocysts at any time during the reproductive cycle.

Keywords: *Eimeria* – rabbits – coccidiosis – reproductive does – litters – OPG

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RESUMEN

La coccidiosis es causa de pérdidas económicas debido a pérdida de peso, retraso del crecimiento, disminución de la eficiencia alimentaria, diarrea y muerte de conejos. El objetivo del trabajo fue evaluar la excreción de ooquistes de *Eimeria* en hembras reproductoras y sus camadas destetadas durante el período de engorde, en una granja para la producción de carne, con antecedentes de infección por coccidiosis. Se recolectó semanalmente una muestra fecal de las conejas para las siguientes condiciones fisiológicas: gestación (G1, G2, G3 y G4) y lactancia (L1, L2, L3 y L4), también se incluyeron aquellas hembras vacías, es decir que no estaban gestando o lactando (V). En conejos destetados, se recolectaron muestras fecales / camadas / semana, hasta alcanzar el peso de faena. Las muestras se examinaron mediante el método de flotación la cámara McMaster. Se observó co-infección con seis especies del género *Eimeria* en todos los animales positivos. La tasa de infección varió según la condición fisiológica de la coneja, siendo 22,7% (G3-L3), 13,4% (G4), 9,1% (G1-L4), 20% (GL), 4,6% (G2-L2). Las cargas parasitarias variaron de 40 a 13820 OPG; los recuentos más altos se observaron en L2 y G3. En la segunda mitad de la lactancia, las hembras tienen siete veces más riesgo de excretar ooquistes en las heces en comparación con la primera mitad. Los conejos excretaron ooquistes durante todo el período de engorde; sin embargo, durante las semanas 3 a 7 la excreción fue mayor. Este es el primer informe en el que se describe la presencia de ooquistes de seis especies de *Eimeria* species (*E. vejdovskyi*, *E. coecicola*, *E. magna, E. exigua*, *E. media* and *E. stiedae)* en conejos de la región. Estos hallazgos muestran que los conejos reproductores son portadores asintomáticos de infecciones por coccidias y pueden arrojar ooquistes en cualquier momento durante el ciclo reproductivo.

Palabras clave: *Eimeria* – conejos – coccidiosis - conejas reproductoras – camadas - OPG

INTRODUCTION

Coccidiosis, epizootic rabbit enteropathy (ERE) and colibacillosis are the main cause of digestive disorders as death in fattening rabbits (Licois, 2004). Rabbit's endoparasitosis are common, mainly in domestic breeding. The genera *Eimeria spp*. is the most frequent parasites found, however, other parasites as *Passarulus ambiguus* (Rudolphi, 1819), *Giardia* spp., *Hymenolepis diminuta* (Rudolphi, 1819), *Trichostrongylus* spp., *Trichuris* spp. and *Cryptosporidium* spp. are detected in feces samples of rabbits (Schafer da Silva *et al.,* 2006; Scialfa, 2020). Mixed infection with nematodes species and *Eimeria* spp. is frequent in farm (Elshahawy & Elgoniemy, 2018), and the prevalence of coccidial oocyst in young rabbits is higher than in adult rabbits. *Eimeria* infection is initiated by oral ingestion of sporulated oocysts by the susceptible rabbit. Concurrent infections with more than one *Eimeria* species were commonly recorded in rabbits infected, and were identified from naturally infected rabbits (Elshahawy & Elgoniemy, 2018): *Eimeria media* (Kessel, 1929), *E. intestinalis* (Cheissin, 1948), *E. coecicola* (Cheissin, 1947), *E. magna* (Pérard, 1925), *E. exigua* (Yakimoff, 1934), *E. perforans* (Leuckart, 1879) Sluiter & Swellengrebel, 1912, *E. irresidua* (Kessel & Jankiewicz, 1931), *E. flavescens* (Marotel & Guilhon, 1941), *E. piriformis* (Kotlán & Pospesch, 1934), *E. stiedae* (Lindemann, 1865) Kisskalt & Hartmann,1907 and *E. vejdovskyi* (Pakandl, 1988).

Coccidiosis is cause of economic losses due to weight loss and growth retardation, decreased food efficiency, diarrhea and death of weaning rabbits (Pakandl, 2009). Intestinal coccidiosis cause more or less severe disease in rabbits, depending mainly on the infective dose, *Eimeria* spp., rabbit immune system and age of animals (Coudert *et al.,* 1995; Pakandl, 2009). Coccidia can be classified according to their pathogenicity: nonpathogenic (*E. coecicola)*, slightly pathogenic (*E. perforans*, *E. exigua* and *E. vejdovskyi*), mildly pathogenic or pathogenic (*E. media*, *E. magna*, *E. piriformis* and *E. irresidua*), highly pathogenic (*E. intestinalis* and *E. flavescens*) (Coudert *et al.,* 1995). *Eimeria stiedae* is associated with liver lesion of rabbits, causing hepatomegaly with numerous and scattered yellowish white nodules on the liver

surface (Singla *et al.,* 2000; Scialfa, 2020; Rampin *et al.,* 2008); these nodules generally are detected in slaughtered rabbits or necropsy findings. The prevalence *Eimeria* spp. infection in rabbit farms is variable (33.9 to 85.1%), dependent of breeding system and risk factors (Li *et al.,* 2010; Okumu *et al.,* 2014; Elshahawy & Elgoniemy, 2018; Tanjung & Rangkuti, 2019).

Local immune response plays more important role in immunity to coccidiosis than the systemic response (Pakandl, 2009). Apparently, the response immune it would associate with the specie of *Eimeria*, being *E. intestinalis* one of the most immunogenic, in contrast with *E. flavescens* and *E. piriformis* (Coudert *et al.,* 1993).

Coccidiosis is mainly controlled by prophylaxis with different anticoccidial drugs, usually mixed in feeding pellets, in drinking water and injected via subcutaneous (Pakandl, 2009; Qamar *et al.,* 2013). Sulphonamides drugs (sulphadimethoxine, sulphadimidine, trimethoprim-sulphamethoxazole and sulphaquinoxaline) are currently used primarily for treatment of coccidiosis outbreak (Pakandl, 2009). The use of these drugs has some disadvantages, such as negative impact on the environment (excretion in faeces), resistance, toxicity for host and residues in meat. Vaccination with live attenuated lines of coccidia is another method for the control of coccidiosis (Pakandl, 2009).

According to the accessible literature, the study of rabbit coccidiosis in Argentina is very limited, and there are few studies that describe the real impact of intestinal *Eimeria* spp. infection in rabbit farms, and even less about *Eimeria* species that affect rabbits in the region. There are few reports about hepatic coccidiosis in domestic as wild rabbit of Argentina (Robles & Bonino, 1985; Scialfa, 2020).

The main objective of the work was to evaluate the dynamic change of *Eimeria* oocyst shedding in reproductive does and their weaned litters during fattening period, in a farm for meat production, with a history of coccidiosis infection. The specific objective was to determine the association between the parasite load (number of *Eimeria* oocysts excreted by g of faeces) and the physiological condition of reproductive does.

MATERIALS AND METHODS

Rabbit farm

This study was carried out at Secondary School of Agricultural Education, Azul, Buenos Aires province, Argentina. Rabbit breeding was developed in a 100 m^2 shed, with a cement floor and no artificial light; temperature, humidity and ventilation were controlled using curtains. Rabbit houses consist of rows of suspended wire mesh cages with shallow pits in the floor for litter. Male (6) and female (14) breeding rabbits were kept in individual cages, while the weaning fattening animals were kept in collective cages (0.25m^2) with the capacity to house 4 rabbits of 2.5-2.6 Kg of live weight. According to the available facilities, it was not possible to make an all-in-all-out system; according to the cleaning system at the end of each cycle, the system was considered continuous. Rabbits were fed a commercial laboratory pelleted feed free of anticoccidial drugs. On the farm, the presence of *Eimeria* infection has been evidenced by flotation methods, and diarrhea and death have eventually been observed in weaned rabbits; chemoprophylaxis had not been applied at the time of the study.

Faecal sample processing

Individual faecal samples (50 g) of 14 reproductive does and their respective weaning litters were collected weekly. Faecal samples were collected by means of plastic sheets placed under the wire mesh of the cage. In reproductive does, a faecal sample of the following physiological condition was obtained weekly: gestation (G1, G2, G3 and G4) and lactation (L1, L2, L3 and L4), those empty females were also included, that is, at some point in the reproductive cycle were not pregnant or not lactating (V). In weaned rabbits, faecal sample/litters were obtained weekly, until reach the slaughter weight (2.5 kg). All faecal samples were conserved with formalin solution (5%). In the Parasitology Laboratory of Rural Zoonotic Department, the samples were examined by flotation method using saturated solution of NaCl (Figure 1). Parasitological objects were observed microscopically under 400x (Figure 1). The invasion intensity of the *Eimeria* species was assessed using McMaster chamber and expressed as the number of oocysts per gram of faeces (OPG index).

Identification of *Eimeria* species

Eimeria species were identified based on the clinical signs, prepatent period, oocyst morphology and molecular methods (Baker, 2007; Taylor *et al.,* 2007). During the study period, litters rabbits were checked daily to observe clinical signs, such as diarrhea, indicating a clinical form of coccidiosis and their weight was recorded at the weaning and at the end of fattening period. The morphological features of the sporulated oocyst, including the size of the oocysts, the presence or absence of residual bodies of the oocyst, the size of the sporocysts, and the clinical manifestation were also used to identify the *Eimeria* species. To determine the size of the oocysts, photomicrographs were taken through the ICC-50HD camera incorporated into the LEICA DM-500 microscope (Figure 1). The interactive measurement module of the LAS V4.5 software was used to carry out the measurements.

For molecular *Eimeria* species identification, total genomic DNA was extracted from stool samples using ADN Puriprep K1210 kit Inbio Highway®). Obtained DNA was quantified using DeNovix Spectrophotometer. Samples were subjected to real-time polymerase chain reaction (qPCR) with generic primers (ITS1-F- 3´-GGGAAGTTGCGTAAATAGA-5´ and ITS1-R CTGCGTCCTTCATCGAT) that amplify all *Eimeria* species of domestic rabbit (Oliveira *et al.,* 2011) generating amplification products from 400bp to 600 bp depending on the specie. The thermal cycling conditions were as follows: an initial denaturation of 3 min at 95° C, and 45 cycles of 94°C 25", 50°C 30", 72°C 40". After amplification, a melting curve analysis to confirm specific amplification of the PCR product was performed. All qPCR reactions were carried out in a thermocycler QTower^3 (Analytic Jena, Germany) in a final volume of 20 µl using EvaGreen® Fluorescent DNA intercalating dye (KAPA Fast, Biosystems, Woburn, E.E.U.U. To corroborate size and amplification specificity, PCR products were resolved on agarose gel. Amplified DNA fragments were purified using the AccuPrep® PCR/Gel Purification Kit (Bioneer, South Korea) and directly sequenced (ABI 3500 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). The sequences similarities were determined by the Basic Local Alignment Search Tool (BLAST, NCBI).

Statistical analysis

In order to compare the oocyst excretion (OPG) in reproductive does, throughout of the different physiological condition (G1, G2, G3, G4, L1, L2, L3, L4 and V), a Chi squared and Fisher Test was performed. However, to compare the oocyst excretion (OPG) by litter's rabbits, throughout the weeks of fattening period, an ANOVA test was performed for repeated measures. The response variable was the log of OPG, and the classification variable was the week with its 12 levels. The repeated measurement of the litters rabbits was contemplated throughout the time that the test lasted (slaughter weight: 2.5 kg). The statistical language R was used for the analysis. The data with negative (OPG value= 0) result or without data were excluded from the analysis, since these values cannot be transformed using logarithm. Spearman coefficient correlation was used for to determine the association between fattening days and OPG, GMD and OPG, and weaning weight and OPG.

Ethic aspects: The authors point out that they fulfilled all national and international ethical aspects.

RESULTS

A total of 119 faecal samples of 14 reproductive does were studied, and the presence of *Eimeria* infection was observed in 10 does (71.4%), which oocyst shedding at certain moments of the reproductive cycle (Table 1). The 16.8% of samples (20/119) were positive, and co-infection with at least three species of the genus *Eimeria* could be observed in all positive animals. The infection rate varied according to the physiological status, being of 25% (G3-L3), 15% (G4), 10% (G1-L4), and 5% (G2-L2). During the first week of lactation (L1), no positivity was observed. Parasitic loads ranged from 40 to 13820 OPG; the highest counts were observed in L2 and G3 (Table 1).

In the second half of gestation period there was a greater probability of finding positive animals, however, the differences were not significant (p= 0.09). During the second half of lactation period the reproductive does are seven times more likely to be positive (p= 0.02). Seven does are found lactating

and pregnant simultaneously, and five were positives; however, the 60% (3/5) of oocyst shedding in feces during this reproductive period (Figure 2). During the study, the age of the reproductive doe, was on average 13 and 15.7 months, for the negative and positive does respectively; respect the number of births, it was 6 and 6.7 for the negative and positive does respectively.

The lactation period was of 31.3 days (range: 27-36 days), and the weaned rabbits weighed of 644 g (range: 486-1000 g). The fattening period was an average of 63.5 days (range: 49-80 days), reaching slaughter weight (2540 g on average) at age of 92.4 days (range: 83-116 days). The 71.4 % of the suckling rabbits reaching slaughter weight at week 8 and 9, with an average weight gain per day of 33.4 g.

A total of 130 faecal samples of 14 weaned litters were analyzed, and the 100% were positive by flotation method. The highs values reached by the different litters during the fattening period were of 12000, 12880, 13460, 15240, 19800, 23240, 25000, 27360, 29500, 30120, 46500, 68000 and 75680 respectively. The 28.6% presented values higher than 30000 OPG.

During all fattening period the weaned litters excreted oocyst; however, at weeks 3 to 7 the oocyst excretion was higher (Figure 3), and the differences observed were statistically significant ($p= 0.01$). The means of oocyst shedding per week of fattening varied from 160 to 16474 OPG, the lowest loads being observed at the beginning and at the end of the fattening period (Figure 3). A high variability of oocyst excretion by litter's rabbits in the different weeks was observed. In 50% of the weaned litters expressed a negative result to the flotation method (Value=0) before or after a positive result with oocyst count (OPG), even after high counts (Figure 4).

Table 1. *Eimeria* infection in reproductive does according to the physiological condition: Negative = flotation methods negative; (-) = without sample; 0 = flotation method positive and counting negative.

				Gestation						Lactation	
Doe	**Age (months)**	**N° birth**	**Empty**	**G1**	**G2**	**G3**	**G4**	**L1**	**L2**	**L3**	**L4**
61	25	12	-	Negative	Negative	**7880**	Negative	Negative	Negative	**480**	Negative
114	7	1	-	Negative	Negative	**700**	**80**	Negative	**13820**	Negative	Negative
41	28	14	-	Negative	**880**	**40**	Negative	Negative	Negative	**880**	Negative
49	26	14	Negative	Negative	Negative	**4860**	**120**	-	-	-	-
80	21	11	-	Negative	Negative	**420**	Negative	Negative	Negative	Negative	Negative
87	19	7	-	Negative	Negative	Negative	Negative	Negative	Negative	**2200**	Negative
113	7	1	**2300**	**0**	Negative	Negative	Negative	Negative	Negative	**0**	**3920**
101	13	5	-	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
104	11	2	Negative	Negative	Negative	Negative	**120**	Negative	Negative	Negative	Negative
91	13	5	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**20**	Negative
107	9	3	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
110	9	2	-	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
109	10	2	-	**160**	Negative	Negative	Negative	Negative	Negative	Negative	**160**
52	24	14	-	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Oocyst shedding by weaned litters during fattening period was very variable (Table 3), regardless of whether or not they came from positive mothers. No association between fattening days and OPG (p= 0.51), GMD and OPG (p= 0.31) and weaning weight and OPG (p= 0.72) was observed. A great variability was observed in oocyst excretion, both between litters and between fattening weeks (Table 3 and 4), however, the differences found were not significant (p= 0.05).

In all analysed stool samples, we detected the presence of *Eimeria* spp. DNA. Three different size PCR products (400 bp ,450 bp and 600 bp) were amplified. BLAST analysis as *E. media* and the 400 bp product as *E. vejdovskyi* identified the 450 bp

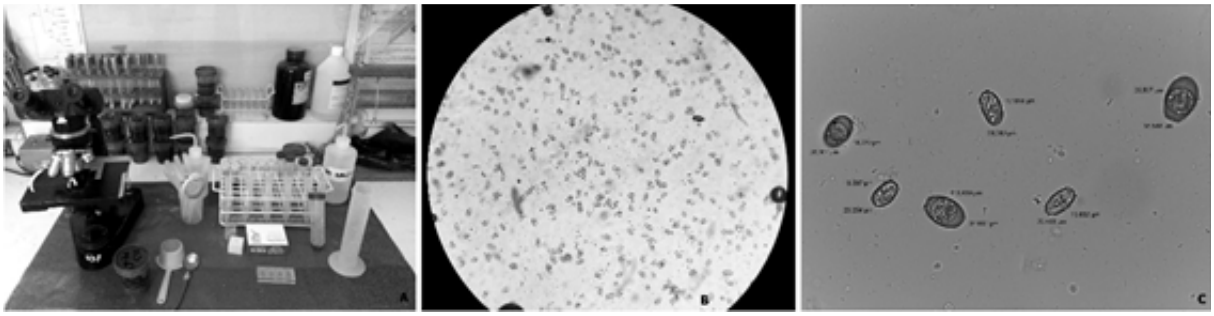

Figure 1. A) Laboratory and faecal sample processing. B) *Eimeria* species observed microscopically under 100x. C) Mixed *Eimeria* oocysts observed by photomicrographs under 400x.

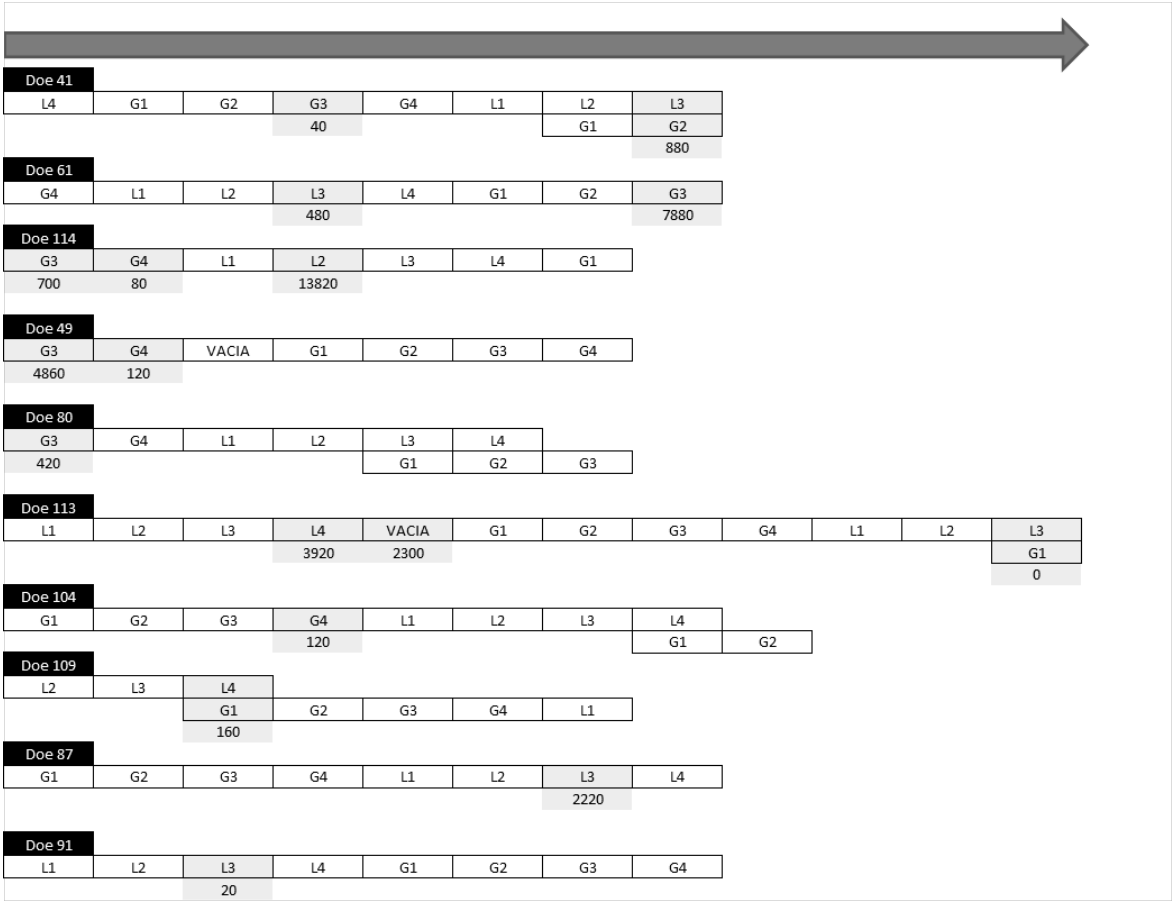

Figure 2. *Eimeria* infection according to the physiological condition of reproductive does. In grey color are observe the moment of oocyst excretion and OPG.

PCR product, although other species that amplify the same molecular weight could be present in the samples. The 600 bp PCR product did not contain enough material for sequencing analysis, although according to the molecular size it could correspond to *E. coecicola* or *E. stiedae.*

According to the distribution of the *Eimeria* species in the rabbit farm, *E. vejdovskyi* was found in all breeding sector, *E. media* in weaning sector, fattening and reproductive does sector, while *E. coecicola* and *E. stiedae*, only in fattening sector.

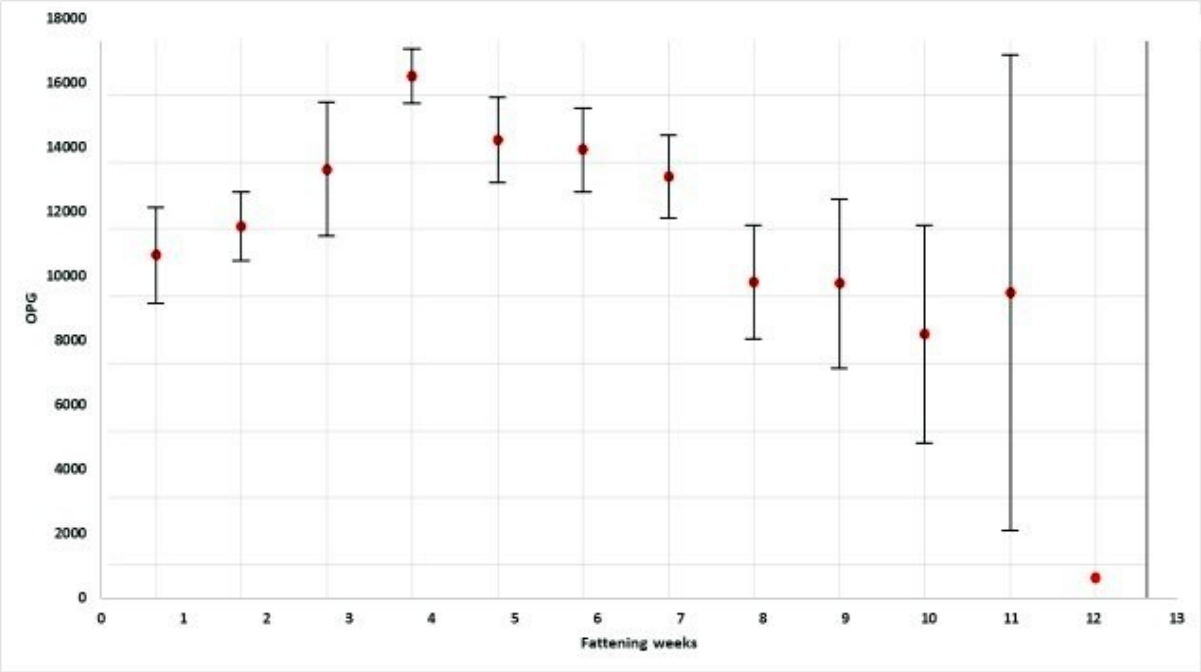

Figure 3. Oocyst excretion (average of oocyst per gram: OPG) by weaned litters during fattening weeks.

Litter doe	Days of lactation	Weaning weight (g)	Fattening weeks 1	2	3	4	5	6	7	8	9	10	11	12
49	35	614	S/D	0	S/D	S/D	9540	76800	1680	18920	880			
104	34	1000	**0**	S/D	S/D	8084	7560	4160	30120	1820				
52	34	800	**0**	740	27360	**0**	11480	3120	2000	4680	20			
41	34	677	280	900	75680	4140	47680	3920	6600	1580	**0**			
109	36	604	**0**	7280	4080	6880	1520	1240	15240	1300	10560	4200	14150	160
101	31	700	**0**	3280	3580	15120	480	2320	5240	**0**	46500			
113	30	630	5360	4080	4560	12640	**0**	12660	4460	13460	6320	2840	240	
91	28	570	960	1160	8600	11240	19800	31000	10400	820	3520			
61	28	550	1600	**0**	2880	23240	5867	19920	14900	4680	3280	2920		
110	28	555	3160	5320	9440	9620	21600	23540	29500	21600	1400	80		
114	27	486	7040	2480	20	68000	8220	9840	1240	2640				
80	31	600	3680	12880	10980	3680	9180	4320	7920	40				
87	31	633	9000	2620	6540	25000	1800	680	280	420				
107	31	600	**0**	12000	10420	10040	**0**	2280	1740	544	540			

Figure 4. Oocyst excretion (oocyst per gram: OPG) in weaning litters during fattening weeks. Litters that expressed a negative result to the flotation method (Value= 0) before or after a positive result with oocysts count (OPG), can be observed.

Table 2. Oocyst excretion (average of oocyst per gram: OPG) in weaning litters during fattening weeks. In addition, the time of the fattening period as the daily weight gain (GMD) can be observed.

Litter doe	*Litter average OPG*	*Fattening weeks*	*GMD*	*OPG range*
80	5131	8	33.9	40 a 12880
87	5796	8	34.2	280 a 25000
109	6056	12	24.6	0 a 15240
113	6662	11	25.6	0 a 13460
52	7057	9	28.6	0 a 27360
61	8809	10	28.3	0 a 23240
91	9722	9	32.3	820 a 19800
104	10349	8	32.7	0 a 30120
101	10931	9	32.1	0 a 46500
114	12435	8	36.0	20 a 68000
110	12526	10	28.2	80 a 29500
41	17598	9	32.6	0 a 75680
49	21564	9	38.5	0 a 76800
107	37564	9	33.1	0 a 12000

Table 3. Variability in the oocyst excretion (oocyst per gram; OPG) by weaning litters during fattening weeks.

Fattening weeks	*OPG average*	*OPG range*
1	3885	0 a 9000
2	4795	0 a 12000
3	13678	20 a 75680
4	16474	0 a 68000
5	12061	0 a 47680
6	13986	680 a 76800
7	9380	280 a 30120
8	5535	0 a 21600
9	8113	0 a 46500
10	2510	80 a 4200
11	7195	240 a 14150
12	160	160

DISCUSSION

The overall prevalence of *Eimeria* spp. of this study (97.2%) is higher than the 78.6%, 70.3%, 64.7% observed in Nigeria, Indonesia and Malaysia respectively (Ola-Fadunsin *et al*., 2019; Hamid *et al*., 2019; Mohamad-Radzi *et al.,* 2021). The prevalence of coccidia oocysts in does and weaned litters were 71.4% and 100% respectively. *Eimeria* infestation in weaned rabbits was similar to that the described in a study of three meat rabbit farms in Malaysia, with a prevalence of 76.5% in young animals; however, in a large-scale farm the prevalence was 100%. Possibly have a stocking density of 5–6 animals per cage is an important factor of the occurrence of coccidia (Mohamad-Radzi *et al.,* 2021). The variation in the prevalence of this study with other studies may be due to study location, environmental conditions and system breeding. Difference between four seasons and infection rate was observed in Egypt, were the highest rates of infection (53.5% and 39%) were recorded in autumn and winter respectively (El-Sayed *et al.*, 2020).

Although the morphological characteristics of coccidia oocyst helped to identify the *Eimeria*

species, it did not allow a good differentiation of the oocysts, mainly in samples with a high load. The unsporulated morphology characteristics between *E. vejdovskyi* and *E. coecicola*, were similar, so it was impossible to differentiate them only on the basis of unsporulated morphology features. In this study, the *E. magna* and *E. exigua* were identified based on the morphological characteristics, but could not be detected by the PCR methods. The complementation of the morphological features of the oocysts with the application of molecular methods allowed to identify *E. vejdovskyi, E. media, E. stiedae* and *E. coecicola*. The molecular technique applied in the present work has the limitation that the number of *Eimeria* species present in each sample can be underestimated. As various species show the same amplicon size, the obtained sequence corresponds to the most prevalent specie in the sample, PCR amplification with specific primers as described by Oliviera *et al*. (2011) could be a more suitable diagnostic assay.

Female rabbits examined sowed two periods de oocyst excretion: in the second half of lactation (preceding weaning of the litter) and after parturition (second half); as being lactating and pregnant simultaneously, the most vulnerable periods. The high nutritional requirements during gestation and lactation may lead to lowered resistance to *Eimeria* infections, increasing the growth of the pathogen at the intestinal level, which is reflected by the presence of oocysts in feces. The period preceding weaning of the litter born was a frequent period of oocyst shedding, and this finding was previously reported (Papeschi *et al.,* 2013).

The 50% of the females eliminated oocysts in a single moment or physiological condition, 30% in two and 20% in three (Figure 1). Although the range of oocyst clearance was wide (20-13820), the 66.7% of the does had counts of 1000 OPG (Median: 480).

Oocyst shedding by litters weaned during fattening period was very variable, with values of 0 to 76800 OPG. A great variability was observed in oocyst excretion, both between litters and between fattening weeks. From weaning moment, the excretion of oocyst increases to week seven (74 d of age) and then it decreases to reaching slaughter weight (83- 116 d age). According previous studies, intestinal *Eimeria* infections occur in kits just after weaning (Papeschi *et al*., 2013). In open-air breeding systems, weaned rabbits reach the maximum oocyst excretion earlier compared to semi-intensive indoor systems, being during the 45 to 60 days of age (Papeschi *et al*., 2003).

Findings from this study suggest that mothers, mainly in the last half of gestation and lactation, may play an important role in *Eimeria* transmission to the litters. Weaning involves changes that can affect your immune response capacity, due to a stressful situation for the animal due to separation from the mother, to the change of accommodation and social group. The fact that all rabbit litters were positive post-weaning, regardless of whether they came from positive does and the negative results between counts of oocysts with high values, this could be due to the sensitivity of the flotation method and McMaster method (sensitivity of 20 oocysts / gram of feces sample). That is, negative results can probably be interpreted as false positives where oocyst counts are less than 20 OPG.

The time of the fattening period as well as the observed daily weight gain (GMD) are the usual ones for the farm, where the litters rabbits reaching slaughter weight (2.5 kg) at week 8 and 9 the litters rabbits reaching slaughter weight with an average of 33.4 gram/day. The growth rate and daily weight gain of current hybrid lines of domestic rabbits, reach the highest level (45-50 g / day) at 8 weeks of age (Gidenne *et al*., 2010). Probably, the daily weight gain in the farm was influenced by feed (formulation of balanced diet, the quality of raw materials and the manufacturing process); however, could be attributed to non-feed factors as genetic, environment and housing.

No evident clinical manifestations in weaning rabbits, even in litters with high counts (mayors of 30000 OPG) could be attributed to moderately and low pathogenic *Eimeria* species observed, being *E. vejdovskyi* the most frequent in the analyzed feces samples. Despite the presence of *E. stiedae* on the farm, no liver lesions were observed in the slaughtered animals, possibly this is due to a low load of these coccidian in relation to *E. vejdovskyi* and *E. media*, and the age of the slaughtered animals.

Although in our study the presence of six *Eimeria* species was observed, similar studies in other countries (Indonesia, Malaysia, Nigeria and Poland) have shown mixed infection with seven and ten *Eimeria* species (Hamid *et al*., 2019; Ola-Fadunsin *et al*., 2019; Balicka-Ramisz *et al*., 2020; Mohamad-Radzi *et al.,* 2021).

This is the first report in which it is described the oocysts mixture of six *Eimeria* species species (*E. vejdovskyi*, *E. coecicola*, *E. magna, E. exigua*, *E. media* and *E. stiedae*) from the rabbit for meat production in the region. These findings show that breeding rabbits are asymptomatic carriers of coccidian infections and can shed oocysts at any time during the reproductive cycle. However, at the time of further development of the fetuses during gestation and at the peak of milk production there is a decrease in resistance to infection by *Eimeria* species, expressed in a greater excretion of oocysts, being a potential source of infection for kits. PCR is an important method for diagnosing rabbit *Eimeria* disease in rabbits and should be complemented by identification based on morphological characteristics. These molecular tests will make it possible to carry out epidemiological surveillance in other rabbit farms of the region, and to know the distribution of *Eimeria* species that affect rabbits. Further studies will aim to evaluate the impact of infection by *Eimeria* on the efficiency of feed conversion in litter rabbits of farm.

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