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Identification and quantification of *Eimeria* spp. in rabbits from Mezquital Valley, Hidalgo

Identificación y cuantificación de *Eimeria* spp en conejos del Valle del Mezquital, Hidalgo

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ABSTRACT

The objective of this study was to identify and quantify species of the genus *Eimeria* present in naturally infected rabbits in the production units of Valle del Mezquital, Hidalgo State. Fecal samples were collected from 10 rabbit production units (RPUs) comprising 320 rabbits aged between 30 and 60 days (fattening period). Identification of *Eimeria* spp. species was carried out using the flotation technique, with criteria of measurement and morphological comparison. Oocysts per gram of feces (OPG) were determined using the McMaster technique. Parasite load data were normalized ($\sqrt{x}$ +0.5) and a principal mean analysis was performed in Minitab® software. *E. stiedae* (hepatic coccidiosis) was identified in 8 RPUs (19-100 %) and intestinal coccidiosis by *E. irresidua* in 5 RPUs (17-63.6 %), *E. exigua* in 4 (23.5-100 %), additionally, *E. media* (14.7-47.6 %) and *E. coecicola* (14.7-19 %) were identified in three RPUs, *E. intestinalis* (24-28 %), *E. magna* (11.7-24 %), and *E. perforans* (4.5-18 %) in two RPUs, and *E. piriformis* (9 %) in one RPU. The parasite load was above average in RPUs 9 (241450 ± 33555) and 8 (56817 ± 3907), only in production unit 10 the amount of OPG (25433 ± 2776) was found within decision limits, the highest amount of OPG was identified in RPUs with poor facility design, as well as those where feeding and supply of good quality water are limited. *Eimeria* spp. is present in all RPUs of Valley of Mezquital, with an OPG quantity that puts the health of the animals at risk.

Keywords: rabbits, *Eimeria* spp. coccidiosis, OPG.

RESUMEN

El objetivo del presente estudio fue identificar y cuantificar especies del género *Eimeria* presentes en conejos naturalmente infectados en las unidades de producción del Valle del Mezquital, estado de Hidalgo. Se colectaron heces en diez unidades de producción cunícola (UPC) de 320 conejos de entre 30 a 60 días de edad (período de engorda). La identificación de especies del género *Eimeria* spp., se realizó con la técnica de flotación, con criterios de medición y comparación morfológica. Los ooquistes por gramo de heces (OPG) se determinaron con la técnica McMaster. Los datos de carga parasitaria se normalizaron

($\sqrt{x}$ +0.5) y se realizó un análisis de media principal en el programa Minitab®. Se identificó, *E. stiedae* (coccidiosis hepática) en 8 UPC (19-100%) y coccidiosis intestinal por *E. irresidua* en 5 UPC (17-63.6%), *E. exigua* en 4 (23.5-100%), además se identificaron en tres UPC *E. media* (14.7-47.6%) y *E. coecicola* (14.7-19%), en dos *E. intestinalis* (24-28%), *E. magna* (11.7-24%) y *E. perforans* (4.5-18%) y en una UPC *E. piriformis* (9%). La carga parasitaria fue superior a la media en las UPC 9 (241 450 ± 33 555) y 8 (56 817 ± 3907), solo en la unidad de producción 10, la cantidad de OPG (25 433 ± 2 776), se encontró dentro de los límites de decisión, la mayor cantidad de OPG se identificó en UPC con deficiente diseño de instalaciones, así como en aquellas en las cuales la alimentación y suministro de agua de buena calidad son limitados. *Eimeria* spp., está presente en todas las UPC del Valle del Mezquital, con una cantidad de OPG que pone en riesgo la salud de los animales.
Palabras clave: conejos, *Eimeria* spp. coccidiosis, OPG.

INTRODUCTION

Rabbit meat plays an important role in health, rural economy, and sustainable development due to its nutritional characteristics, such as high protein content (20.3g/100g) and unsaturated fatty acids (60.5%), low fat content (1.8-8.8g/100g), cholesterol (47mg/100g), and sodium (37–47mg/100g) (Siddiqui *et al*., 2023). However, the production chain faces various problems, mainly related to animal health and product quality. Disease outbreaks, mortality, and feed costs affect profitability (Mukaila, 2023). One of the conditions for successful rabbit farming is ensuring the epizootiological well-being of the RPUs. Parasitosis affects livestock, as it slows the growth of animals, can cause their death, and affects meat quality (Gutyj *et al*., 2023).

Knowledge of risk factors, types of diseases, causes of death, and prevalence rates allows for the implementation of more efficient management strategies (Espinosa *et al*., 2020). Rabbit coccidiosis is a parasitic disease caused by species of the genus *Eimeria* (Xu *et al*., 2022). Affected rabbits present with diarrhea, decreased appetite, dehydration, weight loss, growth retardation, liver and intestinal lesions, and death (Exequiel *et al*., 2021).

Two types of coccidiosis are reported: hepatic coccidiosis caused by *Eimeria stiedae*, a devastating disease with high morbidity and mortality, which has pathological effects on the integrity of hepatocytes and liver function in rabbits (Athanasiou *et al.,* 2023). In turn, *Eimeria* spp. cause intestinal coccidiosis that affects the mucosa, *E. intestinalis* and *E. magna* destroy the intestinal flora, causing changes in metabolites and in the molecular mechanisms of rabbit-parasite interactions (Xu *et al*., 2022). *Eimeria intestinalis* induces intestinal inflammation, loss of goblet cells, alteration of the microbiota (increases *Escherichia* and *Enterococcus* population) and jejunal metabolites, as well as host-microbiome interactions (Xu *et al*., 2022).

Morphological diagnosis remains the tool of choice for diagnosing pathologies associated with this species (Espinosa *et al*., 2020; Cordero del Campillo & Rojo, 2000). Prevention and control are achieved by implementing hygiene measures and using anticoccidial drugs or products (Abd El-Ghany, 2020). The mechanism of action consists of destroying

the intracellular stages of the parasite once it has invaded the host cells. Synthetic products include robenidine, decoquinate, and diclazuril (Kadykalo *et al*., 2018).

The administration of ionophores has been an effective method for controlling *Eimeria* spp. infections; however, due to current political and social pressure, their use in livestock has been reduced and/or prohibited (Ferreira *et al*., 2020). Phytochemical compounds have shown preventive, therapeutic, or immunomodulatory effects against coccidiosis. These treatments are characterized by the absence of coccidial resistance development (Nahed *et al*., 2022).

In Mexico, there are 11,560 RPUs with 1 108 350 rabbits. The states with the most registered RPUs are: the State of Mexico with 3 885 and 293 332 animals, and Hidalgo with 1064 and 274 811 rabbits (SENASICA, 2020). Currently, the health status of the *Eimeria* species prevalent in production units and the parasite load present in them is unknown. Therefore, the objective of this study was to morphologically identify and quantify *Eimeria* species present in naturally infected rabbits in production units in the Mezquital Valley, Hidalgo State.

MATERIAL AND METHODS

The work was carried out in 10 locations in the municipalities of Tezontepec de Aldama (20°11′26″ N, 99°16′27″ W, 2006 m above sea level), Mixquiahuala de Juárez (20°13′49″ N, 99°12′50″ W, 2002 m above sea level), Progreso de Obregón (20°14′50″ N, 99°11′24″ W, 1999 m a.s.l.), Francisco I. Madero (20° 14′ 43″ N, 99° 5′ 28″ W, 1980 m above sea level) and Ajacuba (20° 5′ 33″ N, 99° 7′ 10″ W, 2143 m above sea level) (Figure 1), belonging to the Mezquital Valley, Hidalgo, which due to its characteristics has a semi-arid climate, with irrigated and seasonal soils, an average annual temperature of 18 °C and precipitation of 593 mm (Rosas *et al*., 2015).

Production units

The breed, number of breeding animals, and type of feed were recorded in the RPUs, in addition to the characteristics of the facilities and equipment used.

Sample collection

Fecal samples were collected from 32 rabbits (per unit) in 10 RPUs in 5 municipalities of the Mezquital Valley with cooperating producers, of the Nueva Zelanda Blanco (NZB), California (CAL), Chinchilla (CH), Azteca Negro (AZN), and Satinado (ST), between 30 and 60 days of age (fattening period), housed in galvanized wire cages in groups of 8 animals on average.

To ensure the integrity of the samples, they were transported at a refrigerated temperature (4 ºC) to the laboratory of the Academic Area of Veterinary Medicine and Animal Husbandry (AAMVZ) at the Institute of Agricultural Sciences (ICAp) of the Autonomous University of Hidalgo State (UAEH) for morphological identification and determination of parasite load.

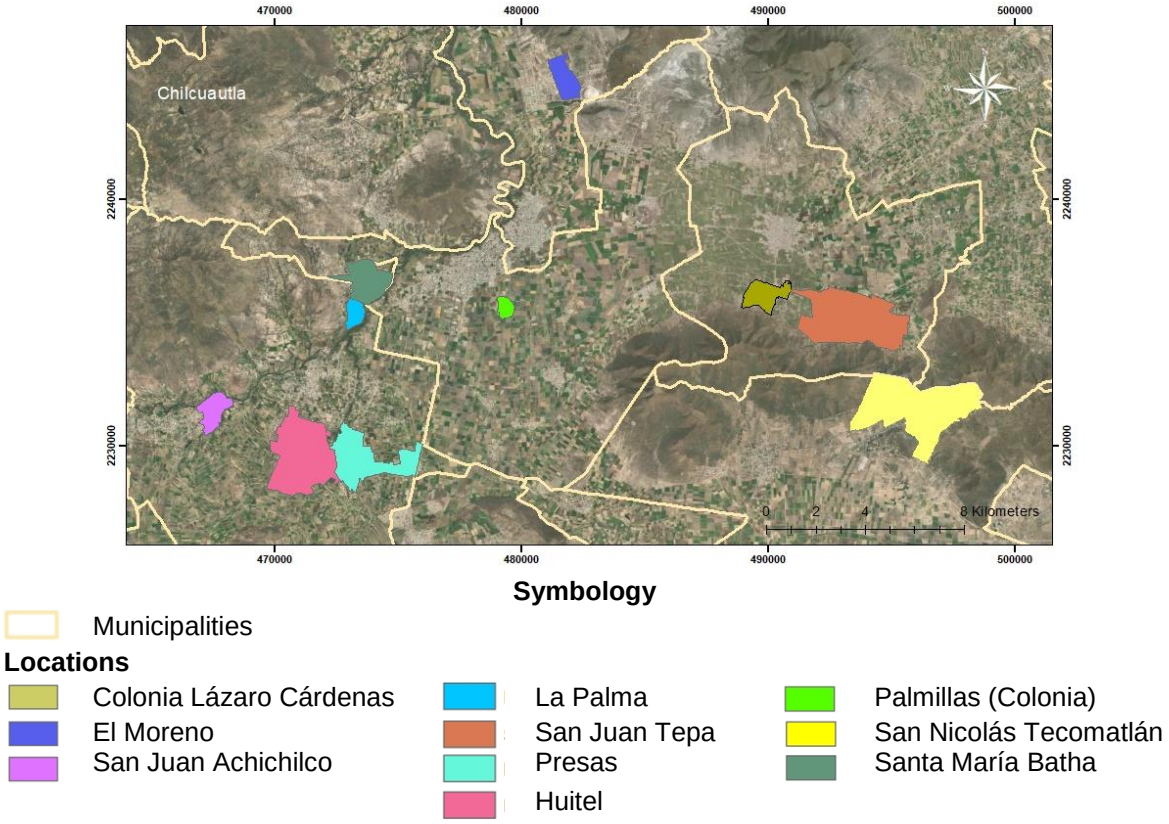

Figure 1. Geographic location of the towns where the RPUs sampled for the study were located

Morphological identification

To identify the species of the genus *Eimeria* present in rabbits, the flotation technique was used, employing a saturated NaCl solution and observing under a microscope at 10X and 40X magnification for qualitative identification using morphological measurement and comparison criteria (Cordero del Campillo & Rojo, 2000).

Determination of parasite load

The McMaster technique was used to determine the number of OPG. The number of OPG was calculated by adding the results of the counts from both chambers and multiplying by 50 (Sandoval *et al*., 2011). Four repetitions were performed per rabbit production unit.

Statistical analysis

The parasite load data were normalized using $\sqrt{x}$+0.5 to perform a principal mean analysis in the Minitab® Statistical Software MiniTab, (2021) program.

RESULTS

According to the analysis of the results, the predominant rabbit breed in the production units was Nueva Zelanda, present in 80% of the farms, followed by the California breed with 50%, corresponding to producers who use crossbreeding with these two breeds. In terms of feeding, seven of the ten farms provide commercial feed in pellet form, while one feeds only green alfalfa, another feeds commercial feed and alfalfa, and one more feeds a combination of commercial concentrate, alfalfa, and nopal (Table 1).

Table 1. Characteristics of rabbit production units in the Mezquital Valley region

	Breed					No. of reproducers			Food				
Farm	Nueva Zelanda	Blanco	California	Chinchilla	Azteca negro	Satinado	Females	Males	Concentrate	Alfalfa	Concentrate + alfalfa	Concentrate + alfalfa + nopal	
1	1				1		13	2		1			
2	1		1				40	5				1	
3	1		1				55	7	1				
4	1		1	1			100	11	1				
5						1	30	5	1				
6						1	30	4	1				
7	1						35	5	1				
8	1		1				36	8	1				
9	1		1				17	3			1		
10	1						36	10	1				
Total	8		5	1	1	2	392	60	7	1	1	1	

With regard to the design of facilities and available equipment, Table 2 shows that most farm roofs (9/10) are made of iron sheets, with only one using asbestos sheets. Fifty percent of farm walls are made of cinder blocks and 50% use cyclone fencing. Sixty percent have cement floors and 40% do not have solid floors (ground).

Eighty percent of the RPUs use American galvanized wire cages, arranged on flat decks (14 gauge), while 20% use galvanized wire modules (12 gauge). Fifty percent use hopper-type feeders that allow for more efficient use of feed, 40% feed in J-type feeders, and only one producer feeds alfalfa on the cages (10%). Seventy percent provide water in plastic or stainless steel containers, and only 30% use automatic nipple drinkers (Table 2).

Table 2. Type of facilities and equipment used in RPU in the Mezquital Valley, Hidalgo

	Roof		Floor		Wall		Cage		Feeder			Waterer		Nests			
Farm	Galvanized sheet	Asbesto	Ground	Concrete	Block	Cyclone mesh	Individual	Galvanized module	J type	Hopper type	Forrage	Plastic	Stainless steel	Automátic	sheet	Plastic	Wood
1	X		X			X	X				X	X				X	
2	X		X		X		X		X			X			X		
3	X			X		X		X		X				X	X		
4	X			X	X		X		X				X			X	
5		X		X	X			X		X				X		X	
6	X		X			X	X			X			X			X	
7	X			X	X		X		X			X					X
8	X		X			X	X			X			X			X	
9	X			X		X	X		X			X				X	
10	X			X	X		X			X				X	X		
Total	**9**	**1**	**4**	**6**	**5**	**5**	**8**	**2**	**4**	**5**	**1**	**4**	**3**	**3**	**3**	**6**	**1**

Identification of species of the genus *Eimeria*

In the RPUs in the study area, two types of coccidiosis were identified: hepatic coccidiosis caused by *E. stiedae* and intestinal coccidiosis caused by different species of *Eimeria* spp (Table 3). *E. stiedae* was identified in 8 RPUs, ranging from 19 to 100%, followed by *E. irresidua*, present in 5 RPUs with a minimum of 17 and a maximum of 63.6%, and *E. exigua*, found in 4 RPUs between 23.5 and 100%. *E. intestinalis* and *E. flavescens* are the species that cause the most severe intestinal coccidiosis and were identified in 2 and 1 RPU, with infection rates of 26 and 9%, respectively. *E. magna*, *E. media*, *E. piriformis*, *E. perforans*, and *E. coecicola* were also identified.

Figure 2. Morphology of oocysts of the genus *Eimeria* species identified in the RPUs of the Mezquital Valley, Hidalgo. A) *E. stiedae*, B) *E. flavescens*, C) *E. intestinalis*, D) *E. magna*, E) *E. media*, F) *E. irresidua*, G) *E. piriformis*, H) *E. perforans*, I) *E. coecicola*, J) *E. exigua*

Table 3. Percentage of species of the genus *Eimeria* in RPU in the Mezquital Valley, Hidalgo

	Rabbit Production Units									
Species	1	2	3	4	5	6	7	8	9	10
E. stiedae	23.4	19		44	45		48	100	30.9	36
E. flavescens	9.3									
E. intestinalis		28					24			
E. magna		24							11.7	
E. media	47.6			39					14.7	
E. irresidua			63.6	17	28		28			31
E. piriformis					9					
E. perforans					18				4.5	
E. coecicola	19.7	29							14.7	
E. exigua			36.4			100			23.5	33

Percentage of infection of *Eimeria* spp. species in RPU

From the 10 RPUs analyzed, 100% tested positive for *Eimeria* spp. Figure 3 shows that there are statistically significant differences in the number of OPG between RPUs, with RPU 9 (241 450 ± 33 555) and 8 (56 817 ± 3 907) having a higher than average number of OPG, while below-average loads were found in RPU 1 (11 417 ± 1 051), 7 (6 367 ± 1 614), 6 (4 500 ± 816), 4 (3 217 ± 437), and 2 (1 583 ± 246), with no significant differences between RPU 5 (583 ± 125) and 3 (433 ± 85), with only RPU 10 presenting an OPG count that fell within the decision limits.

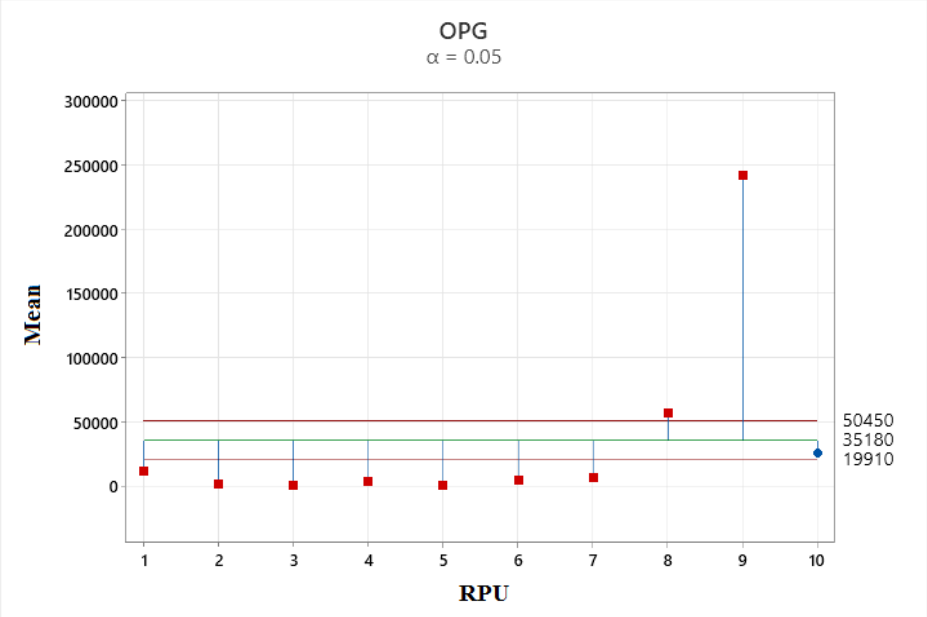

Figure 3. OPG of *Eimeria* spp. in RPU in the Mezquital Valley, Hidalgo

DISCUSSION

Species of the genus *Eimeria* identified in this study correspond to ten of the 11 species reported worldwide that affect rabbits. The presence of several species makes it necessary to determine whether they can act synergistically and whether two or more species increase pathogenicity (García *et al*., 2017). According to Gabriele & Daniel, (2019), *Clostridium spiroforme* and *Eimeria* spp. are associated with gastrointestinal diseases in young rabbits and can cause high morbidity and mortality rates. Serge *et al*., (2020), report that rabbits are susceptible to coccidiosis at the beginning and end of lactation, while the sensitivity of young rabbits is more evident in the days after weaning, where breeding females play an important role in the transmission of coccidiosis.

RPUs were classified as small and medium rabbit farmers according to the classification of Vélez *et al*., (2023), who classify them based on their productive capacity, farmer capacity, and technical efficiency: small family rabbit farmers (37%), medium family rabbit farmers (50%), and commercial rabbit farmers (13%). According to Shkromada & Nedzheria, (2020), in both commercial and family farms, the most common infection is caused by *E. perforans*, *E. magna*, *E. media*, *E. irresidua*, *E. piriformes*, and *E. intestinalis*.

In the Mezquital Valley, the NZB and CAL breeds are the most widely used, and they also have the highest parasite load, which is consistent with the findings of Shola *et al*., (2019), who mention that breed and type of housing are significant risk factors associated with *Eimeria* spp infection. For their part, Pilarczyk *et al*., (2020), report no significant differences in infection by species of the genus *Eimeria* with respect to gender, but do report differences with respect to age and density per cage, with higher infection rates in rabbits younger than 6 months and grouped together, compared to those housed individually.

Cage characteristics (model and size) and equipment (feeders and drinkers) used in the RPUs in the Mezquital Valley allow the reproduction of species of the genus *Eimeria*, which coincides with Legendre *et al.*, (2019), who mention that young rabbits become infected orally by consuming food or water contaminated with oocysts, increasing the possibility of infection when they are fed fodder. Henneb *et al*., (2022), mention that the population of *Eimeria* spp. is significantly higher on farms that do not comply with good hygiene, water, and food quality standards. Gerbil *et al*., (2023), indicate that the type and quality of equipment used in rabbit production must meet food and comfort needs, avoiding contamination with feces.

Shkromada *et al*., (2019), describe that the parasite spreads through sick animals and is well preserved in the external environment. Coccidial oocysts live in cells for a long time, so temporarily removing animals does not prevent infection. Shola *et al*., (2019), report that rabbits kept in “battery” cages have a higher prevalence rate (95.2%) compared to those raised in the “flat-deck” system (71.9%). According to Shkromada & Nedzheria, (2020), management in metal cages, complying with sanitary and hygienic standards and timely removal, reduces the level of infection by *Eimeria* spp. Meanwhile, Hamid *et al*., (2019), mention that the parasites are ubiquitous in the environment and are transmitted via the fecal-oral via.

In the RPUs of the Mezquital Valley, 10 of the 11 species of the genus *Eimeria* reported in rabbits according to Qin *et al*., (2023), were identified. In addition, 8 to 1 species were found in the same RPU, similar to the data reported by Serge *et al.,* (2019), who, after recording the dynamics of oocyst excretion in breeding animals and their offspring for seven months, found high concentrations of oocysts in 100% of the animals, with 7 species of coccidia coexisting in the same individual. Maziz *et al*., (2018), when evaluating

the prevalence of coccidia in rabbit farms in northern Algeria, identified eight species of the genus *Eimeria*, reporting that mixed infections with four species were common, with *E. magna* being the dominant species, compared to *E. media* and *E. irresidua* with respective frequencies of 42.5%, 17.6%, and 14.9% ($p<0.001$).

From the species identified in this study, according to Takami *et al*., (2023)., *E. intestinalis* and *E. flavescens* cause intestinal coccidiosis, and *E. stiedae* causes hepatic coccidiosis, presenting high virulence. In turn, Athanasiou *et al*., (2023), report that *Eimeria stiedae* is a devastating disease with high morbidity and mortality rates. However, Laha & Goswami, (2023), state that only intestinal coccidiosis can cause high mortality in rabbits. Anak & Sarayati, (2023), mention that transmission occurs when rabbits consume food and drink contaminated with oocysts. *Eimeria magna* is characterized as being slightly pathogenic and moderately immunogenic (Geru *et al.*, 2017). It causes lethargy, weight loss, diarrhea, and even death in severe cases (Chen *et al*., 2023).

In the RPUs of the present study, samples were collected from fattening rabbits (30 to 60 days old) because this is a stage where there are large economic losses associated with diarrhea. Elhendy *et al.*, (2018), report a prevalence of 88% in rabbits under 4 months of age. According to studies by El-Ashram *et al*., (2020), 86.50% (198/229) of rabbits after weaning were infected with *E. media*, *E. perforans*, *E. intestinalis*, *E. magna*, *E. coecicola*, *E. exigua*, and *E. flavescens*.

Sun *et al*., (2016), report that species of the genus *Eimeria* in rabbits are only capable of infecting certain hosts and that, during infection, cell division and growth occur, along with inflammation in the intestine to replace damaged enterocytes, requiring cholesterol as an essential constituent of the cell membrane. In turn, Manjunatha *et al*., (2019), observed growth retardation, anorexia, weight loss, diarrhea, abdominal pain, and sudden death in affected rabbits, while blood biochemistry reports an increase in liver enzymes and bilirubin.

Petrova *et al*., (2022), when performing necropsies on rabbits infected with *Eimeria stiedae*, revealed hepatomegaly, multifocal yellowish nodules diffusely distributed over the surface of the liver and in the parenchyma, dilated bile ducts, and biliary hyperplasia. Manjunatha *et al*., (2019), identified numerous *E. stiedae* oocysts, finding multiple areas of coagulative necrosis of liver cells surrounded by inflammatory cells. According to Chatterjee *et al.*, (2023), this clinical situation prevails in different organs such as the pancreas, liver, and cecum.

According to Taraneh *et al.*, (2011), affected animals show weight loss, with reduced fat reserves and muscle atrophy, shaggy hair, and fecal material adhering to the hair in the perineum. Macroscopic pathological changes are observed in the small intestine, distended and filled with a grayish-green semi-solid ingestion, and the intestinal mucosa is severely hyperemic and edematous. Similarly, Sidorenko *et al.,* (2020), identified merozoites, damaged enterocytes, and accumulations of lymphocytes and eosinophils in histological sections of the small intestine.

OPG variability of the RPUs in the Mezquital Valley was high, with only one RPU recording a population of <500 OPG, which, according to Anak y Sarayati *et al*., (2023), is considered a mild infection with no capacity to cause damage. In turn, Sidorenko *et al*., (2020), when evaluating the intensity of infection of *E. perforans* and *E. irresidua* by inoculating 50-60 thousand oocysts per 40-day-old rabbit, reported that maximum live weight gain and carcass yield decrease in F1 Chinchilla x California hybrid rabbits. Balicka *et al*., (2020), report that the number of OPG also shows large fluctuations throughout the year, being highest in May (21 100 OPG).

Tokiwa *et al*., (2022), mention that species of the genus *Eimeria* can be transmitted horizontally through oocysts, which is problematic in closed environments. Meanwhile, Chatterjee *et al*., (2023), report that the right temperature can trigger sporulation and complete their life cycle. In addition, Shkromada *et al*., (2019), point out that it is impossible to completely eliminate coccidia, despite acidifying the water every day, because when the therapeutic concentration is reduced, the rabbits become ill. Acidification with formic, orthophosphoric, sorbic, and citric acids at a pH of 3.5 and an exposure of 60 minutes eliminates 50 to 90 % of oocysts. A pH of 3.5 to 4.5 does not cause destruction of the mucosal epithelium. Similarly, Hamid *et al*., (2019), report that the application of biosecurity measures by eliminating oocysts in feces before they sporulate reduces the number of infectious oocysts.

The high concentration of OPG in at least 9 of the 10 farms evaluated could significantly affect the profits of the RPUs, since according to Chatterjee *et al.*, (2023), approximately 70% of the estimated costs of subclinical coccidiosis are due to the impact on weight gain and feed conversion, making it necessary to implement efficient prevention and control measures to avoid economic losses.

According to Chen *et al*., (2023), one method of preventing coccidiosis in rabbits is to use anticoccidial drugs in the diet; however, there is concern about resistance and the presence of residues (from the drugs) in the carcass. In turn, Xiao *et al*., (2022), recommend the use of vaccines as a preventive medicine strategy. For their part, Rivero *et al*., (2019), propose the use of antimicrobial products obtained from plants and trees such as *Salix babylonica* (weeping willow) as an alternative for the control of coccidiosis in naturally infected rabbits, since the administration of 25 and 50 mg/kg of live weight

reduced OPG elimination, an effect associated with its phytochemical composition (coumarins, triterpenes, flavonoids, sesquiterpene lactones, saponins, terpinen, linalool, thymol, and carvacrol). Meanwhile, Nahed *et al.*, (2022), report that, in chickens, phenolic compounds reduce the oocyst count of *Eimeria* spp. by reacting with the cytoplasmic membranes, causing lysis and destruction of the protozoan.

CONCLUSIONS

Eimeria spp. are present in 100% of the rabbit production units sampled in the Mezquital Valley. In 80% of cases, they were found as mixed infections with the presence of more than one species. The number of OPG (more than 500) poses a risk to animal health and requires the implementation of prevention programs and/or effective controls that consider reducing the use of drugs.

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Errata Erratum

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