Eimeria *spp. in broiler rabbit: seasonal prevalence in the backyard farms of the State of Mexico*

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Summary
Coccidiosis is the most common parasitic disease in rabbits and it can be responsible for severe economic losses in broiler rabbit farms. Eleven species of the *Eimeria* genus, which vary considerably in terms of their morphology and pathogenicity, cause rabbit coccidiosis. The aim of this study was to evaluate the prevalence of *Eimeria* spp. in backyard farms of State of Mexico during the different seasons. Cross-sectional sampling was performed in young rabbits (20 to 60 day old), with clinical histories of diarrhoea, from 3 municipalities located in the Southeastern region of the State of Mexico. Flotation and Mc Master Techniques were used to identify oocysts. The overall prevalence of *Eimeria* in the Southeastern region of the State of Mexico was 48.3%. The highest prevalence was recorded during Winter (88%) in Amecameca, while the lowest prevalence during Spring (5%) in Temamatla. The annual prevalence of the *Eimeria intestinalis,* the most pathogenic species, was 11.3%. The least amount of oocysts per gram of faeces (407) was obtained in Autumn in Temamatla, while the highest quantity was observed in Summer in Cocotitlan (18,330). The highest prevalence of *Eimeria* spp. was detected in Autumn and Winter, making it possible to establish prevention programs in this region according to the season.

*Prevalenza stagionale dell'*Eimeria *spp. in conigli di aziende agricole a conduzione famigliare nello Stato del Messico*

Parole chiave
Prevalenza,
Oocisti,
Medicina preventiva,
Coccidiosi del coniglio.

Riassunto
La coccidiosi è una malattia parassitaria molto comune nei conigli causata da undici specie di *Eimeria* considerevolmente variabili in morfologia e patogenicità. Negli allevamenti di conigli da carne la coccidiosi è in grado di determinare gravi perdite economiche. Questo studio si è posto come obiettivo quello di valutare la prevalenza stagionale di *Eimeria* spp. in aziende agricole a conduzione famigliare dello Stato del Messico. Lo studio basato su un campionamento trasversale è stato condotto su giovani conigli (da 20 a 60 giorni) con storie cliniche di diarrea, provenienti da 3 comuni situati nella regione sud-orientale dello Stato del Messico; per identificare le oocisti sono state utilizzate le tecniche di flottazione e di Mc Master. Nella regione sud-orientale dello Stato del Messico la prevalenza di *Eimeria* è stata complessivamente del 48,3%. Il valore massimo (88%) è stato registrato in Amecameca in inverno, mentre quello minimo (5%) in primavera in Temamatla. La prevalenza annuale di *Eimeria intestinalis*, la specie più patogena, è stata dell'11,3%. La minima quantità di oocisti per grammo di feci (407) si è avuta in autunno a Temamatla, mentre la più alta in estate a Cocotitlan (18.330). La più alta prevalenza di *Eimeria* spp. rilevata in autunno e in inverno rende possibile avviare in questa regione programmi di prevenzione su base stagionale.

In the past few years, broiler rabbit production has grown considerably and has become an important source of animal protein in many developing countries, such as Mexico. The success of the rabbit industry depends on many factors, especially good health conditions (Varga 1982).

Coccidiosis is the most common parasitic disease in rabbits. It can cause serious health problems due to diarrhoea, reduction of food intake, and as a result, loss of body weight, affecting animal metabolism in general (Freitas *et al.* 2010). As such, coccidiosis is responsible for severe economic losses to the industry each year.

Rabbit coccidiosis is caused by at least 11 species of the genus *Eimeria* (*E*): *E. coecicola, E. exigua, E. flavescens, E. intestinalis, E. irresidua, E. magna, E. media, E. perforans, E. piriformis, E. stiedai* and *E. vejdovskyi*. These species vary considerably in terms of their morphology and pathogenicity (Kvicerova *et al.* 2008).

Eimeria spp. transmission is faecal-oral, through contaminated food, water, caging, as well as other fomites, which serve as common means of spreading intestinal coccidian (Kvicerova *et al.* 2008). The morbidity and mortality of this disease, in growing rabbits, are between 60% to 90% (Jing *et al.* 2011, Boag *et al.* 2001, Cere *et al.* 1996).

Rabbit breeding is a livestock activity in full development, in the Southeastern area of the State of Mexico. The aim of this study was to evaluate the prevalence of *Eimeria* spp. in broiler rabbit of backyard farms in this area and during the different seasons.

In 2013, cross-sectional sampling was performed in an area of 21.67 km^2, in the municipalities of Amecameca (19° 06' 42.0" N - 98° 45' 29.5" W), Cocotitlán (19° 13' 58.3" N - 98° 51' 23.7" W), and Temamatla (19° 12' 04.6" N - 98° 51' 50.3" W). All 3 municipalities are located in the Southeastern part of the State of Mexico. In Amecameca, the annual average temperature is 14.1 ºC, ranging from 2.4 ºC during Winter to 24 °C in Spring[1]. In Cocotitlan, the average was 13.6 ºC, going from 19 ºC during Spring to 7 °C in Winter[2]. In Temamatla, the average was 12.5 ºC, with temperature as low as 2- 4 ºC in Autumn and as high as to 24 ºC in Spring and Summer[3].

In addition, in 2013 the annual accumulated rainfall in the State of Mexico was 842.9 mm, with the highest rainfall recorded during Summer (640.2 mm) and the lowest recorded rainfall during Winter, with 20.3 mm[4].

This study took place in backyard farms, in which rabbits presented diarrhoea and where there had been no parasitological diagnosis for a minimum of 3 months. Rabbits in the Cocotitlan and Temamatla farms were fed with alfalfa (*Medicago sativa*) from the region and vegetable leftovers. In Amecameca, rabbits received alfalfa and commercial balanced food. Water was provided *ad libitum* in all farms.

Coccidiosis is one of the main parasitic diseases in domestic rabbits (Yan *et al.* 2013). The rabbits in this study were 20 to 60 day old. According to Papeschi and colleagues (Papeschi *et al.* 2013), it is during this age when the disease is more commonly observed, with *Eimeria* spp. infection appearing more frequently from 46 up to 109 days of life.

Rabbits were kept in collective cages in the farms. For this study, rabbits were placed in individual cages to allow the collection of faeces. After 24 hours they were placed back to the collective cages.

During each of the 4 seasons, faecal samples were obtained from 1,399 rabbits in the fattening stage. Six-hundred-fifty-one samples were collected in Amecameca; 467 in Cocotitlán, and 281 in Temamatla. The samples were obtained from the individual cage floor (Papeschi *et al.* 2013), immediately stored in plastic bags, and then delivered to the laboratory.

Samples were kept at 4 °C until they were analysed for *Eimeria* spp. oocysts by Flotation and Mc Master Techniques, using a 10× microscope objective (Permin and Hansen 1998, Coudert *et al.* 1995). Moreover, oocysts were sporulated, placed in Petri dishes with 2.5% potassium dichromate, at room temperature. The length and width of sporulated oocysts were measured using a light microscope (Carl Zeiss, Oberkochen Germany) equipped with an eyepiece micrometer with 20× objective to identify the species of *Eimeria* in each of the different seasons, according to the morphometric criteria of Kvicerova (Kvicerova *et al.* 2008).

Prevalence was calculated with a confidence interval of 95% (CI 95). Differences in prevalence between seasons and localities were compared using the Kruskal Wallis test. The Mann Whitney test was used to compare the number of oocysts per gram of faeces for each season. The statistical

[1] Instituto Nacional para el Federalismo y el Desarrollo Municipal/ Secretaria de Gobernacion de Mexico. 2015. Enciclopedia de los Municipios y Delegaciones de Mexico. Estado de México. Amecameca. http://www.inafed.gob.mx/work/enciclopedia/EMM15mexico/municipios/15009a.html.

[2] Instituto Nacional para el Federalismo y el Desarrollo Municipal/ Secretaria de Gobernacion de Mexico. 2015. Enciclopedia de los Municipios y Delegaciones de Mexico. Estado de México. Cocotitlán. http://www.inafed.gob.mx/work/enciclopedia/EMM15mexico/municipios/15022a.html.

[3] Instituto Nacional para el Federalismo y el Desarrollo Municipal/ Secretaria de Gobernacion de Mexico. 2015. Enciclopedia de los Municipios y Delegaciones de Mexico. Estado de México. Temamatla. http://www.inafed.gob.mx/work/enciclopedia/EMM15mexico/municipios/15083a.html.

[4] Comision Nacional del Agua/Servicio Meteorológico Nacional (CONAGUA) 2013. Precipitación a Nivel Nacional y por Entidad Federativa. https://www.gob.mx/conagua.

Table I. *Sampling scheme and annual prevalence by municipalities and season.*

		Sampling (n)	Positive (n)	Prevalence (%)	CI 95 (%)
Amecameca	Spring	162	20	12	6-18
	Summer	136	59	43	35-51
	Autumn	213	111	52	49-58
	Winter	140	124	88	82-94
Cocotitlan	Spring	128	37	29	22-36
	Summer	108	49	45	35-55
	Autumn	104	55	53	43-63
	Winter	127	59	47	39-55
Temamatla	Spring	60	3	5	0-11
	Summer	82	50	61	51-71
	Autumn	64	48	75	65-85
	Winter	75	61	81	71-91

CI 95 = Prevalence with a confidence interval of 95%.

analysis was conducted using the Palaeontological Statistics (PAST), ver. 1.81 program, with a significant value of $p < 0.05$.

The annual prevalence in the study area was 48.3% (CI95: 45.7-50.9%). In Amecameca annual prevalence was 48.2% (CI95: 44.4-52.1%); in Cocotitlan it was 42.8% (CI95: 38.3-47.3%), and in Temamatla it was 57.6% (CI95: 51.9-63.4%). There was no significant ($p > 0.05$) difference among these results, as shown in Table I.

Prevalence varied significantly ($p < 0.05$) during the different seasons: in Spring it was 17.1% (CI95: 13.3-21.5%), in Summer 48.1% (CI95: 43.0-53.8%), and 56.2% (CI95: 51.1-61.0%) in Autumn and in Winter 71.3% (CI95: 66.3-75.8%) with significant differences ($p < 0.05$) (Table I and Figure 1).

Regarding the concentration of oocysts per gram of faeces (OPG), we observed that there were no

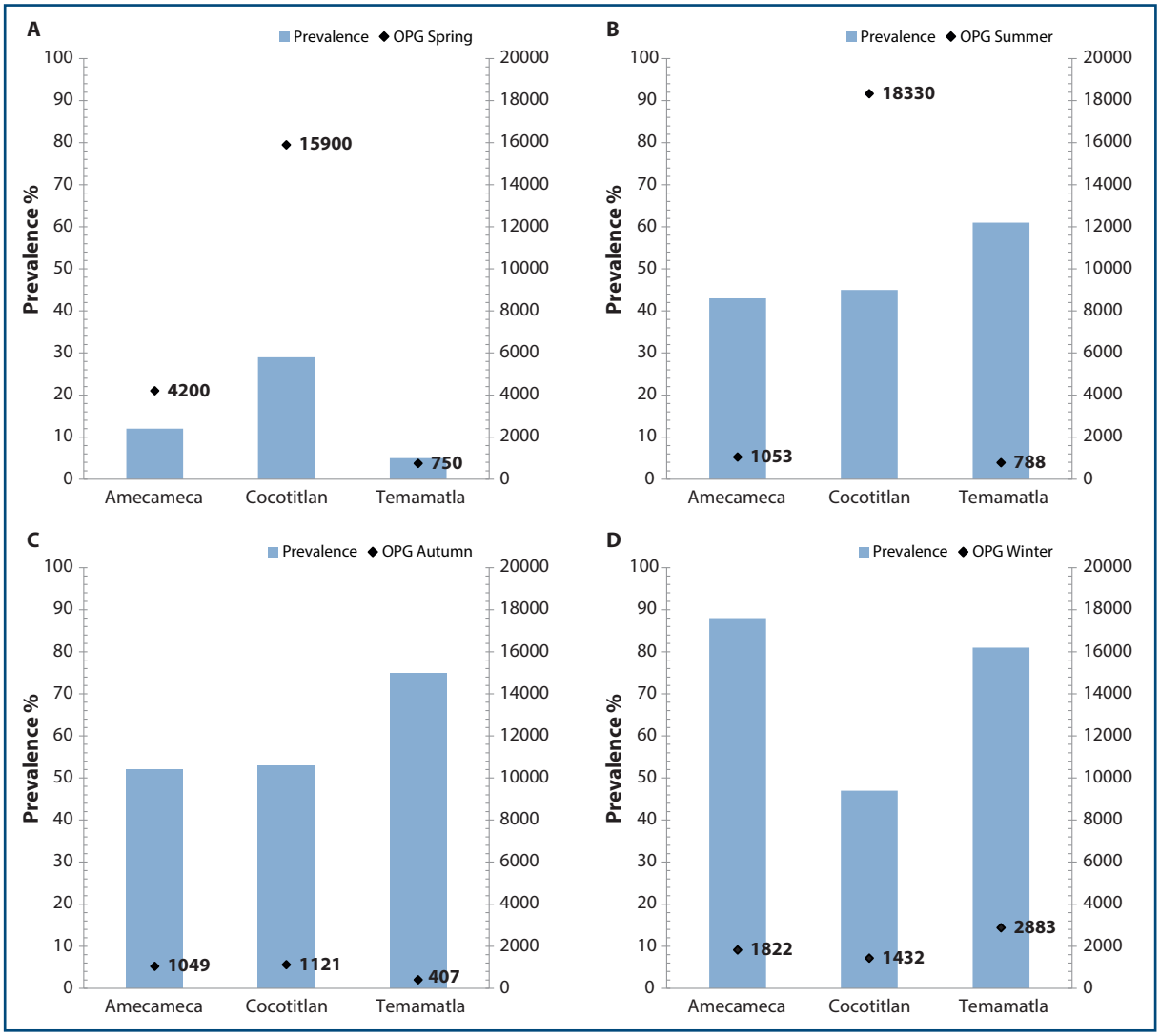

Figure 1. *Prevalence of* Eimeria *spp. identified in rabbit backyard farms in the State of Mexico, México, by number of oocysts per gram of feces (OPG), municipality and season of the year [Spring (A), Summer (B), Autumn (C) and Winter (D)].* Significant differences were detected between prevalence during autumn and winter seasons ($p < 0.05$). Kruskal Wallis Test. No significant differences ($p > 0.05$) were observed in OPG between seasons.

Table II. *Number of rabbits infected with one or more* Eimeria *spp., according to morphometric characteristics, by municipality and season.*

Eimeria* spp. (pathogenicity)	**Oocyst average length × width (µm)**	**Amecameca (n = 651)**	**Cocotitlan (n = 467)**	**Temamatla (n = 281)**
E. intestinalis (high)	25×17.6 (22.5-27.5×12.5-22.7)	92 ^{Sp,Su,A,W}	19 ^{A,W}	47 ^{Su,A,W}
E. vejdovskyi (slight)	32.5×21.2 (32.5×17.5-25)	2 ^{Sp}	11 ^{Sp}	16 ^{Su}
E. coecicola (none)	35×19.7 (35×15-22.5)	77 ^{W}	44 ^{Sp,A}	18 ^{Su}
E. stiedai (mild-high)	37.5×18.7 (37.5×17.5-20)	6 ^{W}	6 ^{Sp}	29 ^{Su}
E. media (mild)	30×17.5 (30×17.5)	-	17 ^{Su,A}	7 ^{Su}
E. perforans (slight)	22.5×16.2 (20-25×12.5-20)	32 ^{Sp,W}	73 ^{Su,A,W}	7 ^{Su}
E. magna (mild)	37.5×22.5 (37.5×20-25)	45 ^{Su}	34 ^{Sp,Su}	72 ^{W}
E. exigua (slight)	15×15 (12.5-17.5×12.5-17)	67 ^{Su,A,W}	-	44 ^{A}
E. irresidua (mild)	38.7×22.5 (37.5-40×20-25)	45 ^{A}	2 ^{Sp}	61 ^{Sp,A}
E. piriformis (mild)	30×17.5 (30×17.5)	-	17 ^{W}	19 ^{W}
E. flavescens (high)	30×25 (30×25)	6 ^{W}	-	-

Numbers in parentheses show the length × width range of different *Eimeria* spp. oocyst. *Pathogenicity, according to the criteria described by Kvicerova and colleagues (Kvicerova *et al.* 2008). Sp = Spring; Su = Summer; A = Autumn; W = Winter.

significant differences ($p > 0.05$) when comparing data pertaining to different seasons (Figure 1).

Species of *Eimeria* were found in different municipalities, and were identified on the basis of their morphology. Seven species of *Eimeria* were found in all municipalities, 3 of them in 2 municipalities, and *E. flavescens* in Amecamenca only. *Eimeria intestinalis,* the most pathogenic species (Oncel *et al.* 2011, Coudert *et al.* 1995), was found in 14.1% of faecal samples (CI95: 11.5-16.8%) from Amecamenca, in 4.0% (CI95: 2.3-5.9%) from Cocotitlan, and in 16.7% (CI95: 12.4-21.1%) from Temamatla (Table II).

In a survey conducted in China, coccidian oocyst prevalence was 61.4% in farms with less than 1,000 rabbits (Jing *et al.* 2012). Likewise, in Saudi Arabia, prevalence of *Eimeria* spp. was reported to range between 73% to 90% (El-Shahawi *et al.* 2012). In both studies, the annual prevalence was greater than the one reported in the present study, although, in terms of seasonal prevalence/year, the results are similar. In this work the amount of OPG was also very variable: the maximum amount of OPG ranged between 407 and 18,330 with the smaller amount being lower than the one reported in China (Jing *et al.* 2012) in farms with less than 1,000 rabbits (23,514 OPG), where coccidiosis occurs as subclinical in the animals.

It has been reported that there is a need for an exposure equivalent to 100,000 OPG to produce 80% of mortality. At the same time, when exposed in an experimental way to 50,000 *E. stiedai* sporulated oocysts for 32 days, rabbits showed a decrease in weight gain (Freitas *et al.* 2010, Pakandl *et al.* 2009).

Likewise, the amount of OPG found by Jing et al. (2012) in China (14,252 OPG) was similar to those reported in Cocotitlan during Spring (15,900 OPG), but lower than those recorded during Summer in the same municipality (18,330 OPG).

Different *Eimeria* species have been reported as the most prevalent ones: *E. perforans* in China (35.2%), followed by *E. media* (31%), and *E. magna* (28%) (Jing *et al.* 2012). In Saudi Arabia, the most frequent were *E. coaecicola* (70%), *E. magna* (60%), *E. perforans* (60%), and *E. media* (55%) (Abdel-Baki and Al-Quraishy 2013). In the present study, the highest prevalence was found for *E. intestinalis* (11.3%) and *E. magna* (11.0%). These species have been recognised as pathogenic. They are associated with the inflammatory lesions in intestines and diarrhoea in rabbits. *E. perforans* (8.0%), that causes decreased feed conversion, was also present (Oncel *et al.* 2011).

A previous study about domestic and wild rabbits indicated that parasite intensity and prevalence could change during the year due to the age of the host and the amount of ingested infective stages (Pakandl *et al.* 2009).

In this study, the highest prevalence (71.3%) was reported in Winter, which is the coldest and driest season; during Autumn, a warm and slightly damp season, prevalence was 56.2%. The lowest prevalence (17.1%) was found during Spring, which was the warmest season. Similar results have been reported in Europe, where the number of cases increases during Spring, Autumn, and Winter, especially in young individuals. Susceptibility to infection is determined by geographic location, hygienic conditions, and population density. Since a higher number of individuals in a given area increases stress, it therefore, decreases the effectiveness of the immune system (Perez-Martinez and Betancourt 2010, Grez *et al.* 2003).

This survey consistently found presence of more than 2 *Eimeria* spp. throughout the samplings. Only

the farm in the Temamatla municipality presented a single type of coccidian, during Spring. This information is consistent with the one reported by El-Shahawi and colleagues (El-Shahawi *et al.* 2012), Jing and colleagues (Jing *et al.* 2012), and Oncel and colleagues (Oncel *et al.* 2011). In all these studies, mixed infections with 3 or more different *Eimeria* spp. were very common. Poor hygienic conditions, which may occur when rabbits are in collective cages on the floor most of the time, with the presence of alfalfa and vegetable waste, facilitate contamination and the emergence of *Eimeria* infection (Jing *et al.* 2012, Pakandl *et al.* 2009).

This study revealed a significant prevalence throughout the year of different types of *Eimeria* spp., in backyard farms located in the Southeastern portion of the State of Mexico. Further studies are necessary to quantify the economic losses due to the presence of this parasite in the farms. In this study, results showed highest prevalence during Autumn and Winter, making it possible to establish a prevention program envisaging improvements in hygiene and use of preventive treatment administrated on a seasonal basis.

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