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ORIGINAL RESEARCH

Prevalence of subclinical coccidiosis in broiler farms in Turkey

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Abstract The presence of *Eimeria* spp. oocysts in fecal samples collected from 1,108 broiler houses in six regions, representing about 12% of all broiler farms in Turkey, was studied using the modified McMaster method. The age of the chickens in the 1,108 pens varied from 1 to 50 days. Oocysts were found in 602 (54.3%) of these broiler houses, and the mean OPG (oocysts per gram of feces) in those samples was 36,498.7 (50–952,000). No indication of clinical coccidiosis or other clinically evident infection or wide mortality was encountered in any of the pens studied. Further study showed that the age of the chickens, the occurrence of diarrhea on the houses and the density of broiler breeding in the area correlated with subclinical coccidiosis prevalence.

Keywords Broiler coccidiosis · Prevalence · Turkey

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Introduction

Coccidiosis, one of the basic problems of poultry farms worldwide, is caused by *Eimeria* spp. It can cause loss of production, diarrhea, hemorrhagic diarrhea and even death. Because of widely implemented prevention and control strategies, lethal coccidiosis is becoming rare. However, due to factors like the monoxenous biology of the pathogen and the inadequacy of disinfection and quarantine for infection control, it has not been possible to eradicate the disease in broiler breeding. As a result, the subclinical form of coccidiosis represents the main cause, today, of losses from this disease (Williams 1999; Rommel et al. 2000; McDougald 2003; Haug et al. 2008).

The prohibition of some of the most frequently used anticoccidial agents in the European Union and several other countries has increased the need for finding more effective measures to fight against this disease, which is already difficult to control (Chapman et al. 2002). It is especially important, in order to ensure the efficacy of the high-budget programs to fight against coccidiosis, to characterize the prevalence and characteristics of the disease in chicken breeding. The aim of this study was to determine the prevalence of coccidiosis and some aspects of its character in broiler breeding, which has been rapidly expanding in Turkey during the last few years.

Materials and methods

Description of the study area and population

This study was performed from September 2006 to September 2007. The chosen study population consisted of the farms declared as usually breeding broilers; the

breeding regions, the provinces composing these areas and the number of pens representing each province were determined. The study sample was selected by a random method from the provinces stratified by geographic areas and proportional to the size of the latter. The sample size was chosen with an approximate sensitivity of 0.02. Before the start of the study, the General Direction of Protection and Control at the Ministry of Agriculture declared that the total of broiler pens across the six regions of Turkey was 11,979 and distributed as follows: Marmara Region, 4,009; Black Sea Region, 3,863; Central Anatolia Region, 1,239; Aegean Region, 1,401; Mediterranean Region, 1,220; Eastern Anatolia Region, 247.0. Based on the relevant data, a total of 1,108 sample size from each of these regions was determined as 424, 274, 148, 125, 117 and 20 pens.

Sample collection

The fecal samples from the farms visited during the study period were collected from different locations in the pens to ensure repeatability. At least one 100-ml plastic stool container was filled for each 1,000 chickens in the pen. Address, sampling date and the age of the chickens were recorded for each sample. Litter and feces samples were examined and the presence or absence of diarrhea and/or blood was also recorded. Samples were kept at +4°C until examination for the presence of oocysts.

Analyzing fecal samples for oocysts

To calculate the number of oocysts per gram of feces (OPG), each collected sample was cleaned of litter and thoroughly homogenized, then samples of the homogenate were analyzed for *Eimeria* oocysts by the modified McMaster method (Anonymous 1986).

In order to acquire general information about *Eimeria* species present in the studied farms, material obtained by mixing equal-volume samples from all positive fecal samples was floated in saturated solution of sodium chloride to obtain purified oocysts. These were poured into Petri dishes in a thin layer of 2.5% potassium dichromate solution. They were kept 10 days at 28°C under regular stirring and control of the potassium dichromate solution amount in order to provide sporulation of oocysts which is necessary for morphological identification. The sporulation ratio was checked on the last day and the obtained sporulated oocysts were identified according to morphological features (Levine 1985; Conway and McKenzie 2007).

Particularities of data analysis

The age groups of the chickens were the basic criterion in the general evaluation of the data. Considering the general biology of *Eimeria* spp. and our results obtained from analyzes of the fecal material, we performed the evaluation of the regional differences and that of the cases of diarrhea mainly in chickens 13 days or older.

Statistical evaluation

A χ^2 test with Bonferroni correction was used to compare the results according to age groups, geographical area and the macroscopic characteristics of the fecal material. A one-way analysis of variance (ANOVA) test with Tamhane's T2 post hoc multiple comparisons were used for testing the significance of the comparisons of OPG values according to the same factors. The statistical software SPSS Version 14.01 was used.

Results

The age of chickens in the 1,108 pens varied from 1 to 50 days (mean, 25.0). *Eimeria* spp. oocysts were detected in 602 samples (54.3%), and the mean OPG in those samples was 36,498.7 (50–952,000). No indication of coccidiosis or other clinically evident infection or wide mortality was encountered in any of the pens studied. Prevalence of positive samples according to age groups is given in Table 1.

The number of positive samples for the Marmara Region, Black Sea Region, Central Anatolia Region, Aegean Region, Mediterranean Region and Eastern Anatolia Region were 265, 178, 57, 61, 40 and 1, respectively. The data of farms with chickens aged 13 days and older are shown in Table 2. The distribution of these data by region, with their respective study population and sample sizes, is shown in Fig. 1.

Oocysts were observed in 449 (63.1%) of the 712 pens containing chickens aged 13–50 days, with a mean OPG of 39,676.0 (50–952,000). Of these, 305 (42.8%) presented diarrhea and 184 of them presented fecal blood too. The data on presence of diarrhea and fecal blood were independently variable (Table 3).

Even though precise species identification only by morphologic criteria is not possible, the investigation showed the presence of *E. tenella*, *E. acervulina*, *E. maxima*, *E. praecox*, *E. necatrix*, *E. mitis* and very few *E. brunetti* similar species in the total oocyst inoculums.

Discussion

Studies performed in different countries showed that the prevalence of coccidiosis is highly variable; it can be very low in some areas while surpassing 90% in others

Table 1 Prevalence of positive samples by age groups

	Age groups (days)					
	0–10	11–20	21–30	31–40	41–50	Total
I	182	230	274	341	81	1,108
	(6.8)	(15.6)	(26.0)	(35.9)	(42.8)	(25.0)
II	2	53	182	301	64	602
	(1.1%)	(23.0%)[a]	(66.4%)[b]	(88.3%)[c]	(79.0%)[c]	(54.3%)
III	125	3,234.0[d]	68,024.2[e]	27,711.0[f]	16,861.7[f]	36,498.7
	(50–200)	(±845.7) (50–35,100)	(±9,748.8) (50–952,000)	(±2,932.6) (50–427,400)	(±3,875.4) (50–212,500)	(50–952,000)

Different lowercase letters in the same row indicate significant differences at $p<0.001$ (χ^2 and one-way ANOVA test)

I Number of samples (and means of chicken age in the groups), *II* number of positive samples, *III* mean OPG values of positive samples (±SEM) (min–max), *SEM* standard error of the mean

(McDougald et al. 1997; Williams et al. 1996; Razmi and Kalideri 2000; Al Natour et al. 2002; Nematollahi et al. 2008). In this study, we determined that 54.3% of the facilities were infected in varying proportions, and that the ratio increased to 66.9% in chickens aged 13 days or older. Such a high prevalence shows that coccidiosis in broiler breeding continues to be important, as it does also in Europe and worldwide (Shirley 2009). On the other hand, the fact that no cases of specific clinical findings or lethal coccidiosis were observed, even in the presence of such high prevalence, suggests that the disease could have a kind of stability. To be precise about the latter point, however, would require a detailed study especially on the pathologic and disease characteristics of the agents.

The prepatent period of the pathogens of coccidiosis in chickens, which have monoxenous biology, varies from 2.5 to 7 days according to the species, while the patent period is 4–19 days (Levine 1985; Williams 2001). The earliest positive cases in this study were seen at 9 days, while widespread infection was observed from 13 days onward. This finding indicates that chickens in many of these pens are infected during the first week of life. Analysis according to age groups showed that the spread of infection increases with age, peaking in days 31–40 and diminishing afterwards. The OPG ratio also increased with age, with a peak in days 21–30 and a definite decrease after that. Published studies suggest that infection of chickens during the first weeks of life provides immunity, which is generally species-specific (Zahner et al. 1994; Jordan and Pattison

Table 2 Data obtained from chicken farms that have 13-day-old or older chickens in the regions and the climatic data of the regions

	Regions (Annual mean air temperature) (Annual mean rainfall)					
	Mediterranean (19°C) (650 mm)	Aegean (16°C) (700 mm)	Central Anatolia (11°C) (350 mm)	Black Sea (14°C) (1,200 mm)	Marmara (15°C) (750 mm)	Eastern Anatolia (12°C) (400 mm)
I	95	99	124	216	347	16
	(28.7)	(31.1)	(28.1)	(29.4)	(29.0)	(23.1)
II	40	61	57	177	264	1
	(42.1%)[a]	(61.6%)[b]	(46.0%)[a]	(81.9%)[c]	(76.1%)[c]	(6.3%)
III	13,846.3[d] (±3,432.0)	18,279.5[d] (±3,330.6)	32,700.0[de] (±8,347.1)	35,127.4[e] (±5,063.5)	46,293.8[e] (±6,691.3)	50
	(50–90,000)	(50–156,800)	(50–320,000)	(50–427,400)	(50–952,000)	

Different lowercase letters in the same row indicate significant differences at $p<0.001$ (χ^2 test), at $p<0.05$ (one-way ANOVA test)

I Number of chicken farms in the regions (and means of chicken ages in the groups), *II* number of *Eimeria* spp positive farms, *III* mean OPG values of positive samples (±SEM) (min–max)

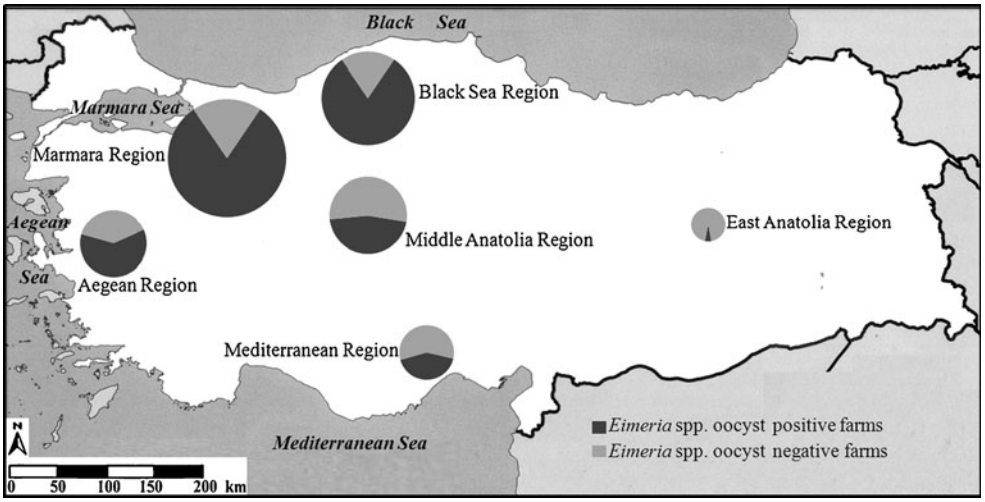

Fig. 1 Results of stool analyzes of the farms that have 13-day-old or older chickens in the regions. (The size of the pie diagrams corresponds to population density and hence the count of the farms examined in the regions)

1996). Parallel to this, the oocyst excretion that follows the first infection reaches a maximum within a delimited period, and then follows a diminishing, and either definitively disappears or continues at lower levels (Suls 2000). On the other hand, in experimental infections it has also been observed that oocyst excretion can follow a variable, undulating course instead of a linear one (Kar et al. 2010). It must be said that each *Eimeria* species has its own characteristics as to ways of inducing immunity, age of the chickens to affect, patent period and prepatent period (Williams 2001; Chapman et al. 2005; Shirley 2000). The ratio of oocyst production for each sporulated oocyst during infection is different (Suls 2000; Shirley 2009). This is probably also, along with the development of immunity, another factor in the age-dependent prevalence and the spread of OPG values that has been shown by the studies.

Diarrhea, with or without bleeding according to the parasite species and some other variables, is among the most typical clinical findings in coccidiosis (Levine 1985; Rommel et al. 2000). Even though no definite finding to suggest clinical coccidiosis was found in our studies, a correlation between diarrhea in the pens and disease prevalence could be shown; the presence of diarrhea, with or without bleeding, increased the probability of diagnosing the disease. On the other hand, disease

Table 3 Information on presence of diarrhea, fecal blood and results of oocysts per gram of feces examination of the samples in the farms

The farms which have;	*n* (%)	Mean age of farms (min–max)	Oocyst-positive farms (%)	Mean age of positive farms (min–max)	Mean OPG of positive farms (±SEM) (min–max)
Diarrhea	305 (42.8%)	30.4 (13–47)	220 (72.1%)[a]	32.8 (14–47)	37,553.2[d] (±6,100.8) (50–952,000)
Blood in stool	284 (39.9%)	30.6 (13–48)	195 (68.7%)[ab]	33.1 (14–48)	36,830.8[d] (±6,975.0) (50–952,000)
Diarrhea without blood	121 (17.0%)	28.6 (13–44)	85 (70.2%)[a]	25.1 (13–41)	28,554.0[d] (±4,579.4) (100–223,000)
Blood without diarrhea	100 (14.0%)	28.8 (13–48)	60 (60.0%)[ab]	33.0 (17–48)	22,456.7[d] (±7,330.2) (50–436,000)
Diarrhea and blood	184 (25.8%)	31.6 (14–47)	135 (73.4%)[a]	33.1 (14–47)	43,219.3[d] (±9,499.1) (50–952,000)
No diarrhea and no blood	307 (43.1%)	27.3 (13–50)	169 (55.0%)[bc]	31.1 (13–50)	48,552.8[d] (±7,862.8) (50–812,000)

Different lowercase letters in the same column indicate significant differences at $p<0.001$

prevalence was lower in facilities with fecal blood in the absence of diarrhea as compared to those with diarrhea, but higher than in those who had neither diarrhea nor bleeding. The lowest mean OPG was in pens with bleeding without diarrhea but a statistically meaningful correlation could not be established. Even though this shows that fecal blood per se has no definite relationship with clinical coccidiosis, the interpretation of the findings concerning OPG values, on the other hand, needs more detailed studies.

The highest prevalence figures in the evaluation based on macroscopic examination of the feces were found in pens with neither diarrhea nor fecal blood. While this finding is consistent with the general characteristics of coccidiosis, the highest OPG value was also obtained in this same group of facilities. The picture suggests that oocyst production could be higher in cases with hidden coccidiosis with no signs at all, including fecal morphology. It has been indicated in published studies that the infection dose and disease intensity, when higher than a given threshold, could inversely influence oocyst generation in the host. Such effect could be related with crowding effect and the development of immunity (Schnitzler and Shirley 1999; Williams 2001; Kar et al. 2010).

It has been reported that the prevalence of coccidiosis in broiler breeding in the same country can show variations among the different regions or even from one year to another (Haug et al. 2008). The character of coccidiosis can vary according to differences in climate (Haug et al. 2008). It can also be influenced by seasonal variations within the same area (Graat et al. 1998; Etuk et al. 2004). In our study, however, no correlation could be shown between the annual rain or temperature averages in given areas with the prevalence of coccidiosis or the fecal OPG values. Our study could indicate that the most important regional factor directly correlated with the mentioned parameters is the density of broiler breeding in the region. The presence of oocysts and constant contagion in areas and farms where more intensive and long-duration broiler breeding has been ongoing could be logically imagined. The fact that chickens are generally infected in the very first week of life indicates that the source could be chicken breeding itself.

This study has shown that subclinical coccidiosis plays a highly significant role in broiler breeding in Turkey, and that there is a direct and proportional relationship between coccidiosis and the density of chicken breeding in a given area. It also showed that the presence of fecal blood should not be confused with hemorrhagic diarrhea due to clinical coccidiosis, while the presence of diarrhea in the farms could provide an important clue to the presence of disease.

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