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ORIGINAL RESEARCH

Seasonal prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan

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Abstract The present paper reports the prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan to determine the occurrence of different species of *Eimeria* in the area and to assess their correlation with the environmental conditions including temperature, humidity and rainfall. The study was conducted from January 2009 to December 2010. Examination of chicken guts revealed 43.89% (n=3,283/7,480) prevalence of coccidiosis. The highest prevalence (27.04%) was recorded of *Eimeria tenella* followed by *Eimeria maxima* (22.42%), *Eimeria acervulina* (19.89%) and *Eimeria necatrix* (4.02%). The prevalence of disease was significantly higher (P<0.05) in autumn (60.02±4.38) followed by summer (47.42±2.92), spring (36.92±2.27) and winter (29.89±3.43). Likewise, prevalence of different species of *Eimeria* also varied (P<0.05) in different seasons of the year except that of *E. necatrix*. A strong correlation (P<0.05) of environmental conditions with the overall and species-wise prevalence of coccidiosis was recorded except *E. necatrix*.

Keywords Coccidiosis · Prevalence · Broilers · Pakistan

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Introduction

Coccidiosis is one of the most common and important diseases that has negative impact on the growth of poultry industry. Poor management practices such as damp litter promoting oocyst sporulation, contaminated feeders and drinkers, poor ventilation facilities and high stocking densities can aggravate the clinical infection (Khan et al. 2006). All the important species of genus *Eimeria* have worldwide distribution. *Eimeria acervulina* and *Eimeria maxima* are the most prevalent, and *Eimeria tenella* is the most common of the highly pathogenic species (Jordan and Pattison 1996). The disease directly influences the production potential of infected chickens due to high mortality, retarded growth and poor feed conversion ratio, causing heavy economic losses up to three billion US dollars annually worldwide (Williams 1999; Dalloul and Lillehoj 2006). On the other hand, in sub-clinical form, it may render the birds immunocompromised and that paves way to secondary disease conditions (Kabell et al. 2006). Thus, management of coccidiosis and maintenance of immune functions for maximum performance, growth and production in poultry industry are primary requirements for profitable farming. Coccidiosis in poultry is becoming more prevalent due to the development of resistant strains against the available anticoccidial agents, being commonly used to avoid or treat the coccidial infection. There is intense need for designing the prevalence surveys to define and quantify the disease burden in broilers. This will help the researchers/clinicians to devise new ways and methodologies for effective control of disease and poultry farmers to adopt in time improved chemo- or immuno-prophylactic approaches to prevent the disease and subsequent production losses associated with the coccidiosis.

The present study was designed to determine the prevalence of coccidiosis in Faisalabad, Punjab, Pakistan

in different seasons of the year and its correlation with different geo-climatic conditions.

Materials and methods

The study was carried out from January 1, 2009 to December 31, 2010 in Faisalabad district, Punjab, Pakistan.

Sampling

A total of 7,480 chicken guts were collected from the poultry sale points and natural outbreak cases of poultry farms in district Faisalabad, Punjab, Pakistan. The samples were collected on alternate days throughout the year to rule out the correlation of the prevalence of disease with a particular season (Table 1). Meteorological data presented in the present study were obtained from the meteorological laboratory of Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan. All the collected guts were brought and investigated at the Immunoparasitology Laboratory, University of Agriculture, Faisalabad, Punjab, Pakistan.

Parasitological examination and techniques

All the guts were cut into different portions (duodenum, jejunum, ilium, large intestine and caeca) with the help of a sharp scissor, and the gut contents of respective portions were examined microscopically (under ×10 and ×40 objective lens) to reveal the presence of coccidial oocysts as described earlier (Soulsby 1982). The results were recorded for the presence or absence of specific species in each gut. Mucosae of the intestines were also examined for gross lesions. A series of scrapings of the duodenum and jejunum were obtained for diagnosis of the subclinical coccidiosis according to the method described by Mattiello (1990). A sample was considered to be negative if three slides from the same sample were observed with no oocysts.

Gut contents of different portions found to be positive for coccidial species were removed and processed for the collection and sporulation of oocysts in separate Petri dishes as described earlier (Reid and Long 1979) with minor modification (Awais et al. 2011). Sporulated oocysts were washed thrice with phosphate-buffered saline and subjected to morphometric analysis by microscopic examination and different species of genus *Eimeria* were identified on the basis of their predilection site, morphology and size (Anonymous 1984).

To confirm the authenticity of different species identified based upon their size and morphology, sporulated oocysts of four different species were administered in 21-day-old broiler chickens (n=60) divided in four different groups each containing 15 chickens. Chickens in all the groups were monitored post-challenge, and on every day from the sixth to the eighth, five chickens from each group were euthanized and examined for species-specific lesions developed and microscopy in respective groups. Lesions on specific predilection sites by specific species (Soulsby 1982) were considered as a confirmation of that species, identified earlier by morphometry.

Statistical analysis

The data thus obtained were subjected to statistical analysis using SAS software (SAS Institute Inc. 2004). The prevalence was calculated as the number of guts found positive for *Eimeria* spp. divided by the total number of guts examined and subjected to two factor complete randomised design and least significant difference to assess the statistical significance. The Pearson correlation coefficient was used to assess the relationship between *Eimeria* spp. and environmental conditions viz. temperature, relative humidity and rainfall.

Results

Four *Eimeria* spp. (*E. tenella, E. maxima, E. acervulina* and *Eimeria necatrix*) were identified in the guts collected from poultry sale points and natural outbreak cases in Faisalabad, Punjab, Pakistan. Examination of chicken guts revealed 43.89% (n=3,283/7,480) prevalence of coccidiosis. The highest prevalence (27.04%) was recorded of *E. tenella* followed by *E. maxima*, *E. acervulina* and *E. necatrix*,

Table 1 Division of the solar year in different seasons with their temperatures, relative humidities and precipitations

Data presented in the table was obtained from meteorological laboratory, Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan

Season	Months	Temperature (°C)		Relative humidity (%)		Rainfall
	From–to	(Min–max)	Mean	(5 p.m.–8 a.m.)	Mean	(mm)
Spring	16 Feb–15 April	13.4–26.2	19.8	45.3–78.1	61.7	10.16
Summer	16 Apr–15 Sept	24.2–40.6	32.4	42.6–62.9	52.75	38.06
Autumn	16 Sept–15 Nov	19.2–31.6	25.4	45.9–80.1	63.0	15.13
Winter	16 Nov–15 Feb	6.6–22.4	14.5	52.4–84.6	68.5	4.4

Table 2 Overall and species-wise prevalence of *Eimeria* among 7,480 guts examined in the year 2009–2010 in Faisalabad district, Punjab, Pakistan

Eimeria spp.	Positive guts	Prevalence (%)
Overall prevalence	3,283	43.89
E. tenella	2,023	27.04
E. maxima	1,677	22.42
E. acervulina	1,488	19.89
E. necatrix	301	4.02

respectively (Table 2). In most of the cases (>80%), guts were found to be infected with more than one species of *Eimeria*.

Samples were collected throughout the year to find out the correlation of occurrence of disease in a particular season with distinct environmental conditions, i.e. temperature, humidity and precipitation/rainfall. Results showed that overall prevalence of disease was higher ($P<0.05$) in autumn (60.02±4.38) compared with summer, spring and winter. There was, however, no difference ($P>0.05$) in

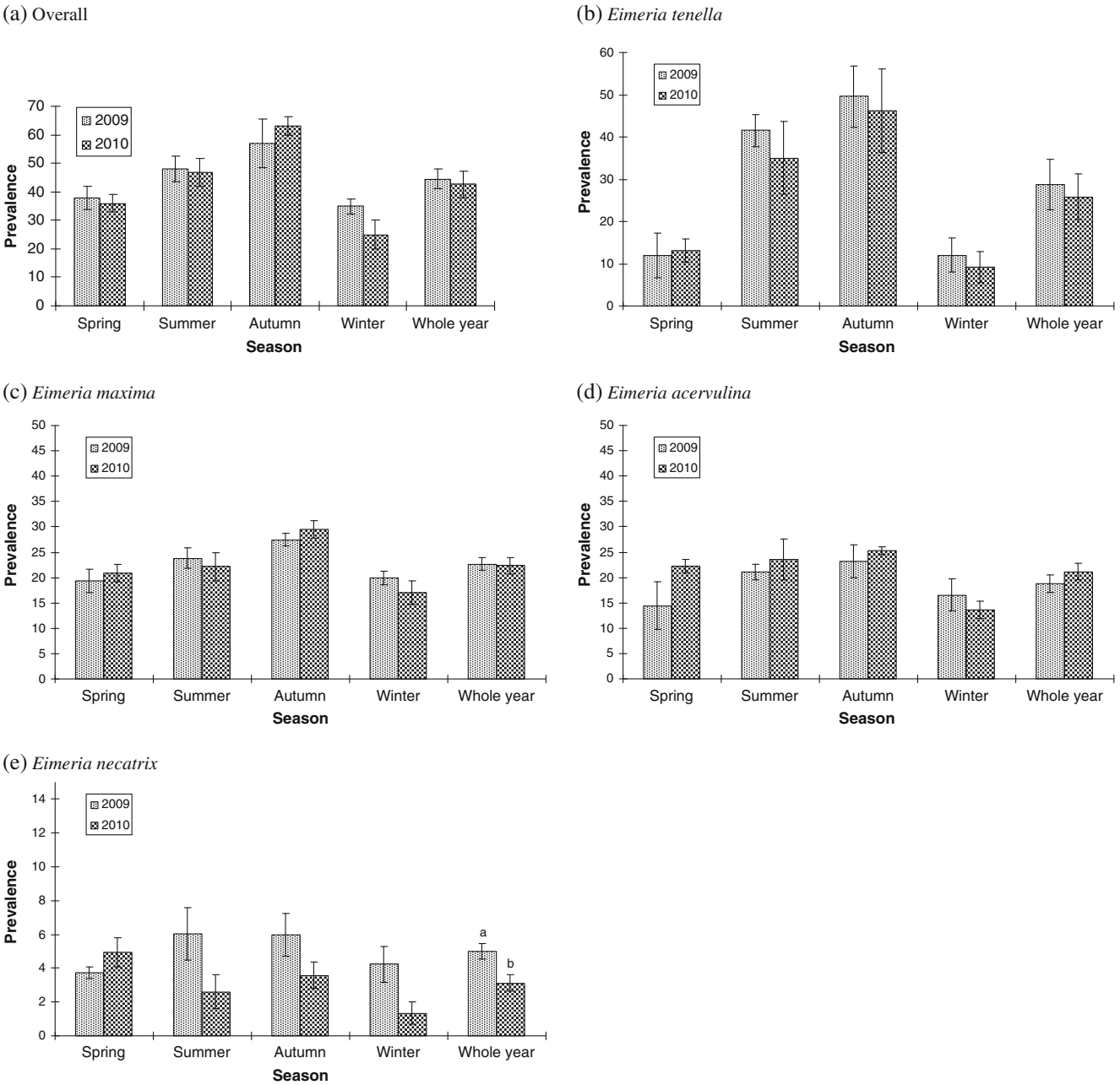

Fig. 1 **a–e** Seasonal prevalence of coccidiosis in broiler chickens in Faisalabad, Punjab, Pakistan. *Bars* present the prevalence (mean±SE) of *Eimeria* spp. in different seasons of the year for 2009 and 2010

prevalence between winter and spring (Fig. 1). The prevalence of *E. tenella* was higher ($P<0.05$) in autumn and summer compared with spring and winter having least prevalence (Fig. 1a–e).

E. maxima was found to be highly prevalent in autumn (28.50±1.06) followed by summer (22.98±1.57) and spring (20.14±1.33). Percent prevalence of *E. maxima* in spring was simultaneously found to be statistically non-significant ($P>0.05$) both with the summer and winter seasons, whereas difference was statistically significant ($P<0.05$) between summer and winter (Fig. 1a–e).

The prevalence of *E. acervulina* was significantly higher ($P<0.05$) in summer (24.19±1.58) and autumn (22.35±1.98) as compared to winter (15.09±1.71), although prevalence in spring (18.33±1.2.79) was statistically similar ($P>0.05$) to those in all other three seasons (Fig. 1a–e).

E. necatrix, with least prevalence rate (4.02%) as compared to all other species identified in the present study was highly prevalent in autumn (4.78±0.70), spring (4.35±0.50) and summer (4.32±0.87) seasons and the difference was non-significant ($P>0.05$) among these three seasons (Fig. 1a–e).

The prevalence of *Eimeria* spp. in the two consecutive years of study (2009 and 2010) was statistically non-significant ($P>0.05$) except *E. necatrix* which showed higher ($P<0.05$) prevalence in year 2009 as compared to year 2010.

Moreover, Pearson correlation coefficients revealed that temperature had a highly significant correlation ($P<0.01$) with the prevalence of *E. tenella* and significant ($P<0.05$) with overall, *E. maxima* and *E. acervulina's* prevalence, whereas no such correlation was found between the prevalence of *E. necatrix* and temperature. With respect to relative humidity, overall and prevalence of *E. tenella* had a highly significant ($P<0.01$) correlation with it, whereas prevalence of *E. maxima* and *E. acervulina* had a significant correlation with relative humidity at a level of $P<0.05$. On the other hand, prevalence *E. necatrix* was independent from this environmental factor. Rainfall was observed for highly significant ($P<0.01$) effect on the overall and *E. tenella* prevalence and significant ($P<0.05$) for *E. acervulina and E. maxima* whereas *E. necatrix* was independent of rainfall for its occurrence (Table 3).

Table 3 Pearson's correlation coefficients for interaction between environmental factors and *Eimeria* spp.

Eimeria spp.	Temperature (°C) (25–35)	Relative humidity (%) (60–80)	Rainfall (mm) (20–40)
Overall prevalence	0.733*	0.578**	0.616**
E. tenella	0.760**	0.541**	0.651**
E. maxima	0.513*	0.422*	0.468*
E. acervulina	0.482*	0.185*	0.479*
E. necatrix	0.381	0.285	0.047

*$P<0.05$ significance; **$P<0.01$ highly significant correlation between the prevalence of different *Eimeria* spp. and environmental conditions

Discussion

Seven different species of *Eimeria* (*E. tenella, E. maxima, E. acervulina, Eimeria mitis, Eimeria brunetti, Eimeria mivati* and *E. necatrix*) have been reported worldwide to cause coccidiosis in chickens (Soulsby 1982; Al-Natour et al. 2002). In the current study, four species of *Eimeria* viz. *E. tenella, E. maxima, E. acervulina* and *E. necatrix* were identified in the industrial broiler chickens in district Faisalabad, Punjab, Pakistan. These results are in conformity with the reports from Iran (Nematollahi et al. 2009), Jordan (Al-Natour et al. 2002), Argentina (McDougald et al. 1997), France (Williams et al. 1996) and Sweden (Thebo et al. 1998) except *E. brunetti and E. mitis* suggesting that these species of *Eimeria* are widespread in most of the countries of the world where poultry industry is a commercial enterprises.

The prevalence of coccidiosis (43.89%) in the present study was higher as compared to the reports of previous studies conducted in Faisalabad, Pakistan, e.g. 10.2% (Dar and Anwar 1981), 30% (Hayat and Hayat 1983), 7.23% (Siddique et al. 1987), 15% (Anjum 1990), 26.3% (Khan et al. 1990) and 37.95% (Ayaz et al. 2003). This higher prevalence might be due to the development of resistance to chemicals used against coccidiosis (Abbas et al. 2011; Usman et al. 2011). On the other hand, Khan et al. (2006) reported the higher prevalence (71.86%) of coccidiosis in poultry birds of Rawalpindi/Islamabad, Pakistan as compared to the results of current study. Prevalence rates of different species were also higher in the findings of Khan and his colleagues who reported *E. tenella* (30.62%), *E. maxima* (34.10%), *E. mitis* (13.95%) and *E. necatrix* (7.75%). This higher prevalence of coccidiosis in Islamabad as compared to Faisalabad may be due to difference in geo-climatic conditions of these two distinct regions of Pakistan as prevalence of coccidiosis is directly related to the environmental conditions that may vary in different geographical regions (Haug et al. 2008; Kaingu et al. 2010). Regional differences in prevalence have also been described previously in other countries (Oikawa et al. 1979; Braunius 1988).

In the present study, data showed that coccidiosis was more prevalent in autumn (60.02±4.38) followed by summer (47.42±2.92), spring (36.92±2.27) and winter (29.89±3.43), and this prevalence pattern of the disease may be correlated with the fact that ambient temperature

(25°C) and relatively higher humidity (>60%) favour the disease by promoting the oocyst sporulation and survivability (Anderson et al. 1976; Razmi and Kalideri 2000). In the autumn, relatively higher humidity (45.9–80.1%) and ambient temperature (mean=25.4°C) might be responsible for increased sporulation and thus high prevalence of disease in this season. Heavy rainfall and afterwards evaporation due to high temperature in the end of summer (season before autumn) are considered to be responsible for high humidity in autumn. In summer, prevalence of disease was lower in the starting months, i.e. from April to mid July (35–40.6°C temperature and 42.5–58.6% relative humidity) that might be due to unfavourable climatic conditions but afterwards prevalence of disease rose up in the rainy months of summer from mid July to September (24–30.2°C temperature and 60–68% relative humidity) when there was heavy rainfall that favoured the developmental stages of coccidial life cycle (Rodríguez-Vivas et al. 1996). Dar and Anwar (1981) and Khan et al. (2006) also found higher prevalence of coccidiosis in the months of the year with higher level of relative humidity. Braunius (1986) and Graat et al. (1998) found coccidial infections to occur more often in autumn with high humidity in Netherlands. Pearson correlation coefficients calculated from study data also exacerbated the previous findings (Razmi and Kalideri 2000; Khan et al. 2006; Haug et al. 2008). According to Pearson correlation coefficients, although environmental factor had individually positive impact on the occurrence of disease, results showed that higher prevalence of diseases was the result of the combined effect of high level relative humidity and ambient temperature, and this was in agreement to the findings of Anderson et al. (1976). Similarly, in the present study, results showed that in spite of high relative humidity in winter, disease showed low prevalence rate that might be attributed to unfavourable temperature (6.6–22.4°C) which is unsuitable for sporulation. Moreover, relative humidity and rainfall are directly proportional to each other, as higher rainfall results in high moisture level in air and thus relative humidity. Furthermore, in developing countries like Pakistan where poultry birds are mostly reared in open sheds, rainfall is a major cause of litter damping that also contributes to sporulation of coccidial oocysts (Nematollahi et al. 2009). These results revealed that ambient temperature in combination with relatively higher humidity and rainfall contributes to the high prevalence rates of disease.

From the results of the study, it was concluded that coccidiosis is highly prevalent in the broiler chickens of Faisalabad, Punjab, Pakistan and *E. tenella* was the most prevalent species. Efforts should be made towards educating the poultry farmers for the effective control of coccidiosis in broiler farms through good management practices and use of appropriate prophylactic or therapeutic anticoccidial drugs. It is also suggested to vaccinate the broiler flocks in the months of July to November during which the prevalence of coccidiosis is higher. Moreover, detailed studies on the countrywide prevalence of coccidiosis are needed to know the present status of disease in Pakistan.

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