RESEARCH ARTICLE

Existence of Coccidiosis and Associated Risk Factors in Broiler Chickens in Southern Punjab, Pakistan

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A B S T R A C T

The present study was carried out to determine the prevalence of coccidiosis in broiler chickens in district Muzaffargarh-Punjab-Pakistan. Out of 500 gut samples examined, during the months of July 2009 to June 2010, the overall prevalence found was 65%. Two hundred fecal droppings from different broiler farms were also investigated during the same period. The prevalence of coccidial infection among adult broiler chickens was 30.18% and among young broiler chickens was 65.95%. Four species of *Eimeria*; *Eimeria tenella* (40.92%), *Eimeria maxima* (31.38%), *Eimeria mitis* (18.15%) and *Eimeria necatrix* (9.53%) were recorded. The prevalence of *Eimeria* infections was highest in the month of September (73.68%), while lowest during April (47.83%). The prevalence of coccidiosis was higher at the farms where the management practices were not satisfactory particularly keeping the litter dry. A strong correlation was found between the prevalence of coccidiosis and age of the chickens.

INTRODUCTION

Poultry industry is one of the most important livestock sectors in Pakistan and is expanding very rapidly particularly for the last decade (Ahad *et al*., 2013). This sector has played a major role in poverty alleviation particularly in the rural areas by generating direct or indirect employment for both male and female community (Islam *et al*., 2012). Poultry meat is contributing about 19% of the total meat production in the country and is providing affordable production of good quality nutritious animal protein. However, intensive poultry production systems are still facing many infectious problems like coccidiosis which have adverse effects on their performance and creating hurdles to get maximum production (Abbas *et al*., 2012a; Sharma *et al*., 2013; Zhang *et al*., 2013; Zhang *et al*., 2014; Zhuang *et al*., 2014). Epidemiological studies have established the economic importance of coccidiosis as a major parasitic disease of poultry in Pakistan (Ayaz *et al*., 2003; Ullah *et al*., 2014).

Avian coccidiosis is a protozoan disease which is caused by genus *Eimeria* localizing in the different parts of the intestine depending on its species. Therefore, this disease is mainly associated in causing intestinal lesions, bloody diarrhea, poor growth performance and severe infections may lead to death (Julie, 1999). According to an economic analysis, United States poultry production is facing about US$127 million annual losses because of coccidiosis (Chapman, 2009). Although in Pakistan estimates are not available; however, same proportionate of economic losses can be expected. Therefore, coccidiosis is considered one of the most expensive diseases (Zaman *et al*., 2012). Furthermore, it is the most consistently reported infectious disease in poultry because *Eimeria* species are capable to survive for long time in infected birds and the environment. In Pakistan, like other parts of the world, control of coccidiosis mainly depends on the application of anticoccidial feed additives, but it is development of drug resistance (Abbas *et al*., 2011), public's awareness about adverse effects of drug residues and proposals of EU commissions to discourage the use of

chemicals and encouraging to shift on alternates to drugs has forced the poultry scientists to find out alternative strategies for controlling avian coccidiosis (Abbas *et al*., 2010, 2012b; Masood *et al*., 2013; Sindhu *et al*., 2014). The approaches like timely diagnosis, as well as knowledge about the epidemiology and intensity of the infection play a vital role for carrying successful and effective control programs (McDougald, 2003).

In Pakistan as a result of increasing growth in broiler production during the last decade, evidenced by increase in the number of poultry farms, a lot of research has been conducted on the experimental infections and anticoccidial drug trials to achieve better control of coccidiosis (Zaman *et al*., 2012). However, epidemiology based studies to find out infection intensity, prevalent infections and frequency levels of different *Eimeria* species in broiler flocks are few and sporadic (Ayaz *et al*., 2003) which could not reflect the *Eimeria* epidemiology in other parts of the country because of variations in husbandry practices, production systems, sample materials, sampling periods, sampling methodologies and control strategies used. Therefore, there is need of more knowledge about the epidemiological aspects of *Eimeria* infections in different geographical territories of Pakistan. This study mainly aimed to get information about the different epidemiological aspects of *Eimeria* species in Muzaffargarh, one of the districts of lower Punjab, which has been among the less privileged areas of Punjab province.

MATERIALS AND METHODS

Study area: Muzaffargarh is a district in the south of the Punjab province of Pakistan. It is spread over an area of 8,249 km². District Muzaffargarh lies in the form of strip between the river Chenab and Indus, which passes along the Eastern and Western boundaries respectively of the district and form a triangle at Alipur Tehsil of the district. District Muzaffargarh has extreme hot and cold climate throughout its area. A large area of the district consists of sand dunes and barren land. Rural households keep few buffaloes, cows, sheep, goats and local chickens.

Sample size estimation: Two stage cluster sampling was done taking union councils as primary units and poultry farms as secondary units. Sample size was estimated by formulae as described by Thursfield (2005). Among 109 union councils of district Muzaffargarh, 47 union councils were screened. Both primary and secondary units were selected randomly by random number table. A total of 500 gut samples of broiler chickens were collected from different poultry farms in district Muzaffargarh from July, 2009 to June, 2010.

Development of questionnaire: A questionnaire was designed for collecting information regarding potential risk factors associated positively or negatively with prevalence of parasitism in poultry. Information regarding age, sex, area, season and management practices like watering methods, feeding methods, nature of litter, frequency of change of litter; were collected through predesigned questionnaire using closed ended and open ended questions. Birds of age 3-4 weeks were categorized

as young while those of age 6 weeks and above were considered as adults.

Parasitological examination: Samples collected were brought to Poultry Production Lab Muzaffargarh. All gut samples were opened and their contents were collected in beaker separately. These contents were macerated overnight in potassium dichromate solution at 37°C. The suspension was filtered through a muslin cloth and allowed to sediment. The supernatant was discarded and the oocysts in the sediment were separated by floatation method in saturated sodium chloride solution. The isolated oocysts were washed to remove salt solution and for the identification of different species they were subjected to micrometry for the measurement of their length (Levine, 1985). For this purpose, ocular micrometer was adjusted at 400x magnification. Fifty oocysts from each positive sample were measured for the identification of *Eimeria* species. The different Eimeria species were identified on the basis of shape and size of sporocysts and sporozoites according to the method described by Levine (1985). The litter samples were also collected from the farms (n=200) to ascertain the source of infection. These samples were processed for isolates of *Eimeria* species by the method described by Levine (1985).

Data analysis: The prevalence of coccidiosis among different seasons and age groups was calculated; furthermore, the prevalence of different *Eimeria* species was also calculated. Data generated were analyzed using descriptive statistics with emphasis on percentages.

RESULTS AND DISCUSSION

Out of 500 gut samples examined, 65% guts were found infective (Table 1). The present study revealed moderately low prevalence rate of coccidiosis in broiler chickens in Muzaffargarh District as compared to previous report (71.8%) on coccidiosis from Rawalpindi District (Khan *et al*., 2006). This difference could be due the existence of more humid weather in District Rawalpindi-Punjab-Pakistan. Because, it is well known fact that humidity plays a vital role for the sporulation of the coccidial oocysts (Haug *et al*., 2008; Bachaya *et al*., 2012). However, the prevalence of coccidiosis in the present study was much higher compared to a previous study (37.9%) conducted in 2000-2001 in District Faisalabad-Punjab-Pakistan (Ayaz *et al*., 2003). After one decade, the higher prevalence of coccidiosis could be due to the development of drug resistance in coccidian species resulting from their frequent and irrational use (Abbas *et al*., 2011). In Pakistan, most of the poultry farmers use salinomycin sodium as anticoccidial drug for the control of avian coccidiosis, and due to its frequent use for a long time resistance could have developed (Abbas *et al*., 2011; Györke *et al*., 2013).

The disease was observed all around the year (Table 1). Among different months, the maximum prevalence of coccidiosis was observed during the months of September (73.7%) and October (73.3%). This may be due to the high level of humidity during these months of the year. Figure 1 is showing the highest prevalence in September
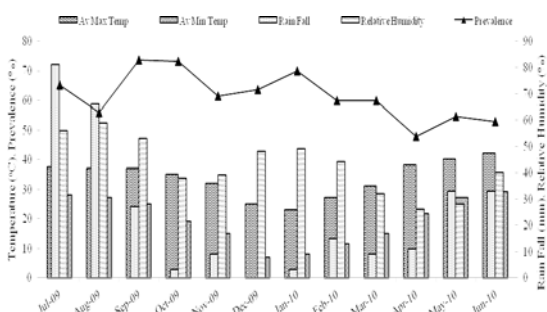
Table 1: Different *Eimeria* species identified and their percentage intensity in broiler chickens

Months	No. of gut samples examined	Total Infected gut samples (No.)	Total Infected gut samples (%age)	*E. maxima* No.	%	*E. tenella* No.	%	*E. mitis* No.	%	*E. necatrix* No.	%
July-9	23	15	65.2	4	26.7	7	46.5	4	26.7	0	0
Aug	25	14	56	3	21.4	4	28.6	4	28.6	3	21.4
Sep	95	70	73.7	22	31.4	25	35.7	13	18.6	10	14.3
Oct	75	55	73.3	15	27.3	30	54.5	7	12.7	3	5.5
Nov	65	40	61.5	17	42.5	15	37.5	5	12.5	3	7.5
Dec	55	35	63.6	9	25.7	16	45.7	8	22.9	2	5.7
Jan-0	40	28	70	9	32.1	11	39.3	3	10.7	5	17.9
Feb	35	21	60	9	42.9	7	33.3	2	9.5	3	14.8
March	25	15	60	4	26.7	6	40	3	20	2	13.3
April	23	11	47.8	4	36.4	4	36.4	3	27.3	0	0
May	22	12	54.6	3	25	4	33.3	5	41.7	0	0
June	17	9	52.9	3	33.3	4	44.4	2	22.2	0	0
Total	500	325	65	102	31.4	133	40.9	59	18.2	31	9.5

Table 2: Prevalence of coccidial infection in broiler chickens of different age groups

No. of fecal samples examined	Young broiler chicken Samples examined	Infected No.	%age	Adult broiler chicken Samples examined	Infected No.	%age
35	15	10	66.7	20	8	40
55	25	18	72	30	10	33.3
20	8	4	50	12	6	50
15	10	7	70	5	2	40
25	10	4	40	15	7	46.7
20	13	9	69.2	7	3	42.9
30	13	10	76.9	17	7	41.2
200	94	62	66	106	32	30.2

Young (3-4 weeks) and Adult (6 weeks and above)

Fig. 1: Association of meteorological factors with prevalence of coccidiosis in poultry of district Muzaffargarh

with higher relative humidity. Highest humidity in August helped sporulation of oocysts which ultimately resulted in peak prevalence during subsequent months (i.e. September and October). Another peak in prevalence was recorded during months of December and January after a slight decline in November. This decline in prevalence coincides with decrease in humidity during this period. Higher prevalence during months of September, October and December, January seems to be associated with higher relative humidity during these months.

These results were in consonance with some of the previous reports (Khan *et al*., 2006) who also reported the maximum occurrence of coccidiosis during these months due to high humidity. However, in contrast to the present study, Awais *et al*. (2012) reported higher prevalence of coccidiosis in autumn season in District Faisalabad-Pakistan. Among different species of *Eimeria* (Table 1), *Eimeria tenella* showed the highest prevalence rate (40.9%), followed by *Eimeria maxima* (31.4%), *Eimeria mitis* (18.2%) and *Eimeria necatrix* (9.5%) in broilers chicken. These findings were in line with previous studies (Ayaz *et al*., 2003; Awais *et al*., 2012), who also reported the highest prevalence of *E. tenella* (50%) in

broiler chickens of District Faisalabad-Punjab-Pakistan. Likewise, *E. tenella* has also been reported the most prevalent species in Iran (Hamidinejat *et al*., 2010). The results obtained in the present study associated with the four species of *Eimeria* support the findings of Khan *et al*. (2006) who identified the *E. maxima*, *E. tenella*, *E. mitis* and *E. necatrix* from poultry litter, but Williams (1995) reported the presence of at least six species of *Eimeria* (e.g., *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunette*, *E. mitis* and *E. praecox*) in the litter from single flock during its first six weeks. It has already been reported that *Eimeria* species and their prevalence vary greatly within the different geographical areas (Chapman, 1997; Györke *et al*., 2013; Zhang *et al*., 2013). The prevalence of coccidial infection among adult broiler chickens was lower compared to young broiler chickens (Table 2). These results were in agreement with the report of Muazu *et al*. (2008) who stated that the prevalence of coccidial infection among adult birds was 36.7% and among young birds was 52.9%. The results obtained in the present study also support the report of Julie (1999) that all ages of poultry are susceptible to infection, but usually resolve itself around 6-8 weeks of age. The results obtained in this study in association with age also support the findings of Etuk *et al*. (2004) that younger birds are more susceptible to infection than older birds.

The overall results of the present study indicate the higher prevalence of coccidiosis in broiler chickens. The reason could be that the coccidial oocysts are ubiquitous and are easily disseminated in the poultry house environment. Further, owing to their high reproduction potential, it is very difficult to keep chickens coccidia free, especially under current intensive rearing conditions (Chapman, 2014). Furthermore, poor management such as wet litter that encourages oocyst sporulation, contaminated drinkers and feeders, poor ventilation, and high stocking density can exacerbate the clinical signs

(Ruff, 1993). However, good management practices including good ventilation, dry and clean litter, cleaning and decontamination of drinkers and feeders and proper stocking density in the farm can minimize the onset of coccidiosis (Abbas *et al*., 2011).

The prevalence of coccidiosis was low on the poultry farms of progressive farmers in the study area because they do a thorough cleanout between the flocks. This practice may also help to control the widely spread threat of coccidiosis. Other control measures such as attendants should change cloths between houses, keeping the wild birds out and the rationale use of anticoccidial drugs are good ways for the effective control of coccidiosis.

Conclusion: The poor management at the poultry farm is the main contributory factor for the onset of coccidiosis such as oocyst sporulation occurs in humid environment, therefore, good management practices are the handy tool to minimize the occurrence and spread of coccidiosis.

Author's contribution: RZA and ZI planed, designed and supervised the experiment. HAB and MAR collected and processed samples. TUR and WB helped in the identification of Eimeria species. RH analyzed the data. All authors read and approved the manuscript.

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