

Prevalence and Associated Risk Factors of *Eimeria* in Sheep of Punjab, Pakistan

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Abstract—A cross-sectional study was carried out to determine the prevalence, species characterization and associated risk factors with *Eimeria* (E.) in sheep of district Toba Tek Singh from April, 2009 to March, 2010. Of the total 486 faecal samples examined for *Eimeria*, 209 (43%) were found infected with five species of *Eimeria*. Amongst the identified species of *Eimeria*, *E. ovinoidalis* was the commonest one (48.32%), followed in order by *E. ahsata*, *E. intricata*, *E. parva* and *E. faurei* with prevalence of 45.45, 28.71, 24.40 and 19.14 percent respectively. Peak prevalence was observed in August. Wet season (rainy and post-rainy) was found to be favourable for *Eimeria* infection. Lambs had significantly higher prevalence ($P < 0.05$) of *Eimeria* than adults. Similarly higher prevalence of *Eimeria* was observed in female as compared to male. Among management and husbandry practices; watering system, housing system, floor type and herd size strongly influenced the prevalence of *Eimeria*. Coccidiosis was more prevalent in closed housing system, non-cemented floor type, pond watered animals and larger herds ($P < 0.05$) as compared to open housing system, partially cemented floor type, tap watered animals and smaller herds respectively. Feeding system, breed and body condition of animals were not found as risk factors ($P > 0.05$) influencing prevalence of *Eimeria*.

Keywords—*Eimeria*, Pakistan Prevalence, Sheep

I. INTRODUCTION

COCCIDIOSIS is an economically important disease which is caused by unicellular protozoa, *Eimeria*. It continues to be a serious threat to animal health and results in lowered productivity due to the associated morbidity, mortality, cost of treatment and control measures. Fifteen *Eimeria* species considered to have the capability of infecting sheep are *E. ahsata*, *E. bakuensis*, *E. crandallis*, *E. faurei*, *E. granulosa*, *E. gonzalezi*, *E. gilruthi*, *E. intricata*, *E. marsica*, *E. ovinoidalis*, *E. pallida*, *E. parva*, *E. weybridgensis*, *E. punctata* and *E. gilruthi* [2], [11], [26]. *E. ovinoidalis*, *E. bakuensis* and *E. ahsata* are the most pathogenic species in small ruminants [18], [22]. All ages of sheep are susceptible to *Eimeria* infection but lambs are most severely affected by clinical coccidiosis [3]. It can be a serious clinical problem of lamb rearing, particularly in pre-weaned and recently weaned lambs [3], causing diarrhea [5].

High stock rates increase the environmental contamination with oocysts, consequently increasing the risk of an infection

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and outbreak of clinical coccidiosis [1]. Under normal conditions lambs are born into an oocyst-contaminated environment. Therefore, lambs usually come into contact with the parasite within the first days of life [16]. The main sources of contaminant at that time are the dirty, oocyst-contaminated udders of the ewes. At a later age, the main sources of infections are the uptake of oocysts containing straw from the floor, originating from excreting mothers and older lambs [16]. The production system may therefore play an important role in the development of subclinical and clinical coccidiosis.

Eimeria species have been investigated in different sheep breeds by various researchers in different countries [7], [8], [11], [22] but there is no single report on *Eimeria* in sheep of Pakistan. Identification of *Eimeria* species is important because of differences in pathogenicity. Information of associated risk factors will assist in designing strategies to minimize losses conferred by clinical and subclinical form of the disease. Taking into account the economics of disease and scarcity of literature, present study was planned with objectives: 1). To identify different *Eimeria* species in sheep, 2). To determine risk factors influencing the prevalence of *Eimeria* in sheep population in Pakistan.

II. MATERIALS AND METHODS

A. Study Area

Study area confined to district T.T.Singh which is located in central Punjab between 30°33' to 31°2' degree north latitudes and 72°08' to 72°48' degree longitudes. The district comprises of three tehsils (Gojra, Kamalia and T.T.Singh) and 82 union councils (UC). It occupies an area of 3252 Km², most of which is lowland that floods during the rainy season. The floods originate from the Ravi River that runs along the southern and southeastern borders. According to Pakistan Livestock Survey (2006), T.T.Singh has an average sheep population of 0.50 million. May, June and July are the hottest months of the year with maximum mean temperature of 40.7°C while December and January are the coldest months of the year with minimum mean temperature of 6°C. An average annual rainfall is 254-381 mm.

B. Sampling Units

Based on two stage cluster random sampling, the number of primary units (UC) and elementary units (animals) was sampled using the formulae as given by Thrusfield [25]. Selection of primary units to be sampled was done using map grid method. Four hundred and eighty six animals were examined in the pr

esent survey.

C. Development of Questionnaire

A questionnaire was developed for collecting necessary information from farmers regarding associated risk factors using closed ended (dichotomous and multiple choice) questions [25]. Information regarding following determinants were collected through questionnaire.

1. Age: Animals were divided into two age categories viz; adults and lambs. The age range of adult and lamb was > 6 months and <6 months respectively.

2. Breed: Kajli, Lohani, Thalli and Cholistani were the breeds under study.

3. Sex: Both sexes were sampled during study.

4. Climate: Season wise prevalence was noted separately. The four well-marked seasons in Pakistan are:-

(i) Cold season (December to March)

(ii) Hot season (April to June)

(iii) Rainy season (July to September)

(iv) Post- rainy season (October and November)

5. Husbandry and Management: Type of feeding system (grazing/ ground feeding/ trough feeding), housing system (open/ close), floor pattern (non-cemented/ partially cemented), watering system (tap water/ pond) and herd size (larger/ smaller) were observed in present study. Herds having more than fifty animals were declared as larger herds whereas herds with less than this were considered as smaller.

D. Collection of Samples

A total of 486 faecal samples were collected. Five grams of faecal samples were collected directly from rectum or immediately after defecation in a wide mouth plastic bottle and preserved in 10% formalin [28]. Collecting bottles were labeled properly for further process.

E. Parasitological Examination

Faecal samples were analyzed using floatation technique with saturated Sodium chloride solution as floatation solution for the presence of oocyst. The procedure was adopted as described by Zajac and Conboy [28]. Quantitative fecal examination was performed by McMaster technique to determine the number of oocysts per gram of feces (OPG) as per the procedures of MAFF [14]. Identification of *Eimeria* species was based on the morphological features of the oocysts (size, shape, color, and texture of oocyst wall, presence or absence of micropyle, polar cap) with the aid of taxonomic keys of Iqbal and Soulsby [9], [23].

F. Meteorological Data

Data regarding meteorological parameters i.e. monthly average temperature, relative humidity and rain fall have been collected from Meteorological Cell, Department of Crop Physiology, University of Agriculture, Faisalabad.

G. Statistical Analyses

Logistic analysis was carried out by using logit model including all variables in the model with backward elimination

procedure. Factors with paired characteristics were analyzed using Odd's Ratio (OR) and Mantel-Haenszel (M.H.) Chi-square. Hosmer-Lemeshow goodness-of-fit test indicated that model fits well. All the analyses were carried out using SAS software package (1998) at 95% confidence level.

The prevalence was calculated for all data by using the following formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of infected individuals (n)}}{\text{Total number of sampled individuals (N)}} \times 100$$

III. RESULTS

Overall prevalence of *Eimeria* was 43% (209/486). Five species of *Eimeria* were identified during coprological examination. *E. ovinoidalis* was the commonest of the identified species (48.32%), followed in order by *E. ahsata*, *E. intricata*, *E. parva* and *E. faurei* with prevalence of 45.45, 28.71, 24.4 and 19.14 percent, respectively (Fig.1). Mean OPG was recorded highest in August (5361) which lies in rainy season in study area (Fig.2). Generally lambs were found to have high mean OPG than adults. Seasonal elevation in OPG was more apparent in lambs (Fig.3).

A. Associated Risk Factors

Analysis of all the hypothesized risk factors by stepwise multivariate logistic regression model and M.H. Chi-Square analysis revealed that age, sex, season, housing system, floor, watering system and herd size were the factors significantly associated ($P < 0.05$) with *Eimeria* infection. *Eimeria* was observed in all age groups. Lambs had significantly higher prevalence (50.70%; 108/213; $\chi^2 = 11.225$) than adults (36.99%; 101/273; OR=0.616) (Table 1;2, Fig.4). *Eimeria* infection was found to be more prevalent in females (49.26%; 168/341; $\chi^2 = 18.252$; OR=0.427) as compared to males (28.27%; 41/145) (Table 1;2, Fig.4). Prevalence of *Eimeria* was not different among four breeds studied i.e., Kajli, Lohani, Thalli and Cholistani (Fig. 5). Peak prevalence was observed in August (64.10%) while least number of animals (23.68%) were found infected with *Eimeria* in March (Fig.2). Higher prevalence was recorded during rainy seasons (56.29%) and post-rainy (55.88%) as compared to cold (34.4%) and hot seasons (32.93%). A relationship between high rain fall; temperature; relative humidity and occurrence of *Eimeria* observed during study is illustrated in Fig. 4.

A strong association ($P < 0.05$) was observed between housing system and risk of *Eimeria* infection. Higher *Eimeria* prevalence was recorded in animals reared under closed type of housing system (53.64%; 140/261; $\chi^2 = 9.334$; OR=2.106) as compared to open type (30.67%; 69/225) (Table 1;2, Fig.5). Statistically different rate of prevalence was found in two floor types. *Eimeria* infection was more prevalent ($P < 0.05$) in non-cemented floor type (48.50%; 129/266; $\chi^2 = 10.7054$) as compared to partially cemented floor (36.36%; 80/220; OR=0.634) (Table 1;2, Fig.5). Multivariate logistic regression analysis and M.H. Chi-Square analysis of watering system with the intensity of infection revealed that there was a highly

significant association between these two. Significantly higher infection rate was observed in sheep watered at pond (48.98%; 121/247; $\chi^2=7.322$; OR=1.674), compared to those given tap water (36.82%; 88/239) (Table 1;2, Fig.5). Prevalence of *Eimeria* was strongly predisposed by the herd size ($P<0.05$). Higher prevalence was observed in larger herds (52.73%; 116/220; $\chi^2=15.472$; OR=2.219) as compared to smaller ones (34.96%; 93/266) (Table 1;2, Fig.5).

Although prevalence of *Eimeria* in sheep ($\chi^2=2.677$; grazing=17.5%; ground feeding=56.02%; trough feeding=38.14%) fed under three feeding systems was different being lowest in grazing sheep, followed in order by trough and ground feeding sheep but statistically insignificant association was found with $P=0.1018$. Moreover, *Eimeria* infection in poor body condition animals was a bit higher than animals with good body condition (Table 3) but there was a statistically non-significant association ($P=0.1889$) between body condition of animals and risk of *Eimeria* infection.

IV. DISCUSSION

Several *Eimeria* species have been reported from Europe, America, Africa and Asia [23]. To author's knowledge; this is the first investigation of *Eimeria* species prevalent in sheep of Pakistan. Species of *Eimeria* identified in present survey have already been documented by Kaya [11] and Yakhchali and Golami [26]. *E. ovinoidalis* has been reported as the most prevalent specie by many researchers in various countries [11], [22], [26]. Higher prevalence in lambs in comparison to adults has been reported by Yakhchali and Golami [26] and Sisodia [21]. The observation that young animals excreted higher number of oocysts compared to adults has also been reported elsewhere [19]. Trend to shed more oocysts in lambs in comparison to adults may be due to acquisition of immunity by adults over periods of time which therefore suppress *Eimeria* infection. Ewes were found to be more susceptible to infection in present survey. Yakhchali and Golami [26] reported that gender significantly influences the prevalence of coccidia in sheep. Same results were recorded in a survey of sheep of another region of Iran which was conducted by Yakhchali and Zarei [27]. Breed susceptibility to *Eimeria* infection has been discussed by Biu [6] who reported that there was no significant difference among prevalence of *Eimeria* infection in different sheep breeds.

Post rainy season peak in prevalence of sheep coccidiosis could be attributed to wet, hot and humid climate which is ideal for reproduction of coccidial infection [24], [26]. Spring and summer coccidiosis was rarely observed by Skirnisson [22]. Oocyst count elevation in rainy season reported by Kusiluka [12] is in line with seasonal dynamics of *Eimeria* in present study.

Poor hygienic situations and overcrowded conditions may have resulted in the development of higher level of infection in non-cemented floor, closed housing system and large herds due to greater contamination [10], [13], [23]. Crowding of animals concentrates the hosts and parasites within a restricted

area. McKellar [15] stated that coccidiosis is most commonly prevalent under conditions of poor sanitation and overcrowding. Bauer [4] described that high stock rates increase the environmental contamination with oocysts and therefore the risk of an infection and outbreak of clinical coccidiosis increases. Coccidiosis is seen universally, most commonly in animals housed or confined in small areas contaminated with oocysts as described by McKellar [15]. Higher prevalence in pond watered animals favours the recommendations described by Radostits [17] and McKellar [15] that watering devices should be clean and protected from faecal contamination.

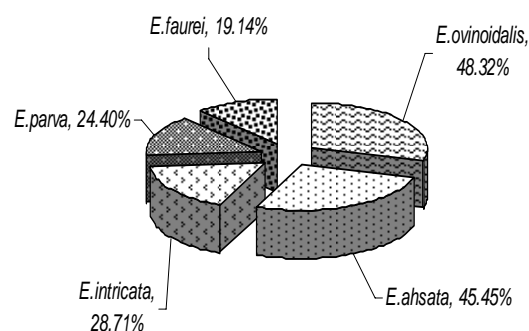


Fig. 1 *Eimeria* species distribution in sheep

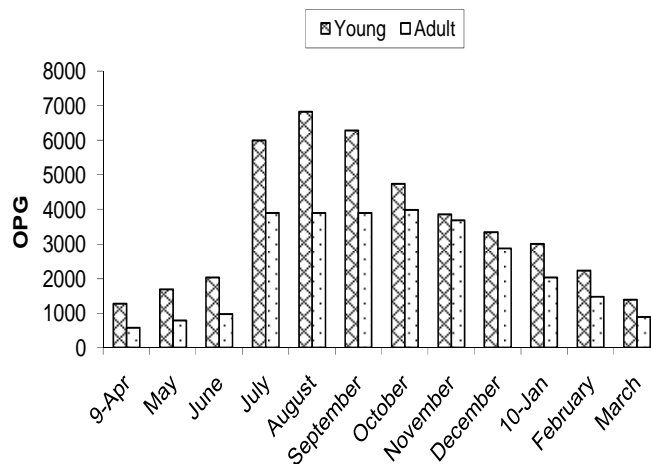


Fig. 2 Month wise prevalence and mean OPG of *Eimeria*

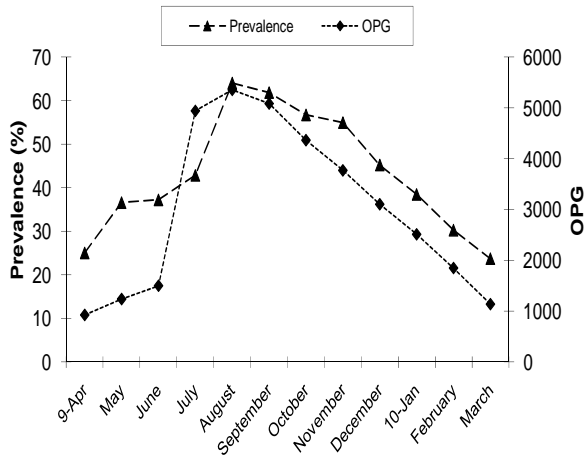


Fig. 3 Month wise mean OPG in young ones and adults

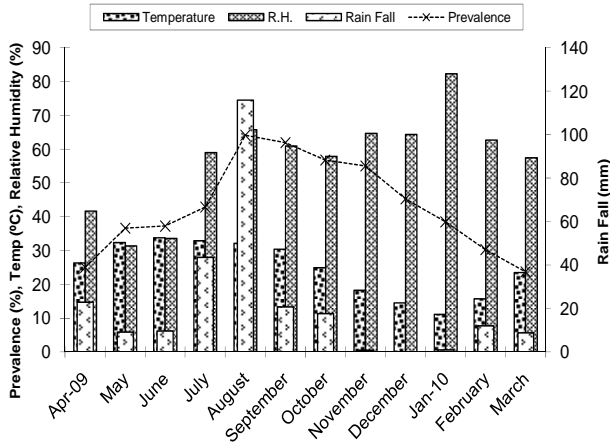


Fig. 4 Association of meteorological parameters with risk of Eimeria in sheep

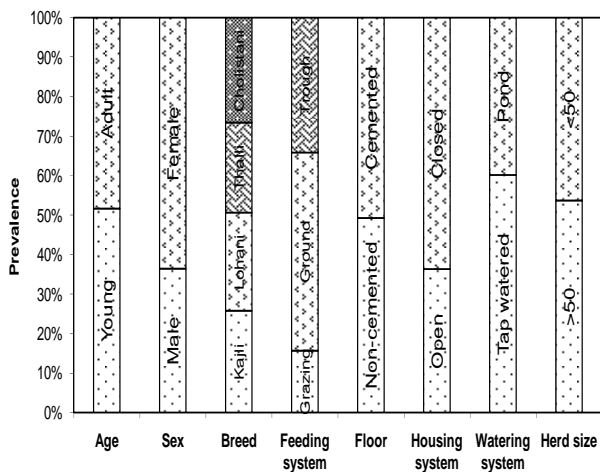


Fig. 5 Association of various risk factors with Eimeria

TABLE I
MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF ASSOCIATED FACTORS WITH RISK OF *EIMERIA* INFECTION IN SHEEP

Term	Odds ratio	C.I.	P- value
Month	1.127	1.063-1.195	0.000
Age	0.616	0.413-0.917	0.017
Sex	0.427	0.273-0.669	0.000
Floor Type	0.634	0.421-0.923	0.000
Housing system	2.106	1.373-3.230	0.001
Watering system	1.674	1.124-2.493	0.011
Herd size	2.219	1.487-3.311	0.000

HOSMER-LEMESHOW GOODNESS-OF-FIT TEST:
P=0.899

TABLE II
MANTEL-HAENSZEL CHI-SQUARE ANALYSIS OF ALL HYPOTHESIZED RISK FACTORS WITH *EIMERIA* INFECTION IN SHEEP

Associated determinants	Variables	Prevalence	Mantel-Haenszel Chi-Square (P-value)
Age	Young	50.70 % (108/213)	11.2239 (0.0008)
	Adult	36.99 % (101/273)	
Sex	Male	28.27 % (41/145)	18.2517 (<.0001)
	Female	49.26 % (168/341)	
Breed	Kajli	43.81 % (46/105)	10.4763 (0.8030)
	Lohani	42.04 % (66/157)	
	Thalli	38.71 % (24/62)	
Feeding system	Grazing	17.5 % (14/80)	2.6773 (0.1018)
	Trough	56.02 % (121/216)	
Floor Type	Non-cemented	48.50 % (129/266)	10.7054 (0.0011)
	Cemented	45.06 % (73/162)	

	Partially cemented	36.36 % (80/220)	
Housing system	Open	30.67 % (69/225)	9.3338 (0.0022)
	Closed	53.64 % (140/261)	
Watering system	Tap water	36.82 % (88/239)	7.3220 (0.0068)
	Pond	48.98 % (121/247)	
Herd size	>50	52.73 % (116/220)	15.4719 (<0.001)
	<50	34.96 % (93/266)	
Body condition	Poor	45.14 % (144/319)	1.7260 (0.1889)
	Good	38.92 % (65/167)	

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