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RESEARCH ARTICLE

Determinants Influencing Prevalence of Coccidiosis in Pakistani Buffaloes

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ABSTRACT

This paper describes a cross-sectional study in buffaloes of district Toba Tek Singh from April, 2009 to March, 2010 which aimed to identify prevalent species of *Eimeria* (E) and risk factors associated with subclinical coccidiosis. Overall prevalence of *Eimeria* in buffaloes was 49.6%. Six species of *Eimeria* were identified in total infected buffaloes. *E. bovis* was the commonest one among the species identified during survey followed in order by *E. zuernii, E. canadensis, E. ellipsoidalis, E. alabamensis* and *E. cylindrica*. Peak prevalence was observed in August. Wet season was found favorable for *Eimeria*. Odds of finding oocysts were higher in young stock and females ($P<0.05$) as compared to those of adults and males respectively. Among management and husbandry practices, five variables were found significantly associated with status. These included housing system, feeding system, watering system, floor type and herd size. Open housing system, trough feeding, tap watering, partially cemented floor type and smaller herds showed protective effect against coccidiosis with Odds of detection being higher in closed housing system, ground feeding, pond watering, non-cemented floor type and larger herd size, respectively. Body condition and breed of buffaloes were not found associated with prevalence of *Eimeria*. Value of feeding in troughs, provision of clean tap water, cementing floor of farms in reducing the extent of infection should be communicated to farmers. Appropriate monitoring and control of the disease is advisable.

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INTRODUCTION

Protozoan diseases are major constraint in progress of dairy farming all over the world, particularly in developing countries (Om *et al*., 2010; Farooq *et al*., 2012). Coccidiosis is one of the most pathogenic intestinal diseases caused by different *Eimeria* species belonging to phylum Apicomplexa. *Eimeria* infections are responsible for huge economic losses to livestock industry in terms of mortality and morbidity particularly in young calves (Nalbantoglu *et al*., 2008). Bloody diarrhea, dehydration, rough hair coat, reduced growth rate, anemia, weakness and weight loss are the signs of coccidiosis in water buffaloes (Bastianetto *et al*., 2007). The disease is present in acute, subacute and chronic forms.

Coccidian species generally have host specificity and in this respect the species of *E. ankarensis, E. azerbaidjanica, E. bareillyi, E. gokaki, E. ovoidalis* and *E. thianethi* have been reported in water buffaloes. Moreover, species found in cattle, such as *E. alabamensis, E. auburnensis, E. bovis, E. brasiliensis, E. bukidnonensis, E. canadensis, E. cylindrica, E. ellipsoidalis, E. subspherica, E. wyomingensis*, and *E. zuernii* have also been reported in the water buffalo (Soulsby, 2006).

Information on water buffaloes parasites particularly on the epidemiology of coccidiosis is very limited and fragmentary in Pakistan. Lot of research has been conducted on coccidiosis in poultry (Ayaz *et al*., 2003; Akhtar *et al*., 2012; Awais *et al*., 2012) but little work has been done on coccidiosis of ruminant in Pakistan (Rehman *et al*., 2011a & b; Mirani *et al*., 2012). Therefore, in preview of significance of the parasite as one of the most important causes of economic losses and the dearth of literature in the country, the present study was designed to determine the prevalence of *Eimeria*

species and associated risk factors with *Eimeria* infections of buffaloes in district Toba Tek Singh (TT Singh), Pakistan.

MATERIALS AND METHODS

Study area and sampling units: District Toba Tek Singh comprised of three tehsils i.e. Gojra, Kamalia and Toba Tek Singh and 82 Union Councils. Buffalo population in the district was 0.44 million (Pakistan Livestock Survey, 2006). The hottest months of the year are May, June and July with maximum mean temperature of 40.7°C while minimum mean temperature of 6°C has been recorded in December and January.

Study was conducted in district Toba Tek Singh. Based on two stage cluster random sampling the number of primary units (Union Council) and elementary units (animals) were sampled using following formulae as given by Thrusfield (2008). Map grid method was used to select primary units to be sampled. Five hundred and eighty five animals were examined in the present survey.

Information regarding association of various factors was gathered by developing and refining questionnaire using closed ended questions (Thrusfield, 2008). Information regarding following determinants were collected through questionnaire.

Animals and their management

Age: Animals were divided into adults and young stock. Adults and young stock were in age range of 5-10 years and 3-5 years, respectively. **Breed:** Breeds under study were Nili Ravi and Kundi. **Sex:** Sampling was done in both sexes. **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). **Husbandry and management:** Regarding husbandry and management practices, variables studied were type of feeding system (ground feeding/trough feeding), housing system (open/close), floor pattern (non-cemented/partially cemented), watering system (tap water/pond) and herd size (larger/smaller). Herds having more than fifty animals were declared as larger herds whereas herds with less than this were considered as smaller.

Collection of samples and parasitological examination: Five hundred and eighty five fecal samples were collected during survey. Fecal samples (5g) were collected in a wide mouth plastic bottle directly from rectum or immediately after defecation and preserved in 10% formalin. Floatation method using saturated Sodium Chloride solution was adopted for coprological examination as described by Rehman *et al*. (2011b). Modified McMaster technique was performed to determine the number of oocysts per gram of feces (OPG) as per the procedures of MAFF (1986). 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 (Soulsby, 2006). Meteorological data have been collected from Meteorological Cell, Department of Crop Physiology, University of Agriculture, Faisalabad.

Statistical analyses: Data obtained were analyzed by multivariate logistic analysis. Odd's Ratio (OR) and Mantel-Haenszel (M.H.) Chi-square were calculated to analyze factors with paired characteristics. 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. Prevalence percentage was calculated.

RESULTS

Overall prevalence of *Eimeria* was 49.6%. Six species of *Eimeira* were identified during coprological examination. *E. bovis* was the commonest of the identified species followed in order *E. zuernii, E. canadensis, E. ellipsoidalis, E. alabamensis* and *E. cylindrica*. The highest mean OPG was observed in August. Generally young stock was found to have higher mean OPG than adults. Seasonal elevation in oocyst count was more apparent in young stock (Fig. 1).

Associated risk factors: Variables identified to be significantly associated with status (coccidiosis) in stepwise multivariate logistic regression model and M.H. Chi-Square analysis included age, sex, season, floor type, housing system, feeding system, watering system and herd size. All age groups showed coccidiosis but negative correlation was estimated between age of animal and incidence of the disease. Young stock had significantly higher prevalence than adults (Fig. 1). Coccidiosis was found to be more prevalent in females as compared to males (Table 1). There were no significant differences among prevalence percentage of *Eimeria* in both breeds (Table 2). Seasonal dynamics study of infection showed a strong positive correlation between month of the year and *Eimeria* infection. Peak prevalence was observed in August while least number of animals was found infected with *Eimeria* in March (Fig. 1). Higher prevalence was recorded during rainy and post-rainy seasons as compared to cold and hot seasons. A relationship between high rain fall; temperature; relative humidity and occurrence of *Eimeria* has been shown in Fig. 2.

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 as compared to open type (Table 2). Cementing floor showed a protective effect with Odds of disease being higher in partially cemented floor as compared to those in non-cemented floor type (Table 1).

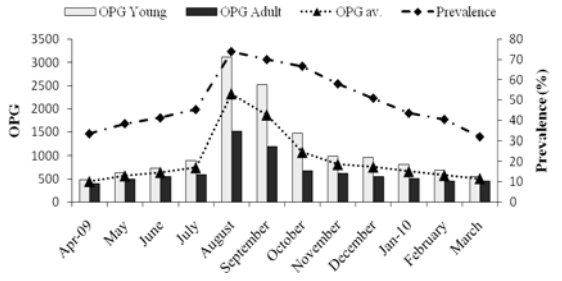

Fig. 1: Comparison of month-wise prevalence and OPG of *Eimeria* in young stock and adults.

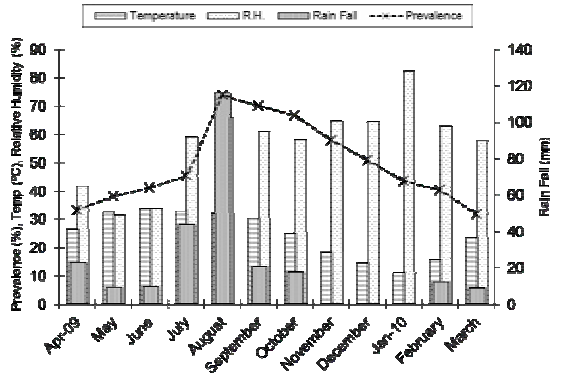

Fig. 2: Association of climatic parameters with prevalence of *Eimeria* in cattle of district T.T. Singh.

Table 1: Multivariate logistic regression analysis of associated factors with risk of *Eimeria* infection in buffalo

Term	Odds ratio	C.I.	P- value
Month	1.132	1.073-1.193	0.000
Age	0.401	0.277-0.579	0.000
Sex	0.628	0.418-0.944	0.025
Feeding system	1.872	1.241-2.823	0.003
Floor	0.545	0.362-0.822	0.004
Housing system	2.216	1.496-3.284	0.000
Watering system	1.663	1.154-2.395	0.006
Herd size	2.006	1.394-2.887	0.000

Hosmer-Lemeshow Goodness-Of-Fit Test: P = 0.731

Table 2: Mantel-Haenszel Chi-Square analysis of all hypothesized risk factors with *Eimeria* infection in buffalo

Associated determinants	Variables	Animals examined	No. of positive cases	Prevalence (%)	Mantel-Haenszel Chi-Square (P-value)
Age	Young	239	154	64.4	34.8291
	Adult	346	136	39.3	(<.0001)
Sex	Male	164	73	44.5	7.1919
	Female	421	217	51.5	(0.0073)
Breed	Nili Ravi	296	149	50.3	0.0845
	Kundi	289	142	49.1	(0.7713)
Feeding	Ground	257	152	59.1	16.1746
system	Trough	328	138	42.1	(<.0001)
Floor	Non-cemented	243	154	63.4	13.7576
	Partially cemented	342	136	39.8	(0.0002)
Housing	Open	321	139	43.3	6.7748
system	Closed	264	151	57.2	(0.0092)
Watering	Tap water	(311	137	44.1	14.1294
system	Pond	274	153	55.8	(0.0002)
Herd size	>20	298	192	64.4	15.4251
	<20	287	98	34.2	(<.0001)
Body condition	Poor	327	182	55.7	0.5212 (0.4703)

The prevalence of *Eimeria* was found to be highly influenced (P<0.05) by feeding system. Ground-fed animals revealed higher risk of infection instead of buffalo fed in trough. Provision of tap water protected animals against coccidiosis. Significantly higher infection rate was observed in buffaloes watered at pond, compared to those given tap water. Prevalence of *Eimeria* was strongly predisposed by the herd size (P<0.05). Higher prevalence was observed in larger herds as compared to smaller ones. Moreover, *Eimeria* infection in animals having poor body condition was a bit higher than animals with good body condition but there was a statistically non-significant association between body condition of animals and risk of *Eimeria* infection (Table 2).

DISCUSSION

This is the first time that a full-scale investigation of various epidemiological aspects of buffalo coccidiosis was carried out in Pakistan. McMaster counting technique was used for quantitative analysis of coccidiosis. This technique is recommended by Vadlejch *et al*. (2013) for the detection and quantification of coccidia oocysts. These results were justified by reports of various researchers in many countries of the world like in India by Jyoti *et al*. (2012) and in Iran by Yakhchali and Zareei (2008) who recorded prevalence as 54.5 and 50.6%, respectively. Overall prevalence of *Eimeria* in present study is higher than previously reported in Pakistan by Mirani *et al*. (2012) who recorded an occurrence rate of 6.6% in buffaloes. This may be due to geographical differences between study areas. The results are lower than the observations of Ahmad and Hasssan (2007) and Nalbantoglu *et al*. (2008). This variation is most likely attributed to the differences in agroecology, management, and husbandry practices of the study animals in different countries. *E. bovis* and *E. zuernii* were recorded as the highest prevalent coccidian species which is in accordance with reports of Nalbantoglu *et al*. (2008) and Priti *et al*. (2008). According to Soulsby (2006), these two species are the most pathogenic of the bovine coccidia.

Higher prevalence observed in young animals is in accordance with findings of Priti *et al*. (2008) who demonstrated that clinical and sub-clinical coccidiosis is more prevalent in younger animals. Young stock has been found to be more susceptible to *Eimeria* infections due to immature immunity. Results of significantly higher oocyst counts in buffalo young stock as compared to adults are compatible with those of Yakhchali and Zareei (2008) who observed maximum number of ova in calves younger than one year-old (582-1302). Higher prevalence of *Eimeria* was recorded in females in the present study. Similar results were reported by Priti *et al*. (2008) from India that female cattle and buffaloes were more susceptible to infection.

The results of month wise prevalence of *Eimeria* in present study were not different from previous reports like de Noronha Jr *et al*. (2009) who reported higher prevalence during months of September to January (months of rain). An increase in prevalence in rainy and post-rainy season could be attributed to increase in rain fall and temperature favoring the progress in development of oocyst (Soulsby, 2006). Lower prevalence of *Eimeria* in hot and cold seasons are justified by the description given by McKellar (2008) that rare "summer coccidiosis" and "winter

coccidiosis" probably results from severe weather stress. The slight variation in the occurrence of coccidiosis in different regions of world may be due to varied seasonal climate. Different breeds were found to have no genetic influence on prevalence of *Eimeria*.

Findings of ground fed animals being at higher risk to *Eimeria* infection than trough fed cattle are in agreement with the findings of Abebe *et al*. (2008) who described a significant association between different feeding systems of cattle calves and the risk of infection with *Eimeria* species. This finding is consistent with the recommendation described by Rehman *et al*. (2011b) and McKellar (2008) to adopt feeding practices that avoid fecal contamination of feed. Priti *et al*. (2008) reported that prevalence of coccidiosis was higher in stall-fed in comparison to free range buffaloes. McKellar (2008) stated that cattle confined to feedlots are susceptible to coccidiosis throughout the year and outbreaks usually occur within the first month of confinement. The observation regarding higher prevalence in animals kept at non-cemented floor may be true because it is easy to clean cemented floor than non-cemented floor. Bangoura *et al.* (2011) reported higher oocyst excretion rates in calves kept on litter compared to rearing on slated floor. Reason for higher prevalence of *Eimeria* in pond-watered animals may be more contamination of water in ponds and overcrowding around a limited water source, which concentrates the hosts and parasites within a restricted area. Our results regarding watering system are in accordance with Siddiki *et al.* (2010) who correlated higher prevalence of enteric protozoa in one of study area (Anowara) to the local practice of providing unsafe water to livestock. Low parasitic burden observation in tap watered animals is justified by the recommendations of McKellar (2008) that all feed and water supplies should be high enough off the ground to avoid fecal contamination. McKellar (2008) described that clinical coccidiosis is more prevalent under conditions of poor sanitation, poor nutrition, and overcrowding. So, lower number of animals was observed infected with *Eimeria* in smaller herds as compared to larger herds in the current study. These results are in accordance with findings of Kusiluka *et al*. (1998) who described that small herd sizes may result in low environmental contamination. Klockiewicz *et al*. (2007) also reported that the highly pathogenic *Eimeria* in cattle occurred more frequently in big farms rather than in small farms. Probable reason for the death of young stock might be ingestion of high dose of oocysts, severity and chronicity of infection.

In preview of significantly high occurrence of pathogenic species of *Eimeria* and economic importance of coccidiosis, results of the present study aim to propose and assess the routine prophylactic administration of anticoccidials at dairy farms well before onset of rainy season. Provisions of clean tap water, trough feeding and cemented capacious housing are suggested on the basis of present investigation.

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