PREVALENCE AND RISK FACTORS OF COCCIDIOSIS IN BUFFALOES AND CATTLE FROM RAVI RIVER REGION, LAHORE, PAKISTAN

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ABSTRACT

The present study was accomplished to determine the prevalence and risk factors of coccidiosis in cattle and buffaloes of Ravi River region, Lahore from October, 2012 to September, 2013. A total of 500 fecal samples (cattle n=250; buffaloes n=250) were randomly collected and coccidial oocysts were identified using Direct Smear Method and Salt Floatation Techniques. Data regarding each sampled animal was entered in a data capture form. The *Eimeria* spp. were identified on the basis of variation in shape, size, texture, color of oocyst wall, polar cap and presence or absence of micropyle of oocysts using taxonomic key. Overall prevalence of coccidiosis in cattle and buffaloes was recorded as 57.2% and 58.8%, respectively. Seven *Eimeria* species identified from cattle were *E. zuernii* (56.64%), *E. bovis* (41.25%), *E. ellipsoidalis* (33.56%), *E. canadensis* (26.57%), *E. cylindrical* (21.67%), *E. alabamensis* (17.48%), and *E. subspherica* (10.45%). On the other hand, common *Eimeria* spp. found in buffaloes were *E. bovis* (52.38%), *E. zurnii* (46.93%), *E. canadensis* (31.29%), *E. ellipsoidalis* (23.12%), *E. alabamensis* (15.64%) and *E. cylindrical* (11.56%). Coccidial infection was significantly higher ($P<0.05$) in females compared to males in both species. Prevalence of *Emeria* was significantly higher ($P<0.05$) in <6 m of age cohorts (cattle, 66.66%; buffaloes, 70.31%) as compared to ≥1 year animals (cattle, 44.87%; buffaloes, 48.91%). Peak prevalence was recorded in August. A significant difference ($P<0.05$) in prevalence was observed in stall feeding confined animals compared to animals with grazing having outdoor access. Prevalence had significantly increased ($P<0.05$) in animals with poor and weak body condition than healthy. A significant difference ($P<0.05$) in prevalence was observed in diarrheic animals compared to normal feces. It is concluded that coccidial infestation is severe in both species and bovine coccidiosis is widely distributed in Ravi River region Lahore. It is suggested that an integrated strategies should be implemented for the effective prevention and control of coccidiosis in cattle and buffaloes in this region.

Keywords: buffaloes, *Bubalus bubalis*, cattle, *Bos taurus*, coccidiosis, Pakistan

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INTRODUCTION

Bovine coccidiosis is a common and worldwide disease of cattle and buffaloes characterized by decreased growth rate, bloody diarrhea and sometimes death, associated with substantial economic losses to the beef and dairy industries (Daugschies and Najdrowski, 2005 and Dedrickson, 2006). This disease is more sever in early-weaned animals, but may occur frequently in calves between six to twelve months of age (Kennedy, 2006). The disease may affect adult animals as well. The calves at six months of age have 100% infestation rate but less than 5% show clinical signs (Dedrickson, 2006). Cicek *et al*. (2007) reported that younger animals have higher prevalence of coccidial infections (27.23%) than older animals (15.65%). Several other species including sheep, goats, cats, dogs, poultry and rabbits are also susceptible to this infection (Nalbantoglu *et al*., 2008). Although, coccidiosis is host specific, every host may be infected with several species of coccidia at the same time (Andrews, 2002).

Stressed conditions like poor hygiene, poor nutrition and overcrowding reduce animal resistance, which may enhance coccidial infections (Oluwadare *et al*., 2010). Coccidiosis is more likely to occur in animals kept under overcrowded and confined areas (Abebe *et al*., 2008). Fecal contaminated feed and water are also the main causes of *Eimeria* transmission (Taylor *et al*., 2007). Diarrhoea and dehydration is the usual outcome of coccidiosis. If weight loss and dehydration are severe enough, cattle may die from coccidiosis (Pilarczyk and Balicka-Ramisz, 2004). Moreover, it results in failure of young stock to gain weight and grow to their full potential (Radostits *et al*., 2007).

The subclinical cases usually remain undiagnosed and cause great production and economic losses to milk and beef industry worldwide (Abebe *et al*., 2008). Ravi River banks of Lahore Municipality are the densely populous region of cattle and buffalo population. Information on the determinants of bovine coccidiosis is extremely lacking. Most of the work has been done on coccidiosis in poultry but little work has been done on coccidiosis of cattle and buffaloes in Pakistan. Therefore, keeping in view of its importance the current study was planned to determine the prevalence and risk factors of coccidiosis in cattle and buffaloes in Ravi River region of Lahore Municipality.

MATERIALS AND METHODS

Study area

The current study was conducted in Ravi River region, Lahore. This city is located at Pakistan India border, close to Ravi River. Its geographical coordinates are, 31°33' North and 74°20' East with an altitude of 214 m (702 ft) from sea. The Northern side is watered by Ravi River originating from India with dense cattle and buffaloes population around river banks. The city of Lahore experiences extreme limits of temperature, though the rainy season is a pleasing time. The climate of the city is a hot semi-arid with wet, long and very hot summers, dry winters, dust storms and heavy rains in monsoon. Weather of city is extreme throughout May, June and July, when the temperature rises to 40 to 48 ^{o}C. The winter months from December to February are the coldest with the mercury level dropping down to may drop to -1^{o}C.

Animals

A total of 500 animals (n=250 cattle; n=250 buffaloes) were examined for the presence of Eimeria oocysts to find out the prevalence of coccidiosis in Ravi River region, Lahore. The data regarding each animal including identification (owner name, address), animal characterization (age, sex, breed), description of home environs (confined/outdoor access, if confined, either paved/dirt yard), feeding system (stall feeding, grazing, both), Body condition score (1=poor, 2=weak, 2.5=medium, 3=good, 4=fat), fecal score (1=normal, 2=soft/does not hold form, 3=runny/ spreads easily, 4=devoid of solid matter) was recorded on a Proforma during sampling.

Collection of fecal samples

About 5 grams of fecal sample was collected directly from the rectum of each animal after wearing disposable plastic gloves from October, 2012 to September, 2013. Fecal samples were then transferred into self-sealing polythene bags, labeled and preserved in ice before transporting to Medicine Laboratory at University of Veterinary and Animal Sciences, Lahore. The samples were refrigerated at 4^{o}C in the Laboratory for maximum 2 days for further processing.

Processing of fecal samples

The fecal samples were first examined by Direct Smear Method and then the negative samples were further processed for the presence or absence of the coccidial oocysts by Salt Floatation Technique (Zajac and Conboy, 2006).

Identification of *Eimeria* species

Eimeria species were identified on the basis of morphology of *Eimeria* oocysts (shape, size, texture, color of oocyst wall, polar cap and presence or absence of micropyle) using taxonomic key (Daugschies and Najdrowski 2005, Levine 1985).

The prevalence of coccidiosis was determined using the formula:

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

Statistical analysis

The data on prevalence was analyzed by Chi-Square test using statistical software package "SPSS, version 17". A probability level of $P<0.05$ was considered as statistically significantly different.

RESULTS

Out of 250 fecal samples each from cattle and buffaloes 143 (57.2%) and 147 (58.8%) were positive for coccidiosis, respectively. Statistically, the difference in prevalence was found non-significant ($P>0.05$) in both species (Table 1). Prevalence of coccidiosis was 45.23% and 59.61% in male and female cattle, respectively. Similarly, prevalence of coccidiosis was 48.8% and 60.36% in male and female buffaloes, respectively. Statistically, the prevalence of coccidiosis was non-significant ($P>0.05$) in male and female animals (Table 2). When prevalence of coccidiosis according to breed of cattle and buffaloes was compared non-significant difference ($P>0.05$) was recorded (Table 3). Prevalence of coccidiosis in cattle and buffaloes was significantly high ($P<0.05$) in animals below 6 m of age than >1 year of age (Table 4). Month-wise prevalence of coccidiosis in cattle and buffaloes is given in Table 5. Significantly higher ($P<0.05$) prevalence of coccidiosis was found in August as compared to September October and July.

Table 1. Prevalence of coccidiosis in cattle and buffaloes in Ravi River region, Lahore.

Animal	No. of samples Examined	No. of samples positive	Prevalence (%)	P-value
Cattle	250	143	57.2[a]	0.3585
Buffalo	250	147	58.8[ab]	

Values in same column having similar superscript letters are non-significantly (P>0.05) different.

Table 2. Sex-wise prevalence of coccidiosis in cattle and buffaloes in Ravi River region, Lahore.

Animal	Sex	Samples examined	Samples positive	Prevalence (%)	P-value
Cattle	Male	42	19	45.23[a]	0.08587
	Female	208	124	59.61[ab]	
Buffalo	Male	33	16	48.48[a]	0.1966
	Female	217	131	60.36[ab]	

Values in same column havingsimilar superscript letters are non-significantly (P>0.05) different.

Table 3. Breed-wise prevalence of coccidiosis in cattle and buffaloes.

Animal	Breed	Samples examined	Samples positive	Prevalence (%)	P-value
Cattle	Sahiwal	76	44	57.89[a]	0.8572
	Friesian	29	16	55.17[ab]	
	Jersey	37	19	51.35[abc]	
	Crossbred	108	64	59.26[abcd]	
Buffalo	Nili Ravi	228	135	59.21[a]	0.6734
	Kundi	22	12	54.54[ab]	

Values in same column havingsimilar superscript letters are non-significantly (P>0.05) different.

Table 4. Age-wise prevalence of coccidiosis in cattle and buffaloes in Ravi River region, Lahore.

Animal	Age	Samples Examined	Samples Positive	Prevalence (%)	P-Value
Cattle	< 6 m	93	62	66.66[a]	0.01592
	6 m - 1 year	79	46	58.22[b]	
	>1 year	78	35	44.87[c]	
Buffalo	< 6 m	64	45	70.31[a]	0.02542
	6 m - 1 year	94	57	60.63[b]	
	>1 year	92	45	48.91[c]	

Values in same column having different superscript letters are significantly different (P<0.05)

Table 5. Month wise prevalence of coccidiosis in cattle and buffaloes in Ravi River region, Lahore.

Animal	Month	Samples examined	Samples positive	Prevalence (%)	P-value
Cattle	July	63	30	47.62[a]	0.03148
	August	74	52	70.27[b]	
	September	66	38	57.57[c]	
	October	47	23	48.93[d]	
Buffalo	July	68	34	50.00[a]	0.01689
	August	63	47	74.60[b]	
	September	71	42	59.15[c]	
	October	48	24	50.00[d]	

Values in same column for each species having different superscript letters are significantly different ($P<0.05$).

Table 6. Prevalence of different *Eimeria* species in cattle in Ravi River region, Lahore.

Eimeria specie	Positive cases out of 143	Prevalence (%)
E. bovis	81	56.64
E. zurnii	59	41.25
E. ellipsoidalis	48	33.56
E. canadensis	38	26.57
E. cylindrica	31	21.67
E. alabamensis	25	17.48
E. subspherica	15	10.45

Table 7. Different *Eimeria* species prevalent in buffaloes in Ravi River region, Lahore.

Eimeria specie	Positive cases out of 147	Prevalence (%)
E. bovis	77	52.38
E. zurnii	69	46.93
E. canadensis	46	31.29
E. ellipsoidalis	34	23.12
E. alabamensis	23	15.64
E. cylindrica	17	11.56

Seven Eimeria species were identified from positive fecal samples of cattle *viz. E. zuernii, E. bovis, E. canadensis, E. ellipsoidalis, E. alabamensis, E. canadensis and E. cylindrica.* Out of them, *E. zuernii and E. bovis* were highly prevalent species with the prevalence of 56.64% and 41.25%, respectively, followed by *E. ellipsoidalis* (33.56%), *E. canadensis* (26.57%), *E. cylindrica* (21.67%), *E. alabamensis* (17.48%) and *E. subspherica* (10.45%, Table 6). Likewise, six *Eimeria* species were identified from positive buffalo fecal samples viz. *E. bovis, E. zurnii, E. ellipsoidalis, E. alabamensis, E. Cylindrica* and *E. canadensis*. *E. bovis* and *E. zurnii*. Most prevalent species among them with the highest prevalence of 52.38% and 46.93% respectively, followed by *E. canadensis* (31.29%), *E. ellipsoidalis* (23.12%), *E. alabamensis* (15.64%) and *E. cylindrica* (11.56%, Table 7). The prevalence of coccidiosis in both the species was significantly higher (P<0.05) in animals housed in confined and dirt yard compare to animals housed on paved floor with outdoor access (Table 8 and Table 9). Data on prevalence of coccidiosis in cattle and buffaloes according to feeding system is given in Table 10. A strong association was found between *Eimeria* infestation and animal feeding systems. Prevalence was higher among stall fed animals in both cattle (71.64%) and buffaloes (73.68%). Grazing animals were infested minimum with the prevalence of 28.58% and 31.91% in cattle and buffaloes, respectively.

Data on prevalence of coccidiosis according to body condition score in cattle and buffaloes are shown in Table 11 and Table 12. *Eimeria* infestation had strong association (P<0.05) with body condition score. Animals with Poor body condition (score 1) were observed to have highest prevalence in both cattle and buffaloes, compared to higher body condition score. Fecal score also had strong association (P<0.05) with coccidiosis both species. Similarly, highest (82.35% and 84.21%) (Table 13) prevalence was found in the diarrheic animals whereas, lowest (36.19% and 42.85%) prevalence was observed in animals with normal fecal score in cattle and buffaloes respectively. Prevalence of coccidiosis between normal and diarrheic feces was found statistically significant (P<0.05) in cattle and buffaloes.

DISCUSSION

Bovine coccidiosis is a common and worldwide disease of cattle and buffaloes and usually associated with decreased growth rate, bloody diarrhea and sometimes death. The disease is caused by intracellular protozoan 'coccidia' which reside in cell linings of intestine, mostly affecting calves resulting in substantial economic losses all over the world to the beef and dairy production. In present study, overall prevalence of coccidiosis in cattle and buffaloes was recorded as 57.2% and 58.8%, respectively. Previously, Afzal (1996) and Rehman *et al*. (2011) mentioned the prevalence as 17% and 47.09% in Pakistan. The findings of the present study are congruent with other researchers from India, 50%, (Harpreet and Daljit, 2008), South Africa, 52% (Matjila and Penzhorn, 2002) and Kenya, 61.4% (Waruiru *et al*., 2000. Similar findings were also reported in Poland (Klockiewicz *et al*., 2007), Turkey (Nalbantoglu *et al*., 2008; Hatice *et al*., 2007), in Argentina (Sanchez *et al*., 2008; Pilarczyk *et al*., 2000; Romaniuk *et al*., 2004) and Ethiopia (Ferid *et al*., 2012). Our results are not in agreement with Klockiewicz *et al*. (2007) and Nalbantoglu *et al*. (2008) and Hatice *et al*. (2007), Sanchez *et al*. (2008), Pilarczyk *et al*. (2000), Romaniuk *et al*.

Table 8. Prevalence of coccidiosis in cattle according to housing system.

Housing system		Samples examined	Samples positive	Prevalence (%)	P-value
Confined	Paved	53	31	58.49[a]	0.01447
	Dirt yard	61	44	72.13[b]	
Outdoor access		136	68	50.00[c]	

Values in same column having different superscript letters are significantly different (P<0.05).

Table 9. Prevalence of coccidiosis in buffaloes according to housing system.

Housing system		Samples examined	Samples positive	Prevalence (%)	P-value
Confined	Paved	49	30	61.22[a]	0.01299
	Dirt yard	58	43	74.13[b]	
Outdoor access		143	74	51.74[c]	

Values in same column having different superscript letters are significantly different (P<0.05).

Table 10. Prevalence of coccidiosis in cattle and buffaloes according to feeding system.

Animal	Feeding system	Samples examined	Samples positive	Prevalence (%)	P-value
Cattle	Stall feeding	67	48	71.64[a]	0.00004706
	Grazing	42	12	28.58[b]	
	Both	141	83	58.86[c]	
Buffalo	Stall feeding	76	56	73.68[a]	0.00002712
	Grazing	47	15	31.91[b]	
	Both	127	76	59.84[c]	

Values in same column having different superscript letters are significantly different (P<0.05)

Table 11. Prevalence of coccidiosis in cattle according to body condition score.

Body condition score	Samples examined	Samples positive	Prevalence (%)	P-value
1 (Poor)	26	19	73.07[a]	0.004617
2 (Weak)	81	54	66.66[b]	
2.5 (Medium)	74	43	58.10[c]	
3 (Good)	48	18	37.5[d]	
4 (Fat)	21	9	42.85[e]	

Values in same column having different superscript letters are significantly different (P<0.05).

Table 12. Prevalence of coccidiosis in buffaloes according to body condition score.

BCS	Samples Examined	Samples Positive	Prevalence (%)	P-value
1 (Poor)	25	18	72.00[a]	0.1240
2 (Weak)	45	31	68.88[ab]	
2.5 (Medium)	84	50	59.52[abc]	
3 (Good)	67	32	47.76[d]	
4 (Fat)	29	16	55.17[cde]	

Values in same column having different superscript letters are significantly different (P<0.05).

Table 13. Prevalence of coccidiosis in cattle according to fecal score.

Animals	Fecal score	Samples examined	Samples positive	Prevalence (%)	P-value
Cattle	1 (Normal)	105	38	36.19[a]	0.000000120
	2 (Soft)	89	60	67.41[b]	
	3 (Runny)	39	31	79.48[c]	
	4 (Devoid of solid matter)	17	14	82.35[cd]	
Buffalo	1 (Normal)	112	48	42.85[a]	0.00001408
	2 (Soft)	83	54	65.06[b]	
	3 (Runny)	36	29	80.55[c]	
	4 (Devoid of solid matter)	19	16	84.21[cd]	

Values in the same column bearing different superscript letters are statistically significantly different (P<0.05).

(2004) and Ferid *et al*. (2012). The variations are attributed to seasonal, climatic and geographical differences, management and husbandry practices in different countries.

Our study demonstrated significantly higher (P<0.05) prevalence of coccidiosis in <6 months age cohorts as compared to >1 year of age. According to Waruiru *et al*. (2000), Cicek *et al*. (2007), Abebe *et al*. (2008), Priti *et al*. (2008), Lassen *et al*. (2009) and Rehman *et al*. (2011) calves are more susceptible to coccidiosis than adults due to immature immune system (Khan *et al*., 2013). Our findings suggested that coccidiosis is more common in August. Similar findings were reported by Kphara and singh (1986) and Woji *et al*. (1994). An increase in prevalence of *Eimeria* in Moon Soon (rainy season) and post-Moon Soon season could be due to increase in high rain fall and temperature favoring the development of oocysts (Soulsby, 2006). Waruiru *et al*. (2000) reported higher occurrence of coccidiosis in calves in wet season than dry season. Lower prevalence in hot and cold seasons as depicted by McKellar (2008) as summer and winter coccidiosis attributed to severe weather stress.

When breed-wise prevalence of coccidiosis in cattle and buffaloes were compared, it was found that breed is not a risk factor for coccidiosis. Similar findings were reported by Rehman *et al*., 2011 and Jäger *et al*. (2005). Seven *Eimeria* species were identified from fecal samples of cattle and six from buffaloes in the study area. The *E. bovis* and *E. zuernii* were found to be most prevalent species among them. Results of the current study were strongly justified by Rehman *et al*. (2011), Klockiewicz *et al*. (2007), Koutny *et al*. (2012), Samson-Himmelstjerna *et al*. (2006) and others (Ernst *et al*., 1984; Kennedy and Kralka, 1987). Cornelissen *et al*. (1995) reported highest prevalence of *E. bovis* followed by *E. zurnii* in both species. The *E. bovis* and *E. zuernii* are the most pathogenic species of cattle and buffaloes (Levine, 1985). The prevalence of coccidiosis in both the species was significantly higher (P<0.05) in confined animals kept at dirt yard compare to animals reared on paved floor with outdoor access because it is easy to clean paved floor than dirt yard. These results are very much similar to Rehman *et al*. (2011) and Ernst *et al*. (1987) who reported coccidiosis in cattle is more common in confined herds than animals kept on pastures. In present study, a strong association was found between *Eimeria* infestation and animal feeding systems. Likewise, coccidiosis was more prevalent in stall fed animals compare to free pasture grazing animals. These results are in agreement with the recommendations of McKellar (2008) and Rehman *et al*. (2011) to adopt feeding system to avoid fecal contamination of feed. Coccidiosis was comparatively more prevalent in animals with poor body condition than in animals with good body condition. Similar findings were reported by Rehman *et al*. (2011) they mentioned that Eimeriosis is higher in animals with poor body condition than good body condition. *Eimeria* infestation in cattle and buffaloes was observed to be highly influenced (P<0.05) by fecal score in present study. Coccidiosis was found more prevalent in diarrheic animals compare to animals with normal feces in both the species. These results were strongly justified by Bangoura *et al*. (2011). They mentioned that high *E. zurnii* and *E. bovis* oocysts were recovered in diarrheic animals than animals with normal feces.

In conclusion, coccidiosis is widely distributed in cattle and buffaloes of Ravi River region, Lahore. Risk factors significantly associated with coccidiosis are age, month, housingand feeding systems, body condition score and fecal

score.

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