PREVALENCE AND DIVERSITY OF *EIMERIA* SPECIES IN DAIRY ANIMALS OF BIKANER, RAJASTHAN[#]

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ABSTRACT

A total of 715 faecal samples comprising of 500 from cattle (290 from native and 210 from crossbred cows) and 215 from buffaloes were collected randomly from eight Tehsil of Bikaner, Rajasthan during March 2015 to January 2016. An overall prevalence of 19.72% (141/715) was recorded for coccidiosis in dairy animals with a significantly higher ($p<0.01$) infection rate in cattle (native; 26.90% and crossbred; 20.00%) in comparison to buffaloes (9.77 %). Quantitative analysis revealed oocysts per gram of faeces ranging from 400-6500 with an average of 2239.29±311.14, 100-2500 (795.24±120.81) and 100-900 (375.00±57.37) in native cattle, crossbred cattle and buffalo populations, respectively. Among the identified species of *Eimeria*, *Eimeria bovis* as the major contributor in coccidia population (22.00%) followed by *E. ellipsoidalis* (19.33%), *E. pellita* (15.33%), *E. subspherica* (14.00%), *E. alabamensis* (12.67%), *E. zuernii* (9.34%) and *E. cylindrica* (7.33%). There was no significant difference between the prevalence rates of infection in dairy animals of different tehsils of Bikaner. The findings of the current study would provide a basis for evolving effective integrated control strategy to prevent and control of coccidiosis in dairy animals of the region.

Key words: Coccidiosis, prevalence, dairy animals, Bikaner

Introduction

The world wide distributed enteric protozoan parasitic disease known as coccidiosis is caused by microscopic apicomplexan organism belongs to genus *Eimeria* sp. affecting several animal species viz. cattle, sheep, goats, buffaloes, rabbits and poultry worldwide (Daugschies and Najdrowski 2005; Almeida *et al.,* 2011). Coccidiosis is responsible for major economic losses in animal husbandry worldwide (Khan *et al.,* 2013). The clinical form of this disease is present mostly in younger animals; however, adult animals are asymptomatic carriers that often serve as a source of infection for juvenile animals, which are more susceptible to infection (Faber *et al.,* 2002; Abede *et al.,* 2008 and Nalbantoglu *et al.,* 2008).

The clinical picture of coccidiosis depends on the innate pathogenicity of different *Eimeria* species. They inhibit in the intestinal epithelial cells, leading to mucosa damage and the appearance of clinical signs, malnutrition, weakness, anaemia, diarrhoea and bloody stool (Yu *et al.,* 2011; Khan *et al.,* 2013). The dairy animals may also be more susceptible to secondary diseases, such as pneumonia, bacterial enteritis and viral infections resulting over-reliance on the drugs (Fox, 1985). Additionally parasite resistance may reduce our ability to successfully control this disease (Harper and Makatouni, 2002). It has been estimated that annual losses due to coccidiosis in cattle and buffaloes approximate $400 million/ year (Matjila and Penzhorn, 2002). Recent studies have shown that more than 20 *Eimeria* sp. have been described in dairy animals, most of which are considered non-pathogenic and mixed infections are generally encountered (Daugschies and Najdrowski, 2005; Bahrami and Alborzi, 2013). *Eimeria bovis* and *E. zuernii*, are particularly more pathogenic to calves and young animals while *E. alabamensis* has been reported to cause clinical disease in older animals. Further, *E. auburnensis* and *E. ellipsoidalis* have also been occasionally observed to cause diarrhoea in adult bovines (Soulsby, 1982). This situation entices the consideration of an investigator to investigate this protozoan parasite. The information regarding epidemiology of coccidiosis in cattle and buffalo populations from Rajasthan state seems to be sporadic (Godara and Sharma, 2010; Swarnakar *et al.,* 2015). However, there is no published data on the species composition of coccidian infection in Bikaner, Rajasthan. Hence the present study was undertaken to determine the species composition (morphotypes) of coccidian species affecting dairy animals.

Materials and Methods

Study area

Bikaner district is located at 27° 11" to 29° 03" North latitude, 71° 54" to 74° 12" East longitude and 238 meters above sea level in North western region of Rajasthan, India. This district has eight tehsils *viz*. Bikaner, Chhatargarh, Khajuwala, Kolayat, Lunkaransar, Nokha, Pugal and Shridungargarh. The ambient mean temperature (32.6°C to 17.64°C) and an annual mean rainfall (53.96 mm) of this area were recorded from Agriculture Research Station, Bikaner during the study year from March 2015 to January 2016.

Collection of samples

A total of 715 faecal samples of dairy animals comprising of 500 from cattle (290 from native and 210 from crossbred cows) and 215 from buffaloes were collected randomly during summer, rainy and winter season with other related information regarding species, age, sex, deworming history, milk yield and location. The samples were placed in sterile polythene bags, properly labeled, kept in a cool transport box and brought to the laboratory for further examination.

Coprological examination

The presence of oocysts in the faecal samples was examined qualitatively by centrifugal floatation technique using

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Sheather's sugar solution (specific gravity 1.27). For quantitative faecal sample examination, standard McMaster's technique was used to calculate the oocysts per gram (OPG) of faeces (Soulsby, 1982). The number of oocysts per one gram feces (OPG) is helpful in confirming coccidiosis as a cause of clinical disease (Almeida *et al*., 2011; Khan *et al.,* 2013). For sporulation, positive samples were placed in Petri dishes, conditioned with a solution of 2.5% potassium dichromate at room temperature and aired daily for up to two weeks. The *Eimeria* species were determined based on morphology of oocysts and sporocysts (size, shape, colour, form index, micropyle and its cap, presence or absence of residual polar granule) and time of sporulation (Soulsby, 1986; Eckert *et al.,* 1995; Yu *et al.,* 2011).

Statistical analysis

All data analyses were performed by using statistical software program (SPSS for Windows, Version 20.0, USA). Association between the prevalence of coccidian infection with various factors (season, Tehsil and breed) was carried out by Chi square (÷^{2}) test. Variables with significant association at Pd"0.05 (two-side) were subjected to the multivariate Binary logistic regression model. The results were each expressed as logistic regression coefficient (B), wald test, Standard error (S.E.) P value and odd ratio (OR) with a 95 % confidence interval (CI 95 %).

Results and Discussion

Of the total 715 faecal samples examined by qualitative faecal examination, 141 were found to be positive for coccidian oocysts, with a prevalence of 19.72% which is in close agreement with the findings of Laha *et al.* (2013). Several other workers (Saravanan *et al.,* 2009; Sreedhar *et al*., 2009; Gupta *et al.*, 2011; Rahman *et al.*, 2012; Murthy and Rao, 2014) have also reported meager infection of coccidia in dairy animals of various parts of India. However, Singh *et al*. (2009) and Haque *et al*. (2011) have recorded a higher prevalence of *Eimeria* sp. in dairy animals. Further, quantitative analysis revealed oocysts per gram of faeces ranging from 400-6500 with an average of 2239.29±311.14, 100-2500 (795.24±120.81) and 100-900 (375.00±57.37) in native cattle, crossbred cattle and buffalo populations, respectively as the susceptibility of animals to coccidiosis depends on genetic predisposition, innate or adaptive immunity, stress level, hygiene conditions, management in farms, breeding of animals, study design and methods as well as on climates and different geographical regions (Hayat *et al.*, 1994; Yu *et al.,* 2011).

Breed wise prevalence of coccidiosis

Higher prevalence (p<0.01) of coccidiosis in cattle (native; 26.90% and crossbred; 20.00%) as compared to buffaloes (9.77%) was reported which unlike to the findings of Manya *et al*. (2008) and Krishnamurthy *et al.* (2014) due to difference in climatic conditions and animal husbandry practices (Table 1). The prevalence of *Eimeria* sp. in healthy animals implies that they can serve as reservoirs of the infection. The native animals are usually resistant to disease and asymptomatic; which play an important role in transmission of infection to other animals (Heidari and Gharekhani, 2014). Statistical analysis using multivariate binary logistic regression for coccidiosis revealed that the odds ratio for breed indicated a negative association in buffalo and crossbred as compared to native cattle i.e. odd ratio of infection decreased by 0.260 and 0.676 in buffalo and crossbred cattle respectively (Table 2).

Season wise prevalence of coccidiosis

The uppermost prevalence was recorded during winter season (25.15%) followed by rainy (20.00%) and summer (14.90%) with a statistically significant difference (p<0.05) which is unlike to the findings of Sreedhar *et al*. (2009) and Das *et al.* (2015) who reported highest seasonal prevalence in rainy season (Table 1). Management practice of keeping the animals altogether in close proximity for longer durations in winter season might be the probable reason for the transmission of infection. Multivariate binary logistic regression analysis for coccidiosis revealed that the odd ratio for season indicated a negative association in summer as compared to winter season i.e. odd ratio of infection decreased by 0.501 in summer season (Table 2).

Tehsil wise prevalence of coccidiosis

A statistically non-significant difference was reported among the different tehsils with maximum prevalence in Khajuwala tehsil (25.00%) followed by Lunkaransar (23.59%), Bikaner (22.12%), Kolayat (20.18%), Nokha (18.37%), Chhatagarh (18.18%), Sridungargarh (16.35%) and Pugal (13.89%) in decreasing order of prevalence (Table 1). Limited and sporadic studies on the prevalence of coccidiosis are at hand but the present study indicated a non significant difference in the prevalence of coccidiosis in the dairy animals among different tehsils of Bikaner.

Identification of morphotypes

The microscopic examination of sporulated oocysts revealed the presence of seven morphotypes of *Eimeria* species in Bikaner district Rajasthan. Results presented *Eimeria bovis* as the major contributor in coccidia population (22.00%) followed by *E. ellipsoidalis* (19.33%), *E. pellita* (15.33%), *E. subspherica* (14.00%), *E. alabamensis* (12.67%), *E. zuernii* (9.34%) and *E. cylindrica* (7.33%) in the decreasing order of prevalence on the basis of their size and morphological characters. The oocysts of *E. bovis* were observed throughout the period of study which is in congruent with the results of Manya *et al*. (2008) and Das *et al.* (2015) in India and Nalbantoglu *et al.* (2008), Bahrami and Alborzi (2013), and Heidari and Gharekhani (2014) in other countries.

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Table: 1. Overall prevalence of coccidiosis of dairy animals in Bikaner

			Examined	*Eimeria* spp.
Breed	Cattle	Native	290	78 (26.90)
		Crossbred	210	42(20.00)
	Buffaloes		215	21 (9.77)
χ^2 value			-	22.897**
Season	Summer		255	38 (14.90)
	Rainy		245	49 (20.00)
	Winter		215	54 (25.12)
χ^2 value			-	7.706*
Tehsil	Bikaner		113	25 (22.12)
	Nokha		98	18 (18.37)
	Sridungargarh		104	17 (16.35)
	Kolayat		109	22 (20.18)
	Lunkaransar		89	21 (23.59)
	Pugal		72	10 (13.89)
	Chhatargarh		66	12 (18.18)
	Khajuwala		64	16 (25.00)
χ^2 value			-	4.905
Total			715	141 (19.72)

Figures in parentheses indicate percentage,*= significant, **= highly significant

Table: 2. Multivariate binary logistic regression for coccidiosis in dairy animals

	Parameter	Logistic regression coefficient (B)	S.E.	Wald test	P value	Odd ratio
Season	Summer	-0.691	0.242	8.174	0.004	0.501
	Rainy	-0.324	0.230	1.984	0.159	0.723
	Winter	-	-	8.178	0.017	-
Tehsil	Bikaner	0.405	0.359	1.273	0.259	1.499
	Chatargarh	-0.138	0.425	0.106	0.745	0.871
	Khajuwala	0.289	0.403	0.517	0.472	1.336
	Kolayat	0.269	0.367	0.539	0.463	1.309
	Lunkaransar	0.497	0.376	1.740	0.187	1.647
	Nokha	0.083	0.382	0.048	0.827	1.087
	Pugal	-0.489	0.442	1.227	0.268	0.613
	Sridungargarh	-	-	7.816	0.349	-
Breed	Buffalo	-1.348	0.273	24.330	0.000	0.260
	Crossbred cattle	-0.391	0.221	3.133	0.077	0.676
	Native cattle	-	-	24.423	0.000	-
Constant		-0.790	0.316	6.263	0.012	0.454

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