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PREVALENCE AND RISK FACTOR ASSESSMENT OF BOVINE *EIMERIOSIS* IN INTERNAL DRAINAGE DRY ZONE OF RAJASTHAN (INDIA)

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ABSTRACT

The examination of 609 faecal samples, randomly collected from Internal drainage dry zone of Rajasthan from September 2016 to August 2017 for prevalence of various species of *Eimeria* affecting the health of dairy animals, revealed an overall prevalence of 32.68%. Risk assessment analysis revealed highest prevalence in native cattle (33.04%), followed by crossbred cattle (32.75%) and buffaloes (32.46%) in decreasing order of prevalence having non-significant difference. Severity analysis based on the estimation of oocysts per gram (OPG) of faeces revealed mild to moderate infection of *Eimeria* spp. ranging from 300-2500 with an average of 1450±334.42 OPG in dairy animals of study area. Microscopic examination of sporulated oocysts revealed the presence of six morphotypes of *Eimeria viz*., *E. bovis* (38.2%) as the major contributor to coccidia population, followed by *E. zuernii* (16.4%), *E. cylindrica* (15.4%), *E. auburnensis* (13.4%), *E. pellita* (11.4%) and *E. alabamensis* (5.2%) in a decreasing order of prevalence.

Key words: Coccidiosis, dairy animals, internal drainage dry zone, morphotypes, risk assessment, strategic control

INTRODUCTION

Bovine coccidiosis is a ubiquitous intestinal protozoan disease of cattle and buffalo incited by various species of *Eimeria* (Almeida *et al.*, 2011) causing major economic losses to animal husbandry worldwide (Nisar-Khan *et al.*, 2013; Teketel *et al.*, 2017). Coccidiosis is a host-specific and self-limiting disease of chronic or subclinical nature (Nalbantoglu *et al.*, 2008). The disease is severe in early-weaned animals and occurs frequently in calves of 6 to 12 months age (Cicek *et al*., 2007). Stress conditions like poor hygiene, malnutrition and overcrowding enhances coccidial infections (Oluwadare *et al*., 2010) resulting into clinical coccidiosis (Bastianetto *et al.*, 2007) and sometimes death of the animal (Daugschies and Najdrowski, 2005; Dedrickson, 2006).

Coccidiosis in adult animals is usually asymptomatic and often serves as a source of infection in juvenile animals which are more susceptible to infection (Faber *et al*., 2002; Abebe *et al*., 2008). Faecal contaminated feed and water are the main causes of *Eimeria* transmission (Taylor *et al*., 2007). Worldwide more than 20 *Eimeria* species with variable pathogenicity have been identified in cattle (Daugschies and Najdrowski, 2005). *Eimeria* spp. develop only in intestinal epithelial cells leading to mucosal damage and appearance of clinical signs, malnutrition, weakness, anaemia, diarrhoea and haemorrhagic faeces (Yu *et al*., 2011; Nisar-Khan *et al*., 2013). Clinical coccidiosis in cattle mainly depends on the factors like the species of *Eimeria*, age of infected animal, number of oocysts ingested, presence of concurrent infections and management practices (Sandhu and

Singla, 2005). Scarcity of information on the occurrence and losses associated with bovine coccidiosis increased gradually in the shadow of studies targeting helminth infections (Wadhawa *et al*., 2011). But there is dearth of information regarding coccidiosis is dry zone of India. Hence, the present study was aimed to assess the prevalence, species composition and associated risk factors of *Eimeria* infections in cattle and buffaloes in internal drainage dry zone of Rajasthan (India).

MATERIALS AND METHODS

Study area

Rajasthan state of India has been divided in 10 major agroclimatic zones. Present work was carried out in Internal Drainage Dry Zone comprising of Jhunjhunu, Sikar, Nagaur and a part of Churu district (Fig. 1). Meteorologically, this zone, on an average, receives annual rainfall of 300-500 mm and has temperature in the range of 5.3 to 39.7°C (DOA, Govt. of Rajasthan, 2016-2017). These regions are enclosed with sand-dunes and sandy plains. The drainage is not intense.

Collection of samples

A total of 609 faecal samples of dairy animals (268 buffaloes and 112 native & 229 crossbred cattle) were randomly collected season-wise directly from rectum or immediately after defecation from the villages of four districts of internal drainage dry zone of Rajasthan during winter, summer and rainy seasons for one year from September 2016 to August 2017. The samples were placed in sterile polythene bags, labeled with information regarding species, age, sex, deworming history and location and brought to the laboratory in a cool transport box for further examination.

Coprological examination

The faecal samples were first subject to standard qualitative faecal examination by using floatation technique for detection of coccidian oocysts. The coccidian parasites were identified on basis of morphological features of oocysts (Soulsby, 1986). For quantitative faecal examination, standard McMaster's technique was used to calculate oocysts per gram (OPG) in faeces (Soulsby, 1986).

Sporulation of oocysts

Faecal samples positive for coccidian oocysts were mixed with 2.5% potassium dichromate in Petri-dishes and kept in an incubator at 25-27°C for sporulation (Gupta and Singla, 2012). The dimensions of oocysts were recorded and identification done on the basis of micrometric analysis and other morphological characters *viz*., thickness of oocysts wall, presence/absence of micropyle, micropylar cap, etc. These parameters were assessed by micrometric analysis using microscopic micrometric calibration method using micrometric slides ocular and stage (ERMA, Tokya Japan) calibrated on Olympus CX21i with the aid of taxonomic keys (Soulsby 1986; Taylor *et al*., 2007).

Statistical analysis

The data was analyzed by using statistical software program (SPSS for Windows, version 20.0, USA). Association between the prevalence of coccidian infection and various factors was carried out by χ^2 test. Variables with significant association at $P \leq 0.05$ (two-sided) were subject to multi-variate logistic regression model. The results were expressed as P value and odds ratio (OR) with a 95% confidence interval (CI 95%).

RESULTS AND DISCUSSION

Out of 609 faecal samples from dairy animals, 199 were found positive, with an overall prevalence of 32.68% for coccidiosis in dairy animals of Internal drainage dry zone of Rajasthan. The findings

are in line with Gupta *et al.* (2015) from India and Chaparro *et al.* (2016), Temesgen (2016) and Teketel *et al.* (2017) from other parts of the world. However, Manya *et al*. (2008), Singh *et al*. (2009) and Krishnamurthy *et al.* (2014) have reported variations in the prevalence rates when compared with data of the present study. This variation may be attributed to the differences in agro-ecology and husbandry practices of the study animals in different countries (Radostits *et al*., 2006).

Risk assessment analysis revealed highest prevalence in native cattle (33.04%) followed by crossbred cattle (32.75%) and buffaloes (32.46%) in a decreasing order of prevalence with a statistically non-significant difference (Table 1). Multivariate binary logistic regression for coccidiosis indicated a positive association of buffalo and crossbred populations as compared to native cattle i.e. odds ratio of infection increased by 1.399 and 1.351, respectively (Table 2). Quantitative analysis based on the estimation of oocysts per gram (OPG) of faeces revealed mild to moderate severity of *Eimeria* spp. infection ranging from 300-2500 with an average opg of 1450 ± 334.42 in dairy animals of Internal drainage dry zone of Rajasthan. The findings are unlike the findings of workers from other parts of India *viz.*, Punjab (Gupta *et al.*, 2015) and Assam (Das *et al.*, 2015). This variation in severity of infection in dairy animals may be due to the fact that 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 methodsas well as on climates and different geographical regions (Yu *et al.,* 2011).

Seasonal dynamics

A highly significant seasonal variation ($p<0.01$) was recorded in the present study with highest during the rainy season (47.78%), followed by winter (26.47%) and summer (23.76%) (Table 1) indicating rainy season having comparatively optimum meteorological conditions for the helminth growth. Multivariate binary logistic regression analysis for coccidiosis indicated a negative association of summer season and positive association of rainy season as compared to winter season i.e. odds ratio of infection decreased by 0.846 in summer and increased by 2.638 in rainy seasons with complete details in Table 2. Similar findings have been reported from different parts of the India (Das *et al.,* 2015; Gupta *et al.,* 2015) and other countries of the world like Brazil, Pakistan (Noronha *et al.,* 2009; Khan *et al.,* 2013) which may be attributed to the fact that higher humidity and temperature in rainy season and post-monsoon season were conducive for optimum sporulation of the oocysts. Moreover, reduced immune-tolerance in rainy season may also be responsible for the higher incidence of coccidiosis during monsoon (Murleedharan, 2005; Gupta *et al.*, 2015).

Table 1: Overall prevalence of coccidiosis in dairy animals of internal drainage dry zone of Rajasthan

			Examined	*Eimeria* (%)
Animal	Cattle	Native	112	37 (33.04)
		Crossbred	229	75 (32.75)
	Buffaloes		268	87 (32.46)
χ^2 value			-	0.13
Season	Winter		204	54 (26.47)
	Summer		202	48 (23.76)
	Rainy		203	97 (47.78)
χ^2 value			-	31.927**
District	Jhunjhunu		152	51 (33.55)
	Sikar		152	45 (29.61)
	Churu		154	45 (29.22)
	Nagaur		151	58 (38.41)
χ^2 value			-	3.978
Total			609	199 (32.68)

The figures in parentheses show percentage;
*= significant, **= highly significant at ($p<0.05$) and ($p<0.01$) respective levels

District-wise prevalence

A statistically non-significant difference ($p>0.05$) among different districts indicate almost similar perpetuating conditions with maximum prevalence in Nagaur district (38.41%), followed by Jhunjhunu (33.55%), Sikar (29.61%) and Churu district (29.22%) in decreasing order of prevalence (Table 1). However, multivariate binary logistic regression analysis for coccidiosis indicated a positive association of Jhunjhunu, Nagaur and negative

Table 2: Multivariate binary logistic regression for coccidiosis in dairy animals of internal drainage dry zone of Rajasthan (India)

	Parameter		Logistic regression coefficient	S.E.	Wald test	P value	Odds ratio
Season	Winter				32.553	0.000	-
	Summer		-0.167	0.232	0.517	0.472	0.846
	Rainy		0.970	0.215	20.356	0.000	2.638
District	Sikar		-	-	5.244	0.155	-
	Jhunjhunu		0.189	0.255	0.547	0.459	1.208
	Churu		-0.006	0.260	0.001	0.981	0.994
	Nagaur		0.511	0.259	3.876	0.049	1.667
Animal	Cattle	Native	-	-	1.723	0.422	-
		Crossbred	0.301	0.267	1.275	0.259	1.351
	Buffaloes		0.336	0.263	1.623	0.203	1.399
Constant			-1.467	0.317	21.484	0.000	0.231

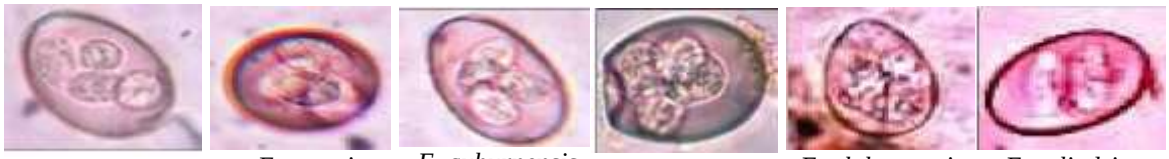
association of Churu districts when compared to Sikar district *i.e*. odds ratio of infection increased by 1.208, 1.667 in Jhunjhunu, Nagaur and decreased by 0.994 in Churu districts, respectively (table 2). Variation in prevalence of coccidiosis in dairy animals with in the zone may be due to differences in animal husbandry practices, pasture availability as well as management of dairy animals and difference in the deworming programs. Limited and sporadic regional studies on the prevalence of coccidiosis are at hand but the present study indicated a non-significant difference in the prevalence of coccidiosis in dairy animals among different districts of internal drainage dry zone of Rajasthan which is in concordant to the findings of Renwal *et al*., 2017 and Monika, 2017 from the state.

Identification of morphotypes

Identification of morphotypes assists in the better understanding of the composition and epizootiological severity of the infection. Faecal samples with high coccidial infection, selected on the basis of OPG >700, were subjected to sporulation and identification was done on the basis of micrometric analysis using microscope (Olympus) and other morphological characters *viz*. shape of oocyst, single or double wall, thickness of oocysts wall, presence/absence of micropyle, micropylar cap, etc. which revealed the presence of six morphotypes of *Eimeria* spp. in the study area (Table 3). Results presented *E. bovis* (38.2%) as the major contributor in coccidia population followed by *E. zuernii* (16.4%), *E. cylindrica* (15.4%), *E. auburnensis* (13.4%), *E. pellita* (11.4%) and *E. alabamensis* (5.2%) in decreasing order of prevalence. Among coccidia population, dominance of

Table 3: Mean measurements (µm) of sporulated coccidian oocysts in dairy animals of Internal Drainage Dry Zone of Rajasthan (mean ± S.E.)

Coccidia	Length (mean ± S.E.)	Width (mean ± S.E.)	Shape	Salient features
Eimeria bovis	27.7±1.14 (24.50-31.0)	20.1±0.65 (17.65-21.35)	Ovoid to subspherical	Smooth wall with inconspicuous micropyle
E. zuernii	17.5±0.97 (15.5-21.0)	16.28±1.14 (14.67-20.75)	Subspherical to round	Colourless smooth wall with no micropyle or oocysts residuum
E. cylindrica	21.64±1.46 (17.0-25.18)	12.51±0.87 (10.89-15.65)	Elongated cylindrical	smooth wall without micropyle
E. pellita	39.3±1.19 (35.85-42.15)	25.95±0.74 (23.75-28.0)	Egg shaped	Very thick wall with a micropyle
E. alabamensis	17.5±0.50 (17.0-18.0)	12.6±0.73 (11.87-13.33)	Ovoid	Smooth wall without micropyle
E. auburnensis	35.30±2.71 (32.49-38.0)	24.5±2.85 (21.65-27.35)	Ovoid to ellipsoidal	Smooth wall with narrow micropyle



E. bovis *E. zuerni* *E. auburnensis* *E. peltita* *E. alabamensis* *E. cylindrica*

Fig. 1: Various morphotypes of *Eimeria* from dairy animals of internal drainage dry zone of Rajesthan (40X)

Eimeria bovis is in harmony to the findings Renwal *et al*. (2017) and Monika (2017) from other parts of the state as well as from different parts of India (Das *et al.*, 2015; Gupta *et al.*, 2015) and other countries of the world like Ethiopia, Brazil, Pakistan, Western Iran (Abebe *et al.*, 2008; Bruhn *et al.*, 2011; Qamar *et al.*, 2011; Bahrami and Alborzi, 2013; Heidari and Gharekhani, 2014). Also, the oocysts of *E. bovis* were observed throughout the study period while rest of the species were encountered less frequent**.**

The prevalence rate of 32.68% reported in the present study in dairy animals of the study zone indicates an alarming prevalence while presence of six different morphotypes of *Eimeria* indicating the complexity of the infection, demand serious attention. Findings of significant seasonal variation and mild variation in between the study area will be used to formulate the area specific control strategies against coccidial infection in the dairy animals of internal drainage dry zone.

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