

PREVALENCE OF *EIMERIA* SPECIES IN BUFFALO CALVES OF HARYANA

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Received: 28.01.2017; Accepted: 11.06.2017

ABSTRACT

To study the prevalence of *Eimeria* species in buffalo-calves in Haryana, a total of 427 faecal samples were collected from four districts of Haryana namely, Fatehabad, Hisar, Bhiwani and Sirsa. Parasitological examination was carried out using saturated salt (NaCl) solution and sporulation was done using 2.5% potassium dichromate solution at 27°C to identify the species. Overall prevalence of *Eimeria* species was 57.84%. Based on the morphological characteristics and sporulation time, eleven species of *Eimeria* were identified, which included *E. bareillyi* (38.46%), *E. bovis* (34.81%), *E. zuernii* (31.98%), *E. subspherica* (29.96%), *E. canadensis* (19.43%), *E. alabamensis* (12.9%), *E. ellipsoidalis* (9.31%), *E. cylindrica* (8.09%), *E. auburnensis* (7.29%), *E. wyomingensis* (6.07%) and *E. pellita* (4.45%). Prevalence among male calves (69.39%) was significantly higher ($p < 0.01$) as compared to females (48.05%). Similarly, calves of 0-3 months of age showed significantly higher ($p < 0.01$) infection (63.14%) than 4-6 months old calves (43.47%). Out of 247 positive samples, 118 had mono-infection, 40 had two *Eimeria* species, 65 had three species and 24 had multiple species with 47.77%, 16.19%, 26.31% and 9.72% prevalence, respectively. *Eimeria pellita* has been recorded for the first time in Haryana.

Key words: *Eimeria*, coccidiosis, prevalence, buffalo calves, Haryana

In most Asian countries livestock has been an integral component of traditional agriculture sector since centuries. In these countries, buffalo's contribution to milk, meat, hides and draft power for agriculture sector plays an important role in overall social development of farmers. The buffaloes in India constitute about 57% of world's population and contribute a major chunk of milk towards total milk production. In Haryana, buffaloes are the major milk producers and their population has increased from 43.72 lacs in 1992 to 60.35 lacs in 2003 (FAO, 2003).

One of the major hindrances in buffalo production is calf diarrhoea, which may be of bacterial, viral or parasitic origin. Of various parasitic causes, coccidiosis caused by *Eimeria* species plays a significant role in calf diarrhoea. Coccidiosis is a generalized term used for a group of sporozoa in the family Eimeriidae that are commonly parasitic in the host's intestinal tract, but occasionally also found in liver and kidneys. Infection by coccidia causes enteritis in all species, however, the clinical picture varies between species, depending upon their pathogenesis. In calves, the infection is characterized by acute invasion and destruction of intestinal mucosa, anorexia, weight loss, diarrhoea, emaciation and sometimes death (Coetzer and Justin, 2004). The coccidia infection infers clinical form either in heavy infection or in immunocompromised conditions. Low level of challenge can

actually be beneficial for stimulating protective immune responses in the host (Catchpole *et al.*, 1993).

In order to formulate suitable control strategies against any parasite, its epidemiology including prevalence is to be studied continuously. Sanyal *et al.* (1985) reported the prevalence of *Eimeria* species from Hisar. After this report in 1985, no work seems to have been carried out on prevalence of *Eimeria* species in buffalo calves of Haryana.

MATERIALS AND METHODS

The study was conducted in four districts of Haryana viz. Fatehabad, Bhiwani, Hisar and Sirsa. A total of 427 faecal samples were collected from buffalo calves from different villages, organized farms including Buffalo Farm and Veterinary Clinic of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar from November, 2015 to July, 2016. Samples collected from Teaching Veterinary Clinical Complex solely comprised of diarrhoeic cases. Data generated from the study was statistically analysed using SPSS (Statistical Package for Social Sciences) software.

Faecal Sample Collection: A total of 427 fresh faecal samples were collected directly from rectum of each healthy calf using sterile disposable plastic gloves. The samples were placed in a labelled clean polythene cover and transported to the Parasitology Department on the

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same day of collection and were preserved at 4°C in a refrigerator until processing. At the time of sampling, the name of the farm (owner), date of sampling, consistency of the faeces (soft, watery or normal), age, sex, breed, address and management system were recorded for each calf on a data recording sheet.

Parasitological Examination: A 5-10 g portion of faecal sample was mixed with a little amount of saturated salt solution (NaCl) and triturated with pestle and mortar. The mixture was then sieved into a flat bottom floatation tube and filled up to the top with saturated salt solution and then covered with a glass slide. The slide was removed after 20 min. with a jerk, inverted and covered with cover slip and examined for the presence of coccidia oocysts under a microscope at 10x and 40x. Photographs of different *Eimeria* oocysts were taken and micrometry was done with the help of trinocular digital microscope.

Sporulation of *Eimeria* Oocysts: A solution of 2.5% potassium dichromate was added to each positive faecal sample and mixed thoroughly with a wooden applicator in a Petri dish. Each Petri dish was left in a BOD incubator at 28°C to allow sporulation. Thereafter, every 24 h, the culture of oocysts was mixed thoroughly with the aid of Pasteur pipette and a drop of the culture was examined under the microscope to determine the sporulation. Sporulation of oocysts was completed after 17 days. The Petri dish containing oocysts was stored in a refrigerator at 4°C until needed.

Identification of Coccidia Oocysts: The identification of different oocysts was done based on the morphological features of the sporulated oocysts (size, shape, colour and texture of oocyst wall, presence or absence of micropyle, polar cap) and time of sporulation with the help of taxonomic keys (Soulsby, 1982; Kennedy and Kalka, 1987; Sommer, 1998).

RESULTS AND DISCUSSION

Overall prevalence of *Eimeria* species in buffalo calves was found to be 57.84% (Table 1), whereas, Sanyal *et al.* (1985) had reported an overall prevalence of 51.19% (172/336) in buffalo calves of Hisar district. In another study, Rana *et al.* (2011) reported a prevalence rate of 16.10% (24/142) in neonatal buffalo calves of upto one month of age at an organised farm in Hisar. More or less similar positivity rate (54.55%) in buffalo calves of Punjab was reported by Jyoti *et al.* (2012). However, a higher positivity rate of 73.2% (711/971) of coccidiosis was reported in cow-calves of Kashmir valley by Pandit in 2009.

Prevalence in Relation to Various Risk Factors:

The buffalo calves of the university farm showed the highest rate of prevalence, which was 78% and consisted of all the species (Table 1). Cornelissen *et al.* (1995) in Dutch reported higher prevalence in organized farms than the unorganized sector. This might be due to the fact that chances of contamination are more in organized farm as compared to unorganized sector where the less number of animals are kept per house which reduces the chance of contamination with *Eimeria* oocysts and the same has been reflected in our findings from village Mochiwali, where the prevalence rate was the minimum (40%). Moreover, clinical cases of coccidiosis have been observed where high concentration of ammonia, CO₂ and moisture was present due to lack of aeration in pens and groups or accumulation of faeces on the ground due to unsuitable slatted floor (Grafner *et al.*, 1985) and these conditions are comparatively more prevalent in organized farms. The low prevalence (40%) of *Eimeria* species in buffalo-calves of Veterinary Clinic (TVCC) of LUVAS, Hisar might be due to the fact that the animals brought to the Clinic are pre-treated in the field by field veterinarians against parasites including *Eimeria* species.

Sex-wise prevalence of *Eimeria* species (Table 2) revealed that males had significantly higher prevalence (69.39%) as compared to females (48.05%). This finding is contrary to one reported earlier by Sanyal *et al.* (1985) who recorded more or less similar prevalence in males and females. However, Ahmed and Hassan (2007) from Egypt reported higher prevalence of coccidiosis in male

Table 1
Area wise prevalence of *Eimeria* species in buffalo calves of Haryana

District	Village / Area	Total samples examined	Total samples positive (%)	District-wise prevalence
Fatehabad	Kanheri	30	15 (50.0)	55.0%
	Lali	30	18 (60.0)	
Hisar	Masudpur	23	10 (43.4)	62.25%
	Datta	25	15 (60.0)	
	Durjanpur	20	12 (60.0)	
	LUVAS	100	78 (78.0)	
	Buffalo Farm			
	TVCC	15	06 (40.0)	
	Mangali	25	14 (56.0)	
	Umra	27	12 (44.4)	
	Ladwa	22	13 (59.0)	
Sirsa	Mochiwali	20	08 (40.0)	42.50%
	Ding	20	09 (45.0)	
Bhiwani	Milakpur	25	11 (44.0)	52.85%
	Bawanikhera	20	13 (65.0)	
	Jeetakheri	25	13	
Total		427	247	

Table 2
Prevalence of *Eimeria* species in buffalo calves in relation to various risk factors

Risk factor	Object	Total samples examined	Positive samples (%)	P1	P2	Z value	Significance level
Sex	Male	196	136 (69.39)	69.387	48.519	4.449	**
	Female	231	111 (48.05)				
Age group	0-3 months	312	197 (63.14)	63.141	43.478	3.650	**
	4-6 months	115	50 (43.47)				
Type of farm	Organized	127	90 (70.86)	70.866	52.333	3.545	**
	Unorganized	300	157 (52.33)				

**p<(0.01)

buffalo calves (70.5%) as compared to female calves (61.5%). Similarly, Heidari *et al.* (2014) from Iran also reported comparatively higher prevalence rate in males (10.7%) than females (8.2%). The possible reason for higher prevalence rate in males in our study might be the better management of females than males keeping in view their economic importance.

Calves of 0-3 months of age had significantly (p<0.01) higher prevalence rate (63.14%) than those of age group 3-6 months (43.47%; Table 2). Sanyal *et al.* (1985) had also reported higher incidence (65.71%) in buffalo calves of ½ to 6 months of age as compared to adult calves i.e. 27.77% in 6 months to one year age group, 25.92% in 1 year to 2½ years old cattle. Chhabra (1960) observed 89.8% infection in buffalo calves below three months of age, 78 % in 3-6 months old, 75.3% in 6 months–1 year old and 68.5% in animals above one year of age. However, Ahmed and Hassan (2007) in their studies carried out in Egypt recorded highest prevalence (64.30%) of *Eimeria* sp. in 6-9 months old calves and least in one week - 3 months old calves (22.20%). Heidari *et al.* (2014) from Iran recorded the highest prevalence in calves of less than 1 year of age (20.5%) and least in calves of more than 1 year of age (4.1%). These

findings indicate that animals of any age are susceptible to coccidia infections but their susceptibility tends to decrease in older animals resulting in lower prevalence of the parasites in adult buffaloes.

Based on the morphological characteristics of sporulated oocysts, eleven species of *Eimeria* were identified (Table 3), which included *E. bareillyi* (38.46%), *E. bovis* (34.81%), *E. zuernii* (31.98%), *E. subspherica* (29.96%), *E. canadensis* (19.43%), *E. alabamensis* (12.9%), *E. ellipsoidalis* (9.31%), *E. cylindrica* (8.09%), *E. auburnensis* (7.29%), *E. wyomingensis* (6.07%) and *E. pellita* (4.45%). (Figs. 1, 2). The most prevalent *Eimeria* species in our study included *E. bareillyi*, *E. bovis*, *E. zuernii* and *E. subspherica*. These findings are in agreement with the earlier findings of Sanyal *et al.* (1985), who had also reported the occurrence of these species in more or less same percentage i.e. *E. bareillyi* (33.13%), *E. bovis* (30.23%), *E. zuernii* (20.34%) and *E. subspherica* (27.90%). However, Manyal *et al.* (2008) reported the occurrence of four *Eimeria* species in bovines of Patna. The predominant species was *E. zuernii* (62%) followed by *E. bovis* (45%), *E. subspherica* (10%) and *E. ellipsoidalis* (4%). *Eimeria pellita* has been recorded for the first time in Haryana.

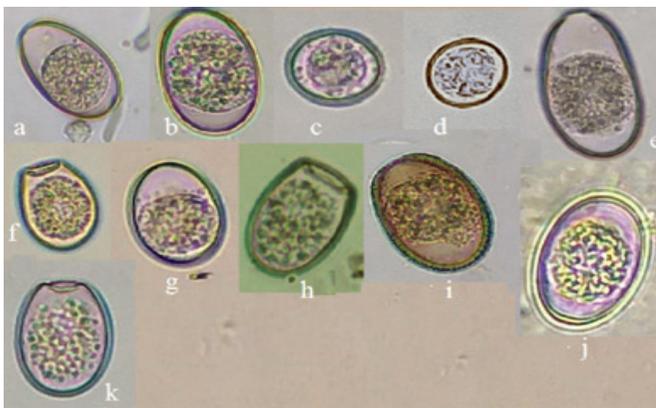


Fig 1. Photomicrographs of unsporulated oocysts of *Eimeria* species (40x)
a=*E. auburnensis*; b=*E. bovis*; c=*E. zuernii*; d=*E. subspherica*;
e=*E. wyomingensis*; f=*E. bareillyi*; g=*E. alabamensis*; h=*E. cylindrica*;
i=*E. pellita*; j=*E. ellipsoidalis*; k=*E. canadensis*

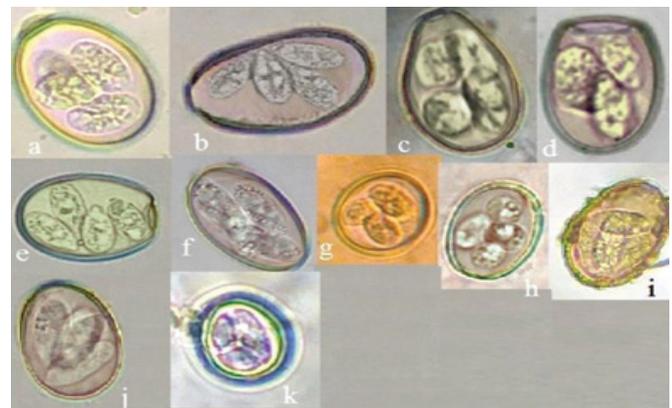


Fig 2. Photomicrographs of sporulated oocysts of *Eimeria* species (40x)
a=*E. alabamensis*; b=*E. wyomingensis*; c=*E. bareillyi*; d=*E. cylindrica*;
e=*E. canadensis*; f=*E. auburnensis*; g=*E. zuernii*; h=*E. ellipsoidalis*;
i=*E. pellita*; j=*E. bovis*; k=*E. subspherica*

Table 3
Oocysts size and sporulation time of different *Eimeria* reported from buffalo calves

Species	Sporulation time (hours)	Breadth (μ)	Length (μ)
<i>E. alabamensis</i>	72–96	11.03–16.77	13.11–24.57
<i>E. auburnensis</i>	72–96	20.01–25.67	20.2–46.03
<i>E. bareillyi</i>	72–96	15.3–25.65	24.07–35.22
<i>E. bovis</i>	48–72	17.01–23.4	23.21–34.05
<i>E. canadensis</i>	72–96	20.2–22.38	28.21–37.2
<i>E. cylindrica</i>	36–48	12.01–15.3	16.11–27.29
<i>E. ellipsoidalis</i>	48–72	12.3–17.4	20.30–26.4
<i>E. pellita</i>	17–18days	26.18–30.12	36.19–38.96
<i>E. subspherica</i>	72–96	8.7–14.19	8.9–16.13
<i>E. wyomingensis</i>	72–96	26.4–31.38	37.2–45.21
<i>E. zuernii</i>	48–72	13.21–23.2	14.13–26.12

Out of 247 positive samples, 118 had mono-infection, 40 had two *Eimeria* species, 65 had three species and 24 had multiple species with 47.77%, 16.19%, 26.31% and 9.72% prevalence respectively. Nyberg *et al.* (1967) from Western Oregon (USA) found 25 of the 86 samples having mono-infection. All *Eimeria* species except *E. subspherica* and *E. alabamensis* were recorded singly. Fabiyi and Bawa (1995) from Bauchi (Nigeria) found the commonest combinations of 3 to 4 *Eimeria* species in trade cattle, though they also observed the combinations ranging from 2 to 8 *Eimeria* species. Kasim and Alshawa (1985) in Saudi Arabia observed 2 to 4 *Eimeria* species. This indicates that bovine *Eimeria* species can be found in various combinations.

From the above studies it can be concluded that the prevalence of bovine coccidiosis in Haryana is showing an increasing trend as it was 51.19% in 1985 and now it is 57.87%. More focus should be on improving management conditions particularly at the organized farms as it is mainly a management problem. Further, male calves should be given same attention as the female calves as the prevalence was significantly higher in male calves. Young calves upto 3 months of age require special attention.

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