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Prevalence of *Eimeria* species in Swamp Buffaloes of Guwahati, Assam

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ABSTRACT

To determine the prevalence of *Eimeria* spp. in swamp buffaloes of Guwahati, Assam, a total of 1258 nos. of fecal samples of calves (246), heifer (265) and adult (747) swamp buffaloes were screened for detection of *Eimeria* oocysts by flotation and modified McMaster techniques. Sporulation of the oocyst was done in 2.5% potassium dichromate solution for identification of the *Eimeria* spp. Examination of fecal samples revealed *Eimeria* oocysts in 57 nos. of samples (4.53%). Three species of *Eimeria* viz. *E. bovis*, *E. zuernii* and *E. bukidnonensis* were identified based on their morphological characters. Prevalence varied with age and season: 5.28% (13/246) in calves, 15.09% (40/265) in heifer and 0.53% (4/747) in adults ($P < 0.05$); 7.04% (15/213) in post-monsoon, 7.0% (28/400) in monsoon, 2.88% (9/312) in winter and 1.50% (5/333) pre-monsoon seasons ($P > 0.05$). *E. bovis* was recorded in all the four seasons while *E. zuernii* and *E. bukidnonensis* were recorded during monsoon, post-monsoon and winter seasons.

1. Introduction

Buffaloes are known as the black gold of South Asia. The domestic Asian water buffalo (*Bubalus bubalis*) is found on all five continents, with a global population of some 202 million (Zhang *et al.*, 2020). On the basis of morphological and behavioral criteria, Macgregor (1941) first recognized the two types of domestic Asian water buffalo. The river buffalo is native to the Indian subcontinent and has spread west as far as the Balkans, Greece, Egypt and Italy within recorded historical times, whereas swamp buffalo are found throughout south-east Asia, from Assam and Bangladesh in the west to the Yangtze valley of China (Cockrill, 1974). North-eastern states of India form the continuous land link with East Asian countries which is the home of swamp buffaloes (Singh *et al.*, 2021). Recently Singh *et al.* (2020) has shown the existence of swamp buffalo in the state Meghalaya.

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 plays a significant role in calf diarrhoea (Nain *et al.*, 2017). Coccidiosis is one of the most pathogenic intestinal diseases caused by different species of *Eimeria* and is often

underemphasized in Indian scenario. Bloody diarrhoea, dehydration, rough hair coat, reduced growth rate, anaemia, weakness and weight loss are the signs of coccidiosis (Bastianetto *et al.*, 2007). Adult animals are usually asymptomatic carriers and often serve as a source of infection for more susceptible juvenile animals (Faber *et al.*, 2002; Abebe *et al.*, 2008). 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). According to Nalbantoglu *et al.* (2008) they are responsible for huge economic losses to livestock industry in terms of mortality and morbidity in young calves. Overcrowding and lack of sanitation increases the chance of high rate of infection. The prevalence of *Eimeria* spp. in buffaloes have been reported from various states of India such as Uttar Pradesh (Singh and Agrawal, 2003), Punjab (Jyoti *et al.*, 2012), Haryana (Nain *et al.*, 2017) and Rajasthan (Sodha *et al.*, 2021). Since, no detail reports on coccidiosis in swamp buffaloes are available from the state of Assam, thus the present study was designed to find out the prevalence of *Eimeria* spp. in swamp buffaloes of Guwahati, Assam.

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2. Materials and Methods

2.1 Study area

The present study was conducted in Guwahati, the capital city of the state of Assam that lies within the latitude of 26°11'0"N and longitude 91°44'0"E. The city is situated on an undulating plain with varying altitudes of 49.5-55.5m above mean sea level. The southern and eastern sides of the city are surrounded by hillocks.

2.2 Study design

A total of 1258 fecal samples of swamp buffaloes were collected from the Government (1no.) and Private farms (9nos.) located in Khanapara, Bonda and Panikhaiti area of Guwahati, Assam for one calendar year (2014). The selected animals were categorized according to age viz. calves (246), heifer (265) and adult (747). The study period was divided into four seasons viz. pre-monsoon (March, April, May), monsoon (June, July, August, September), post-monsoon (October, November) and winter (December, January, February). Fecal samples were collected directly from the rectum of the individual animal and kept in marked plastic pouch/vials. Three grams of fecal samples were examined by direct flotation technique using saturated salt and sucrose solution (Pyziel and Demiaszkiewicz, 2013). Positive samples were then quantified to estimate the oocysts per gram (OPG) of feces by using modified McMaster technique (MAFF, 1986). Samples not being examined on the same day were preserved in 2.5% potassium dichromate solution and stored at refrigerated temperature (4°C) for next day examination. Sporulation of the oocyst was done by mixing positive fecal sample containing oocyst of *Eimeria* spp. with 2.5% potassium dichromate solution in a ratio of 1:5 volume as per the procedure described by Duszynski and Wilber (1997) and incubated at room temperature for 4-7 days, checked daily. Morphological characterization and measurement of oocysts was done as per the guidelines of Duszynski and Wilber (1997) and (Soulsby, 1982) by using an Olympus BX51 light microscope at ×200 and ×400 magnifications.

2.3 Statistical analysis

Data were statistically analyzed by Analysis of Variance (ANOVA) for significance using SPSS 15 version.

3. Results and Discussion

The prevalence of *Eimeria* spp. in swamp buffaloes of Guwahati, Assam was 4.53%. Age wise, 5.28%, 15.09% and 0.53% infections of *Eimeria* spp. were recorded in calves, heifer and adult buffaloes, respectively. Highest infections was observed in heifer (70.2%) followed by calves (22.8%) and adult (7.01%), statistically significant ($P < 0.05$) by Chi Square test (Table 1). Three species of *Eimeria* viz. *E. bovis*, *E. zuernii* and *E. bukidnonensis* were observed in the present study (Fig.1). The length×width (Mean±SE) of each species was *E. zuernii* (16.4±0.43×14.3±0.35µm), *E. bovis* (25.4±0.52×19.4±0.72µm) and *E. bukidnonensis* (43.4±0.23×30.1±0.48µm). In heifer, *E. bovis* (77.5%), *E. bukidnonensis* (17.5%) and *E. zuernii* (5.0%) were recorded while in calves *E. bovis* (69.2%) and *E. zuernii* (30.8%) were recorded. In adult buffalo only *E. bovis* (100%) was observed (Fig.2). Season wise variation in the prevalence of *Eimeria* spp. in swamp buffaloes was observed and highest during post-monsoon (7.04%) followed by monsoon (7.0%), winter (2.88%) and pre-monsoon (1.50%) (Table 2). *E. bovis* was recorded in all the four seasons while *E. zuernii* and *E. bukidnonensis* were recorded during monsoon, post-monsoon and winter seasons.

Table 1. Prevalence of *Eimeria* spp. in swamp buffaloes

<i>Eimeria</i> species	Calves	Heifer	Adult	Chi sq value
<i>Eimeria bovis</i>	9 (69.2)	31 (77.5)	4 (100)	14.97*
<i>Eimeria zuernii</i>	4 (30.8)	2 (5.0)	-	
<i>Eimeria bukidnonensis</i>	-	7 (17.5)	-	
Overall	13 (22.8)	40 (70.2)	4 (7.01)	

*P (<0.05), - (Negative)

Figures in parentheses indicates percent positivity



Eimeria bovis



Eimeria zuernii



Eimeria bukidnonensis

Fig 1. Microphotographs of oocysts of *Eimeria* spp. in buffaloes (20x)

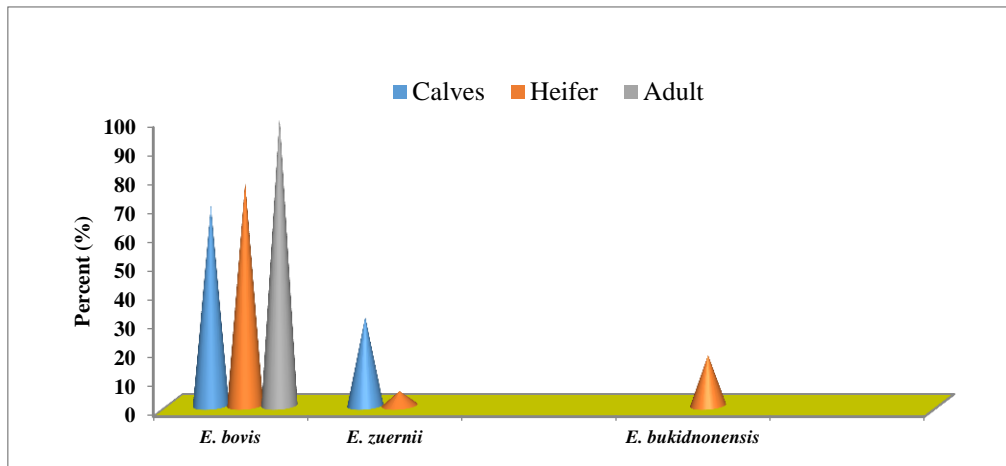


Fig 2. Age wise prevalence of *Eimeria* spp. in buffaloes

Table 2. Seasonal prevalence of *Eimeria* spp. in different age groups of buffaloes

Season	Sample Screened	<i>Eimeria bovis</i>	<i>Eimeria zuernii</i>	<i>Eimeria bukidnonensis</i>	Total Positive
Pre-monsoon	Calves	2	-	-	2
	Heifer	3	-	-	3
	Adult	-	-	-	-
	333	5 (100)	-	-	5 (1.50)
Monsoon	Calves	4	2	-	6
	Heifer	13	2	5	20
	Adult	2	-	-	2
	400	19 (67.85)	4 (14.28)	5 (17.85)	28 (7.00)
Post-monsoon	Calves	2	1	-	3
	Heifer	9	-	1	10
	Adult	2	-	-	2
	213	13 (86.66)	1 (6.66)	1 (6.66)	15 (7.04)
Winter	Calves	1	1	-	2
	Heifer	6	-	1	7
	Adult	-	-	-	-
	312	7 (77.77)	1 (11.11)	1 (11.11)	9 (2.88)
X² value	5.88^{NS}				

NS (Non-significant), - (Negative)

Figures in parentheses indicates percent positivity

Intensity serve as an index of the worm burdens and the counts are used as useful criteria for assessing the nature of the parasitism such as acute/chronic or sub-clinical infection. Fecal samples found positive for *Eimeria* infection were further evaluated quantitatively to estimate the oocyst per gram (OPG) of feces by modified McMaster technique. In buffalo, season wise maximum OPG (Least Sq Mean±SE) was recorded during monsoon (35.35±3.38) followed by pre-monsoon (10.47±3.94), post-monsoon (10.06±4.56) and winter (7.14±3.98) (Table 3). Statistical analysis revealed that there was significant difference (P<0.05) in the intensity of

Eimeria infection during monsoon season. However, according to age, statistically there was no difference in the OPG of calves (15.3±4.02), heifer (30.54±3.77) and adult (1.43±2.31) animals. ANOVA showed highly significant (P<0.01) effect of age x season on OPG of buffalo (Table 4).

Table 3. Season wise mean OPG of Eimeriosis in buffaloes

Age group	Pre-monsoon (Least Sq Mean±SE)	Monsoon (Least Sq Mean±SE)	Post-monsoon (Least Sq Mean±SE)	Winter (Least Sq Mean±SE)	Total (Least Sq Mean±SE)
Calves	15.32 ^{bc} ±7.72	30.23 ^b ±6.56	8.75 ^{bc} ±9.61	6.9 ^{bc} ±7.98	15.3 ^B ±4.02
Heifer	16.1 ^{bc} ±7.92	73.21 ^a ±6.63	18.33 ^{bc} ±7.85	14.52 ^{bc} ±7.72	30.54 ^A ±3.77
Adult	1 ^c ±4.18	2.61 ^c ±4.01	3.1 ^{bc} ±5.72	1 ^c ±4.39	1.43 ^C ±2.31
Total	10.47 ^b ±3.94	35.35 ^a ±3.38	10.06 ^b ±4.56	7.14 ^b ±3.98	11.41 ±1.79

Means with same superscripts are not significantly different (P>0.05)

Total means (season-wise) with same scripts are not significantly different (P>0.05)

Total means (age-wise) with same superscripts are not significantly different (P>0.05)

Table 4. Season wise ANOVA of OPG (Eimeriosis) in different age groups of buffalo

Source	d.f.	Sum of Squares	Mean Square	F Ratio	Prob > F
Age	2	166477.21	83238.61	22.5122	<.0001**
Season	3	149415.20	49805.07	13.47	<.0001**
Season × Age	6	130708.33	21784.72	5.8918	<.0001**
Error	1246	4607073.00	3697.50		
Total	1257	5043810.00			

**P (<0.01)

In the present study, *Eimeria* infections were observed in the 4.53% swamp buffaloes. Earlier studies revealed that *Eimeria* infections in buffaloes ranged from a minimum of 8% (Singh and Agrawal, 2003) to as high as 55.1% (Nalbantoglu *et al.*, 2008). Jyoti *et al.* (2013), Sodha *et al.* (2021) and Nain *et al.* (2017) from Punjab, Rajasthan and Haryana reported 54.55%, 22.58% and 57.84% infections in buffaloes, respectively. The variation in percent prevalence as well as number of species from the present study might be due to different geographical region, climatic conditions, housing and feeding system, herd size and other managemental factors. In the present study, *E. bovis* was recorded highest followed by *E. zuernii* and *E. bukidonensis* which was in agreement with the findings of Nain *et al.* (2017), Bahrami and Alborzi (2013) and Adhikari *et al.* (2021). Sanyal *et al.* (1985) from Northern India reported ten species viz. *E. zuernii*, *E. bovis*, *E. cylindrica*, *E. subspherica*, *E. auburnensis*, *E. ellipsoidalis*, *E. bukidonensis*, *E. bareillyi*, *E. wyomingensis* and *E. canadensis* in buffaloes. Similarly, Nain *et al.* (2017) from Haryana reported eleven species of *Eimeria* viz. *E. bareillyi*, *E. bovis*, *E. zuernii*, *E. subspherica*, *E. canadensis*, *E. alabamensis*, *E. ellipsoidalis*, *E. cylindrica*, *E. auburnensis*, *E. wyomingensis* and *E. pellita* based on the morphological characteristics. Das *et al.* (2017) from hilly region of Meghalaya reported four species of *Eimeria*, *E. bareillyi*, *E. zuernii*, *E. bovis* and *E. ellipsoidalis* in buffaloes. Recently, Ramakrishnan *et al.* (2020) from Chennai and Port Blair reported *E. bareillyi*, *E. braziliensis*, *Eimeria canadensis*, *E. zuernii*, *E. wyomingensis*, *E. bukidonensis* and *E. ellipsoidalis* in buffaloes.

It was observed that the younger age groups are more susceptible to infections than adults. Sanyal *et al.* (1985) also reported higher incidence in buffalo calves (65.71%) than adult (25.92%). Clinical coccidiosis in buffaloes has been reported from India, The Netherlands and Brazil (Shastri *et al.*, 1974; Bastianetto *et al.*, 2008; Dubey *et al.*, 2008; De Meireles *et al.*, 2012). Dubey *et al.* (2008) reported fatal intestinal coccidiosis in a three week old buffalo calf. According to Nambiar and Devada (2002), clinical coccidiosis is commonly prevalent in animals under the age of one year and susceptibility to infection gradually declines with the advancement of age due to developed immunity.

In the present study, it was observed that the intensity of *Eimeria* infection was statistically significant (P<0.05) during monsoon season. Sreedhar *et al.* (2009), Das *et al.* (2015) and Gupta *et al.* (2015) also revealed higher prevalence of coccidiosis during rainy season. This may be due to optimum temperature and high humidity which favors sporulation of the oocysts during monsoon season. As far as our knowledge is concerned there are no reports on the prevalence of *Eimeria* species in swamp buffaloes of Guwahati, Assam and this report may be considered as the first report. The present findings have significance because swamp buffaloes are prevalent in many states of Northeastern region including Meghalaya, Manipur and Nagaland and farmers are not aware of the coccidia infections in animals.

4. Conclusions

The present study revealed prevalence of three species of *Eimeria* i.e. *E. bovis*, *E. zuernii* and *E. bukidnonensis* in calves, heifer and adult swamp buffaloes of Guwahati, Assam. Younger age groups are more susceptible to the infection than adults and infection is prevalent throughout the year. Thus, fecal samples of animals should be regularly screened and anticoccidial drugs should be used for treatment and control of coccidiosis in swamp buffaloes.

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