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M Das
Senior Scientist, Division of Animal Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India

R Laha
Principal Scientist, Division of Animal Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India

G Kharguria
Chief Technical Officer (T7), Division of Livestock Production, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India

Corresponding Author:
M Das
Senior Scientist, Division of Animal Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India

Gastrointestinal protozoan parasites in pigs of hilly region of Meghalaya, India

M Das, R Laha and G Kharguria

Abstract
To determine the prevalence of gastrointestinal (G.I.) protozoan parasitic infections in pigs of hilly region of Meghalaya, a total of 2574 fecal samples of pigs from different age groups <6 months (881 nos.), 6-12 months (857 nos.) and >12 months (836 nos.) were collected and examined by Sheather's sucrose flotation, modified McMaster and Ziehl-Neelsen staining techniques. The overall prevalence of G.I. protozoan parasitic infections in pigs of hilly region of Meghalaya was 29.02%. *Balantidium coli* (39.36%), *Entamoeba polecki* (5.62%), *Giardia* sp. (2.54%), *Cryptosporidium* sp. (10.04%), *Eimeria* sp. (35.34%) and *Isospora suis* (7.10%) recorded. Highest and lowest infections was recorded in the month of December (34.42%) and March (17.96%), respectively. Season wise infection was recorded highest during rainy season (32.49%) followed by cool (31.93%), cold (25.50%) and hot (21.85%) season. Age wise, 31.56%, 35.01% and 20.22% infections were recorded in <6 months, 6-12 months and >12 months old pigs, respectively.

Keywords: Pig, gastrointestinal, protozoa, Meghalaya

Introduction
Animal husbandry is an important subsector of agriculture in hilly region of Meghalaya. Pigs are one of the most important livestock reared by tribal population of Meghalaya, a state situated in the North-Eastern region of India. Pigs grow fast, have high fecundity rates and short generation intervals resulting in quick generation of cash for farmers ILRI [18]. In Meghalaya, pig farming emerged as a major source of additional income for the small and marginal farmers because it requires very low input cost. Moreover, tribal people of Meghalaya prefer to have pork in their meals every day. Thus, livelihood of the tribal farmers can be improved by increasing their income through pig farming. But prevalence of gastrointestinal (G.I.) parasitic infections in pigs is very common [19, 15]. The economic losses which occur due to G.I. parasites are mainly due to subclinical effects and are usually not noticed by the farmers. Subclinical infections are mainly responsible for high morbidity in young pigs and enormous production loss in adult pigs. Production loss usually occurs in terms of reduce growth rate and feed conversion, substantial reproductive loss and poor reproductive performance [31]. The parasites also injures some vital organs which play key role in metabolic activities and causes anorexia, poor growth rate, anaemia, emaciation, infertility and condemnation of affected organs [32].

Protozoans are unicellular organisms under the Kingdom Protista; three important phyla under this are Sarcomastigophora, Ciliophora and Apicomplexa. According to Lekule and Kyvsgaard [24], pigs are considered to be important reservoir hosts for zoonotic intestinal protozoa such as *Cryptosporodium* sp. and *Giardia* sp. which are responsible for diarrhea in animals and human beings [38]. In immune compromised animals *Cryptosporodium* causes severe diarrhea, anorexia and weight loss. Similarly, *Entamoeba polecki* in piglet causes severe ulcerative colitis and it acts as reservoirs for human infections. Although there are reports on the prevalence of G.I. parasitic infections specially helminths in pigs of Meghalaya [26, 27, 7, 34] but reports on different species of G.I. protozoan parasites in pigs of this region are not available. Though anthelmintics are used regularly in farms but they will mostly act against G.I. helminths not against G.I. protozoa [12]. Diarrhea is the main clinical signs of the G.I. protozoan infections which generally do not respond to antibiotic treatment. Specific anti-protozoal drugs will be required to control G.I. protozoan infections in pigs. Thus, taking into account the significance of the G.I. protozoan infections in pigs, the present study was designed to determine the prevalence of different species of G.I. protozoan parasites in pigs of hilly region of Meghalaya.

2. Materials and Methods

2.1 Study area: The present study was conducted in three districts of Meghalaya, *viz.* Ri Bhoi, East Khasi Hills and Jaintia Hills. Ri Bhoi district lies between 25°15´ and 26°15´ North latitudes and 91°45´ and 92°15´ East longitudes (https://en.wikipedia.org/wiki/Ri-Bhoi_district). East Khasi Hills district present in the central part of Meghalaya and lies between 25°07´ and 25°41´ North latitudes and 91°21´ and 92°09´ East longitudes (https://en.wikipedia.org/wiki/East_Khasi_Hills_district).
Jaintia Hills district is the eastern most part of the Meghalaya which lies between 20°58′ and 26°3′ North latitudes and 91°59′ and 92°51′ East longitudes (http://westjaintiahills.gov.in/MapAnthem.htm).

2.2 Study period: The study was conducted for three years (2015-18) and divided into four seasons, *viz.* hot (March, April), rainy (May, June, July, August, September), cool (October, November) and cold (December, January, February).

2.3 Sample collection: The selected animals were categorized according to age *viz.* <6 months, 6-12 months and >12 months. Fecal samples were collected directly from the rectum of the individual animal and kept in marked plastic pouch/vials. A total of 2574 fecal samples of pigs were collected from three districts of Meghalaya and examined for detection of different species of G.I. protozoan parasites in pigs of hilly region of Meghalaya.

2.4 Parasitological techniques: To detect G.I. protozoan parasites in pigs of hilly region of Meghalaya, three grams of fecal samples were examined by direct flotation technique using saturated salt (sp.gr. 1.20) and sucrose (sp.gr. 1.27) solution [29]. Positive samples were then quantified to estimate the oocysts per gram (OPG) of feces by using modified McMaster technique [36]. Samples not being examined on the same day were preserved at refrigerated temperature (4°C) for next day examination. Sheather's sucrose flotation method used for concentration of *Cryptosporidium* oocysts [1]. The positive sample were then subjected to modified Ziehl-Neelsen staining technique [17] and examined under microscope (400x, 1000x) for detection of *Cryptosporidium* oocysts.

3. Results and Discussion

The overall prevalence of G.I. protozoan parasitic infections in pigs of Meghalaya was 29.02% which was in accordance with the findings of Esrony *et al.* [14] and Pakandl [33]. Highest percentage of infection was recorded in Ri Bhoi district (40.73%) followed by East Khasi Hills (39.26%) and Jaintia Hills (20%). Six species of G.I. protozoan parasites were observed *viz. Balantidium coli* (39.36%), *Eimeria* sp. (35.34%), *Cryptosporidium* sp. (10.04%), *Isospora suis* (7.10%), *Entamoeba polecki* (5.62%) and *Giardia* sp. (2.54%) (Fig.1).
In the present study, *B. coli* infection was recorded highest (39.36%) in pigs which were in agreement with the findings of Ybanez *et al.* [40], Mendoza-Gomez *et al.* [30] and Yui *et al.* [39] from Phillippines, Colombia and Japan, respectively. Earlier from North East region of India, Deka *et al.* [8], Rajkhowa *et al.* [35] and Laha *et al.* [26] from Mizoram, Nagaland and Meghalaya reported prevalence of *B. coli* infections in pigs, respectively. *B. coli* infections in pigs were also reported from West Bengal [9], Nagpur [4], Rajasthan [16], Jharkhand [2], Karnataka [22] and Mumbai [13], respectively. In the present study, *Eimeria* sp. was recorded in 35.34% pigs which were in accordance with the findings of Kochanowski *et al.* [23] from Poland (42.9%) and Karaye *et al.* [21] from Nigeria (14%). From Nagaland, 16.25%, 34.7% and 42.95% *Eimeria* infections in pigs were reported earlier by Ebibeni *et al.* [15], Borkotoky *et al.* [5] and Laha *et al.* [28], respectively. The variation in the percent prevalence of *Eimeria* sp. from the present study may be attributed due to different geographical distributions, host factors, climatic conditions required for their development as well as management practices adopted in different regions [11].
In the present study, more than one species of G.I. protozoan parasites *viz. Balantidium coli*, *Entamoeba polecki*, *Giardia* sp., *Cryptosporidium* sp., *Eimeria* sp. and *Isospora suis* were recorded in pigs of Meghalaya. Prevalence of more than one species of G.I. protozoan parasites in the present study in pigs was in agreement with the findings of Barbosa *et al.* [6] from Brazil; Lai *et al.* [25] from China; Uysal *et al.* [37] from Turkey and Bornay-Llinares *et al.* [3] from Spain. Prevalence of *Balantidium coli*, *Entamoeba polecki* and *Cryptosporidium* sp. in the pigs of Meghalaya is important because all the protozoan parasites are zonootic and has public health significance. So, infected pigs required to be treated immediately with anti-protozoal drugs to prevent spread of infections to the susceptible pigs and pig farmers of this region.
Monthwise highest infections was recorded in the month of December (34.42%) which was in accordance with the findings of Kumsa and Kifle [20]. The oocyst per gram (OPG) of feces ranges from 50-1550. Maximum and minimum OPG was recorded in the month of July (425.17) and February (122.22), respectively (Fig. 2). Season wise infection was recorded highest during rainy season (32.49%) followed by cool (31.93%), cold (25.50%) and hot (21.85%) season (Table 1). This may be due to high intensity of infections acquired during the rainy season and non-administration of anti-protozoal drug in infected animals. Age wise G.I. protozoan parasitic infections was recorded in all age groups of pigs *viz.* <6 months (31.56%), 6-12 months (35.01%) and >12 months (20.22%) (Table 2). The prevalence of *Balantidium coli* was recorded highest in 6-12 months (12.83%) followed by >12 months (11.84%) and <6 months (9.65%) old pigs. *Cryptosporidium* sp. was observed in <6 months (8.51%) old pigs while *Entamoeba polecki* was recorded in 6-12 months (4.90%) pigs. However, *Giardia* sp. was recorded in both <6 months (0.68%) and 6-12 months (1.52%) old pigs. The prevalence of *Eimeria* sp. was highest in 6-12 months (11.44%), followed by <6 months (11.01%) and >12 months (8.25%). However, *I. suis* was recorded highest in 6-12 months (4.32%), followed by <6 months (1.70%) and >12 months (0.12%). It was observed that the G.I. protozoan infections in young animals were higher in comparison to adult pigs which might be due to variation in the immune status of pigs of different age groups. Similarly, Kumsa and Kifle [20] from Ethiopia and Damriyasa and Bauer [10] from Germany observed age wise variations in the prevalence of G.I. protozoan parasites. The present study revealed that different species of G.I. protozoan parasites are prevalent in pigs of hilly region of Meghalaya throughout the year, some of which are zoonotic and has public health significance. Reports on the prevalence of *Entamoeba polecki*, *Cryptosporidium* sp. and *Giardia* sp. in pigs of hilly region of

Meghalaya may be considered as the first report from this region. This might be due to favorable environmental conditions for propagation and perpetuation of the parasites, lack of awareness about G.I. protozoan parasites and non-administration of anti-protozoan drugs by the pig farmers. Other factors which might be responsible are constant exposure to infections, continuous deposit of infections by the adult carrier animals as well as poor animal husbandry practices adopted by the pig farmers of the hilly region of Meghalaya.

Table 1: Season-wise prevalence of G.I. protozoan parasites in pigs of Meghalaya

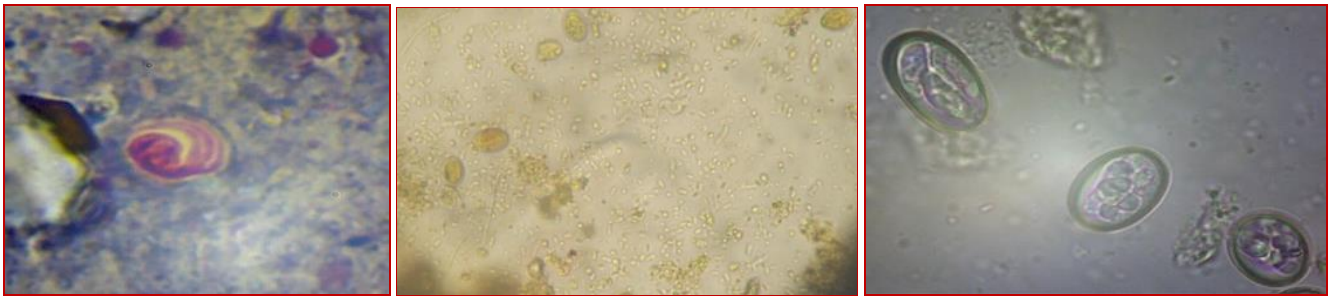
Season	Sample examined	Sample positive	Ciliophora	Sarcomastigophora		Apicomplexa			OPG
			Balantidium coli	*Entamoeba polecki*	*Giardia* sp.	*Eimeria* sp.	*Isospora suis*	*Cryptosporidium* sp.	
Hot	421	92 (21.85)	36 (39.13)	4 (4.35)	0	40 (43.48)	2 (2.17)	10 (10.87)	214.99
Rainy	1034	336 (32.49)	144 (42.86)	18 (5.36)	7 (2.08)	105 (31.25)	26 (7.74)	36 (10.71)	365.33
Cool	523	167 (31.93)	64 (38.32)	7 (4.19)	3 (1.80)	69 (41.32)	12 (7.19)	12 (7.19)	343.41
Cold	596	152 (25.50)	50 (32.89)	13 (8.55)	9 (5.92)	50 (32.89)	13 (8.55)	17 (11.18)	167.62

Figures in parentheses indicates percent positivity

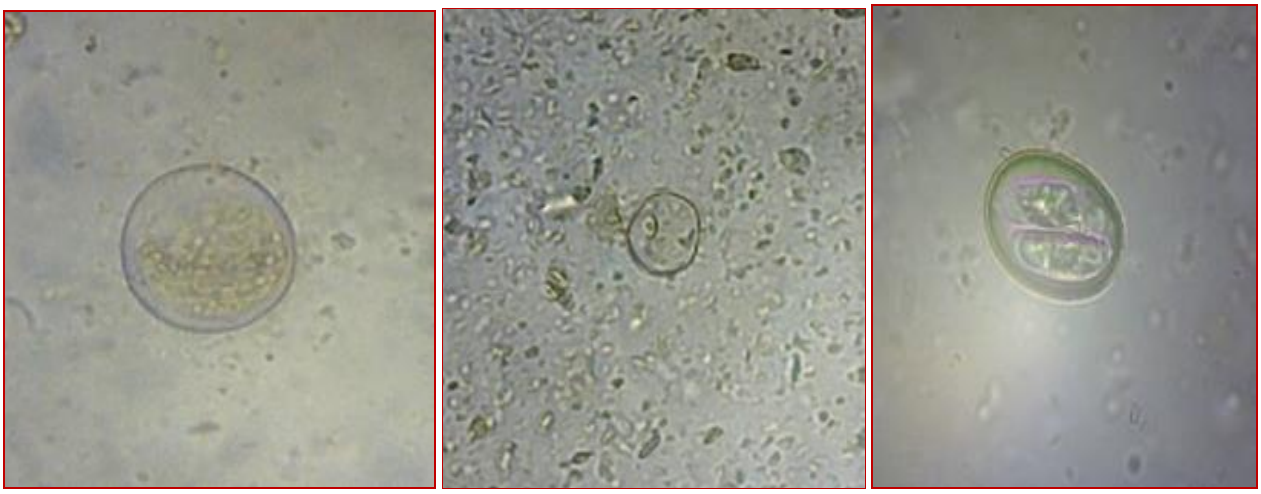
Table 2: Age-wise prevalence of G.I. protozoan parasites in pigs of Meghalaya

Age (months)	Sample examined	Sample positive	Ciliophora	Sarcomastigophora		Apicomplexa		
			B. coli	*E. polecki*	*Giardia* spp.	*Eimeria* spp.	*I. suis*	*Cryptosporidium* spp.
<6	881	278 (31.56)	85 (9.65)	0	6 (0.68)	97 (11.01)	15 (1.70)	75 (8.51)
6-12	857	300 (35.01)	110 (12.83)	42 (4.90)	13 (1.52)	98 (11.44)	37 (4.32)	0
>12	836	169 (20.22)	99 (11.84)	0	0	69 (8.25)	1 (0.12)	0

Figures in parentheses indicates percent positivity



Cryptosporidium sp. *Giardia* sp. *Eimeria* sp.



Balantidium coli *Entamoeba polecki* *Isospora suis*

Fig 1: Different species of G.I. protozoan parasites in pigs

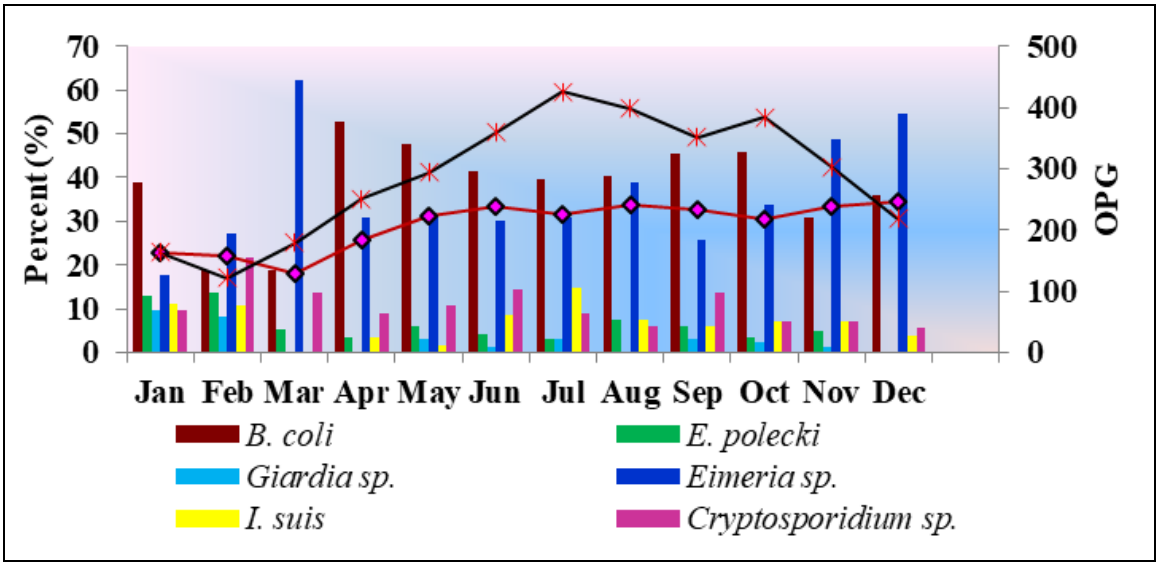


Fig 2: Month-wise prevalence and intensity of G.I. protozoan parasites in pigs

4. Conclusion

The present study revealed that six species of gastrointestinal protozoan parasites i.e. *Balantidium coli*, *Entamoeba polecki*, *Giardia* sp., *Cryptosporidium* sp., *Eimeria* sp. and *Isospora suis* are prevalent in the pigs of hilly region of Meghalaya. Few species are zoonotic and thus has public health significance. Infected pigs should be treated immediately with anti-protozoal drugs to prevent spread of infections.

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