Coccidiosis in pigs of subtropical hilly region of Meghalaya, India

M Das, R Laha, G Khargharia and A Sen

Abstract
To determine the prevalence of coccidia infections in pigs of subtropical hilly region of Meghalaya, a total of 2574 Fecal samples of pigs from different age groups <6 months (706 nos.), 6-12 months (896 nos.) and >12 months (972 nos.) were collected and screened by flotation techniques. Oocysts of Cryptosporidium spp. were examined by Sheather’s sucrose flotation, Malachite green staining (0.2%) and modified Ziehl-Neelsen staining techniques. The overall prevalence of coccidia infections in pigs of Meghalaya was 16.86%. Three different species of coccidia were observed, Eimeria spp. (11.50%), Isospora suis (2.45%) and Cryptosporidium spp. (2.91%). Age wise, 26.35%, 15.74%, and 11% infections were recorded in <6 months, 6-12 months and >12 months old pigs, respectively. Highest and lowest infections was recorded in the month of August (32.83%) and January (6.93%), respectively. Season wise infection was recorded highest during rainy season (21.95%) followed by cool (18.16%), hot (12.35%) and cold (10.07%) season. Six species of Eimeria were recorded viz. E. debliecki (42.23%), E. porci (13.85%), E. suis (27.36%), E. perminuta (12.5%), E. cerdonis (1.69%) and E. spinosa (2.36%). The oocyst count per gram of feces ranged from 50.27-1826.64 in infected pigs.

Keywords: Pigs, coccidia, species, Meghalaya

Introduction
Animal husbandry is an important subsector of agriculture and pig rearing is one of the important livestock components of the tribal population of Meghalaya. It is a major source of income for the landless and marginal farmers which directly influence their socio-economic status. The consumption of pork is increasing due to per capita income, urbanization, changes in life style and food habit [39]. So, the tribal population of this region may increase their income through pig rearing. But one of the major constraints in pig rearing is the gastrointestinal (GI) parasitic infections [21]. Coccidiosis is one of the most pathogenic GI parasitic diseases caused by the different species of Phylum-Apicomplexa [1]. It is a common problem of confined animals kept under intensive husbandry practices. The disease is characterized by scours, dehydration, rough hair coat, reduced growth rate, weakness, weight loss, variable mortality and morbidity and is not responsive to most antibacterial therapy. It is caused by small parasites that multiply inside the host cell mainly in the intestinal tract viz. Eimeria spp., Isospora suis and Cryptosporidium spp. [33]. Coccidiosis is more common and wide spread in sucking piglets but is seen occasionally in growing and finishing pigs and boars when they are moved or housed into continually populated and infected pens [36]. It is suspected in young pigs suffering from continuous diarrhoea which does not respond to antibiotic therapy. In early stage, diarrhoea is the main clinical signs and in later stage consistency of feces varies and colour varies from yellow to grey green or bloody according to the severity of the condition. According to Davies et al. [10] and Ruprah [31] oocysts of coccidia are passed in feces and may not cause a significant mortality but can certainly cause a clinical disease or can at least retard the growth of pigs. Environmental factors play a pivotal role in the dissemination and prevalence of coccidia infections in susceptible animals. It is mostly transmitted by ingestion of contaminated feed and water with oocysts of coccidia. Although several authors studied the prevalence of GI parasitic infections in pigs of Meghalaya [21, 22, 43, 6] but detailed reports on the prevalence of different species of coccidia have not been mentioned. In piglets, coccidiosis causes poor performance during the fattening period as well diarrhoea, and also predisposes the animal to secondary bacterial and viral infections [20, 25]. This is mainly due to disruption of small intestine villi and surface area which interrupts with normal absorption process of nutrients. Therefore, taking into account the significance of the
coccidia parasites as one of the most important causes of economic losses in pigs, the present study was designed to determine the prevalence of different species of coccidia 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 occupies an area of 2378 km² and 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 forms a central part of Meghalaya and covers a total geographical area of 2748 km². It 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 with a geographical area of 3819 Km². It lies between 20°58’ and 26°3’ North latitudes and 91°59’ and 92°51’ East longitudes. (http://westjaintiahills.gov.in/MapAnthem.htm). The study area was situated at an altitude of about 400-1800m above mean sea level where average monthly minimum and maximum temperature were 6.5°C and 30.8°C, respectively. The average monthly relative humidity prevailed during the study period was 61.6% (minimum) to 88.9% (maximum) with average annual total rainfall 2891 mm.

2.2 Study period

The study was conducted for two years (2015-16, 2016-17) 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 size

A total of 2574 fecal samples of pigs were collected from both organized and unorganized farms present in three districts of Meghalaya and screened for detection of coccidia infections.

2.4 Study method

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. Three grams of fecal samples were examined by direct flotation technique using saturated salt (specific gravity: 1.20) and sucrose (specific gravity: 1.27) solution [27]. Samples found positive for *Eimeria* spp. and *Isospora suis* were then quantified to estimate the oocysts per gram (OPG) of feces by using modified McMaster technique [33]. 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. and *Isospora suis* with 2.5% potassium dichromate solution in a ratio of 1:5 volume as per the standard procedure [27, 32]. Morphological characterization of the oocysts was done as per the guidelines of Daugschies et al. [9] and Soulsby [33] by using an Olympus BX51 light microscope at 200x and 400x magnifications. For detection of *Cryptosporidium* spp. in fecal samples of pigs, fecal samples were examined at first by Sheather’s sucrose flotation method for concentration of *Cryptosporidium* oocysts as per the procedure described by Barr [4]. Malachite green staining technique was also used for the detection of *Cryptosporidium* spp. as per the method described by Elliot et al. [15] with slight modifications. A drop of fecal sediment and a drop of malachite green stain (0.2%) were mixed on a glass slide, evenly spread and air dried and examined under magnification (400x) and oil immersion (1000x). The positive sample was then subjected to modified Ziehl-Neelsen staining technique [34] and examined under microscope (400x, 1000x) for detection of *Cryptosporidium* oocysts.

3. Results and Discussion

The overall prevalence of coccidiosis in pig of hilly region of Meghalaya was 16.86%. Three different species of coccidia were observed under Phylum-Apicomplexa in the present study viz. *Eimeria* spp. (11.50%), *Isospora suis* (2.45%) and *Cryptosporidium* spp. (2.91%) (Table 1). In the present findings, it has been observed that the prevalence of different species of coccidia infection followed an age pattern. Age wise, 26.35%, 15.74%, and 11% infections were recorded in <6 months, 6-12 months and >12 months pigs, respectively. The prevalence of *Eimeria* spp. was highest in 6-12 months (13.06%), followed by <6 months (11.47%) and >12 months (10.08%). However, *I. suis* was recorded highest in <6 months (4.25%), followed by 6-12 months (2.68%) and >12 months (0.93%). *Cryptosporidium* spp. was recorded only in <6 months (10.62%) old pigs (Fig. 1). District wise highest prevalence of coccidiosis was recorded in Ri Bhoi (21.25%) followed by East Khasi Hills (15.02%) and Jaintia Hills (12.62%) (Fig. 2). Month wise highest and lowest infections was recorded in the month of August (32.83%) and January (6.93%), respectively (Fig. 3). Maximum and minimum OPG was recorded in the month of July (425.17) and February (122.22), respectively. Season wise infection was recorded highest during rainy season (21.95%) followed by cool (18.16%), hot (12.35%) and cold (10.07%) season (Table 2). The OPG of feces ranged from 502.87-1826.64 in infected pig. In the present study, variations in the percent prevalence of different species of *Eimeria* spp. were observed according to age. Six species of *Eimeria* were recorded viz. *E. debliecki* (42.23%), *E. porci* (13.85%), *E. suis* (27.36%), *E. perminuta* (12.5%), *E. cerdonis* (1.69%) and *E. spinosa* (2.36%) (Table 3). All the species of *Eimeria* were identified on the basis of their morphological characters (Fig. 4) for the first time from the hilly region of Meghalaya. In <6 months old pigs, *E. porci* (34.15%) was highest followed by *E. suis* (33.33%), *E. debliecki* (30.4%) and *E. perminuta* (27.02%). However, in 6-12 months old pigs, *E. perminuta* (51.35%) were recorded highest followed by *E. debliecki* (49.6%), *E. suis* (41.98%), *E. porci* (41.46%), *E. spinosa* (2.36%) and *E. cerdonis* (1.69%). In >12 months old pigs, *E. suis* (24.69%) was highest followed by *E. porci* (24.39%), *E. perminuta* (21.62%) and *E. debliecki* (20%).

The present study revealed prevalence of 16.86% coccidia infections in pigs of hilly region of Meghalaya which was in accordance with the findings of Kaur et al. [17] from Punjab and Krishnamurthy et al. [18] from Karnataka. Similarly, prevalence of coccidiosis in pigs from different parts of the world are reported by Roesel et al. [19] from Uganda (40.7%), Jufare et al. [16] from Ethiopia (12%), Geresu et al. [12] from Ethiopia (11.8%), Abdu and Gasahaw [2] from Ethiopia (5.6%), Matsubayashi et al. [22] from Japan (40.3%), Tiwari et al. [18] from West Indies (88%), Weka and Ikek [40] from Nigeria (15.6%) and Weng et al. [41] from China (47.2%). The
variation in the prevalence from the present study may be attributed due to different geographical distributions, host factors, climatic conditions required for their development [7] as well as management practices adopted in different regions. In the present study it was observed that the percentage of coccidia infections in young animals (<6 months, 6-12 months) were highest and maximum during the month of August. Similar reports on the maximum prevalence of coccidiosis were reported by Tsunda et al. [36]. This may be due to immature immunity in younger animals and continuous dissemination of infection in the environment by adult carrier animals which make young animals more susceptible towards infection. The OPG of feces ranged from 502.87-1826.64 in infected pig which was in agreement with the findings of Chhabra and Mafukidze [5].

Reports on the prevalence of different species of coccidia in pigs of India are extremely meagre. The first report was of Ahlunwalia [3] from Uttar Pradesh followed by Gill [13] from Izatnagar, Patnaik [29] from Orissa, Mishra [28] from Uttar Pradesh, Shrivastav and Shah [34] from Madhya Pradesh and Tayo et al. [37] from Arunachal Pradesh. In the present study, different species of coccidia (Eimeria spp., Isospora suis, Cryptosporidium spp.) were identified which was in conformity with the findings from Azerbaijan [14], China [24] and Poland [19]. In the present study, six different species of Eimeria spp. were identified. In contradiction to our findings, Tsunda et al. [36] from Nigeria and Daugschies et al. [8] from Germany reported prevalence of different species of Eimeria in pigs viz. E. perminuta, E. scabra, E. spinosa, E. debliecki, E. polita, E. suis and E. porci. Leyton et al. [23] from Ontario, Canada reported high rate of Isospora suis (26.4%) infections in suckling pigs. Tomass et al. [35] and Yin et al. [42] from Ethiopia and China reported 7% and 17% Cryptosporidium spp. in pigs, respectively. As far as our knowledge is concerned there are no reports on the prevalence of different Eimeria spp. and Cryptosporidium spp. in pigs of Meghalaya and this report on the prevalence of six species i.e. E. debliecki, E. porci, E. suis, E. perminuita, E. cerdonis, E. spinosa and Cryptosporidium spp. may be considered as the first report from Meghalaya.

The present study revealed that coccidia infections in pigs are common and prevalent throughout the year in Meghalaya which was in agreement with the findings of Tsunda et al. [36]. This might be due to favorable environmental conditions for propagation and perpetuation of the parasites and non-administration of coccidiostat or coccidicidal drugs by the 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.

Table 1: Age wise prevalence of coccidiosis in pigs of Meghalaya

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Sample examined</th>
<th>Sample positive</th>
<th>Eimeria spp.</th>
<th>Isospora suis</th>
<th>Cryptosporidium spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>706</td>
<td>186 (26.35)</td>
<td>81 (11.47)</td>
<td>30 (4.25)</td>
<td>75 (10.62)</td>
</tr>
<tr>
<td>6-12</td>
<td>896</td>
<td>141 (15.74)</td>
<td>117 (13.06)</td>
<td>24 (2.68)</td>
<td>---</td>
</tr>
<tr>
<td>&gt;12</td>
<td>972</td>
<td>107 (11)</td>
<td>98 (10.08)</td>
<td>9 (0.93)</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td>2574</td>
<td>434 (16.86)</td>
<td>296 (11.50)</td>
<td>63 (2.45)</td>
<td>75 (2.91)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicates percent positivity

Table 2: Season wise prevalence of coccidiosis in pigs of Meghalaya

<table>
<thead>
<tr>
<th>Season</th>
<th>Sample examined</th>
<th>Sample positive</th>
<th>Eimeria spp.</th>
<th>Isospora suis</th>
<th>Cryptosporidium spp.</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot (Mar, Apr)</td>
<td>421</td>
<td>52 (12.35)</td>
<td>40 (9.50)</td>
<td>2 (0.48)</td>
<td>10 (2.38)</td>
<td>429.98</td>
</tr>
<tr>
<td>Rainy (May, Jun, Jul, Aug, Sep)</td>
<td>1034</td>
<td>227 (21.95)</td>
<td>147 (14.22)</td>
<td>37 (3.58)</td>
<td>43 (4.16)</td>
<td>1826.64</td>
</tr>
<tr>
<td>Cool (Oct, Nov)</td>
<td>523</td>
<td>95 (18.16)</td>
<td>69 (13.19)</td>
<td>14 (2.68)</td>
<td>12 (2.29)</td>
<td>686.81</td>
</tr>
<tr>
<td>Cold (Dec, Jan, Feb)</td>
<td>596</td>
<td>60 (10.07)</td>
<td>40 (6.71)</td>
<td>10 (1.68)</td>
<td>10 (1.68)</td>
<td>502.87</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of different species of Eimeria in pigs of Meghalaya

<table>
<thead>
<tr>
<th>Eimeria spp.</th>
<th>&lt;6 months</th>
<th>6-12 months</th>
<th>&gt;12 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. debliecki</td>
<td>38 (30.4)</td>
<td>62 (49.6)</td>
<td>25 (20)</td>
<td>125 (42.23)</td>
</tr>
<tr>
<td>E. porci</td>
<td>14 (34.15)</td>
<td>17 (41.46)</td>
<td>10 (24.39)</td>
<td>41 (13.85)</td>
</tr>
<tr>
<td>E. suis</td>
<td>27 (33.33)</td>
<td>34 (41.98)</td>
<td>20 (24.69)</td>
<td>81 (27.36)</td>
</tr>
<tr>
<td>E. perminuita</td>
<td>10 (27.02)</td>
<td>19 (51.35)</td>
<td>8 (21.62)</td>
<td>37 (12.5)</td>
</tr>
<tr>
<td>E. cerdonis</td>
<td>-</td>
<td>5 (1.69)</td>
<td>-</td>
<td>5 (1.69)</td>
</tr>
<tr>
<td>E. spinosa</td>
<td>-</td>
<td>7 (2.36)</td>
<td>-</td>
<td>7 (2.36)</td>
</tr>
</tbody>
</table>

Fig 1: Oocysts of Cryptosporidium spp. in pigs
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4. Conclusion
The present study revealed prevalence of three types of coccidia i.e. *Eimeria* spp., *Isospora suis* and *Cryptosporidium* spp. in pigs of Meghalaya and mostly prevalent during rainy season. Young animals are mostly susceptible. Six species of *Eimeria* spp. were also observed in pigs throughout the year.

5. Acknowledgement
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6. References


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