Original Research

Risk Factor Assessment for Prevalence of Coccidiosis in Domestic Pigs (*Sus Scrofa Domesticus*) from Punjab, India

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Abstract
The copro-prevalence and assessment of risk factors for coccidiosis in pigs was studied from 36 organised and backyard farms representing 5 agro-climatic zones of Punjab. An overall prevalence of 9.41% was recorded with maximum prevalence in western plain zone (22.6%). Of the farms screened, 63.88% (23/36) were found positive for coccidiosis with mixed infections of two or more species of Eimeria having oocysts per gram ranging from 200-62000. Significantly (P<0.05) higher infection was recorded in winter and rainy season. Further, piglets and growers were at a higher risk as compared to adults. Eight species of Eimeria were recorded viz. E. polita, E. spinosa, E. scabra, E. perminuta, E. suis, E. debliecki, E. neodebliecki and E. porci. Morpho-micrometric parameters were recorded for phenotypic characterisation of Eimeria.

Key words: Coccidiosis, *Eimeria*, Pigs, Prevalence, Punjab, Risk factors

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Introduction

Genus *Eimeria* (Family: Eimeriidae) is an enteric monoxenous coccidian parasite responsible for causing coccidiosis characterised by profuse diarrhoea, anorexia and weight loss in livestock including pigs (Henry and Tokach, 1995). The disease has worldwide prevalence mostly affecting sucking piglets but occasionally in growing and finishing pigs. In piglets, clinical signs result from the invasive action of endogenous developmental stages in the intestinal mucosa resulting in diarrhoea mostly in the second week of life with watery or pasty yellowish faeces tinged with blood accompanied by dehydration (Ruprah, 1985). The coccidian oocysts are transmitted *via* faeco-oral route leading to retarded growth in pigs, lesser weight gains and rare mortality (Davies *et al*., 1963). Environmental factors *viz*. relative humidity, rainfall, temperature and microclimate plays an important role in the survival and maturation of pre-parasitic and infective stages of Coccidia (Tsunda *et al*., 2013).

In previous decades, coccidiosis in pigs has been reported worldwide *viz*. Estonia (Toivo, 2003), Germany (Daugschies *et al*., 2004), Brazil (Alynne *et al*., 2015), Mozambique (Chilundo *et al*., 2017) and China (Junhui *et al*., 2015). However, perusal of literature indicated that the information on the epidemiology of porcine coccidiosis is very limited and fragmentary in India (Laha *et al*., 2014; Kaur *et al*., 2017; Singh *et al*., 2017). Moreover, there is no comprehensive study from the state of Punjab. Nonetheless, all-inclusive knowledge on the epidemiology and associated risk factors of porcine coccidiosis is vital for designing effective prophylaxis measures and control of disease. Therefore, in preview of significance of this parasite as one of the most important causes of economic losses to pig industry, the present study was conducted.

Materials and Methods

Study Area

Punjab state extends from the latitude 29°30'N to 32°32'N and longitudes 73°55'E to 76°50'E in the north-west region of India. It covers geographical area of 50,362 $km^2$and lie between altitudes 180 meters and 300 meters above mean sea level. The state is divided into five-agro climatic zones based on the cropping pattern, geography, rainfall and soil texture *viz*. sub-mountain undulating zone, undulating plain zone, central plain zone, western plain zone and western zone.

Collection of Samples

The sample size was calculated by employing Software EpiTool (http://epitools.ausvet.com.au), proportional to the state pig population of approximately 32,221 heads (DAHP, 2016) with an expected prevalence of 50%, margin of error as 3.1% (≤5%), and a 95% confidence interval which gave sample size of approximately 320. However, additional convenient sampling was done to include more samples in the study. Thereafter, cluster sampling was employed where each of five agro-climatic zones namely sub-mountain undulating zone (I), undulating plain zone (II), central plain zone (III), western plain zone (IV) and western zone (V) formed a single cluster each and a systematic sampling comprising of villages representing each cluster was done. A proportional sampling scheme was adopted for the samples collected from the suckling piglets and weaner piglets (<4months), growers (4-8months) and adults (>8months) from both males and females. Information regarding various determinants hypothesized to be associated with the risk of GI infection i.e. type of farm management (organised/ unorganised), water consumption (whether owner had the control on consumption of water or not, controlled/ uncontrolled), deworming (present/absent) and cleanliness status of the farm (present/absent) etc. was collected through questionnaire.

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Coprological Examination

The faecal samples were collected immediately after defecation for detection of coccidian oocysts by qualitative faecal assays (concentration-flotation and sedimentation techniques) as per standard protocols (Soulsby, 1982). For quantification of the infection Modified McMaster technique was employed for the estimation of mean oocyst per gram (OPG) as per Soulsby (1982). Sporulation studies were conducted as described by Yang *et al*. (2014). Morpho-micrometric parameters were recorded by software Image J. Confirmation of the Emirian species was done by matching the dimensions of the oocyst to the available literature along with other morphological features like shape, colour, shape index, thickness of the outer wall, micropyle characteristics as per Taylor *et al*. (2007) and Ramirez *et al*. (2008).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics for Windows, Version 22.0. Armonk, NY, IBM was employed and bivariate association between each hypothesized determinant and coccidiosis in pigs was determined using the Pearson chi-square test. A binary logistic regression analysis model was constructed and the relationship between the determinants significantly associated with the risk of coccidiosis was evaluated using coprological status (positive/negative) as the dependent variable. The variables introduced in the model included: location, agro-climatic zone, age, sex, season, type of farm, water consumption, deworming status and cleanliness status of the pens. The effect of each factor on the likelihood of infection was quantified by the Odds ratio (OR) which was computed as the exponent of the respective regression coefficient. The results were presented as odds ratios (OR) along with their 95% confidence intervals (CI). The probability level (P) of <0.05 was regarded as statistically significant.

Results and Discussion

Results of the coproscopic examination revealed a comparatively low (9.41%) level of coccidiosis in pigs of Punjab. Of the 839 samples, 79 (9.41%) were positive for coccidian oocysts (Table 1). Similar low level of porcine coccidiosis had been reported from various geographical locations worldwide *viz*. 8.6% from Germany (Daugschies *et al.*, 2004), 8.0% from Mozambique (Chilundo *et al.*, 2017), 6.38% from China (Junhui *et al.*, 2015) and 15.4% from Ludhiana, Punjab (Kaur *et al.*, 2017). However, high prevalence rate has been documented from north-eastern state of Meghalaya, India (37.96%) (Laha *et al*., 2014) and Madhya Pradesh, India (92.4%) (Singh *et al.*, 2017).

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Table 1: Prevalence of coccidiosis in pigs and mean oocyst per gram (OPG) values

Category	Number of Samples Examined	Number of Positives Samples (%)	Mean OPG±SE	Range
Piglet(<4m)	341	44(12.9)	4302.5±2050.27	200-60000
Grower(4-8m)	228	16(7.0)	5553.33±2080.68	400-21500
Adult(>8m)	270	19(7.0)	1130.77±247.65	600-3300
P value		0.01		
Rainy	397	48(12.1)	7571.43± 2568.85	400-60000
Summer	321	10(3.1)	1050 ±152	600-2600
Winter	121	21(17.4)	2817.39±1266.53	200-22000
P value		0.00		
Total	839	79(9.4)		

A significant variation in the prevalence rate of coccidiosis was recorded ($P<0.05$) in various agro-climatic zones of Punjab, the highest being in the western plain zone (22.6%) followed by central plain (11.4%), western (10.2%), undulating plain (5.5%) and the lowest in sub mountain undulating zone (3.1%). Further, as compared to the western zone, pigs in central (OR: 1.11; CI 95%:0.59-2.48) and western plain zone (OR: 1.47; CI 95%: 0.55-3.98) had increased odds of infection with coccidia (Table 2).

Table 2: Binary logistic regression analysis of factors associated with the prevalence of coccidiosis in pigs

Variable	Reference	OR (Odds Ratio)	*P* value	Confidence Interval (CI 95%)
Season	Summer		0	
Rainy		4.43	0	2.15-9.09
Winter		5.11	0	2.21-11.81
Age	Adult		0.02	
Piglet(<4m)		2.12	0.01	1.18-3.82
Grower(4-8m)		1.21	0.59	0.59-2.48
Agro-climatic Zone	Western		0.03	
Sub-mountain undulating		0.37	0.08	0.12-1.13
Undulating plain		0.44	0.09	0.16-1.15
Central plain		1.36	2.77	0.77-2.41
Western plain		1.47	0.44	0.55-3.98

The difference in prevalence rates might be possibly due to variation in topography, sampling methods, managemental practices and study period (Roepstorff *et al.*, 1998). Further, alteration in micro-climatic conditions of the shed affected by managemental practices of delayed manure and bedding removal, drainage would provide a suitable environment for the development of pre-parasitic stages, hence varied infection rates. Age had a non-significant ($P>0.05$) effect on prevalence rates. Within the age category, the piglets had the highest prevalence followed by growers and adults (Table 1). Compared to the adults, both piglets (OR: 2.12; CI 95%: 1.18-3.82) and growers (OR: 1.11, CI 95%: 0.59-2.48) were at a higher risk of disease (Table 2). The intensity of infection/ mean oocysts per gram (OPG) was the highest in growers

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followed by piglets and adults (Table1, Fig. 1). Similar high prevalence rates in young piglets had been reported earlier (Oneil and Perfitt, 1976).

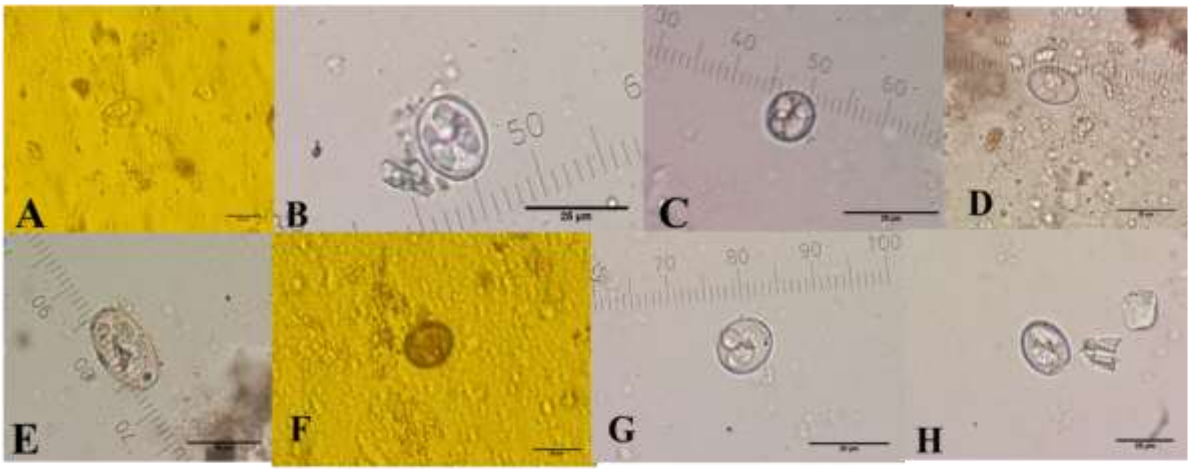

Fig. 1: Oocysts of various *Eimeria* species (40X) A. *E. deblieck* (10X), B. *E. neodebliecki* (40X), C. *E. perminuta* (10X), D. *E. polita(*10X), E. *E. scabra* (10X), F. *E. spinosa* (10X), G. *E. suis* (10X), H. *E. porci*(10X)

However, Ruprah (1985) reported that the piglets were resistant to natural infection for the first three weeks of life due to innate immunity but were susceptible thereafter and older pigs were the carriers of the disease corroborating our findings. Further, a slightly higher prevalence rate of 10.8% (54/498) was recorded in females in comparison to males 7.3% (25/341) but the variation was statistically non-significant (P>0.05). A significant (P<0.05) seasonal variation was recorded with the highest prevalence in the winter season followed by rainy and summer. The pigs had more odds of acquiring the infection during winter (OR: 5.11; CI 95%: 2.21-11.81) and rainy (OR: 4.43; CI 95%: 2.15-9.09) seasons (Table 2). This may due to the decrease in immunity of animals associated with severe weather stress due to cold environmental conditions (Khan *et al.*, 2013). Amongst the managemental factors, the source of water for consumption in pigs significantly (P<0.05) influenced the prevalence rate of coccidiosis (Table 3). However, other determinants studied *viz*. type of farm management (organised/unorganised), deworming status (present/absent) and cleanliness status of the pens (present/absent) had a non-significant (P>0.05) effect. Thus, habit of coprophagy in pigs and drinking water from unknown sources make them prone to coccidiosis as reported earlier by Boes *et al*. (2000). The morphometric studies of the coccidian oocysts revealed eight species of *Eimeria* in pigs of Punjab, India *viz. Eimeria polita*, *E. scabra*, *E. porci*, *E. debliecki*, *E. spinosa*, *E. suis*, *E. neodebliecki* and *E. perminuta* (Fig. 2).

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Table 3: Prevalence of coccidia in pigs under different management systems in the state Punjab

Management		Examined (n)	Coccidia (%)
Type of farm	Organised	372	32(8.6)
	Unorganised	409	42(10.3)
	p value		0.42
Deworming	Absent	348	41(11.8)
	Present	418	33(7.9)
	p value		0.07
Cleanliness	Present	375	31(8.3)
	Absent	391	43(11)
	p value		0.201
Water consumption	Controlled	675	61(9)
	Uncontrolled	41	10(24.4)
	p value		0.001

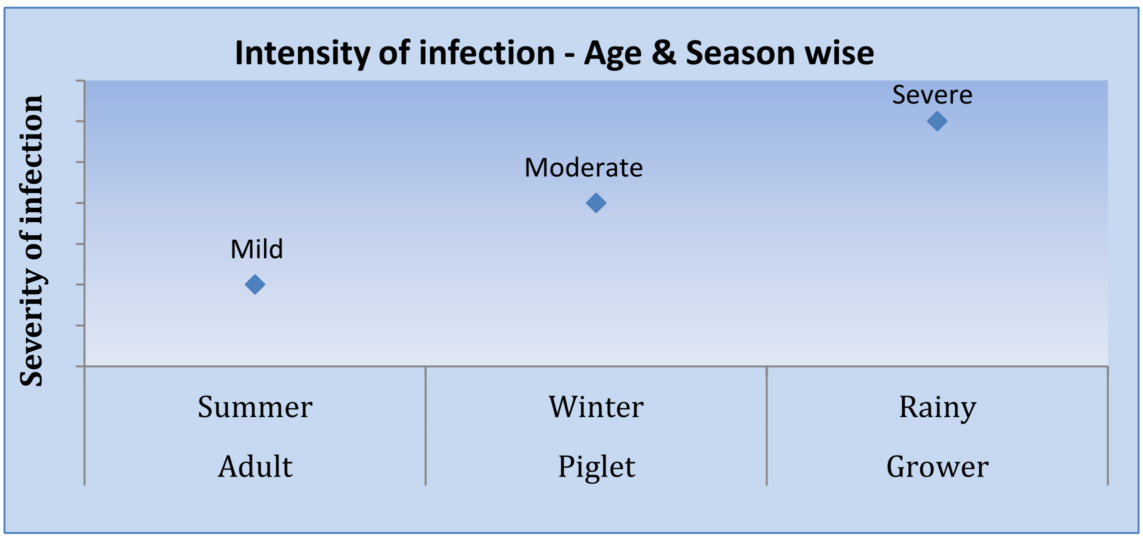

Fig. 2: Age wise and season wise intensity of infection

Out of 36 farms screened, 23 (63.88%) were found positive for coccidiosis and in all the farms, mixed infections were recorded with a minimum of at least two species of *Eimeria* with OPG ranging from 200-62000. Out of 1046 oocysts examined, *E. polita* was the most dominant species with the highest prevalence (42.2%), followed by *E. spinosa* (18.4%), *E. scabra* (13.7%), *E. suis* (7.0%), *E. debliecki* (5.2%), *E. neodebliecki* (3.9%), *E. porci* (3.7%) and *E. perminuta* (2.3%). The details of morphological characteristics of various porcine *Eimeria* species are presented in Table 4. Oocysts of *E. scabra* was the largest in size measuring 29.46±0.39 µm x 20.24±0.33 µm and was ovoid with shape index of 1.46. Also, the oocysts were yellowish-brown in colour with thick outer wall with a micropyle. *E. perminuta* oocyst was the smallest (13.05±13.05 µm x11.15±0.35 µm), spherical in shape, colourless with thick outer wall without a

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micropyle. *E. spinosa* oocysts were brownish, spherical with thick outer layer having distinct spines whereas, all other species had a smooth outer layer.

Table 4: Morpho-metric description of various *Eimeria* sp. infecting pigs

Species	Av Length ±SE (Range) (µm)	Av Width ±SE (Range) (µm)	Outer Wall	Shape	Micropyle	Shape Index*	Sporulation Time (days)
E. polita	22.79±0.25 (18.40-27.59)	16.58±0.29 (11.37-22.45)	Thick	Ovoid	-	1.4	7-10
E. debliecki	28.37±0.75 (18.84-38.38)	16.33±0.35 (12.49-21.10)	Thick	Ellipsoidal /Oval	-	1.74	6-10
E. porci	22.44±0.45 (20.08-28.28)	14.85±0.45 (11.11-20.57)	Thin	Oval	+	1.52	7-10
E. scabra	29.46±0.39 (25.75-39.98)	20.24±0.33 (16.9-29.61)	Thick	Ovoid	+	1.46	8-10
E. suis	17.69±0.45 (14.17-24.89)	16.08±0.47 (12.54-24.38)	Thick	Spherical	-	1.11	5-7
E. perminuta	13.05±0.42 (11.37-15.72)	11.15±0.35 (9.24-13.66)	Thick	Spherical	-	1.18	5-8
E. neodebliecki	18.53±0.25 (11.70-22.81)	13.94±0.19 (9.6-17.21)	Thin	Ovoid	-	1.33	6-8
E. spinosa	26.92±0.82 (23.02-34.31)	24.20±0.65 (20.47-29.32)	Thick with spines	Spherical	-	1.11	10-12

**Length / width*

Eimeria polita was the most dominant species however, reports from other parts of the world describe *E. debliecki* (Chhabra and Mafukidze, 1992; Varghese, 1986; Ramirez *et al.*, 2008) and *E. perminuta* (Tsunda *et al.*, 2013) as the most common porcine Emirian species. Oocyst of *E. scabra* was the largest and *E. perminuta* was the smallest amongst all the porcine *Eimeria* species recorded in the current study and the results are similar to the observations of Tsunda *et al.* (2013). The characteristics of the oocysts described in our study are similar to the reports of Daugschies *et al.* (1999) and Taylor *et al.* (2007).

Conclusion

In conclusion, subclinical coccidiosis is prevalent in the pigs of Punjab state, India. The baseline data generated in the current study would be of immense help for the formulation and implementation of control strategies against the coccidiosis in pigs. As observed in the current study that pigs ingest the infective sporulated coccidian oocysts from environment through contaminated water and feed. Therefore, regular cleaning and disinfection of pig farms, regular monitoring, provision of quality food/water and strategic anti-coccidial regimens would play a constructive role in disease control.

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