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Prevalence of gastrointestinal helminths and assessment of associated risk factors in pigs from Rajasthan districts, India

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Abstract
The aim of this study was to determine the prevalence and associated risk factors of gastrointestinal helminthic infections in swine population of Rajasthan, India. A total of 769 faecal samples (including 398 from farm and 371 from stray pigs) were collected from pigs of various districts representing different agro-climatic zones of Rajasthan from September 2017 to August 2018 and examined for gastrointestinal helminths. Coprological examination revealed an overall prevalence of 56.95% (farm: 34.67%, stray: 80.86%) while mixed infection was found in 29.90% population with statistically a highly significant difference (p< .01). The parasites reported in the present study with their respective prevalence rates were *Ascaris suum* (29.51%), *Trichuris* sp. (17%), Strongyles (10.79%), *Fasciolopsis buski* (4.46%), *Strongyloides* sp. (3.51%), *Fasciola* sp. (1.04%). Multivariate binary logistic regression analysis revealed highly significant difference (p< .01) in the prevalence rates of gastrointestinal helminths. Seasonal analysis revealed statistically highly significant difference (p< .01) among the seasons with rainy season (B=2.582, OR=13.222) to be the most conducive for the growth of gastrointestinal helminth infections. Similarly, a highly significant statistical difference was observed management wise analysis with farm animals (B= - 2.252, OR= .105) at minimum risk of infection when compared to stray animals. Zone wise and district wise analysis revealed that hot and humid conditions of district Kota (B= 3.715, OR= 41.052) in the humid south eastern plain zone of Rajasthan, provided the most favourable niche to gastrointestinal helminths of swine population in the state. Coproculture analysis revealed the higher prevalence rate of *Hyostrongylus* sp. to *Oesophagostomum* sp. Quantitative analysis in the present study presented a severe gastrointestinal parasitic infection in terms of EPG (eggs per gram of feces) for *Ascaris suum* 400-25,600 (5411.76 ± 984.24), *Fasciolopsis buski* 800-7100 (2736.36 ± 532.42) and *Trichuris* sp. 600-5600 (1842.85 ± 662.19).

Keywords: swine, coproculture, gastrointestinal helminthic infections, prevalence, Rajasthan

Introduction
Pigs are fast growing and one of the most prolific livestock species. They are even toed ungulates of Genus *Sus* under family Suidae. India is an agro-climatically diversified country of which, Rajasthan is geographically the largest state. Pig farming in India is catching business particularly for the persons belonging to lower socioeconomic groups as they are inexpensive to raise with quicker and higher returns on their investment, resulting in quick generation of cash to farmers [7]. Pig industry consequently has led to the production of more animal origin protein, increased employment opportunities, alleviation of poverty, contribution to the National Gross Domestic Product and generation of foreign exchange for economic development [22].

GI parasites are among the most devastating disease causing organisms of swine. Recently, substantial loss of productivity (inefficient feed conversion, reduced weight gain, stunted growth, delayed fertility, decreased litter size and the condemnation of affected organs after slaughter) is recorded annually in swine husbandry worldwide due to bewildering status of gastrointestinal helminth infections [31]. Infected pigs rarely show ominous clinical symptoms, hence, treatment is often delayed [24]. Feeding habit of these ungulates such as scavenging, cannibalism and rearing system adopted are major accountable factors of infection [25].

Although, such studies have been started in different parts of country by various workers [3, 6, 15, 16, 19, 29] but there is dearth of studies and lack of database in regards to the epizootiology of swine gastrointestinal helminth infections in Rajasthan.

As per the best of our knowledge, the present study is the pioneer study planned to generate epizootiological data and associated risk factors analysis of various GI parasites in pig population to frame their sustainable control strategies in the region. Results would help increase the awareness of local swine producers and veterinary practitioners in the area and will add knowledge to the swine parasitic epidemiology in the country.

Material and Methods

The present cross sectional study was conducted for aperiod of one year from September 2017 to August 2018. Sampling was done homogenously covering all three seasons (winter, summer and rainy) to determine the prevalence of gastrointestinal tract helminth infections in the major part of swine population from seven districts represent into six agro-climatic zones of Rajasthan state.

Study area

Rajasthan is located at 27.0238° North latitude, 74.2179° East longitude and 309 m above the sea level on the western side of the country. The Rajasthan state has been divided in 10 major agroclimatic zones. Present work has been carried out covering six out of the 10 agro-climatic zones comprising Semi Arid Eastern Plain, Flood Prone Eastern Plain, Internal Drainage Dry, Hyper Arid Partial Irrigated, Transitional Plain of Luni Basin and Humid South Eastern Zones (Fig. 1) with an average rainfall 100-1000 mm and temperature ranging between 4 °C to 48 °C [9].

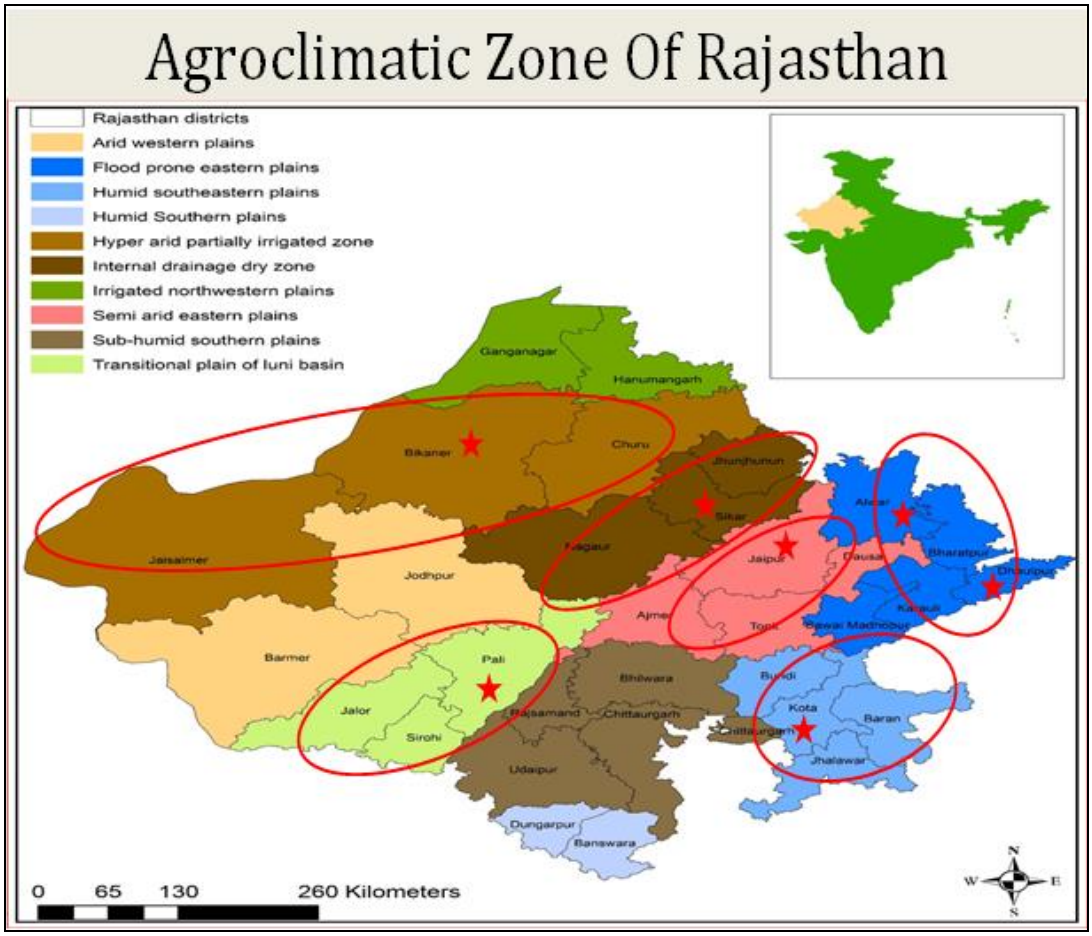

Fig 1: Different Agroclimatic zones of Rajasthan indicating study area.

Collection of samples

A total of 769 faecal samples were collected per rectally or immediately after defecation from swine randomly selected from both free ranging population (371) and farm pigs (398) from the study area selected for the study. Collection of samples done seasonally for a period of one year from September 2017 to August 2018. The samples were placed in sterile polythene bags, properly labeled the information regarding species, age, sex, deworming history and location kept in a cool transport box and brought to the Post graduate laboratory of Department of Veterinary Parasitology, CVAS, Bikaner, for further examination.

Coprological examination

The fecal samples were first subjected to standard qualitative fecal sample examination by using floatation and sedimentation techniques [29] for detection of helminth eggs and quantitatively by modified McMaster egg counting technique and Stoll's technique [5]. Coproculture study was also performed to harvest and identify infective strongyle larvae [1, 30].

Statistical analysis

Statistical analysis was performed by using SPSS 20.0 software by applying Chi Square ($\chi 2$) test and subjected to the multivariate binary logistic regression model with significant association at $p \leq .05$ (two-sided).

Results

Out of 769 samples examined, 438 samples were found positive with an overall prevalence rate of 56.95% for gastrointestinal helminth infections (Table 1). Mixed infection

was found in 29.90% population with highly significant difference (p< .01) with complete details in table 1. Among the various parasitic infections reported in the study, *Ascaris suum* (29.51%) showed the highest prevalence followed by *Trichuris* sp. (17%), strongyles (10.79%), *Fasciolopsis buski* (4.46%), *Strongyloides* sp. (3.51%), *Fasciola* sp. (1.04%) in the decreasing order of prevalence.

Quantitative analysis presented a severe gastrointestinal infection in terms of EPG (eggs per gram) for *Ascaris suum* ranging from 400-25,600 (average 5411.76 ± 984.24) for *Trichuris* sp. ranging from 600-5600 (average 1842.85 ± 662.19) and for *Fasciolopsis buski* ranging from 800-7100 (average 2736.36 ± 532.42).

Table 1: Overall prevalence of GIT helminths in swine in six agro-climatic zones of Rajasthan

		Examined (%)	Infected (%)	Mixed (%)	*Ascaris suum* (%)	Strongyle (%)	*Strongyloides* (%)	*Trichuris suis* (%)	*Fasciola gigantica* (%)	*Fasciolopsis buski* (%)
Animal group	Stray	371	300 (80.86)	219 (59.03)	221 (59.57)	74 (19.95)	28 (7.55)	84 (22.64)	8 (2.16)	34 (9.16)
	Farm	398	138 (34.67)	11 (2.76)	6 (1.5)	9 (2.26)	-	47 (11.8)	-	-
χ^2 Value			167.09**	289.97**	311.12**	62.37**	-	15.94**	-	-
Total		769	438 (56.96)	230 (29.90)	227 (29.51)	83 (10.79)	28 (3.64)	131 (17)	8 (1.04)	34 (4.42)

Management wise analysis

Management wise prevalence analysis revealed statistically a highly significant difference (p<0.01) in the prevalence rates of gastrointestinal helminth infections in stray pigs (80.86%) than farm pigs (34.67%) with complete details in table 1 & 4. Multivariate binary logistic regression model indicated the manamgement plays a very important role in regulating the infection rate as the farm animals were reported to be in the minumum risk of infection (B= -2.252, OR=0.105) when compared to stray animals.

Seasonal dynamics

A highly significant difference (p<0.01) was reported in the season wise analysis with maximum prevalence of gastrointestinal helminth infections during the rainy season (92.60%) followed by summer (41.29%) and winter (38.26%) with highest prevalence rate of *Ascaris suum* and strongyle infections. Statistical analysis using multivariate binary logistic regression revealed a positive association (B=2.58) of rainy season with odds ratio of 13.222 as the most favourable season for GI helminthoses as compared to summer and winter seasons (Table 3).

Table 3: Season and district wise prevalence of GIT helminths in swine in six agroclimatic zones of Rajasthan

		Examined (%)	Infected (%)	Mixed (%)	*Ascaris suum* (%)	Strongyle (%)	*Strongyloides* (%)	*Trichuris suis* (%)	*Fasciola gigantica* (%)	*Fasciolopsis buski* (%)
Season	Winter	311	119 (38.26)	23 (7.4)	10 (3.22)	11 (3.54)	-	25 (8.04)	-	-
	Summer	201	81 (40.30)	14 (6.97)	28 (13.93)	4 (1.99)	-	49 (24.38)	-	-
	Rainy	257	238 (92.60)	193 (75.09)	189 (73.54)	68 (26.46)	28 (10.89)	57 (22.18)	8 (3.11)	34 (13.23)
χ^2 Value			200.31**	376**	457.74**	98.69**	-	30.29**	-	-
District	Alwar	78	33 (42.31)	13 (16.67)	10 (12.82)	2 (2.56)	0	15 (19.23)	0	0
	Jaipur	85	56 (65.88)	8 (9.41)	0	9 (10.59)	0	2 (2.35)	0	0
	Sikar	148	30 (20.27)	1 (0.68)	0	0	0	8 (5.41)	0	0
	Dholpur	50	13 (26)	0	0	0	0	13 (26)	0	0
	Pali	75	30 (40)	2 (2.67)	6 (8)	0	0	17 (22.67)	0	0
	Bikaner	96	47 (48.96)	16 (16.67)	30 (31.25)	4 (4.17)	0	19 (19.79)	3 (3.13)	0
	Kota	237	229 (96.62)	189 (79.75)	181 (76.37)	68 (28.69)	28 (11.81)	57 (24.05)	5 (2.11)	34 (14.35)
χ^2 Value			273.80**	419.41**	395.86**	121.75**	-	40.69**	11.26	-

Zone wise prevalence

A highly significant difference (p<0.01) in the prevalence of gastrointestinal helminth infection in pigs was reported among six agro-climatic zones with highest prevalence in humid south eastern zone (96.62%) (Table 2).

Table 2: Zone wise prevalence of GIT helminths in swine in six agro-climatic zones of Rajasthan

		Examined (%)	Infected (%)	Mixed (%)	*Ascaris suum* (%)	Strongyle (%)	*Strongyloides* (%)	*Trichuris suis* (%)	*Fasciola gigantica* (%)	*Fasciolopsis buski* (%)
Zone	Flood prone eastern plain	128	46 (35.93)	13 (10.16)	10 (7.8)	2 (1.56)	0	28 (21.88)	0	0
	Semi arid eastern	85	56 (65.88)	8 (9.41)	0	9 (10.59)	0	2 (2.35)	0	0
	Internal drainage dry zone	148	30 (20.27)	1 (0.68)	0	0	0	8 (5.41)	0	0
	Hyper arid partial irrigated	96	47 (48.96)	16 (16.67)	30 (31.25)	4 (4.17)	0	19 (19.79)	3 (3.13)	0
	Transitional plain of luni basin	75	30 (40)	2 (2.67)	6 (8)	0	0	17 (22.67)	0	0
	Humid south eastern	237	229 (96.62)	189 (79.75)	181 (76.37)	68 (28.69)	28 (11.81)	57 (24.05)	5 (2.11)	34 (14.35)
χ^2 Value			270.50**	414.73**	393.46**	121.55**	-	38.63**	11.27*	-

District wise prevalence

Similarly, a highly significant difference (p<0.01) in the prevalence rate of gastrointestinal helminth infection in pigs from seven districts of the study area was observed with the highest prevalence in Kota district (96.62%) followed by Jaipur (65.88%), Bikaner (48.96%), Alwar (42.31%), Pali (40%), Dholpur (26%) and Sikar (20.27%) in the decreasing order of prevalence rates. Statistical analysis using multivariate binary logistic regression revealed Kota district at highest risk (B=3.71, OR=41.05) and Jaipur district found statistically at the lowest risk (Table 4).

Table 4: Multivariate binary logistic regression for helminthosis in swine in six agroclimatic zones of Rajasthan

	Parameter	Logistic regression coefficient (B)	S.E	Wald test	P value	Odds ratio
Animal group	Farm	-2.252	.139	261.914	.000	.105
	Constant	-1.168	.055	457.965	.000	.311
District	Sikar			345.023	.000	
	Alwar	1.918	.408	22.135	.000	6.806
	Bikaner	2.430	.384	40.008	.000	11.357
	Dholpur	1.613	.456	12.542	.000	5.019
	Jaipur	.978	.461	4.507	.034	2.659
	Kota	3.715	.361	105.899	.000	41.052
	Pali	1.788	.416	18.517	.000	5.979
	Constant	-4.516	.355	161.414	.000	.011
Season	Winter			341.516	.000	
	Rainy	2.582	.160	259.327	.000	13.222
	Summer	1.034	.188	30.237	.000	2.813
	Constant	-3.468	.148	548.309	.000	.031

Coproculture studies

The strongyle positive faecal samples were subjected to coproculture and L_3 stage were recovered from fecal cultures in the present investigation, were identified on the basis of measurements of their total length, extension of tail sheath beyond the tip of the larvae (µm), intestinal cell number and shape and some morphological characters. The larvae were identified as *Hyostrongylus* sp. as the major contributor to nematode population (68%) followed by *Oesophagostomum* sp. (32%). The coproculture study revealed few other larvae reported could not be identified due to the lack of concerned literature, hence not included in the study (Table 5).

Table 5: Mean measurements (µm) of 3rd stage strongyle larvae of swine in six agroclimatic zones of Rajasthan (Mean±S.E.)

Nematodes	Total length (Range)	Extension of tail sheath beyond tail (Range)	Intestinal cell no. and shape	Salient features
Hyostrongylus rubidus	786.18±5.8 (728-868)	60.78±1.14 (44.8-74.2)	16 Triangular	Head rounded, oesophagous strongyliform, buccal cavity spearhead shaped, tip of tail with a digitiform process.
Oesophagostomum sp.	636.56±8.4 (560-700)	137.11±2.14 (117.6-156.8)	16 Triangular	Head and oesophagous as in *H. rubidus*, buccal cavity with lining drawn out into thin strands of interlacing fibers,tail long hair like tapering to a fine point.

Discussion

Several studies reporting the prevalence of gastrointestinal helminths of swine population have been conducted in other parts of India as well other countries of the world. Overall prevalence rate reported in the study is in close agreement to the findings of [10] in West Bengal, [6] in Mumbai region as well as [20] in Japan and [24] in Nigeria. Various studies had earlier reported the higher prevalence rates of gastrointestinal parasites in pigs from different parts of India viz. Jammu [11, 12, 16, 29]. In contrast to the present study lower prevalence rates were also reported from India viz. Mizoram [3], Northeast states of India [19] and Punjab [15].

Higher prevalence of *Ascaris suum* is in congruent to the findings of various other workers from India [15, 19] as well as from other countries [34]. This could be due to the high survival rate of A. suum egg in the environment for many years (ability to survive for longer time and produce large numbers of eggs) hence reported more in outdoor pigs [17].

EPG as a result of quantitative analysis was in concordant to the findings of [8] whereas, it was unlike to other investigators viz. [17, 27]. The probable reason for variation in severity of infection might be due to management practices adopted, wet and humid conditions and the number of samples included in the study. Also host's immunity leads to the inhibition of the fecundity of female worms so that the number of eggs laid decreases in proportion to the increase of the host's resistance [33].

The higher prevalence found in scavenging pigs than farm animals is in concordant with the findings of [10] from other parts of the country as well as [13] from other countries of the world. It could be attributed to the fact that majority of pigs are reared by tribal people under backyard conditions without proper introduction of scientific management like housing, feeding and deworming practices [22] as well as their free access to potentially contaminated areas [15]. Moreover, underfed animals have every possibility to suffer from nutritional deficiency which is a predisposing factor for parasitic infections, besides this, untreated animals remain a potential source of infection. However, study of immune status of animal is very essential for executing future parasite control programme [2].

The reason for high prevalence rate of different parasites in rainy season might be due to suitable macro and microclimatic conditions (adequate moisture and optimum temperature), agro ecology favouring the growth, survival and dissemination of infective stages in the pasture, leading to higher infections in pigs [29]. Several other workers from other parts of the country have also reported higher prevalence of gastrointestinal helminths in rainy season viz [10, 19]. However [3] reported lower prevalence of infection during rainy season.

To the best of our knowledge the present study seems to be the pioneer study studying the zone and district wise gastrointestinal helminth prevalence in the pig population of the state. However, intrazonal studies have been conducted earlier in the state by other workers in case of dairy animals viz. [21, 27] who reported a highly significant difference among districts and a non significant difference was reported by Choudhary *et al*. [4] from the state. These differences among the districts of various zones of Rajasthan may be primarily due to variation in annual rainfall which is recorded higher in Kota district [9] providing more conducive conditions for parasitic perpetuation and secondarily due to variation in topography, management, husbandry practices and deworming program [21, 27] which are comparatively better in capital region of the state.

The nematodes encountered in the present study are in concordant with the findings of other workers [35]. The present study reported comparatively higher prevalence rates which are in contrary to the findings of other workers viz. [11,33] which may be attributed to the fact that eggs of Hyostrongylus rubidus develop better in humid pastures.[18] However, some gastrointestinal parasites are apparently influenced by management changes due to differences in the basic biological requirements of the pre-infective developmental stages, differences in transmission characteristics and immunogenicity of the different parasitic species [14]

Conclusion

The study was concluded that among different types of animals screened during the study, stray animals were reported to have maximum gastrointestinal helminth infection with *Ascaris suam* as the most dominant spp. Seasonally, rainy season has been reported as the most desirable season for the perpetuation of helminth infection. In zone wise analysis, Humid south eastern zone was reported to be at higher risk for gastrointestinal helminth infection. Also, the study reported moderate severity of strongyle infection in the swine population of the region.

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