RESEARCH ARTICLE

Viral Pathogens, Risk Factors and Spatial Clustering of Porcine Respiratory Disease Complex in Hassan District of Karnataka State, India

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Abstract

A pilot study was conducted to identify the prevailing viral agents associated with porcine respiratory disease complex, probable risk factors and spatial clustering of these pathogens in Hassan district. A total of 225 blood samples were collected randomly from 27 different pig farms of the district. The detection of antibodies for viral pathogens like Porcine Circovirus 2 (PCV 2), Porcine Parvovirus (PPV), Classical Swine Fever Virus (CSFV) and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was done using commercial ELISA kits. An overall of 31.11 % samples were positive for viral co-seropositivity. The farm prevalence of viral co-seropositivity was found to be 64 %. Among the co-seropositive samples, PPV and PCV 2 antibodies were found in 97.14 % and 94.28 %, respectively and were appeared as dominant viral pathogens. The risk factors like large farm size, poor ventilation, movement of pigs between the pens, exchange of live pigs between the farms, feedback type of feeding, visitors movements, inadequate watering, location of nearby farms, high farm density, etc., were significantly influencing the higher occurrence of PRDC in the district. The analysis of spatial pattern of PRDC in the district revealed no clustering of infection.

Keywords: Hassan, PRDC, Risk factors, Seroprevalence, Spatial clustering.

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Introduction

Pigs hold a significant position among India's livestock species since they are raised by the socioeconomically disadvantaged segments of the population. In India, native pigs make up more than 70% of the total population. Nonetheless, India's pig population growth rate has been trending downward over the last 15 years (Indian Livestock Census Report, 2019). There may be several factors at play. The mortality rate from infectious diseases is one of the major contributing causes (Thomas *et al*., 2021). Because of the interplay of infectious agents (bacteria, mycoplasma, and viruses), host variables, and environmental conditions, respiratory illnesses continue to be one of the most difficult issues facing the global swine industry (Przyborowska-Zhalniarovich *et al.,* 2021). One of the most prevalent pig diseases, Porcine Respiratory Disease Complex (PRDC), results in significant financial losses for pig farmers worldwide (Qin *et al.,* 2018). Numerous aetiological agents contribute to the disease's complex etiology. The main function of viral infections is to harm the respiratory system, which encourages secondary bacterial colonization and ultimately leads to PRDC (Fablet *et al.,* 2012; Schmidt *et al*., 2016).

Porcine respiratory disorders caused by a single causative agent have been the subject of few investigations in India; however, there have been no systematic studies to comprehend PRDC caused by several causative agents. Because Hassan district is one of the main districts in Karnataka state in southern India for pig farming and pork consumption, the current study was conducted with the aim of finding the most common viral agents, risk factors, and geographical clustering of PRDC in this district.

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Materials and Methods

Selection of Study Area and Pig Farms

This clinical study was conducted from April 2019 to March 2020 in and around the Hassan district (12° 13′ and 13° 33′ N and 75° 33′ and 76°38′ E) located in the Southern part of Karnataka state (India). Detailed survey on the piggery farms in the district revealed the existence of a small house hold farms with less than 50 pigs to commercial bigger farms with 300-350 pigs.

The pig farms were identified personally by contacting the various resource persons/ organisations like Hassan District Pig Cooperative Society, Department of Animal Husbandry and Veterinary Services, Government of Karnataka, Department of Livestock Farm Complex and Department of Animal Husbandry and Veterinary Extension of Veterinary College, Hassan, through contacting farmers and local pork vendors. A total of 27 such farms were selected for the sample collection. The representative herds from all the eight Talukas (Administrative blocks bellow district level) of Hassan district were selected. A total of 225 pigs belonging to two months to two years from 27 farms were chosen randomly.

Collection of Herd Data

The data were obtained after thorough inspection of the pigs and the pig units, and face-to-face interviews of the pig farmers. A self designed questionnaire with precise definitions containing various sections pertaining to farms general information, herd information, common clinical features in the farm, biosecurity practices and breeding information was used to record the observations to identify the potential risk indicators of PRDC. The farm size was categorised as small, medium and large. The farm density was calculated based on the floor space available per pig in the unit. The farm ventilation was considered as good, poor and bad.

Seasons were classified in to four categories according to the standards mentioned by Indian Meteorological Department (IMD) as Winter (January, February), Pre-monsoon (March, April and May), South-West Monsoon (June, July, August and September) and Post-Monsoon (October, November and December).

Blood and Faecal Sample Collection

Approximately 10 mL of blood sample was collected from jugular vein, ear vein or cephalic veins depending on convenience of site of blood collection. The fresh faecal samples were collected for *A. suum* parasite screening as per the method described by Foreyt (2001) and the detection of the parasite was done within 2 h of sample collection.

Detection of Antibodies against Viral Pathogens

Antibodies against the viral pathogens were detected using commercial ELISA kits. The antibodies against PRRSV (PrioCHECK™ PRRSV Ab porcine, Thermo Fisher Scientific Inc.), PCV2 (GENELISA™ Porcine Circovirus (PCV2) antibody ELISA kit, Krishgen Bio Systems), PPV (PrioCHECK™ Porcine Parvovirus Ab Plate Kit Thermo Fisher Scientific Inc.) and CSFV (PrioCHECK™ CSFV Ab 2.0 Plate ELISA Kit Thermo Fisher Scientific Inc.) were determined as per the manufacturers' recommendations. The co-seropositivity for two or more viral pathogens in the single sample was considered as the case of PRDC.

Statistical Analysis and Spatial Clustering of Pathogens

The risk factors associated with PRDC were analysed by SPSS version 19.0 software and the automatic method for feature selection provided by the caret R package, called Recursive Feature Elimination (RFE), which is wrapper type variable selection algorithm. The RFE works by searching for a subset of features by starting with all features in the training dataset and successfully removing features until the desired number remains.

The spatial autocorrelation to examine the relationship between PRDC high prevalent farms that are placed at defined distance was analysed using GeoDa™ version 0.9.05-i5 software. The extent of clustering, in terms of prevalence/percent positivity for PRDC, that exists in a dataset was examined by K-nearest neighbour method using GeoDa™ software. Moran's *I* was used as measure of spatial autocorrelation where Moran's *I* obtained is interpreted as positive spatial auto-correlation (Moran's *I* is 1), negative spatial auto-correlation (Moran's *I* is -1) and no pattern (Moran's *I* is equal to 0).

Results and Discussion

Seroprevalence

The co-seropositivity of two, three and four viral pathogens was observed in the study. Among the 27 farms, seropositivity for PRDC was found in 16 farms (64%). In an overall, 31.11 % of co-seropositivity of viral pathogens was observed. The numbers of 02, 03 and 04 pathogen co-seropositivity observed were 60 (81.71%), 08 (11.43%) and 02 (2.86%), respectively. Among these samples, the PPV was involved in highest number with 68 (97.45%) followed by PCV 2 with 66 (94.28%) appeared as the first and second viral pathogens involved in PRDC followed by other pathogens. The reports on incidence of PRDC in different geographic region of India were sparse till date and this is the first systematic report on PRDC seroprevalence and associated managemental risk factors. In Punjab, according to Bhat *et al.* (2018), 18.89 % of pigs had two or more co-infections of *P. multocida, M. hyopneumoniae*, and PRRSV. In Ugandan smallholder pig farms, Dione *et al.* (2018) found that PCV 2 and *M. hyopneumoniae* had a greater impact on PRDC, and that the two main pathogens causing PRDC were PCV2 and

PPV. Li *et al*. (2016) found that PRRSV had the highest infection rate in China, followed by PCV2. They also believed that the epidemiological features of PRDC varied by region. Qin *et al*. (2018 and Tregaskis *et al*. (2020) have also recorded cases of PPV participation in PRDC in many parts of the world The results of this investigation made it abundantly evident that the Hassan district's pig farms had a significant incidence of PRDC viral pathogen infection.

Risk Factors

The influence of managemental risk factors on PRDC seroprevalence during the study period was analysed using logistic regression (Table 1). The univariate logistic regression of the selected risk factors on seroprevalence revealed that the season had a significant (p=0.005) influence. Out of four different seasons, pre-monsoon (p=0.003) and South West monsoon (p= 0.032) had the significantly lesser occurrence. The farm size also had the (p= 0.001) influence on seroprevalence with significantly lesser in small (p= 0.000) and medium (p= 0.008) sized farms compared to large farms. The prevalence was less in farms having good ventilation (p= 0.034) than poor ventilation.

The seroprevalence was significantly lower (p= 0.000) in the farms where the movement of pigs between pens was restricted. Similarly, farms in which the exchange of pigs between the neighbouring farms had taken place, seroprevalence was significantly (p=0.001) higher. The exchange of pigs between the farms also had the significant (p=0.001) effect on the higher seroprevalence. The farms which are not practicing the 'feedback' type of feeding showed the significantly (p=0.000) lower seroprevalence compared to the farms which are practicing 'feedback' system of feeding. The seroprevalence was significantly (p=0.000) higher in the farms practicing the watering the animals only once compared to the farms watering twice. The farms which were surrounded by any nearby other farms had the significantly (p=0.005) higher seroprevalence compared to the isolated farms. Farms with high density pig population showed significantly (p=0.000) higher seroprevalence than farms with low density of population. The flexible entry of visitors to the farms showed significantly (p=0.001) higher seroprevalence than farms with restricted entry.

The selection of variables using Recursive feature selection by outer resampling method for the seroprevalence of PRDC was made. Out of these variables, the top eight variables were utilized for decision tree models (Fig. 1) and logistic regression analysis (Table 2). Among the different variables related to host, managemental and climatic factors, a set of eight variables were shown the 80.04 % accuracy which was the highest among all other sets. Among the eight variables, top four variables such as season, movement of pigs between the pen, visitors and feeding in the farm were selected. The Logistic Regression analysis using these selected variables revealed significant influence of movement between the pen, visitors and breeds (Table 3). The movement of pigs between the pens variable had the significant (p= 0.000389) influence on PRDC seroprevalence. The visitor movement to the farm (Family members, veterinarians, vehicle movement and pig traders) had the significant (p= 0.004231) influence on seroprevalence. The findings confirm the findings of stark (2000), who found that farms with a history of unauthorized automobile and person admission had high PRDC occurrence.

The univariate regression analysis revealed that the season had a significant influence on PRDC prevalence. Given that the district's year-round temperatures range between 20 and 24 °C (Ramachandra *et al.,* 2004) and that the lower temperature range is ideal for the development of respiratory diseases in pigs (Fablet *et al*., 2012), this result suggested that there may be a chance of PRDC occurring in any season. All of the pig farm buildings in the study areas had open ventilation systems. The health of farm animals may be directly impacted by seasonal temperature fluctuations. The health of farm animals may therefore be directly impacted by seasonal temperature fluctuations. Although there are more small and medium-sized herds in the district, the prevalence of disease was far lower on these farms than on large ones. The same findings were reported by several researchers, who concluded that the growing herd size and wider age differences in the same premise have a major impact on PRDC (Bochev, 2007; Fablet *et al*., 2012).

Farms with adequate ventilation had a noticeably decreased prevalence of PRDC. The cause might be that pig respiratory illnesses and aerosol contamination were directly linked to inadequate ventilation (Stärk, 2000). The greater seroprevalence was influenced by the indiscriminate movement of pigs between the pens on the same farm. The results are in accordance with various other reports elsewhere (Bochev, 2007).

Farms that exchanged live animals had a greater seroprevalence of PRDC than farms that did not; this difference was significant. Combining animals from various sources with unknown disease status could raise the likelihood of pathogens entering the farms and causing a higher risk of contagious pathogens being introduced into the new herd. The current results are consistent with those of other researchers (Hurnik *et al.,* 1994). Increased social stress was blamed for this, as it may impair an animal's immune system and raise its risk of illness (Fablet *et al*., 2012). The farms that used the feedback style of feeding had a greater seroprevalence of PRDC. According to Alawneh *et al*. (2018), farms that used "feedback" were at risk of having a high pleurisy score. Similarly, farms that only watered their pigs once a day led to inadequate water supply, which may have caused dehydration stress and immunosuppression as reported by Bochev (2007). Comparing isolated farms to those in close proximity to other farms, the former had a substantially lower risk of contracting PRDC. The current results are consistent with those of Stark (2000). High farm lung and pleurisy scores were linked to the presence of another farm within 500 meters (Alawneh *et al*. 2018).

The current findings are consistent with those of Constable *et al*. (2017), who found that pigs' respiratory disorders are significantly influenced by overcrowding. According to Alawneh *et al*. (2018), a high animal density raises the likelihood that infections may be expelled, increasing the environmental load. However, according to Lo´ Pez-Soria *et al*. (2010) the seroprevalences and co-seropositivity of PRRSV, SIV, PCV2, PRV, and PPV were not significantly impacted by the farm's animal density

Table 1: Univariate logistic regression for association of seroprevalence of PRDC with the host and managemental risk factors

Risk factors	Variable Name	Univariate logistic regression		
		OR	95% CI	p-value
Season (*p*=0.005)	Winter	0.000	0.000-0.000	0.9980
	Pre-Monsoon	0.045	0.006-0.343	0.003
	S-W Monsoon	0.409	0.180-0.928	0.032
	Post-Monsoon	1.000		
Farm size (p=0.001)	Small	0.203	0.088-0.466	0.000
	Medium	0.154	0.154-0.756	0.008
	Large	1.000		
Ventilation (p=0.050)	Good	0.090	0.010-0.832	0.034
	Poor	1.000		
Movement between pens	No	0.204	0.102-0.410	0.000
	Yes	1.000		
Exchange of pigs between farms	No	0.379	0.212-0.676	0.001
	Yes	1.000		
Feed Back	No	0.315	0.172-0.576	0.000
	Yes	1.000		
Watering	Once	3.361	1.838-6.147	0.000
	Twice	1.000		
Nearby pig farm	No	0.416	0.227-0.763	0.005
	Yes	1.000		
Farm Density	High	3.750	1.929-7.292	0.000
	Low	1.000		
Visitors	Flexible	8.077	0.00-0.00	0.001
	Restricted entry	1.000		0.999
A. suum	Yes	1.287	0.604-2.744	0.513
	No	1.000		

Table 2: Recursive feature selection using Outer resampling method (Cross-Validated, 10- fold) and resampling performance over subset size for the seroprevalence of PRDC

Variables	Accuracy	Kappa	Accuracy SD	Kappa SD
4	0.7605	0.3913	0.05392	0.1607
8	0.8004	0.5066	0.06947	0.1809*
16	0.7913	0.4933	0.05038	0.1220
45	0.7915	0.4889	0.07217	0.1855

The seroprevalence of PRDC was not significantly impacted by the prevalence of *Ascaris suum* infection. However, the odds ratio was more than one. White (2011) and Bernardo *et al*. (1990) provided evidence in support of the current findings. The Hassan district's farms had a higher overall prevalence of *A. suum* (51.19%), according to Satheesha *et al*. (2020). This could be a contributing factor for the occurrence of PRDC in this region.

Spatial Clustering of Farms with High Risk of PRDC

Among the eight Talukas in the district, maximum numbers of farms were concentrated at one region (Hassan Taluka region), however, the farms in other Talukas were scattering and found very few. Most of the farms in the district were well connected with the primary and econdary roads. The spatial autocorrelation was examined in the present study using *k*-nearest neighbour method implemented in the GeoDa™ software. The Moran's *I* value (Fig. 2), plotted against the seroprevalence of PRDC in pig farms of Hassan district was found to be 0.138139 which is near to the zeroand considered that no clustering of the PRDC positive farms occurred in

Hassan district. However, the GIS maps indicated the aggregation of infected farms at one Taluka (Hassan) possibly due to the availability of feed and marketing facility and probably no other factors may be involved and further detailed analysis using large sample size over long period of time may through a light on this aspect

Table 3: Logistic regression using two selected variables for the seroprevalence of PRDC

Variables		Estimate	Standard Error	Z value	Pr (>|Z|)
Intercept		18.9142	1427.6864	-0.013	0.989430
Season	Pre-monsoon	14.3971	1427.6867	0.010	0.991954
	South-west monsoon	15.8190	1427.6864	0.011	0.991159
	Post monsoon	19.3696	1427.6863	0.014	0.989175
Movement between the pen		3.0515	0.8602	3.547	0.000389***
Visitors movement		-3.9123	1.3678	-2.860	0.004231**
Feed	Feeding garbage + concentrate	-0.6756	1.0416	-0.649	0.516568
	Feeding garbage +poultry slaughter wastes (Swill feed)	-0.1794	1.4085	-0.127	0.898634
Farms maintaining	Large white Yorkshire and other cross breeds (Breed 1)	-4.7060	2.3055	-2.041	0.041227*
	local pigs only (Breed 2)	-3.7329	1.4260	-2.618	0.008852**

Significance: ***p<0 .001, **p<0.01, *p<0.05

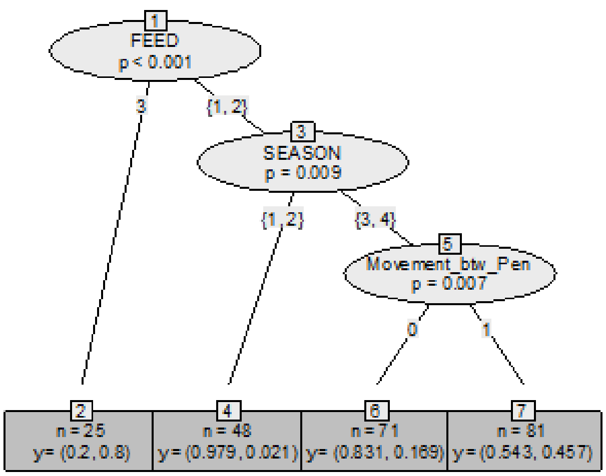

Fig. 1: Decision tree for the risk factors associated with seroprevalence of PRDC in Hassan district during the period from April 2019 to March 2020

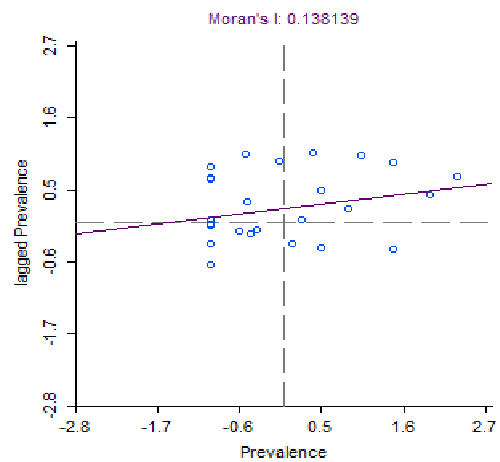

Fig. 2: The differential Moran's scatter spots for the seroprevalence of PRDC in Hassan district during the period from April 2019 to March 2020

Conclusion

The prevalence of PRDC was high in Hassan district during the period of study. The PPV and PCV 2 were the most common viral pathogens in the district. Analysis of various risk factors associated with the occurrence of two or more PRDC viral pathogens revealed that the movement of pigs between pen, feedback system of feeding, visitors flexible movement, high density farms, less water supply to the animals and location of farm adjacent to nearby farms were the significantly influencing factors. The spatial clustering of PRDC infection was not observed during the study period at Hassan district.

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