



Risk Factors Associated with Porcine Leptospirosis in Uttar Pradesh, India

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ABSTRACT

Porcine leptospirosis is a zoonotic disease. Pigs act as reservoir host for various *Leptospira* serovars. A study aimed to pinpoint the risk factors of porcine leptospirosis was designed on pigs of three agro-climatic zones- Midwestern plain zone (Bareilly), Eastern Plain zone (Barabanki) and Southwestern semi- arid zone (Aligarh) of Uttar Pradesh. Risk factor analysis was done using pretested structured questionnaire. The univariate analysis of the variables of interest was done using Fisher's exact test/ Pearson's chi-square. Further, multivariate analysis was done through logistic regression model using serological status of animal in Microscopic Agglutination Test (MAT) as dependent variable. The overall seropositivity of 23.81% (70/294; 95% CI: 18.94% - 28.67%) was recorded in MAT with Icterohaemorrhagiae as the leading serovar. Multivariate risk factor analysis revealed that accessibility of swine to unsanitary wallowing ponds (Odds ratio: 8.58; 95% CI: 3.34-21.93; P<0.001), contact of domesticated pigs with wild/feral pigs and water buffaloes (Odds ratio: 12.83; 95% CI: 3.72-44.26; P<0.001) and accessibility of swine to garbage pits (Odds ratio: 3.97; 95% CI: 1.28-12.26; P<0.016) were statistically significant. Further, risk factor analysis revealed that mature pigs of >2 years age had higher chance of contracting leptospirosis than young pigs of <2 years (Odds ratio: 12.09; 95% CI: 2.92-50.03; P<0.001) owing to higher probability for exposure to above mentioned risk factors. Domesticated pigs reared under mixed farming system that gets access to wallow in unsanitary ponds frequented by feral pigs and water buffaloes and pigs that access garbage pits frequented by rodents were at high risk of contracting leptospirosis.

HIGHLIGHTS

- ❶ Faulty swine husbandry practices are responsible for spread of porcine leptospirosis.
- ❷ Exacerbating factor responsible for porcine leptospirosis is providing pigs access to wallowing ponds frequented by water buffaloes.

Keywords: Leptospirosis; pig; risk factor; wallowing pond; water buffalo

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Leptospirosis is a zoonosis of ubiquitous distribution, found in virtually all tropical and temperate areas of the world, and possesses a broad host range affecting almost all mammals (Verma *et al.*, 2020). This emerging public health problem is caused by spirochetes which are currently divided into 35 species classified into three phylogenetic clusters, which supposedly correlate with the virulence of the bacteria (Vincent *et al.*, 2019). Leptospirosis is a burden to swine husbandry since it cause heavy economic losses due to a plethora of clinical manifestations spanning from infertility to spontaneous abortions which occur 2-4 weeks before completion of gestation period, stillbirth, foetal mummification and maceration, increased weaning-to-oestrus interval, decreased number of piglets per litter, birth of runt piglets, agalactia and reduced productivity (Wasinski, 2014; Divers, 2018; Pozzi *et al.*, 2020). Swine serve as maintenance/reservoir hosts for serovars such as Bratislava, Pomona, Tarassovi Mitis and Muenchen (Lee *et al.*, 2019; Bertasio *et al.*, 2020), while serovars Canicola, Grippotyphosa, Icterohaemorrhagiae and Hardjo commonly cause incidental infections in pigs (Ellis, 1999; Strutzberg-Minder *et al.*, 2018). In humans involved in swine husbandry, leptospirosis is known as “swine herder’s disease” (Bhatia and Umaphathy, 2015).

Risk factor analysis plays a pivotal role in pinpointing the managerial practices in swine husbandry which needs to be corrected in order to check the threat posed by porcine leptospirosis. Several studies performed in various countries highlighted the role of various risk factors such as rodents control failure, water mismanagement practices, higher number of female pigs in a breeding stock, sampling period especially during warm and wet weather, presence of swampy areas for pigs to wallow, pig farms housing other species of livestock, higher proportion of mature pigs, absence of quarantine facilities, stagnant water source, pig farms where healthy swine were bred with sick pigs, farm premises with flooded areas, and artificial insemination utilizing asymptomatic boars as aggravating factors associated with porcine leptospirosis (Valenca *et al.*, 2013; dos Santos *et al.*, 2019; Ngugi *et al.*, 2019; Atherstone *et al.*, 2020).

Perusal of literature revealed that porcine leptospirosis has been reported mostly in southern states of India such as Andhra Pradesh (Rani Prameela *et al.*, 2013), Tamil Nadu (Bojiraj *et al.*, 2017) and Kerala (Reshma *et al.*, 2018). At present, there is a shortage of information

with regard to seroprevalence of porcine leptospirosis in Northern India except for one study conducted in Uttar Pradesh (Behera *et al.*, 2014). In India, where vaccination against porcine leptospirosis is not routinely practiced, the immediate requirement is identification of the risk factors and taking appropriate preventive measures to contain porcine leptospirosis. Hence, the present study was designed to study the seroprevalence of leptospirosis in the pigs of three agro-climatic zones- Midwestern plain zone (Bareilly), Eastern Plain zone (Barabanki) and Southwestern semi- arid zone (Aligarh) of Uttar Pradesh and to identify the risk factors responsible for porcine leptospirosis in these areas.

MATERIALS AND METHODS

Study area, Target population and serum collection

The study was approved by Institutional Animal Ethics Committee of ICAR – IVRI (Research Permit No: F.26-1/2015-16/JD(R) and the protocols followed during serum collection were strictly adhered according to protocols laid by Institutional Animal Ethics Committee of ICAR-IVRI. Based on multistage cluster sampling techniques, serum samples were collected from selected pigs of semi-intensive and backyard farms from one district each of three agro-climatic zones of Uttar Pradesh namely Southwestern semi- arid zone (Aligarh), Midwestern plain zone (Bareilly) and Eastern Plain zone (Barabanki) for a period of one year from July 2016 to June 2017. A total of 294 sera samples, including 212 sera samples from rural locations and 82 sera samples from peri-urban region were collected. Out of 212 sera sample of rural locations, the 61 were collected from Gursaina, HastpurIglas, Kajroth and Gandhi Gram villages of Aligarh, 36 from Fatehpur, Triveniganj and Dewa villages of Barabanki and 115 from Nekpur, Bhojipura, Richha, Faridpur and Baheri villages of Bareilly Districts (Fig. 1). The 82 sera samples from peri-urban region of Bareilly district included 32 sera samples from Chaupla & Beharipur pig farms and 50 from pig farm located at Indian Veterinary Research Institute/IVRI (Fig. 1). The study sample comprised of 230 male pigs and 64 female pigs of which 172 pigs were <2 years old while 122 pigs were >2 years old. A total of 113 pigs belonged to Large White Yorkshire breed while the rest 181 pigs were indigenous breeds.

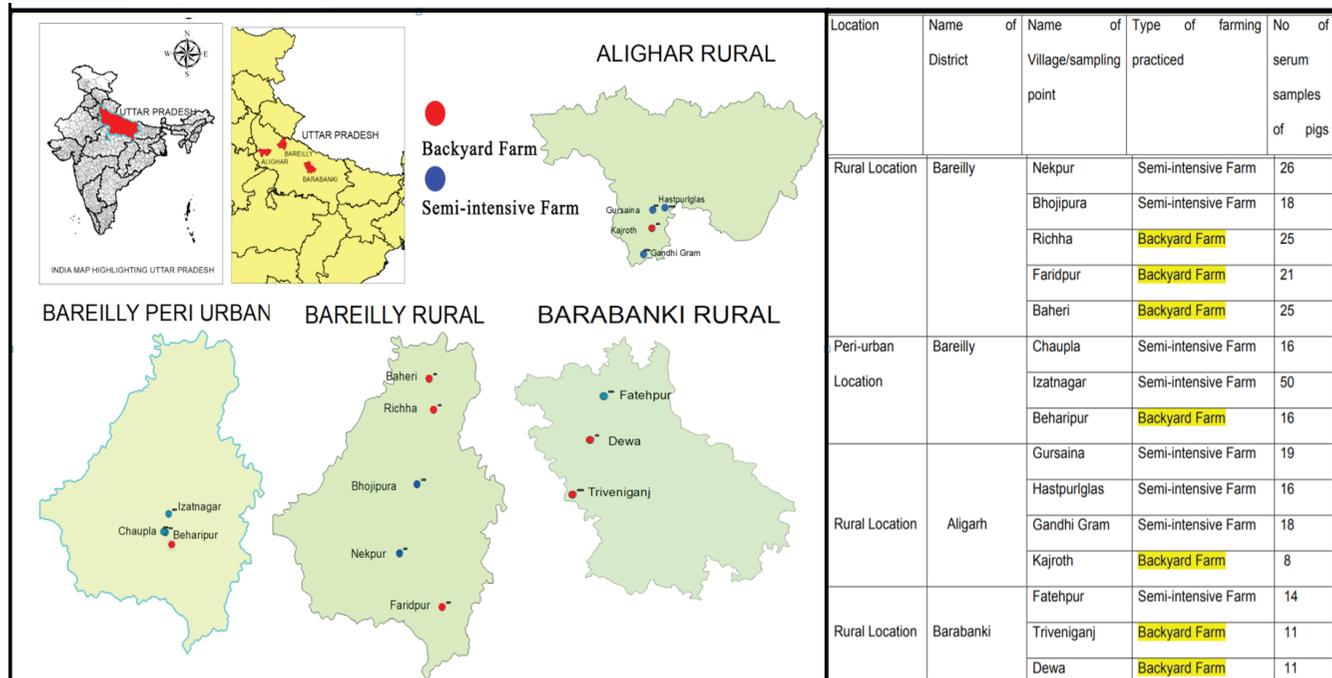


Fig. 1: Showing the location of pig farms where sampling has been done and type of pig farming adopted at each sampling point

Study of risk factors

A survey was conducted using a questionnaire with regard to general swine husbandry practices (rodent pest control, presence of other livestock species and accessibility of pigs for wallowing and scavenging in garbage pits) and demographic variables (breed, sex, age). The risk factor analysis was conducted using the data collected with the questionnaire tool. Multi-level logistic regression models were used to explore the association between risk factors for leptospirosis seropositivity in pigs and binary outcomes of MAT for each animal. A positive outcome in MAT was defined as any pig positive for at least one serovar in MAT at 1 in 100 titre. In this study, a pre-selection was performed in which all the variables were tested using models with just one explanatory variable at a time (univariate models) and afterwards included in the multivariate model only those significant variables that have shown a relaxed P-value (P value ≤ 0.25). This pre-selection strategy using relaxed P-value criterion allowed in reducing the initial number of variables in the model reducing the risk of missing important variables (Sperandei, 2014). This model was adopted by epidemiologists while evaluating prognosis factors and risk factors for leptospirosis seropositivity in Guadeloupe and western Kenya respectively (Herrmann-

Storck *et al.*, 2010; Cook *et al.*, 2017). The univariate analysis of the variables of interest was done using either Fisher’s exact test or Pearson’s chi-square, with the level of significance 95% C.I. and 5% error rate in order to pinpoint the risk factors associated with *Leptospira* spp. seropositivity. Data were entered in Microsoft Excel 2013 and analyzed using SPSS version 22.

Leptospira serovars and strains used in Microscopic agglutination test (MAT)

A battery of 16 leptospiral serovars namely, *Leptospira interrogans* serovar Australis strain Ballico, *L. interrogans* serovar Autumnalis strain Akiyami A, *L. interrogans* serovar Ballum strain S102, *L. interrogans* serovar Bataviae strain van Tienen, *L. interrogans* serovar Canicola strain Hond Utrecht IV, *L. kirschneri* serovar Cynopteri strain 3522C, *L. interrogans* serovar Djasiman strain Djasiman, *L. kirschneri* serovar Grippotyphosa strain Moskva V, *L. borgpetersenii* serovar Hardjo-bovis strain JB197, *L. interrogans* serovar Hebdomadis strain Hebdomadis, *L. interrogans* serovar Icterohaemorrhagiae strain RGA, *L. borgpetersenii* serovar Javanica strain Veldrat Batavia 46, *L. noguchii* serovar Louisiana strain

**Table 1:** Seroprevalence of Leptospiraserovars and serogroups by microscopic agglutination test (titre>1:100) among 294 porcine serum samples in Uttar Pradesh, India

Genomo- Species	Serogroup/Serovar/Strain	Positive (N) †	Prevalence (%)	95% CI
<i>L. interrogans</i>	Icterohaemorrhagiae/Icterohaemorrhagiae/RGA	49	16.67	12.40-20.90
	Pomona/Pomona/Pomona	31	10.54	7.0-14.10
	Australis/Australis/Ballico	06	2.04	0.4-3.70
<i>L. kirshneri</i>	Grippotyphosa/Grippotyphosa/Moskova V	32	10.88	7.30-14.40
<i>L. borgpetersenii</i>	Tarassovi/Tarassovi/Perepelitsin	28	9.52	6.20-12.90

n, number of positive sera by MAT; †Cumulative figure (N = 146) exceed total MAT positive sera (n = 70) since several porcine sera tested positive for multiple serovars; CI, Confidence Interval of 95%.

LSU 1945, *L. interrogans* serovar Pomona strain Pomona, *L. interrogans* serovar Pyrogenes strain Salinem and *L. borgpetersenii* serovar Tarassovi strain Perepelitsin were employed for performing MAT.

Microscopic agglutination test (MAT)

Porcine sera were screened for anti-leptospiral antibodies by MAT as previously described (USDA, 1987). Briefly, serum samples were diluted 1 in 50 in phosphate buffer saline (PBS) and a volume of leptospiral antigen, equal to the diluted serum volume, was added to each well, making the final serum dilution 1 in 100. The 4-8 days old live leptospiral antigens (approx. 2×10^8 leptospores/ml) of 16 reference serovars were used. The microtitre plates were incubated for 2 hours at 29°C and the serum-antigen mixtures were examined using dark field microscopy. A positive outcome in MAT suggestive of exposure/seropositivity was defined as any single serum sample found to have >50% reduction in the number of free non-agglutinable leptospores in the test when compared to the control at 1 in 100 serum dilution for at least one leptospiral serovar.

RESULTS

Out of 294 sera tested at Aligarh, Bareilly and Barabanki districts of Uttar Pradesh, 70 sera tested positive in MAT and maximum seropositivity was detected for serovar Icterohaemorrhagiae (n=49), followed by Grippotyphosa (n=32), Pomona (n=31), Tarassovi (n=28) and Australis (n=06) as shown in Table 1. Cumulative figure of sera positive for various serovars (n=146) exceed total MAT positive sera (n=70) since several sera reacted with

multiple leptospiral serovars. The MAT titres against various serovars ranged between 1:100 and 1:3200.

Higher frequency of seropositives were observed in sows/gilts (32.81%; 21/64) in comparison to boars (21.30%; 49/230), mature pigs of >2 years (42.62%; 52/122) in comparison to young pigs of <2 years (10.47%; 18/172), cross bred pigs (29.20%; 33/113) in comparison to indigenous pigs (20.44%; 37/181), rural pigs (24.06%; 51/212) in comparison to peri-urban pigs (23.17%; 19/82), pigs reared in extensive farming system (32.48%; 38/117) in comparison to counterparts in semi-intensive system (18.08%; 32/177) and swine which had access to wallowing ponds (29.56%; 47/159) than pigs without wallowing access (17.04%; 23/135). The higher frequency of seropositives were also observed in pigs which resorted to scavenging (39.56%; 36/91) than pigs which were fed concentrates/corn byproducts (16.75%; 34/203), swine which had no access to garbage pits (30.23%; 26/86) than pigs with garbage access (21.15%; 44/208), swine which had contact with other animals (37.65%; 32/85) than pigs without animal contact (18.18%; 38/209), swine which had no history of reproductive problems (24.03%; 62/258) than swine with history of reproductive problems (22.22%; 8/36) and swine reared in farms where rodents had access to feeders and waterers (29.63%; 16/54) than in farms with limited rodent access (22.50%; 54/240) (Table 2).

The complete univariate analysis for risk factors for leptospirosis seropositivity in pigs reared in both extensive and semi-intensive system included 11 potential exposure variables. The individual variables that had a p value <0.25 in the univariate analysis are highlighted in italics in Table 2. Variables that were significantly associated with

Table 2: Characteristics of pigs, proportion of MAT results, sero-prevalence and associated odds ratios by their demographic characteristics, (Antibody titre cut-off >100), Uttar Pradesh, North India, July 2015 to June 2016 (n=294)

Variables	Variable Categories	N (%)	Positive Cases by MAT (%)	Univariate Analysis	
				Unadjusted 'OR' (95% CI)	P Value*
Age	Young (< 2 years)	172 (58.50)	18 (10.47)	1 (reference)	0.001*
	Mature (> 2 years)	122 (41.50)	52 (42.62)	6.356 (3.467, 11.469)	
Sex	Female	64 (21.77)	21 (32.81)	1 (reference)	0.056*
	Male	230 (78.23)	49 (21.30)	0.544 (0.301, 1.020)	
Breed	Cross Bred (Large White Yorkshire)	113 (38.44)	33 (29.20)	1 (reference)	0.086*
	Non Descript/Local Breed	181 (61.56)	37 (20.44)	0.623 (0.362, 1.072)	
Location	Rural	212 (72.11)	51 (24.06)	1 (reference)	0.873
	Peri-Urban	82 (27.89)	19 (23.17)	0.952 (0.521, 1.738)	
Type of Farming	Semi-Intensive	177 (60.20)	32 (18.08)	1 (reference)	0.005*
	Extensive/backyard	117 (39.80)	38 (32.48)	2.180 (1.265, 3.756)	
Source of Water	River/Tap Water	135 (45.92)	23 (17.04)	1 (reference)	0.012*
	Wallowing pond	159 (54.08)	47 (29.56)	2.043 (1.163, 3.589)	
Source of Feed	Scavenging/ Hotel/kitchen waste	91 (30.95)	36 (39.56)	1 (reference)	0.001*
	Concentrate Feed/Corn byproducts	203 (69.05)	34 (16.75)	0.307 (0.176, 0.537)	
Access to Garbage area	No	86 (29.25)	26 (30.23)	1 (reference)	0.096*
	Yes	208 (70.75)	44 (21.15)	0.619 (0.351, 1.092)	
Contact with other animals (Water Buffalo, feral pigs)	No	209 (71.09)	38 (18.18)	1 (reference)	0.001*
	Yes	85 (28.91)	32 (37.65)	2.717 (1.549, 4.767)	
History of Reproductive problems	No	258 (87.76)	62 (24.03)	1 (reference)	0.811
	Yes	36 (12.24)	8 (22.22)	0.903 (0.391, 2.084)	
Rodent Access to Feeder and waterers	No	240 (81.63)	54 (22.50)	1 (reference)	0.266
	Yes	54 (18.37)	16 (29.63)	1.450 (0.751, 2.800)	

N, number of animals; total animals=294 swine; OR, 'Odds Ratio'; CI, Confidence Interval of 95%; * Statistically significant ≤ 0.25 .

seropositivity for porcine leptospirosis after univariate analysis were: mature pigs of >2 year old (OR 6.356; 95% CI 3.467 to 11.469; p value <0.001); extensive farming system (OR 2.180; 95% CI 1.265 to 3.756; p value 0.005); access to garbage area (OR 3.97; 95% CI 1.286 to 12.261; p value 0.016); access to wallowing pond (OR 2.043; 95% CI 1.163 to 3.589; p value 0.012) and contact with other animals such as feral pigs and water buffaloes (OR 2.717; 95% CI 1.549 to 4.767; p value <0.001). Demographic variables such as male sex (OR 0.544; 95% CI 0.301 to 1.020; p value 0.056) and non-descript breed (OR 0.623; 95% CI 0.362 to 1.072; p value 0.086) were found protective against leptospirosis seropositivity. Concentrates/corn

byproducts as a source of feed (OR 0.307; 95% CI 0.176-0.537; p value 0.001) was also found protective against leptospirosis seropositivity. Interestingly, access of pigs to garbage area (OR 0.619; 95% CI 0.351-1.092; p value 0.096) was found as a mitigating factor against leptospirosis seropositivity. After univariate analysis, three variables such as location of study, rodent access to feeder and waterer and history of reproductive problems were excluded from multivariable model since these variables were not statistically associated (p value > 0.25).

The multivariate analysis for risk factors for leptospirosis seropositivity in pigs included 8 potential exposure



variables. Risk factors that were significantly associated with seropositivity for porcine leptospirosis after multivariate analysis were: contact with feral pigs and water buffaloes (OR 12.83; 95% CI 3.72 to 44.26; p value <0.001), mature pigs of >2 year old (OR 12.09; 95% CI 2.92 to 50.03; p value <0.001), access to wallowing (OR 8.58; 95% CI 3.34 to 21.93; p value <0.001) and access to garbage pits (OR 3.97; 95% CI 1.29 to 12.26; p value 0.016) as shown in Table 3. Demographic variable such as non-descript breed (OR 0.22; 95% CI 0.05 to 0.95; p value 0.043) was found protective against leptospirosis seropositivity. Three variables such as type of farming system, source of feed and sex were not statistically significant (p value > 0.05).

DISCUSSION

Global analyses of emerging infectious diseases (EIDs) has identified India as one of the several 'EID hotspots' with leptospirosis included as an important zoonotic disease requiring continuous attention of disease investigators for devising intervention strategies for controlling the disease (Chatterjee *et al.*, 2017). In this study, the clinical signs suggestive of leptospirosis in breeding sows/gilts were restricted to reproductive problems such as higher number of still births and runts per litter, abortion and increase in oestrus interval post weaning. Out of 36 breeding sows showing reproductive problems, only 8 sows showed seropositivity in MAT. Seropositive breeding sows (n = 6) with lower MAT titre of $\leq 1:400$ for both *Icterohaemorrhagiae* and *Grippotyphosa* presented clinically at farrowing time with higher number of stillborns per litter and the live piglets born were mostly runts with low birth weight. The presence of stillborns or runts is a typical sign of chronic infection of breeding sows possessing lower MAT titre which cause huge economical losses to piggy units (Ellis, 1999). Two seropositive gilts introduced newly into the breeding stock aborted and had high MAT titre of 1:1600 and 1:3200 for serovar *Icterohaemorrhagiae*. The high MAT titre in gilts which had aborted suggests that these animals never had prior contact with the infectious agent before being introduced to the herd (Gummowa *et al.*, 1999). Four out of six sows which had still born fetuses and runts showed an increase in oestrus interval post weaning. This finding suggests a detrimental effect of leptospirosis on reproductive organs such as ovaries which control post-weaning

oestrus occurrence. The present findings of involvement of serovars *Icterohaemorrhagiae* and *Grippotyphosa* with post weaning increase in oestrus interval corroborate with findings of a previous study where an association was drawn between increase in weaning-to-oestrus interval and seroprevalence of serovar *Grippotyphosa* (Valenca *et al.*, 2013).

The male pigs presented for slaughter showed no clinical signs suggestive of leptospirosis during ante-mortem examination. However, post mortem examination revealed white spots in a few kidney samples in animals seropositive for leptospirosis. It was noticed that the presence of white spots is an unreliable indicator of the presence of agglutinins against leptospirosis since only 66% (16/24) of kidney samples of pigs seropositive for leptospirosis showed white spots in kidneys. Our findings are in concordance with observations of other disease investigators who also reported 'white-spotted kidneys' during post mortem examination in seropositive cases of porcine and ovine leptospirosis respectively (Boqvist *et al.*, 2003; Jarad *et al.*, 2020).

Multivariate analysis by means of non-conditional logistic regression analysis revealed that wallowing in unsanitary ponds and contact of domesticated pigs with wild/feral pigs and water buffaloes were key risk factors for contracting porcine leptospirosis. In the multivariate analysis, it was revealed that the odds for seropositivity in pigs that had contact with these animals were 12.83 times the odds of swine that had no contact with these animals. This observation is in concordance to findings of a previous study in Kenya where higher incidence of leptospirosis was reported in pigs having contact with other animals (Ngugi *et al.*, 2019). Moreover, it was observed that the odds for seropositivity in pigs that had access to wallowing were 8.56 times the odds of swine that had no access to wallowing. Similar report of higher incidence of porcine leptospirosis with access to wallowing area has been reported from Nigeria (Adah *et al.*, 2018).

In this study, we are able to demonstrate that a significant number of pigs (n = 28) from U.P. were seropositive for serovar *Tarassovi*. Pigs are usually considered as the reservoir host for serovar *Tarassovi* (Lee *et al.*, 2019; Bertasio *et al.*, 2020). However, the authors have demonstrated in a previous study the presence of antibodies against serovar *Tarassovi* in a few water

Table 3: Multivariate analysis for risk factors relevant or non relevant with seropositivity by MAT in Swine samples, Uttar Pradesh (July 2015 to June 2016)

Variables	β coefficients	S.E.	odds ratio	95% C.I. for 'OR'		P-value*
				Lower	Upper	
Age						
<2 Years	0	0	1 (reference)	—	—	0.001*
>2 Years	2.492	0.725	12.09	2.921	50.033	0.001*
Sex						
Female	0	0	1 (reference)	—	—	0.162
Male	-0.869	0.621	0.42	0.124	1.416	0.162
Breed						
CB	0	0	1 (reference)	—	—	0.043*
Non Descript	-1.51	0.745	0.22	0.051	0.952	0.043*
Farming Type						
Semi-intensive	0	0	1 (reference)	—	—	0.911
Extensive	0.078	0.699	1.08	0.275	4.255	0.911
Access to garbage area						
No	0	0	1 (reference)	—	—	0.016*
Yes	1.379	0.575	3.97	1.286	12.261	0.016*
Contact with other animals (Wild pigs and Water Buffalo)						
No	0	0	1 (reference)	—	—	0.001*
Yes	2.552	0.632	12.83	3.72	44.259	0.001*
Source of Water						
Tap Water/River	0	0	1 (reference)	—	—	0.001*
Wallowing Pond	2.147	0.48	8.58	3.338	21.931	0.001*
Source of Feed						
Scavenging/ Hotel/kitchen waste	0	0	1 (reference)	—	—	0.594
Concentrate feed/Corn leftover (Farm waste)	0.288	0.541	1.33	0.462	3.849	0.594
Constant	-4.129	1.209	—	—	—	—

OR, 'Odds Ratio'; CI, Confidence Interval of 95%; * Statistically significant ≤ 0.05 .

buffalo serum samples (n = 15) in the same geographical location (Deneke *et al.*, 2014). Moreover, there are reports of pigs in India contracting serovar Hardjo (Prameela *et al.*, 2013) whose maintenance host is cattle and water buffalo. Based on this serological evidence, the authors hypothesize that the mixed, backyard type of farming where water buffaloes get chance to wallow in sewage ponds frequented by domestic and feral pigs has led to cross species disease transmission with serovar Tarassovi

and serovar Hardjo finding ample opportunity to break the host species barrier to infect water buffalo and pigs respectively. Similar reports of cross species leptospirosis transmission in mixed farming involving cattle and pigs with documented reproductive disorders are available in Belgium (Mori *et al.*, 2017). This trend of wallowing ponds becoming prime hot spots for leptospiral disease transmission is not only restricted to cross species disease transmission but also intra-species disease transmission

as domesticated pigs can also contract leptospirosis when these animals wallow in sewage ponds frequented by feral pigs. Thus, feral pigs can serve as reservoir hosts for various serovars of *Leptospira* and poses danger for interspecies transmission of leptospire to humans and other domestic livestock species (Brown *et al.*, 2018).

Multivariate analysis revealed that age of swine was a significant risk factor since mature pigs have 12.09 times the odds of seropositivity than young pigs. The relatively higher number of mature pigs (>2 years) is due to the fact that most of the pigs reared under extensive farming system require longer time period to reach desired slaughter weight due to multi-factorial reasons such as poor nutritive diet, genetic predisposition and high gastro-intestinal parasitic load. These non-descript pigs have ample opportunity for exposure to key risk factors such as access to wallowing in ponds frequented by feral pigs and water buffaloes. Cross bred pigs show good feed conversion ability under semi-intensive care and reaches slaughter weight within a year and has minimum opportunity for exposure to key risk factors during their short life span. Results of significant association of age of swine with seropositivity corroborate with the findings of other disease investigators who suggested the reason to be increased exposure over time (Lee *et al.*, 2019; Ngugi *et al.*, 2019).

Multivariate analysis revealed that access to garbage area might be a significant variable since pigs with access to garbage have 3.97 times the odds of seropositivity than pigs without access to garbage. The garbage pits are frequented by rodents which act as reservoir host for several leptospiral serovars. The sniffing habit of swine exposes their nasal and buccal mucosa as well as mucosa of eyes to rodent urine laden with pathogenic *Leptospira*. The leptospiral organisms gain entry through minor abrasions in mucous membranes of pigs which frequent rodent infested garbage.

Multivariate analysis seems to suggest that cross bred pigs are apparently at higher risk of contracting leptospirosis than indigenous breeds. This result is in discordance to the findings of Ngugi *et al.* (2019) who observed no association between porcine breed and *Leptospira* seropositivity. However, several disease investigators have suggested breed as a risk factor for leptospirosis in bovines and small ruminants. In India, sero-epidemiological studies on bovine leptospirosis have

revealed that the risk of leptospirosis was more in exotic breeds followed by indigenous breeds and cross breeds (Balakrishnan *et al.*, 2011). In a sero-epidemiological study in Brazil on ovine leptospirosis, low prevalence of *Leptospira* spp. was observed by serology and PCR which suggested that the rusticity of the mixed breed sheep may have contributed to the low occurrence of leptospirosis in comparison to pure bred sheep (da Costa *et al.*, 2017). Hence, more studies need to be done to ascertain breed susceptibility in contracting porcine leptospirosis.

Even though multivariate analysis suggests that type of farming is not a significant risk factor (Odds ratio: 1.08; 95% CI: 0.275-4.255; P 0.911), it is clear that type of farming as a variable may be confounded by the other factors given the difference in results from univariable to multivariable analysis for this variable. The present study revealed that pigs reared in extensive farming system had significantly higher proportion of leptospiral antibodies (32.48%; 38/117) in contrast to their counterparts reared under semi-intensive system (18.08%; 32/177). Pigs grown under backyard farming (n = 117) forage on municipal garbage, have access to sewage for wallowing, reach slaughter weight at older age and are in constant contact with other species of livestock especially water buffaloes due to the mixed farming system adopted in rural areas. Hence, it could be argued that extensive farming system is part of the causal pathway, since this may determine the other significant risk factors discussed above. Moreover, pigs reared under semi-intensive system were not exposed to significant risk factors in the present study such as wallowing, scavenging in garbage pits and contact with other animals which meant that these pigs were less prone to be exposed to leptospirosis.

The Planning Commission of India (1989) has categorized India into 15 agro-climatic zones and U.P. falls in the agro-climatic zone termed Upper Gangetic Plains (Vikaspedia, 2019). Different serovars of *Leptospira* spp have shown predilection to thrive in specific agro-climatic zones of India (Spatial Variation) (Sabarinath *et al.*, 2018). The present study has revealed spatial variation when comparison was done with previous seroepidemiological studies conducted in other agro climatic zones of India. The serovars Autumnalis (n = 11), Canicola (n = 10) and Hardjo (n = 15) agglutinins present in 133 porcine sera samples in Andhra Pradesh (Prameela *et al.*, 2013) (Agro climatic zone: Southern Plateau and Hills) were all missing in two

seroepidemiological studies conducted in U.P. from 2011-2012 and 2016-2017. Further, the presence of agglutinins against serovars Autumnalis (n = 7), Canicola (n = 6) and Hebdomadis (n = 4) in 177 porcine sera samples in Tamil Nadu (Bojiraj *et al.*, 2017) (Agro climatic zone: East Coast Plains and Hills) and presence of agglutinins against serovar Canicola (n = 14) in 103 porcine sera samples in Kerala (Reshma *et al.*, 2018) (Agro climatic zone: West Coast Plains and Hills), were all missing in U.P. Thus, the present study will further enhance the knowledge regarding the predilection of various leptospiral serovars for different agro-climatic zones of India.

In the present study, we could detect 32 serum samples positive for leptospiral serovar Grippotyphosa. However, a seroepidemiological study conducted in 2011-12 by the same author (Behera *et al.*, 2014) in U.P. failed to detect the presence of serovar Grippotyphosa in all 85 serum samples tested. Moreover, the 2011-12 study detected the presence of agglutinins against serovar Javanica in 12 serum samples which was missing in all the sera samples (n=294) tested in the present study. The above data suggests the relevance of conducting periodic seroprevalence studies on pigs on a regular basis as it helps to unravel temporal variation due to emergence of newer serovars over a period of time which will be missing from existing commercial vaccines and the inclusion of these new serovars help in the preparation of effective vaccines (Klaasen and Adler, 2015). At present, there are no indigenous vaccines available in India and vaccination against porcine leptospirosis is not routinely practiced. This is the reason vaccination was not included as one of the variables while assessing risk factors.

In conclusions, the present study sheds light on the role played by faulty swine husbandry practices such as providing swine accessibility to unsanitary wallowing ponds and practicing mixed farming in which water buffaloes are kept in pig farm premises as the precipitating factors in the spread of porcine leptospirosis. Further, feral pigs and water buffaloes pose serious threat for cross-species transmission of leptospires to domesticated pigs.

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