SEROLOGICAL EVIDENCE OF SWINE LEPTOSPIROSIS IN AN ORGANISED FARM

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ABSTRACT

The present study was undertaken to assess the prevalence of anti-leptospiral antibodies in swine reared in an organized farm located in the outskirts of Chennai city. Out of 63 samples collected, 57 (90.47 per cent) samples were found positive by Microscopic agglutination test with the prevalence of *Australis* (74.60 per cent), followed by *Hardjo* (30.16 per cent), *Pomona* (25.40 per cent), *Autumnalis* (23.81 per cent), *Pyrogenes* (17.46 per cent), *Hebdomadis* (6.35 per cent), I*cterohaemorrhagiae* (4.76 per cent), *Canicola* (3.17 per cent), *Grippotyphosa* (3.17 per cent), *Javanica* (3.17 per cent) and *Tarassovi* (3.17 per cent). The presence of antibodies indicated the circulation of leptospires among pigs. Hence this study alerts for the implementation of adequate sanitary measures on the farm to prevent the transmission of the infection to human in contacts.

KEY WORDS: Swine - Leptospirosis - Seroprevalence - Tamil Nadu

INTRODUCTION

Leptospirosis is a worldwide zoonosis affecting domestic animals, pet animals, wild animals and human beings (Balakrishnan Govindan, 2014,2015). Intensification of cattle and pig farms is a major risk factor for leptospiral infection. Domestic pigs are considered to be a potential source of leptospirosis and poses public health risks among those working with pigs, processing pork and veterinarians. *Leptospira* infection among pigs proceeds most commonly without clinical signs. This subclinical infection is commonly seen in growing pigs which may constitute a health hazard for more susceptible piglets and pregnant sows (Michna, 1970). Leptospirosis in pigs results in economically significant losses due to fetal death, abortion, infertility and birth of weak piglets (Ellis, 1999). Hence immunization of pigs against leptospirosis is essential to control the infection. Immunity is serovar specific. Hence the seroprevalence studies are essential to design a vaccine to control leptospirosis. In India, studies on leptospirosis among pigs in organized farms are scanty. The objective of the present study was to assess the seroprevalence of leptospirosis among pigs reared in an organized farm located in the outskirts of Chennai city, Tamil Nadu.

MATERIALS AND METHODS

An organized pig farm at the outskirts of Chennai city holding about 700 pigs under intensive management system was selected for the present study. The farm also housed cattle, buffaloes, sheep and rabbits. Blood samples were collected from the ear vein of the pigs using blood collection system (Plain Vacutainer, Becton Dickinson). About 1.5 ml of blood was collected and left undisturbed in a slanting position for clot formation. After three hours, the samples were processed in the laboratory by centrifugation at 3500 rpm for 10 minutes. The clear serum was aspirated and stored in serum vials at – 80^{o}C deep freezer.

Microscopic agglutination test (MAT)

A 5-8 day old liquid culture of live leptospires incubated at 29 ± 1^0 C, containing density of $2x10^8$ leptospires per ml was used. The leptospiral cultures without clumps were used as antigens in MAT. Twelve *Leptospira* reference strains obtained from National Reference Laboratory, ICMR, Andaman and Nicobar Islands, India were used as antigens (Table 1).

This test was conducted as per OIE (2008) in 96 well 'U' bottom titration plates (M/s. Laxbro, India). Quantitative assay was carried out in 'U' bottom microtitration plates against the reacting serovars of leptospires. All the 96 wells were charged with 20 µl PBS. In the first well of each row, 20 µl of 1: 25 diluted (Initially diluted in PBS in a separate deep well dilution plate) serum samples were added and mixed well. Then equal volume (20 µl) was serially transferred upto 9 wells. From 9^{th} well 20 µl was discarded. A constant volume of 20 µl of the respective *Leptospira* antigen (2 x 10^8 per ml) was added in each row and incubated at 37^0 C for 2 h. All final dilution mixtures (50, 100, 200, 400, 800, 1600, 3200, 6400 and 12800) were observed under dark field microscope and the results recorded as before. The reciprocal of the highest dilution which showed 50 per cent reduction in the number of free leptospires comparable to the respective antigen control with or without agglutination was recorded as the respective titre.

Table 1 : Reference strains of Leptospires* used in the study

S.No	Serogroup	Serovar	Strain
1	Australis	*australis*	Ballico
2	Autumnalis	*rachmati*	Rachmati
3	Ballum	*ballum*	Mus127
4	Canicola	*canicola*	HondUtrecht IV
5	Grippotyphosa	*grippotyphosa*	Moskva V
6	Hebdomadis	*hebdomadis*	Hebdomadis
7	Icterohaemorrhagiae	*icterohaemorrhagiae*	RGA
8	Javanica	*poi*	Poi
9	Pomona	*pomona*	Pomona
10	Pyrogenes	*pyrogenes*	Salinem
11	Sejroe	*hardjo*	Hardjoprajitno
12	Tarassovi	*tarassovi*	Peripellicin

**Obtained from National Reference laboratory, ICMR, Andaman Nicobar Islands, India.*

RESULTS AND DISCUSSION

Out of 63 samples collected, 57 (90.47 per cent) samples were positive by MAT at 1 : 100 dilution with the prevalence of Australis (74.60 per cent), followed by Hardjo (30.16 per cent), Pomona (25.40 per cent), Autumnalis (23.81 per cent), Pyrogenes (17.46 per cent), Hebdomadis (6.35 per cent), Icterohaemorrhagiae (4.76 per cent), Canicola (3.17 per cent), Grippotyphosa (3.17 per cent),

Javanica (3.17 per cent) and Tarassovi (3.17 per cent).Out of 57 samples, 24 samples showed positive titres for single serogroup followed by 33 samples for more than single serogroup, 17 for more than 2 serogroups, 7 for more than 3 serogroups, 3 for more than 4 serogroups, 3 for more than 5 serogroups, 2 for more than 6 serogroups, 2 for more than 7 serogroups and 2 for more than 8 serogroups. The MAT titres ranged from 1:100 to 1: 12800.

Table 2 : Detection of antileptospiral antibodies swine in an organized farm

S.No	Serogroups (In No.)	Total Positive (In Percentage)	Total Positive
1	Australis	47	74.60
2	Rachmati	15	23.81
3	Ballum	--	--
4	Canicola	2	3.17
5	Grippotyphosa	2	3.17
6	Hardjo	19	30.16
7	Hebdomadis	4	6.35
8	Icterohaemorrhagiae	3	4.76
9	Javanica	2	3.17
10	Pomona	16	25.40
11	Pyrogenes	11	17.46
12	Tarassovi	2	3.17
Total seropositivity		**57**	**90.47**

Several authors have reported prevalence of antibodies against *Pomona* and *Tarassovi* in pigs (Vicente *et al.,* 2002). It has been suggested that swine is an important maintenance host for serovars belonging to serogroups *Pomona*, *Tarassovi* and *Australis* (Ellis, 1999). Hence the prevalence of *Pomona* (25.40 per cent) *Tarassovi* (3.17 per cent) and *Australis* (74.60 per cent) in the present study substantiates the pigs to be the reservoirs of these serovars. The infection of *Pyrogenes* is considered as incidental for pigs. The incidental infections are determined by the opportunity that prevailing social, management and environmental factors provide for contact and transmission of leptospires from other species to pigs. Prevalence of *Autumnalis* in this study has also been reported earlier among pigs showing signs of reproductive failure (Rocha, 1998). The predominance of *Hebdomadis* was recorded in cattle and buffaloes (Balakrishnan *et al*., 2011) whereas the present study recorded Hebdomadis in pigs. This indicated that no animal species specificity was observed with regards to the distribution of *Leptospira* serogroups. Hence a continuous systematic seroprevalence study is essential to ascertain the circulating serovars / serogroups in different species of animals in different geographical area to identify the source of infection and disease transmission. The serogroups *Grippotyphosa* and *Icterohaemorrhagiae*, reported in this study have been shown to cause clinical disease in pigs (Hathaway, 1985). These

two serovars have also been reported to be maintained by rodents (Faine *et al.,* 1999). These are widely distributed throughout the country and become the natural foci of leptospirosis of these serogroups any where in the country. Hence the pig can get infected as evidenced in our study. The prevalence of *Canicola* in this study might be due to dog, the maintenance host for this serogroup which acts as probable vector whereby this serogroup enters a piggery. The occurrence of *Grippotyphosa*, *Icterohaemorrhagiae* and *Canicola* are commonly identified as incidental infections in swine. Sero group *Hardjo* infection is maintained by cattle worldwide. The chances of pigs contracting the infection are more where cattle and pigs come in close contact (Ellis, 1999). The farm in which the present study undertaken comprises both cattle and pigs. Hence the presence of antibodies against *Hardjo* in pigs was probably due to contact with cattle since the predominance of *Hardjo* was recorded earlier among cattle and sheep (Balakrishnan *et al*., 2008a). The present study recorded the prevalence of *Javanica* in pigs whereas Balakrishnan *et al*. (2008b) have reported *Javanica* in rabbits. Hence it is construed that the prevalence of Javanica among pigs might be due to direct or indirect contact with rabbits or due to movement of farm workers. However a thorough seroprevalence study is essential to establish the species specificity of *Javanica*.

The high seropositivity of leptospirosis (90.40 per cent) was found in swine in an organized farm located in the outskirts of Chennai city has potentially important implications for public health, because the farm workers were exposed to the occupational risk of infection and alerts for the adequate sanitary measures in the farms to prevent the transmission of the infection. This study further warranted a thorough epidemiological study to ascertain the source of infection, transmission and animal species specificity of *Leptospira*. However the present study identified and indicated the circulation of *Australis*, followed by *Hardjo, Pomona, Autumnalis, Pyrogenes, Hebdomadis, Icterohaemorrhagiae, Canicola, Grippotyphosa, Javanica* and *Tarassovi* among pigs. In turn it paved a way to chalk out suitable control strategy by developing appropriate vaccines for swine leptospirosis. The present study further considered all the pigs both MAT positive and negative belonging to a single herd as carriers. Hence it is recommended to administer single dose of Dihydrostreptomycin at the rate of 25 mg/kg body weight intramuscularly or Oxytetracycline at the rate of 800 g / tone in feed for 10 days to eliminate carrier status and leptospiruria. Chemotherapy should be supplemented with practically feasible biosecurity measures with rodent / stray dog control. The animal attendants were advised to wear cumboots, wash hands and feet with soap and water every time they handle animals and to attend any cuts / injuries if any immediately to avoid the occupational risk of infection.

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