www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(9): 101-103

www.thepharmajournal.com
Received: 25-07-2018
Accepted: 27-08-2018

PS Reshma
MVSc Scholar, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

M Mini
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

R Ambily
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

S Joseph
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

AP Usha
Directorate of Farms, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

RM Reji
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

N AIswarya
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

TS Akkara
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Correspondence
PS Reshma
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Seroprevalence of leptospirosis among swine in Kerala, India

PS Reshma, M Mini, R Ambily, S Joseph, AP Usha, RM Reji, N AIswarya and TS Akkara

Abstract
The present study was undertaken to corroborate the seroprevalence of leptospirosis among pigs of Central Kerala, India. A total of 103 serum samples collected from pigs were screened for the presence of anti-*Leptospira* antibodies by Microscopic Agglutination Test (MAT). A seroprevalence of 35.92 percent was observed. Among a battery of 10 *Leptospira* reference serovars used in the study, *Leptospira interrogans* serovar Pomona was identified as the most prevalent serovar (45.95 percent) in the sample population followed by, Grippotyphosa (24.32 percent), Canicola (13.51 percent), Icterohaemorrhagiae (10.81 percent) and Tarassovi (5.41 percent). The results reveal the significant prevalence of anti-*Leptospira* antibodies among pigs of Kerala.

Keywords: *Leptospira*, seroprevalence, microscopic agglutination test (mat), Pomona, kerala, India, pigs, swine

1. Introduction
Leptospirosis is a continuing global crisis, especially in developing countries like India whose tropical climate and other natural phenomenon favours the growth and survival of the causative organism. Caused by the *Spirochaete, Leptospira*, the disease affects a wide variety of mammals including humans, cattle, dogs, pigs, horses, sheep and goats. Human beings are considered the end hosts who acquire the infection via contact with water and soil contaminated with urine of infected animals as well as via direct contact. With 22 known species of *Leptospira* having more than 300 serovars including pathogenic, intermediate and saprophytic species, the outspread of the disease is noteworthy (Picardeau, 2017) [1].
There exists serovar-wise variation in the clinical presentation of the disease in pigs. Various serovars have been reported to cause reproductive disorders in pigs such as *L. borgpetersenii* serovar Tarassovi (Kemenes, 1984) [2], *L. interrogans* serovar Bratislava (Ellis and Thiermann, 1986; Bolin and Cassells, 1990; Bolin *et al.*, 1991) [3, 4, 5], *L. interrogans* serovars Canicola (Paz-Soldan *et al.*, 1991) [6] and most importantly, *L. interrogans* serovar Pomona (Chappel *et al.*, 1992; Gummow *et al.*, 1999) [7, 8]. Microscopic agglutination test enables serovar specific and quantitative diagnosis of leptospirosis based on antibody detection and is the gold standard test for the same (OIE, 2014) [9].
Swine leptospirosis, characterised by anorexia, weakness, icterus and complications with reproductive problems, have been reported from many parts of India. Yet, further epidemiological studies for identification of the prevalent serovars of *Leptospira* and implementation of proper control strategies are far from priorities. The present study prospects to identify the important serovars of *Leptospira* prevalent in Central Kerala, India as groundwork for future research.

2. Materials and Methods
A farm wise cross-sectional study was conducted from seven organised pig farms in Thrissur district, Kerala, India. The cases were pigs showing mild to severe clinical signs from weakness, fever, icterus and haemoglobinuria to abortions and stillbirths. Representative samples from seemingly normal pigs were also included.
Blood samples of two to three millilitres were collected in clot activator vials. The samples were centrifuged for separating serum and stored at -20 °C. All the samples were subjected to MAT employing a panel of 10 live reference serovars of *Leptospira*, representing 10 serovars, as antigens.

These reference cultures procured from the National Leptospirosis Reference Centre (NLRC), Regional Medical Research Centre (RMRC PB), Port Blair, Andaman and Nicobar Islands, India were maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Thrissur, Kerala, India. The serovars included in the antigen panel were *Leptospira interrogans* serovars Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Pyrogenes and *Leptospira borgpetersenii* serovars Javanica, Tarassovi and Sejroe. A 1:100 serum dilution was prepared in sterile PBS (Hi Media, India), 30 µL of which was taken in 96 well microtitre plates (Tarsons) and mixed with 30 µL of each of the five to ten day-old live *Leptospira* serovars separately. Antigen controls were set with 30 µL sterile PBS and 30 µL of different live *Leptospira* serovars and the plates were incubated at 37 °C for two to four hours. After incubation, the results were read by examining a drop of the serum-antigen mixture from each well under low power of a dark field microscope for agglutination of leptospires. The end point was recorded as the highest dilution of the serum showing 50 percent agglutination or reduction in number of organisms in comparison to the respective antigen control.

Further, quantitative assay was carried out in 96 well microtitre plates against the reacting serovars of leptospires. All the 96 wells were filled with 30 µL PBS. In the first well of each row, 30 µl of 1 in 50 dilution serum samples were added and mixed. Then, serial double fold dilutions were made up to eight wells and 30 µL was discarded from the last well. A constant volume of 30 µL of a particular serovar with a density of 2 x 10^8 per mL was added in each row and incubated at 37 °C for two to four hours. All the final dilution mixtures (100, 200, 400, 800, 1600, 3200, 6400, 12800) were observed under a dark field microscope and the results were recorded. The reciprocal of the highest dilution of the serum which showed 50 percent agglutination or 50 percent reduction in the number of free leptospires in comparison to the control was considered as the respective titre.

3. Results

Pigs from seven farms in Thrissur district located at Mannuthy, Kolazhy, Erumapetty, Kodakara, Cherpu, Kattilapoovam and Ponganangadu were included in this study. The majority of the subjects were sows, farrowing sows or dry sows. One hundred-and-three porcine serum samples were subjected to MAT, out of which, thirty-seven samples were positive (35.92 percent). The most predominant serovar was found to be Pomona (45.95 percent), followed by, Grippotyphosa (24.32 percent), Canicola (13.51 percent), Icterohaemorrhagiae (10.81 percent) and Tarassovi (5.41 percent). The titres ranged from 1: 100 to 1:800 (Figure 1).

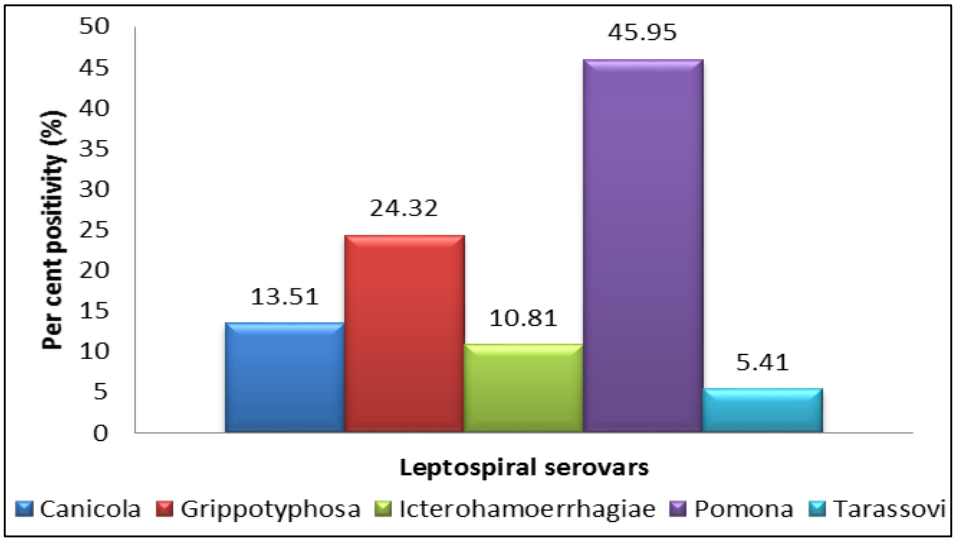

Fig 1: Seroprevalence of leptospirosis using MAT

4. Discussion

The studies on swine leptospirosis is critical as far as the pig industry is concerned, since it is one among the infectious etiologies associated with abortions worldwide. Porcine leptospirosis is mainly characterised by the occurrence of abortions, still births and birth of weak piglets (Michna, 1970) [10]. Diagnosis includes culture techniques, serological and molecular methods. Microscopic agglutination test is the gold standard test for the diagnosis of leptospirosis. It is a useful herd test which also enables identification of the prevalent serovars in an area, thereby providing an epidemiological database for further studies. In the present study, 103 serum samples were tested for occurrence of leptospirosis among pigs using MAT and 37 (35.92 percent) samples were found to have anti-*Leptospira* antibody titres in the range of 1:100 to 1:800. Antibodies were detected in both clinically ill as well as seemingly healthy animals. Hence, occurrence of serologically positive animals could be detected in the present study. According to Ellis (1999) [11], *Leptospira* antibodies in pigs could be detected for long time periods and pigs possess a long duration of urinary shedding of leptospires. Hence, they play a major role in transmission of the disease to other animals and humans.

The serovar Pomona (45.95 percent) was found to be the most prevalent serovar in the present study followed by Grippotyphosa (24.32 percent), Canicola (13.51 percent), Icterohaemorrhagiae (10.81 percent) and Tarassovi (5.41 percent). Previously, Soman *et al*. (2014) [12] had reported similar results on the seroprevalence of porcine leptospirosis in Kerala. According to Chappel *et al.* (1992) [7], Pomona was found to be the most important serovar causing infections in swine. Other studies from India also reported the predominance of serovar Pomona infection in pigs and were associated with swine abortions (Bhagwat, 1964; Rajasekhar and Nanjiah, 1971) [13, 14]. Serovars Icterohaemorrhagiae and Canicola were found to be associated with still births and

weak newborn piglets (Paz-Soldan *et al.*, 1991; Ramos *et al*., 2006) [6, 15] while serovars Tarassovi were known to cause carriage of dead piglets (Kemenes, 1984) [2].

Among the seven farms studied, five farms reported history of abortions. Of these, only animals from three farms had a history of clinical signs similar to porcine leptospirosis. However, the remaining two farms which showed not much noticeable signs were also found to have agglutinins to *Leptospira* serovars. Here, it is possible that the pigs could be just serologically positive during this period. Ellis (1999) [11] reported a prolonged urinary shedding of leptospires in swine and high antibody titres. Also, acute cases of leptospirosis only occurs if the flock is newly infected or due to other conditions threatening the immune status of the animal (Boqvist *et al*., 2002) [16].

As per Ellis (1992) [17], a seropositivity of 10 percent or more might indicate endemicity of the disease. With an overall seroprevalence rate of 35.92 percent, the present study suggests that proper management practices may be encouraged in pig farms in the study area to control further transmission of infections and more elaborate epidemiological studies covering a larger area may be conducted. This is important owing to the zoonotic potential of the disease as serovars which are maintained in one host may cause disease incidentally in another.

5. Conclusion

The present study reports the prevalence of *Leptospira* serovars among pigs in Thrissur, Kerala which are associated with abortions in pigs. The study exposed animals which may be acutely infected as well as serologically positive carrier animals. This could increase the occurrence of incidental infections in both man and animals. Most studies on porcine leptospirosis so far in Kerala are concentrated to discrete regions. Hence, more elaborate studies including isolation of *Leptospira* from the environment and animal reservoirs must be undertaken in addition to serology for a better understanding of the epidemiology of the infection in the area.

6. Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India for providing necessary infrastructure for the study.

7. References

1. Picardeau M. Virulence of the zoonotic agent of leptospirosis: still terra incognita?. Nature Reviews Microbiology. 2017; 15(5):297.
2. Kemenes F. *Leptospira* abortions of sows: new data. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology. 1984; 257(4):544-7.
3. Ellis WA, Thiermann AB. Isolation of leptospires from the genital tracts of Iowa cows. American journal of veterinary research. 1986; 47(8):1694-6.
4. Bolin CA, Cassells JA. Isolation of *Leptospira* interrogans serovar bratislava from stillborn and weak pigs in Iowa. Journal of the American Veterinary Medical Association. 1990; 196(10):1601-4.
5. Bolin CA, Cassells JA, Hill HT, Frantz JC, Nielsen JN. Reproductive failure associated with *Leptospira* interrogans serovar bratislava infection of swine. Journal of Veterinary Diagnostic Investigation. 1991; 3(2):152-4.
6. Paz-Soldán SV, Dianderas MT, Windsor RS. *Leptospira* interrogans serovar Canicola: A causal agent of sow abortions in Arequipa, Peru. Tropical animal health and production. 1991; 23(4):233-40.
7. Chappel RJ, Prime RW, Millar BD, Mead LJ, Jones RT, Adler B. Comparison of diagnostic procedures for porcine leptospirosis. Veterinary Microbiology. 1992; 30(2-3):151-63.
8. Gummow B, My burgh JG, Thompson PN, Van Der Lugt JJ, Spencer BT. Three case studies involving Leptospira interrogans serovar Pomona infection in mixed farming units: case report. Journal of the South African Veterinary Association. 1999; 70(1):29-34.
9. International Office of Epizootics. Aquatic Animal Health Standards Commission. Manual of diagnostic tests for aquatic animals. Office international des epizooties, 2014.
10. Michna SW. Leptospirosis. The veterinary record. 1970; 86:484-496.
11. Ellis WA. Leptospirosis. In: Straw BE, D'Allaire S, Mengeling WL and Taylor DJ, eds. Diseases of swine, 8th ed. Ames, Iowa: Iowa State Univ Pr, 1999, 483-493.
12. Soman M, Jayaprakasan V, Mini M. Seroprevalence of leptospirosis in human beings and animals in Central and North Kerala. Journal of agricultural and veterinary science. 2014; **7:**38-41.
13. Bhagwat SV. Serological evidence of *Leptospira* Pomona infection in pigs in India (Aurungabad). Indian veterinary journal. 1964; 41:792-796.
14. Rajasekhar M, Nanjiah RD. Animal leptospirosis in Mysore state: A serological study. Indian veterinary journal, 1971.
15. Ramos AC, Souza GN, Lilenbaum W. Influence of leptospirosis on reproductive performance of sows in Brazil. Theriogenology. 2006; 66(4):1021-5.
16. Boqvist S, Thu HT, Vågsholm I, Magnusson U. The impact of Leptospira seropositivity on reproductive performance in sows in southern Viet Nam. Theriogenology. 2002; 58(7):1327-35.
17. Ellis WA. Leptospirosis in pigs. Pig veterinary journal, 1992; 28:24-34.