RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.1.65-69

Seroprevalence of leptospirosis among slaughtered cattle in Thrissur, Kerala#

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Citation: Murugavelu, M., Vrinda, M. K., Latha, C., Deepa, J. and Vinodkumar, K. 2022. Seroprevalence of leptospirosis among slaughtered cattle in Thrissur, Kerala. *J. Vet. Anim. Sci.* **53**(1): 65-69
DOI: https://doi.org/10.51966/jvas.2022.53.1.65-69

Received: 09.06.2021 *Accepted: 09.07.2021* *Published: 31.03.2022*

Abstract

Leptospirosis is anunderdiagnosed bacterial anthropozoonotic disease that is considered as a major public health concern in India. The present study was conducted to detect the presence of antibodies against different leptospiral serovars in slaughtered cattle in Thrissur. A total of 165 blood samples were collected from slaughtered cattle of two slaughterhouses viz., Meat Technology Unit (MTU), KVASU, Mannuthy (n=85) and Thrissur Municipal Slaughterhouse (TMS), Thrissur (n=80). The serum samples from the slaughtered cattle were subjected to Microscopic Agglutination Test (MAT). The overall occurrence of leptospirosis from both the slaughterhouses was 44.42 per cent. A seropositivity of 37.65 and 51.25 per cent in slaughtered cattle respectively were found by MAT from samples collected from MTU, Mannuthy and TMS, Thrissur. The predominant serovars detected in slaughtered cattle were Sejroe (28.76 per cent), Grippotyphosa (21.91 per cent) and Australis (15.06 per cent).The results revealed that the apparently healthy slaughtered cattle had been exposed to leptospirosis and could act as a source of infection to the slaughterhouse workers.

Keywords: *Leptospirosis, slaughtered cattle, microscopic agglutination test (MAT)*

Leptospirosis is a widespread re-emerging bacterial zoonotic disease caused by spirochete of the genus *Leptospira*. *Leptospira* is a long thin spiral shaped, obligate aerobic, Gram negative and slow growing fastidious bacterium. The genus *Leptospira* is divided into 22 species which include pathogenic, intermediates and saprophytic species and encompasses more than 300 serovars (Thibeaux *et al.,* 2018). The disease has been recognised as a major

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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public health concern in both developed and developing countries. Most mammalian species get infected by *Leptospira* but only few act as efficient reservoirs. In the reservoir host, the organism is capable of establishing itself as a long term kidney coloniser and shedding *Leptospira* in urine. Livestock and companions animals are the major source of infection to human beings. Asymptomatic carrier cattle shed the pathogenic *Leptospira* in urine leading to contamination of the environment and spread of infection to other susceptible animals and humans. The kidney is the prime organ for the localization of Leptospira; mainly in the proximal convoluted tubules of kidney and the organism can be excreted through urine from 10 to 118 days (Vegad and Katiyar, 2001). Cattle act as maintenance host, but rodents act as the most important reservoirs for leptospirosis. According to the reports of Directorate of Health Services (DHS), Kerala (2019), 1211 confirmed cases of leptospirosis were reported in humans with 57 deaths, of which 16 cases were from Thrissur district with 56.25 per cent mortality. Leptospirosis is known as one the most significant occupationally acquired zoonotic diseases. Slaughterhouse workers handling potentially infected animal are at risk of acquiring this diseases.The prime route of transmission of infection is mainly through direct contact with an infected animal and indirect contact by soil and water contaminated with body fluids of infected animals (Haake and Levett, 2015). The antigen and antibody detection methods are used as effective diagnostic tools for the detection of leptospirosis. Microscopic agglutination test (MAT) is a serological diagnostic method which remains thegold standard test for diagnosis of leptospirosis. The MAT is used to identify the infecting serogroups or serovars of *Leptospira*. The present investigation was undertaken to study the seroprevalence of leptospirosis in slaughtered cattle in Thrissur.

Materials and methods

A total of 165 blood samples of slaughtered cattle were collected from both slaughterhouses *viz.,* MTU, Mannuthy (n=85) and TMS, Thrissur (n=80) during the period July 2019 to December 2020. All the blood samples were collected from apparently healthy cattle present in the two slaughterhouses. The blood samples were collected aseptically from pre-slaughtered cattle by intravenous puncture and then centrifuged for serum separation and the serum samples were stored at -20°C until use for MAT. All collected serum samples were screened for anti-leptospiral antibodies by Microscopic Agglutination Test (MAT) as per the procedure described by Faine (1982) with a slight modification at a dilution of 1:50. A panel of twelve live *Leptospira* serovarswere used as antigen in MAT *viz.,*Australis, Autumnalis, Bataviae, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Sejroe and Tarassovi. Further, quantitative assay was performed for the MAT confirmed serovars up to a dilution of 1:6400 (Faine, 1982). The reciprocal of the highest dilution of the serum which revealed fifty per cent reduction or agglutination in the number of free leptospires in comparison with the control was considered as the positive titre.

Results and discussion

The study was conducted on apparently healthy slaughtered cattle. The overall seropositivity of leptospirosis in slaughtered cattle in samples collected from both slaughterhouses was 44.24 per cent and the antibody titre ranged between 1: 50 to 1:400 as shown in Fig.1. Among the serum samples analysed, 45, 19, six and three samples revealed an antibody titre of 1:50, 1: 100, 1:200 and 1: 400, respectively. The predominant serovars detected in MAT were Sejroe (28.76 per cent) followed by Grippotyphosa (21.91 per cent), Australis (15.06 per cent), Pomona (9.58 per cent), Autumnalis (6.84 per cent), Bataviea (5.47 per cent), Hebdomadis (4.10 per cent), Icterohaemorrhagiae and Tarassovi (2.73 per cent each) and Javanica and Pyrogenes (1.36 per cent each) as shown in Fig.2. The serum samples positive in MAT revealed that 63 samples showed the presence of only one serovar, whereas seven, two and one serum samples showed the presence of two, three and four serovars, respectively. Statistical analysis of data using chi-square test revealed that there was no significant difference ($p>0.05$) between the seroprevalence of leptospirosis among

Fig. 1. Microscopic agglutination test

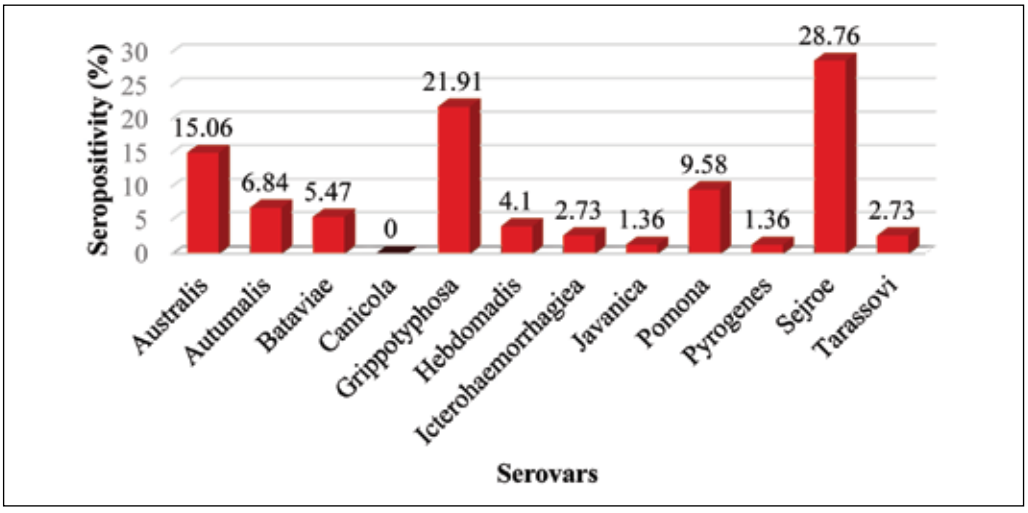

Fig. 2 Predominant serovars detected from slaughtered cattle

slaughtered cattle in MTU, Mannuthy and TMS, Thrissur.

According to OIE (2018), in animals, antibody titres of 1:100 and 1: 400 are considered as positive in non-endemic and endemic regions respectively, for leptospirosis. However, because of the high specificity of MAT, a lower antibody titre could be taken as evidence of past exposure to *Leptospira* spp. (Favero *et al*., 2017). In the present study, the antibody titre of 1: 50 was taken as a positive titre to understand exposure to the disease. Favero *et al*. (2017) and Shivakumar (2008) have also suggested a titre cutoff of 1:50 to test exposure to *Leptospira* spp. by MAT.

The overall occurrence of leptospirosis was found to be 44.24 per cent by MAT in serum samples collected from slaughtered cattle of both slaughterhouses with an antibody titre ranging from 1:50 to 1:400. The occurrence of leptospirosis in Thrissur Municipal Slaughterhouse (TMS), Thrissur (51.25 per cent) was higher when compared to Meat Technology Unit (MTU), Mannuthy (37.65 per cent). The samples collected from slaughtered cattle in TMS were mainly from animals which were brought for slaughter from neighbouring states of Kerala whereas in the MTU, Mannuthy, most of the cattle were from government farms within the state. The reports by Natarajanseenivasan *et al*. (2011) found 87 per cent of anti-leptospiral antibodies by MAT in cattle in southern states. Hence, there might be higher prevalence of anti-leptospiral antibodies in cattle of other states which could have led to increased seroprevalence of leptospirosis in cattle slaughtered at TMS, Thrissur. Moon*etal*. (2019)reported a seroprevalence of 45.11 per cent in cattle in Nagpur, which was in accordance with the present study. Similar

results were obtained by Sreekutty (2019), who found 40per cent seropositivity by MAT with an antibody titre of 1: 50 in cattle inAlappuzha, Kerala. However, Nally *et al*. (2018) obtained only 20per cent seroprevalence of leptospirosis in slaughtered cattle by MAT with anantibodyti trerangingfrom1:25to1:800 in United States of America. The prevalence in temperate countries like USA is significantly lower than that reported from tropical countries like India and this could be attributed to the longer survival of leptospires in warm environments and humid conditions which could expose the susceptible animals to the organism. Balamurugan *et al*. (2018) found 70.51 per cent seroprevalence of leptospirosis by using MAT in cattle in different states of India. The difference in seroprevalence of leptospirosis in different studies could be due to the variation in cut off titres considered as positive for leptospirosis in the study.

The predominant serovars obtained in the present investigation were Sejroe (28.76 per cent), Grippotyphosa (21.91 per cent) and Australis (15.06 per cent). Serovar Canicola was not detected in the study. The serovar Hardjo-bovis (serogroup Sejroe) is a host adopted serovar in cattle. Cattle act as classic reservoirs of serovar Hardjo (Rajala *et al.,* 2017; Loureiro and Lilenbaum, 2020). A similar observation was reported by Abiayi *et al*. (2015) in slaughtered cattle in Nigeria where the predominant serovars were Hardjo (27.8 per cent) and Grippotyphosa (25.6 per cent) with the seroprevalence of 57.9 per cent. Natarajaseenivasan *et al*. (2011) also found that, serovar Hardjo was one of the predominant serovars in cattle from samples collected from southern India. Sharma *et al*. (2014) reported that, serovars Grippotyphosa, Icterohaemorrhagiae and Hebdomadis were the predominant serovars of cattle in Andaman Island. Bojiraj *et al*. (2017) reported that, the predominant serovars detected in cattle from different regions of Tamil Nadu were Australis and Autumnalis. In Alappuzha district of Kerala, Sreekutty *et al.* (2020) reported that the predominant serovars obtained in cattle were Grippotyphosa, Sejroe and Autumnalis. The predominant serovars detected in cattle were serovar Pomona, Hebdomadis, Javanica, Icterohaemorrhagiae and Sejroe by Moon *et al.* (2019), in Nagpur.

Soman (2004) reported that the predominant serovars among cattle in Thrissur district, Kerala were Sejroe, Potac and Pomona. The comparison of the predominant serovars detected in cattle in the present study with the result of Soman (2004) revealed that there was a change in the occurrence of serovars over a period of time in the same region. Moreover, the comparison of the present study with the various reports of different research workers revealed that serovars of *Leptospira* varied across regions over a period of time and hence regular studies are required to understand the prevalent serovars in different species in the regions.

Conclusion

The present study revealed that, apparently healthy slaughtered cattle can act as a source of infection to the slaughterhouse workers and contaminate the environment. Hence, preventive measures should be taken by slaughterhouse workers to reduce the occupational risk due to the disease.

Acknowledgement

The authors are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities needed to carry out the research.

Conflict of interest

The authors declare that they have no conflict of interest.

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