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Short Communication

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.4.845-847

Isolation and molecular detection of *Leptospira* from pigs

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Citation: Reshma, T., Ambily, R., Mani, B.K., Priya, P.M., Menon, K.V., Ajith, Y. and Reshma, P.S. 2024. Isolation and molecular detection of Leptospira from pigs.
J. Vet. Anim. Sci. **55** (4):845-847

Received: 30.09.2024 Accepted: 23.10.2024 Published: 31.12.2024

Abstract

Leptospirosis is a globally significant zoonotic disease caused by Leptospira spp., affecting animals and humans. In animals, different serovars of Leptospira exist depending on varied geographic locations. Leptospirosis in pigs can lead to a range of health issues, including reproductive failure, abortions, and stillbirths. It may also cause neonatal mortality, as infected piglets are highly susceptible, leading to significant economic losses in swine production due to reduced litter sizes and poor health outcomes. Hence, this study was undertaken to detect Leptospira from pigs with history of abortions. Serum and whole blood samples were collected from of 23 pigs with history of abortion from some organised pig farms in Kerala. Blood samples were screened for the presence of Leptospira using polymerase chain reaction (PCR) targeting the loa22 gene followed by isolation in Ellinghausen McCullough Johnson Harris (EMJH) media. For the detection of antileptospiral antibodies, MAT was carried out. Two out of 23 serum samples were positive for MAT and the serovars positive were Australis and Pomona (1:200 dilution). The same samples were tested positive in PCR. One isolate was obtained and confirmed using loa22 gene-specific PCR.

Keywords: *Leptospirosis*, *Isolates*, *MAT, PCR*

Leptospirosis is a zoonotic disease caused by pathogenic spirochaetes of the genus *Leptospira*. It affects a wide range of domestic and wild animals, including pigs and is considered a significant cause of reproductive loss in swine herds. Pigs are recognised as carriers of leptospires, which can be shed through urine, contaminating the environment and causing risk to both animals and humans. In pigs, leptospirosis is often subclinical, making it difficult to detect without targeted diagnostic methods (Bolin and Alt, 2001). However, clinical signs such as abortion, stillbirths and reduced fertility are common indicators of leptospiral infection (Bolin and Cassells, 1992). Venereal and placental transmission in pigs resulted in reproductive problems, *viz*. abortion and stillbirth (Ellis, 1994). In many cases, leptospirosis in pigs is diagnosed using serological methods like MAT, which is the gold standard for detecting antibodies (Faine *et al*., 1999). However, isolation and molecular techniques such as PCR are more definitive diagnostic methods. Successful isolation contributes to the characterisation of local strains, which can be essential for vaccine development and improving diagnostic accuracy. This study aims to detect the presence of *Leptospira* in blood samples collected from pigs with history of abortion by PCR.

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Twenty-three whole blood and sera samples were collected from aborted pigs from organised pig farms. Serum samples collected in clot activator vials were utilised for MAT and whole blood samples, collected in EDTA vials, were subjected to PCR targeting *loa22* gene. The blood samples were inoculated into EMJH semisolid and liquid media for isolation. The serum samples were screened by MAT using a battery of 12 serovars of *Leptospira interrogans viz*., serovars Australis, Autumnalis, Bataviae, Canicola, Djasiman, Hardjo, Hebdomadis, Grippotyphosa, Icterohaemorrhagiae, Javanica, Pomona and Pyrogenes as per the method described by (Faine *et al*., 1999). The DNA was extracted from the whole blood samples using a DNA isolation kit (Qiagen, Courtaboeuf, France) following the instructions of the manufacturers. The concentration and purity of the DNA were assessed using a NanoDrop spectrometer, and the samples were stored at -20°C. A PCR targeting the *loa22* gene of *Leptospira* was conducted using the primers enlisted in Table 1(Roshni *et al*., 2023). A 6.25 µL 2X PCR master mix, one microlitre of 10 pM/µL of each forward and reverse primer, 3 µL of template DNA and 1.25 µL nuclease-free water were prepared. The amplification protocol used was: initial denaturation at 94°C for 4 min. followed by 30 cycles of denaturation (94°C for 1 min.), annealing (60°C for 45 sec.), and extension (72°C for 2 min.), the final extension was done for 10 min. at 72°C followed by hold at 4°C. Identification of PCR product was done in a submerged agarose gel electrophoresis system using one per cent agarose stained with ethidium bromide, and Tris Borate EDTA buffer was used as the matrix at a voltage of 90V. The gel was visualised under a UV transilluminator and results were documented in the Gel Doc system (Biorad, USA).

The PCR positive whole blood samples were inoculated into EMJH semi-solid and liquid media containing 5-Fluorouracil (500 µg/mL) to prevent contamination. The samples were then incubated at 28°C to 30°C and monitored for leptospira growth at weekly intervals using dark field microscopy (DFM) for up to three months (Ellis and Thiermann, 1986).

In pigs, two out of 23 sera (8.69 per cent) were positive for leptospirosis, with a MAT titre of 1:200. The positive serovars identified were Pomona and Australis, accounting for 50 per cent of the positive cases, this result is as per Zamir *et al*. (2022), who observed Pomona as the predominant serovar. The MAT positive samples were also positive for PCR targeting the *loa22* gene targeting pathogenic *Leptospira*. Among the two PCR positive blood samples that were inoculated into EMJH semisolid and liquid media isolation was successful from one sample. This was later confirmed as pathogenic using *loa22* gene-specific PCR (Fig. 1). In a seroprevalence investigation, Reshma *et al*. (2018) reported a 35.92 per cent seroprevalence among the pig population in central Kerala with Pomona having the highest seroprevalence and conducted isolation trials and could not isolate leptospires from suspected pig samples.

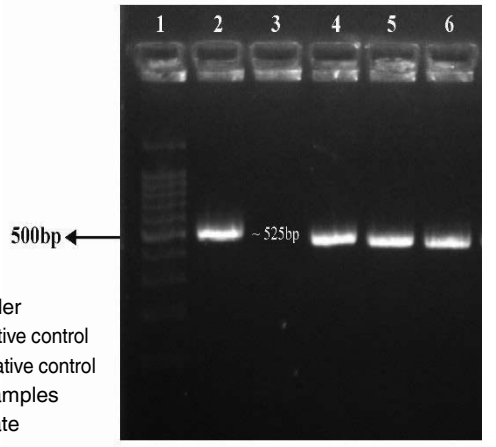

Fig. 1. Amplification of *loa22* gene from clinical samples and isolate (Amplicon size - 525bp)

Summary

This is the first report of isolation of *Leptospira* from pigs in Kerala. *Leptospira interrogans* serovars Australis and Pomona were the serovars observed in pigs. The isolate obtained was confirmed as pathogenic using PCR targeting the pathogenic marker gene *loa22*. The isolate was identified as pathogenic *Leptospira*, underscoring the presence of virulent strains in pig populations. The results highlight the importance of both serological and molecular diagnostic methods for the detection of leptospires. The isolation of a pathogenic strain emphasises the potential zoonotic risk posed by infected pigs. These findings underline the need for enhanced surveillance and control measures to mitigate the spread of leptospirosis in pig populations. Future studies should focus on expanding the sample size and exploring the genetic diversity of *Leptospira* strains circulating in pigs, particularly due to their potential role in neonatal mortality, to aid in vaccine development and prevention strategies.

Table 1. Primers used for the amplification of the *loa22* gene of *Leptospira*

Primers	Sequence	Amplicon size
loa22 F	5' - AGAGGAGAATTCAGCTCCTGAGC - 3'	525 bp
loa22 R	5' - TGGTGCCTGCAGCGCAAAACGGA - 3'	

Acknowledgment

The financial support provided by Kerala Veterinary and Animal Sciences University and Indian Council of Medical Research (ICMR) is acknowledged.

Conflict of Interest

The authors declare that they have no conflict of interest.

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