8. CONCLUSION

Leptospirosis is an infectious disease prevalent in the livestock predominated Salem and Namakkal districts of Tamil Nadu. Livestock activities coupled with farming practices had paved way for the entry of this pathogen into human population particularly in integrated farm workers. The seroprevalence analysis of leptospirosis showed an ergonomic relationship and workers involved in farm and livestock activities stood first to carry this pathogen. An age wise and sex wise differentiation was observed in the seroprevalence. Males particularly, in the age group 51-60 years were much affected. The people complaining of intermittent fever and jaundice symptoms had more leptospiral positive condition. Hence such clinical symptoms cannot be easily ignored and such person must be screened for leptospirosis.

About five serovars antibodies (Autumnalis, Australis, Icterohaemorrhagiae, Grippotyphosa and Louisiana) were found from the case patients. Of the different serovars, Leptospira interrogans serovar Louisiana was more common and prevalent than the other serovars in the samples tested.

Both direct diagnostic techniques like dark field microscopy, Fontana staining, EMJHI cultivation and polymerase chain reaction study and indirect methods such as IgM ELISA, MAT assay, MSAT study, Lepto Dri-dot, LITPO Tek Dip-Stick assay and Lepto Lateral-flow assay were applied to find out the infection. Persons with symptoms for leptospirosis for ten days and beyond ten days and also after antibiotic treatment were screened by these methods. Performance evaluation of different methods showed that PCR method is ideal for early diagnosis within 10 days.
after the onset of illness. In the persons who had illness over 10 days, serodiagnostic methods were much useful. As the antibody development needs time, these tests were good for cases whom had illness beyond 10 days. Serodiagnostic methods using agglutination principle was not effective after three or four weeks of illness as the antibody titre will be reduced by natural immune reaction in such cases.

For early and rapid investigation, MAT, MSAT and PCR techniques were found ideal. The sensitivity and specificity of different methods were compared with the standard techniques like MAT and PCR. The results revealed that except PCR technique, all the other serological techniques hold good value for cases having illness over 10 days. The urine analysis of the case patients was compared with control cases by applying different direct analysis. For the urine sample study also, PCR technique was very sensitive and reliable. The applicability of broad spectrum leptospirosis diagnosis kits like LEPTO Dip-S-tick, Lepto Dri-dot and Lepto Lateral-flow etc., did not yield cent percent positivity as desired by the pioneers of the kits. This is because of the antigenic variations in the serovar Louisiana which is the prevalent serovar in the local population.

In the present study, RAPD fingerprinting of two isolates were carried out and it was compared with the genotypic analysis of five genospecies (12 strains). The isolates K₁ and K₂ had 85% similarity in DNA bands with the strain Rachmat as per dendogram analysis. The strain Rachmat had been included under the genospecies Leptospira interrogans. This molecular analysis confirms the isolates K₁ and K₂ belong to the genospecies Leptospira interrogans.
The isolates K₁ and K₂ were subjected SDS-PAGE protein analysis to find out whether they were pathogenic or nonpathogenic. SDS-PAGE analysis revealed that the isolates K₁ and K₂ were pathogens. The isolates K₁ and K₂ had intense band at 110 KDa as it was present in the pathogenic serovar Australis. The molecular analysis clearly confirmed that the leptospires isolated from the Salem and Namakkal population were pathogenic leptospires. SDS-PAGE analysis is useful in identifying additional targets for vaccine and drug development to aid in controlling leptospirosis.

To develop safe antileptospiral drug, pharmacological studies were carried out on four medicinal plants viz. *Adhatoda vasica*, *Azadirachta indica*, *Andrographis paniculata* and *Phyllanthus niruri*. Methanolic, ethanolic, and aqueous extracts of the plants were tested for antileptospiral activity. Of the four plants tested, the ethanolic extract of the plant *Adhatoda vasica* was found very effective in curtailing the motility and growth of the spirochaetes. The comparison of antileptospiral activity of *Adhatoda vasica* extracts with standard antibiotic, penicillin showed positive signs for the development of a plant drug using the bio-active compounds in *Adhatoda vasica*. The pathogenicity of *Adhatoda vasica* extracts on the *Leptospira* isolates was evaluated using electron microscopic analysis. In the *Adhatoda* treated leptospires, inclusion body, a characteristic feature for virulence in leptospires was absent. Further the lengthy leptospire showed breaks at several sties in its body. Cellular damage caused by *Adhatoda* extracts had inhibited the motility and growth of the leptospires as evident from minimum inhibitory concentration analysis.