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Immunohistochemical localisation of YKL-40 in multiple organ dysfunction associated with leptospirosis in a dog#

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Abstract

Canine leptospirosis is a bacterial infection which has serious consequences in dogs. This study focused on the pathology of leptospira infection in a dog and the immunohistochemical localisation of YKL-40, a biomarker known for its association with inflammation in various tissues. The post-mortem examination revealed severe damage in several organs, particularly the liver, kidneys, lungs, heart and spleen. Histopathological analysis confirmed substantial inflammatory changes such as interstitial pneumonia, and interstitial nephritis with glomerular atrophy and tubular necrosis while PCR and Warthin-Starry staining identified the presence of Leptospira in kidney tissues. Significant localisation of YKL-40 could be noticed in the liver, lungs and kidneys suggesting its potential as a biomarker for early inflammation in leptospirosis. The study confirmed that leptospirosis is one of the infectious causes which could cause multiple organ dysfunction in dogs. YKL-40 plays an important role in diagnosing inflammatory changes associated with infectious diseases like leptospirosis, emphasising its potential for future research into the inflammatory response and offering valuable insights for developing treatments in both animals and humans.

Keywords: *Canine leptospirosis, YKL-40, multiple organ dysfunction, histopathological analysis, PCR, Warthin-Starry staining*

Canine leptospirosis is a bacterial illness caused by spirochetes from the *Leptospira* genus. While it mainly affects dogs, it can also be passed on to humans and other animals. The infection is commonly transmitted through exposure to water or soil contaminated by the urine of infected animals. Leptospirosis can result in both acute and chronic conditions, with the severity of the disease influenced by the virulence of the pathogen, the host's susceptibility, and the specific species affected (Radostits *et al*., 2000). In acute leptospirosis, the initial clinical signs include pyrexia (103-104°F), trembling and widespread muscle soreness. These are soon followed by signs such as vomiting, quick onset of dehydration, and compromised peripheral circulation (Greene, 1998).

YKL-40, also called chitinase 3-like protein 1, is an acute-phase protein with important roles in inflammation and infectious diseases (Çeliktürk *et al.,* 2023). Numerous cell types, particularly neutrophils, chondrocytes, macrophages,

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certain synovial cells, hepatic cells, colon epithelial cells, and airway epithelial cells, release the YKL-40 protein (Çeliktürk *et al.,* 2023). There is increased expression of YKL-40 in both acute and chronic inflammations. Thus YKL-40 can be used as an early biomarker for inflammation. Also, elevated levels of YKL-40 have been demonstrated in the serum of patients with various infectious conditions like bacterial pneumonia, skin and urinary tract infections, sepsis, bacterial diarrhoea etc. In this study, an attempt has been made to analyse the tissue localisation of YKL-40 in a case of multiple organ dysfunction associated with leptospirosis in a dog.

Materials and methods

The carcass of a non-descript male dog approximately five years of age was submitted for necropsy to the Department of Veterinary Pathology at CVAS, Mannuthy. A detailed post-mortem examination was conducted and the gross lesions were documented. Tissue samples were carefully collected, labelled, and fixed in 10 per cent neutral buffered formalin for 48 hours. They were then processed using the routine paraffin embedding method (Spencer and Bancroft, 2013). The tissue sections were cut into 5-micron-thick slices and stained using the standard haematoxylin and eosin (H&E) staining method (Suvarna *et al.,* 2018). Representative tissue samples were collected from these carcasses in RNase-free cryogenic vials for PCR and immediately stored at -80°C with proper labelling until processing. DNA was extracted from the pooled lymphoid organ samples using commercially available DNA isolation kit (Origin Genomic DNA Kit), as per the manufacturer's instructions and screened for leptospirosis by PCR targeting 767 bp of the *lipl 32* region using the published primers (F-5'-CGC GCT GCA GTT ACT TAG TCG CGT CAG AAG-3' and R-5'- CGC GGT CGA CGC TTT CGG TGG TCT CTG CCA AGC-3') (Amutha *et al*., 2007). The annealing temperature of the *lipl 32* gene was standardised at 60°C for 45 sec. The final volume of the reaction mixture was made up to 12.5µL. After amplification, visualisation of the PCR product was carried out after performing agarose gel (1.5 per cent agarose gel in Tris boric acid EDTA (TBE) electrophoresis buffer (1X)) electrophoresis at 80V for 50 minutes. The gel images were documented in the BIO-RAD gel documentation system. The DNA isolated from a standard culture of *Leptospira* spp. was used as positive control.

Warthin-Starry special staining was done to demonstrate the spirochetes in tissues. Immunohistochemistry was done to localise the protein YKL-40 in formalin-fixed paraffin-embedded tissues according to the procedure described by Ramos-Vara (2005).

Results and discussion

Clinical signs

The animal was severely debilitated. A detailed history obtained from the owner revealed that the dog had exhibited anorexia, vomiting, and neurological signs.

Gross lesions

Post-mortem examination of the carcass revealed pale and severely collapsed lungs and focal areas of pulmonary emphysema were also evident. The heart was paler than normal with noticeable variation in shape. Hepatic congestion with diffuse pale areas was

Fig 1. Gross lesions. 1a. Debilitated animal. 1b. Pale and collapsed lungs with pulmonary emphysema.1c. Misshapen heart. 1d. Congested liver with diffuse pale areas on the surface. 1e. Spleen with splenic infarcts. 1f. severely contracted kidneys. 1g. oesophagus showing distal dilatation.

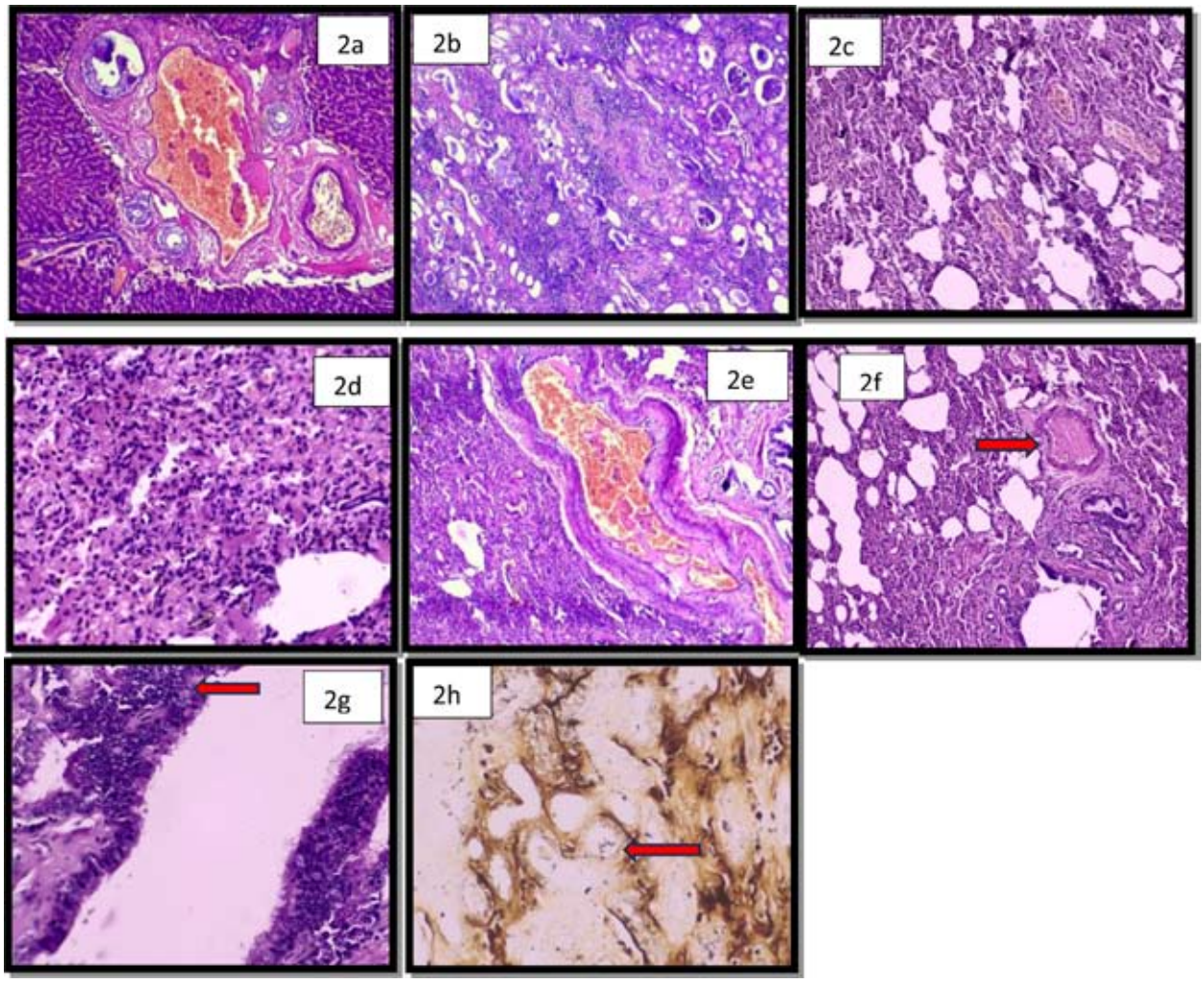

Fig 2. Histopathological lesions. Portal area showing thrombus formation in portal vein and desquamation of bile duct epithelium. (2a) (H&E x100). Atrophic changes in glomerulus, degenerating tubules, cast formation and infiltration of mononuclear cells and interstitial fibrosis (2b) (H&E x100). Pulmonary emphysema with congestion in blood vessels (2c) (H&E x100). Infiltration of inflammatory cell the pulmonary interstitium (2d) (H&E x400). Pulmonary congestion (2e) (H&E x100). Fibrin thrombi in lungs (2f) (H&E x100). Hyperplasia of bronchiolar epithelial cells (2g) (H&E x400). Warthin-Starry special staining showing Leptospira organism in kidney tubules (2h) (x400)

observed on the liver surface. Spleen showed focal areas of infarction. Kidneys were severely contracted and had irregular surfaces. The distal end of the oesophagus was dilatated more than normal.

Histopathology

Histopathological examination of the liver revealed marked degeneration of hepatocytes and desquamation of bile duct epithelium. Thrombi formation was noticed in the portal vein (Fig 2a). Multiple lesions including vascular, degenerative and inflammatory changes in the liver of dogs affected with leptospirosis were described earlier also by Chandrasekaran *et al.* (2011). The kidney showed severe chronic changes. Glomerular atrophy, necrosis of tubular epithelial cells, cast formation, extensive interstitial fibrosis and infiltration of inflammatory cells predominantly mononuclear type, were evident (Fig 2b). These pathological changes are consistent with the findings of Winaya *et al*. (2018), Ashna *et al*. (2019), Idrees *et al*. (2023) and Joseph *et al*. (2023), who reported vascular and degenerative alterations in

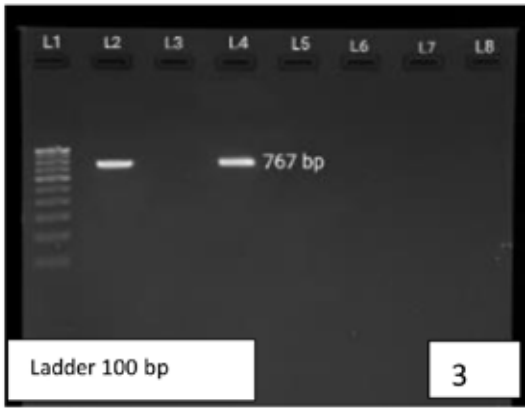

Fig 3. Molecular detection of leptospira. Agarose gel electrophoresis showing PCR product of Leptospira. Ll Ladder, L2 Positive control, L3 Negative control, L4 Positive sample, L5-L8 negative samples

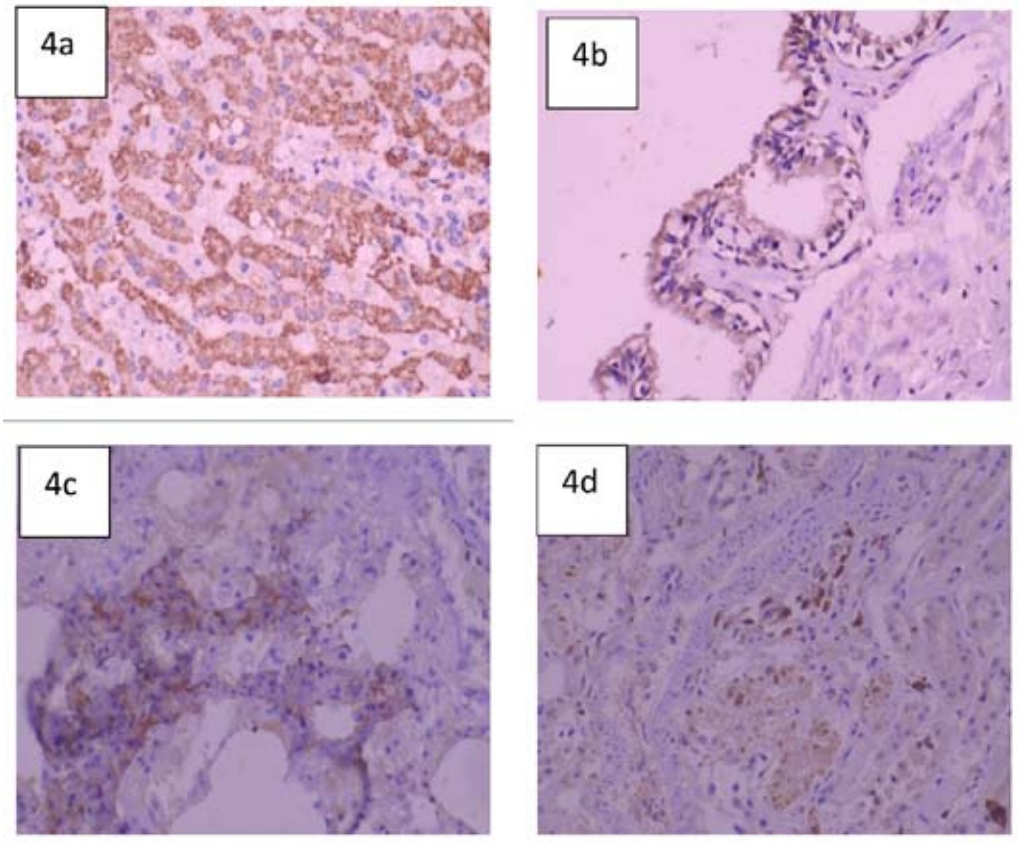

Fig 4. Immunohistochemistry of tissues of dog with leptospirosis. Liver- strong immunostaining (4a) (IHC x400). bronchial epithelium- moderate immunostaining (4b) (IHC x400). pulmonary interstitium moderate immunostaining (4c) (IHC x400). kidney- strong immunostaining (4d) (IHC x400).

the liver and extensive renal damage due to interstitial nephritis in leptospirosis. Lungs showed severe interstitial pneumonia with infiltration of inflammatory cells (Fig 2d), pulmonary congestion (Fig 2c and 2e) and fibrin thrombi formation (Fig 2f). Bronchiolar epithelial cell hyperplasia was also noticed (Fig 2g). These findings were similar to the observations of Tochetto *et al*. (2012).

The molecular confirmation of leptospirosis was done using PCR and got 767 bp amplicons specific for leptospira organism (Fig. 3). Warthin-Starry special staining revealed leptospira organisms within the kidney tubules (2h). Previous studies, such as those by Azizi *et al*. (2014) and Umesh *et al*. (2014), also reported a special affinity of leptospira for renal tissues.

Immunohistochemical staining of tissues revealed immunopositivity for YKL-40 in the cytoplasm of liver, kidney and lungs (4a-4d). The increased expression of YKL-40 in inflamed tissues indicates its potential as a biomarker for the early detection of inflammation in infectious diseases like leptospirosis, presenting a new avenue for diagnosis (Çeliktürk *et al.,* 2023).

Conclusion

To sum up, this study confirms the widespread impact of leptospirosis in dogs and highlights YKL-40 as a significant marker in the disease's inflammatory response. The evidence gathered here suggests that YKL-40 could be pivotal for future research into new diagnostic and treatment strategies for infectious diseases, potentially leading to better outcomes for both animals and humans affected by the disease.

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

Amutha, R., Chaudhury, P., Garg, A.P., Vasan, P., Cheema, P.S. and Srivastava, S.K. 2007. Cloning and sequence analysis of the gene encoding LipL32 of *Leptospira interrogans* serovar Sejroe. *Vet. Res. Commun.* **31**: 513-519.

Ashna, S., Devi, S.S., Sajitha, I. S., Reshma, P. S. and Mammen, J. A. 2019. Acquired hepatic encephalopathy due to leptospirosis in dog: A case report. *J. Entomol. Zool. Stud*. **7**: 777-779.

Azizi, S., Kheirandish, R. and Rahimi, E. 2014. Comparison of polymerase chain reaction and Warthin-Starry techniques to detect *Leptospira* spp. in kidneys of slaughtered cattle. *Onderstepoort J. Vet. Res.* **81**: 1-6.

Çeliktürk, E., Salt, Ö., Sayhan, M.B. and Dıbırdık, İ. 2023. A novel biomarker in acute cholecystitis: YKL-40. *Asian J. Surg.* **46**: 1564-1570.

Chandrasekaran, D. Prathaban, S. Dhanapalan, P. Venkataraman, K.S. and Balachandran, C. 2011. Occurrence of new *Leptospira* serovars in vaccinated dogs. *Indian J. Vet. Med.* **31**: 1-3.

Greene C.E. 1998. Infectious Disease of the Dog and Cat. W.B. Saunders Co. Philadelphia,1320p.

Idrees, A., Deepa, P.M., Vinodkumar, K., Rathish, R.L. and Pradeep, M. 2023. Assessment of acute kidney injury in dogs suffering from babesiosis and leptospirosis. *J. Vet. Anim. Sci.* **54**: 921-926.

Joseph, A., Vinodkumar, K., Tresamol, P.V., Vijayakumar, K. and Sajitha, I.S. 2023. Aetiopathology of canine hepatitis in Kerala. *J. Vet. Anim. Sci.* **54**: 477-485.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. 2000. *Vet. Med.* W.B. Saunders London, 2156p.

Ramos-Vara, J.A. 2005. Technical aspects of immunohistochemistry. *Vet. Pathol.* **42**: 405-426.

Spencer, L.T. and Bancroft, J.D. 2013. Tissue processing. In: Bancroft, J.D., Layton, C. and Suvarna, S.K. (Eds.) *Bancroft's Theory and Practice of Histological Techniques.* Churchill Livingstone Edinburgh, pp. 114-123.

Suvarna, K., Layton, C. and Bancroft, J.D. 2018. *Bancroft's Theory and Practice of Histological Techniques.* Elsevier Health Sciences Churchill, 584p.

Tochetto, C., Flores, M.M., Kommers, G.D., Barros, C.S. and Fighera, R.A. 2012. Anatomopathological aspects of leptospirosis in dogs: 53 cases (1965-2011). *Braz. Vet. Res.* **32**: 430-443.

Umesh, S., Chandan, S., Gopinath, S. M. and Shareef, M. I. 2014. Detection of Leptospira in Canine Samples using Polymerase Chain Reaction based on LipL32gene and Biochemical Profiles in Canine Leptospirosis. *J. Cell Sci. Mol*. Biol. **1**: 1-5.

Winaya, I.B.O., Berata, I.K., Kardena, I.M., Adi, A.M. and Rompis, A.L.T. 2018. Histopathological description of liver and kidney of dogs suspected of leptospirosis in Denpasar City. *Indonesian j. Vet. Sci.* **19**: 298-303. ■