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Diagnosis of canine leptospirosis by Immunocomb dot ELISA testing

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Abstract
The present study was conducted to diagnose Leptospirosis infection in dogs presented to Out patient department of Veterinary College Hospital, Hebbal with the clinical manifestations of anorexia, lethargy/malaise, vomiting, lymphadenopathy, dark yellow urine, pyrexia, conjunctivitis, ascites, melena, polyuria/polydipsia, recumbency and epistaxis. Eighteen dogs which were screened prior via Rapid test kit and PCR were further tested by Dot ELISA test Kit for serological evaluation of IgG antibodies.

Keywords: Immunocomb, Dot, ELISA

Introduction
Emerging infectious diseases are primarily of zoonotic significance having greater impact on public health besides global providence (Chen 2022; Pal *et al.*, 2021) [2, 17]. Disease leptospirosis is usually overlooked zoonotic ailment caused by the spirochete of genus *leptospira*, having complex transmission cycle amongst humans, animals and environment. "One Health" Approach is required for the effective preventive and control policies (Pal *et al.*, 2021) [17]. The proposition is related to epidemiological studies in case of companion animals owing to knit interconnection between dogs, cats and humans with main route of contraction of infection via urine contaminated soil/water (Francey *et al.*, 2023; Overgaauw *et al.*, 2020) [7, 16]. Presently, the methods for diagnosing the animal leptospirosis as recommended by World Organisation for Animal Health (OIE), directs to either detection of the agent itself or the immune response elicited by prior past exposure (Musso and La Scola, 2013; Pinto *et al.*, 2022) [13, 18]. For the detection of the infection in early and chronic phases, PCR is the recommended gold-standard test talking of agent detection whereas in case of serum antibody detection, MAT using the live serovars (should reflect the serovars present in the study population) or ELISAs with protein antigens are suggested for surveillance (Pinto *et al.*, 2022) [18]. MAT being most commonly used test in veterinary practice has several limitations with respect to sensitivity and specificity. Certain studies have used MAT to test the asymptomatic carriers in dogs and cats but right now there is no consensus with respect to the standard MAT titre, serovars included in the diagnostic panel and technical interpretation of the results (Esteves *et al.*, 2023) [6]. Therefore the alternative diagnostics should be standardized like ELISAs and Rapid serological tests which help at timely arriving at the confirmative diagnostics.

Materials and Methods
Clinical animals
Dogs (n = 18) presented to Veterinary College Hospital, Hebbal, Bengaluru with the clinical signs of anorexia, lethargy/malaise, vomiting, lymphadenopathy, dark yellow urine, pyrexia, conjunctivitis, ascites, melena, polyuria/polydipsia, recumbency and epistaxis were tested with Dot ELISA (Immunocomb, Biogal, Israel). These dogs hence included in present study were initially screened with Rapid Antibody test Kit followed by molecular detection with PCR testing as well for Leptospirosis.

Dot ELISA (Immunocomb, Biogal, Israel)

The Immunocomb test is a modified ELISA described as an enzyme labeled "dot-assay which detects antibody levels in serum, plasma or whole blood. On the day of presentation of animals, blood samples were collected aseptically from either cephalic/saphenous vein of dogs in clot activator vials and further centrifugation was done to collect the serum which was subjected to testing.

Before performing the test, developing plate was brought to room temperature by removing all kit components from the kit carton and placed on the work bench for 60-120 minutes and Assay was performed at room temperature of 20°-25 °C. Reagents were mixed by gently shaking the developing plate few times before use. Tweezers were used to pierce the protective aluminium cover of row A. Procedure was commenced by depositing 5µl of sample into a well in row A of the multi-compartment developing plate with raising and lowering pipette plunger several times to achieve proper mixing along with avoid spillage and cross-contamination of solutions. Thereafter, Comb was removed from its protective envelope without touching the teeth of ImmunoComb card followed by inserting comb into open wells in row A. Incubation was done for 5 minutes. Subsequently, after completion of the color development in row F comb was moved back to row E for 2 minutes for color fixation. Final step was undertaken by Taking the Comb out and letting it dry for 5 minutes prior to reading the results**.** At the end of the developing process, a purple-grey color results were developed in all Positive Reference spots and in any positive sample tested spot. The intensity of the color result corresponds directly to the antibody level in the test specimen. Results were scored using the Positive Reference spot and CombScale.

Interpretation of Result

The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3 which is considered as "cut-off" level of IgG antibodies roughly equivalent to a positive immune response at a titer of 1:400 by the Microscopic Agglutination Assay-MAT. Color tone of the anti Leptospira serovars mix spot (bottom one) was compared with the Positive Reference spot (upper one). A clear, visible purple-grey dot indicated a positive response. To evaluate the antibodies score, CombScale provided in the kit was used.

Results

Dot ELISA (Immunocomb, Biogal, Israel)

Among 18 animals 2 (11.11%) were strong positive at the combscale value of S3 which is equivalent to 1:400 M.A.T titre and 3 (16.66%) were faint positive at the combscale value of S1 corresponding to 1:100 M.A.T titre. Overall positivity was recorded as 27.77% based on ELISA testing (Plate 1).

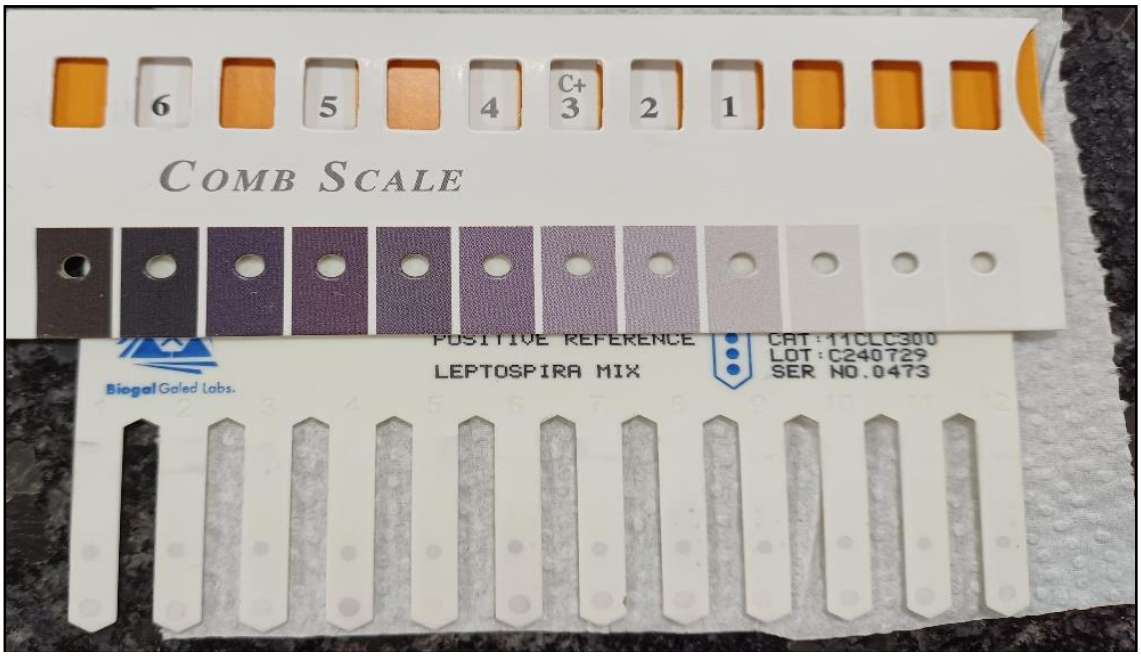

Plate 1: Immunocomb results for IgG antibodies

Discussion

The present finding is in accordance with the findings of Tuemmers *et al.* (2013) [20], Dhliwayo (2014) [4], Ojha *et al.* (2018) [15], Desai *et al.* (2020) [3], who documented the positivity rate on the basis of ELISA testing as 21.3%, 15.6%, 11.4%, 36.95% respectively. Odontsetseg *et al.* (2005) [14], Jimenez-Coello *et al.* (2008) [10], and Shah *et al.* (2018) [19] delineated the appointment of ELISA as serological test in the diagnostic panel of Leptospirosis in dogs. Similarly, Joshi and Joshi (2000) [11], Gautam *et al.* (2010) [8] reported the positivity on the basis of ELISA testing for leptospirosis in dogs as 5.5% and 8.1% respectively. On the contrary, Dreyfus *et al.* (2021) [5] and Kanthala *et al.* (2023) [12] documented the positivity of 47.6%, and 49.07% respectively while employing ELISA testing as diagnostic tool for Canine leptospirosis. According to Bora *et al.* (2022) [1] Immunocomb assay is a sensitive simplified form of ELISA which can be delineated as "dot assay" detecting antibody levels in serum or whole blood without the need for sophisticated classical ELISA which needs technical expertise and moreover time elapsed is also greater.

In the present study IgG antibody levels detected in case of confirmed cases of Leptospirosis were low owing to low

positivity which could be attributed to the fact that the generation of IgG antibodies takes alteast 2 weeks as opined by Desai *et al.* (2020) [3] to develop when the disease is moving towards chronic phase and most of the positive cases encountered in the present study were acute ones in which mostly IgM antibodies would be predominated on contrary to IgG antibodies. Moreover only 18 confirmed leptospira samples were subjected to IgG testing which could also be underestimated due to less inclusion. Hence conjunction with other molecular testing can help at arriving definitive diagnosis. Furthermore, the kinetics of IgM and IgG antibodies might also vary between different animals residing in different geographical areas owing to higher positivity of one than the other.

Conclusion

Present study proposes the use of IgG testing preferably during the chronic phase of the disease and not during the acute phase of the disease when mostly IgM antibodies are predominant. Hence the serological test should always be combined with the other diagnostics like Molecular assays as in natural infections the stage of the infection is unknown which points to the multimodal approach of diagnostics with the consequent timely management of disease overall.

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