Leptospira hardjo serodagnosis: a comparison of MAT, ELISA and Immunocomb


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BACTERIOLOGICAL methods for the isolation and identification of pathogenic Leptospira serovars are slow, labour intensive, relatively insensitive and are not used for routine diagnosis (Ellis 1986). There are over 200 serovars placed in 23 serogroups according to the extent of antigenic cross-reactivity between isolates (Kmety and Dikken 1988) although contemporary phylogenetic approaches (Ramadass and others 1992) recognize six pathogenic genospecies. A variety of molecular methods have been developed for the specific detection of particular pathogenic Leptospira serovars from clinical samples, such as DNA probes (Terpstra and others 1986, Le Febvre 1987, Millar and others 1987, Van Eys and others 1988, Zuerner and Bolin 1988, Paccarini and others 1992), polymerase chain reaction (PCR) (Van Eys and others 1989, Woodward and Sullivan 1991) and variants of the PCR methodology (Woodward and Redstone 1993, Redstone and Woodward 1996). These methods require technical expertise and equipment not currently available in many laboratories. Thus, given the constraints of bacteriological and molecular approaches to diagnosis, indirect evidence of infection by a serological approach is the historical approach and remains the option of choice for most routine diagnostic laboratories. The microscopical agglutination test (MAT) using live antigen is considered the international standard methodology (Faivre 1994).

Leptospira interrogans serovar hardjo hardjo-bovis is associated with milk drop syndrome and abortion in cattle and is of significant zoonotic potential causing human infection via infected fetal material or urine (Ellis and others 1982, Thiermann 1984, Chappel and others 1989, Le Cour and others 1989). Thus, in recent years, novel serological methods for detecting infection in man, such as an IgM based ‘immuno-dipstick’ (Gussenhoven and others 1997) and a proteinase-K resistant antigen dot ELISA (Ribeiro and others 1995), an erythrocyte activation ELISA (Shamardin and others 1995) and a one-point microplate agglutination test (Armitage and others 1994), have been developed. ELISAs for both veterinary diagnostic and surveillance needs have been developed also. Bercovich and others (1990) evaluated an ELISA for the diagnosis of L hardjo-bovis infection in cattle in comparison with the MAT and demonstrated a 90 per cent correlation between the two tests using positive and negative field sera from 704 adult cattle from 90 separate farms. Additionally, the ELISA was demonstrated to be specific in reacting with only three of 227 sera known to be seropositive arising from heterologous leptospiral infections.

Less favourable comparisons were shown by Staal and others (1990) who compared the complement fixation test, MAT and ELISA on a limited selection of test sera to establish a close correlation between the complement fixation test and MAT on the one hand and 74 per cent correlation between MAT and ELISA on the other. Other ELISA formats have been developed and evaluated. For example, Path and others (1991) monitored the fate of maternal antibody and the response of calves to vaccination by MAT and ELISA at week 0 and 8 weeks after vaccination. Goddard and others (1991) detected circulating IgM and IgG by ELISA compared with MAT to monitor the immune responses in vaccinated bovines. Sting and Dura (1994) used 2 per cent sodium taurocholate at 50°C to extract antigens from eight leptospira species for use in an ELISA which performed well in comparative studies with the MAT and the immunofluorescence test on sera from cattle, pigs, horses and dogs.

Gerritsen and others (1994a,b) compared MAT, PCR and ELISA to study the effect of dihydrostreptomycin on L hardjo-bovis shedding in cattle and used MAT and ELISA to study sheep as a maintenance host for the transfer of L hardjo-bovis to cattle. More recently, Dhaliwal and others (1996a,b) compared the immunofluorescence test, MAT, and IgG and IgA ELISAs on cervical-vaginal mucous, postcalving discharges and serum from naturally infected cows to assess detection of infection. In all the cases cited above, ELISA formats compared very favourably with the other tests, with MAT and ELISA being regarded by some authors as comparable, whereas the immunofluorescence test was regarded as particularly insensitive. Interestingly, mass screening of cattle in California by ELISA without recourse to MAT indicated 22 per cent prevalence of Leptospira species (Behmer and others 1991) and yet, ELISA alone is rarely used in the veterinary field for either diagnosis or surveillance.

The availability of a commercially produced ELISA (L hardjo ELISA cedilis; ID-DLO, Institute for Animal Health and Science, Lelystad, The Netherlands) and the Immunocomb (Immunocomb Leptospiriosis kit; Biogal) systems, which detect L hardjo antibodies, prompted a comparative test against the standard laboratory MAT employed within the Central Veterinary Laboratory, part of the Veterinary Laboratories Agency. A total of 2144 cattle sera were selected at random from the annual submission of approximately 40,000 cattle sera during 1996. ELISA and Immunocomb tests were performed exactly as per the manufacturers’ instructions, including the reading of ELISA cut-off points by optical density and Immunocomb by colour development, by one operator and checked independently by a second. All sera were tested by MAT, by the routine diagnostic section at the Veterinary Laboratories Agency and 50 per cent agglutination was scored positive at dilutions of 1/50 or more. The data were collated and analysed statistically and the test results are shown in Table 1.

For statistical analyses, the MAT result in line with Office International des Epizooties recommendations was taken as the reference standard against which the other two tests were compared using the exact significance probabilities for McNemar’s test for paired samples. The performance of the ELISA and Immunocomb in terms of sensitivity, specificity and predictive values together with 95 per cent confidence limits are shown in Table 2.

The percentages of positive results obtained by the Immunocomb (31.5 per cent) and ELISA (31.3 per cent) both exceeded significantly (P<0.004) the 29.3 per cent obtained by the MAT. However, comparison between the Immunocomb and ELISA tests based on results diagnosed as positive and negative by MAT, showed that the ELISA was significantly more sensitive (54.4 per cent compared with 54.6 per cent, P<0.01) and also more specific (92.4 per cent compared with 90.6 per cent, P=0.007) than the Immunocomb.

In conclusion, the Immunocomb and ELISA tests evaluated were easily performed and safe tests, bearing in mind the hazards associated with the use of live antigen in the MAT, and compare favourably with each other, and with the accepted standard. Additionally, these findings for ELISA sensitivities and specificities are in line with those published by Bercovich and others (1990) and this would indicate no essential differences between these tests as detected by this ELISA in UK cattle with those in Europe, as might be anticipated. To the authors’ knowledge, this is the first extensive report of the use of the Immunocomb in
cattle. Although the ELISA appeared to be marginally better than the Immunocomb, care in interpretation should be taken because comparisons were made against the MAT only, itself a less than accurate test. It may have been informative to have undertaken the statistical analyses using varying cut-off points in the MAT but, for the purposes of this study, 50 per cent agglutination at a serum dilution of 1:50, the Office International des Epizooties recommended figure, was used. Importantly, there is a need for these tests to be evaluated in sera from known carrier animals.

Finally, on a practical note, the Immunocomb test was very simple and quick to use requiring no sophisticated equipment and designed for both high and particularly low volume use. However, readings of the test were by eye and required verification by two independent operators although the authors are aware that an automated reading device is in development, so that numerical values may be given from test results. The ELISA, although simple to use, required a significant reaction time and other reagents but was well suited for automation and high volume use. With the development and successful evaluation of tests such as these, questions could be raised as to the continued reliance on the MAT as the accepted international standard for L. hardjo serodiagnosis. The same argument applies equally to other serovars, such as L. pomona, where appropriate alternative tests exist (Rubi 1996).

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References

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Abstracts

Gastrointestinal transit times in healthy cats

NORMAL gastrointestinal transit times were measured in 12 healthy cats by taking radiographs at intervals after the cats had been fed 60 g of a canned food containing 30 small (1-5 mm) and 10 large (5 mm) barium-impregnated polyethylene spheres (BIPS). Six of the cats had been sedated and the other six had not. For the small bars the median gastric emptying time (first exit from the stomach) was six hours (range 3 to 8) in the sedated cats, and two-and-a-half hours (range 2 to 6) in the unsedated cats. There were no significant differences between the two groups for other transit times and the pooled data gave the following median values and ranges: a 50 per cent gastric emptying time of 6-4 hours (2-5 to 10-9), a complete gastric emptying time of 12 hours (6 to 27), an orocecal transit time (first appearance in the colon) of 6-5 hours (4-0 to 12-0), and a 50 per cent orocecal transit time of 8-8 hours (4-6 to 12-8). The transit times of the large bars were significantly longer except for the complete gastric emptying time.


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