Enzyme-Linked Immunosorbent Assay for the Detection of Antileptospiral Antibodies in Swine Serum

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Introduction

Leptospirosis, particularly in swine, poses a serious health and economic threat because of losses due to abortion, stillbirth, and neonatal mortality. Thus the rapid diagnosis of leptospirosis is necessary for adequate control of the disease. The microscopic agglutination test (MAT) is currently the diagnostic test of choice, however, the MAT is laborious, hazardous, and serotype specific. The enzyme-linked immunosorbent assay (ELISA), for antibody detection, has proven to be sensitive, specific, simple, rapid, and relatively inexpensive.

Adler et al. (1980) developed an ELISA for detecting anti-leptospiral antibody in human sera. They utilized sonicated antigen preparations of Leptospira interrogans serovars pomona, hardjo, and copenhageni adsorbed to polyvinyl microtiter plates. Their ELISA proved to be useful and tended to give cross reactivity. Terpstra et al. (1980) developed a genus-specific ELISA for human sera and noted a 95% agreement between the MAT and ELISA.

The purpose of this study was the development of the ELISA to serve as a diagnostic screening test for swine leptospirosis.

Materials & Methods

The ELISA technique consists basically of four steps, each separated by washing procedures: (0.05% tween in phosphate buffered saline): 1. The physical adsorption of the antigen to the solid phase; 2. The addition and incubation of the test antisera; 3. The addition and incubation of the enzyme-linked anti-Ig conjugate (i.e. goat anti-swine IgG-horseradish peroxidase); 4. The addition and incubation of the enzyme substrate (i.e. o-phenylenediamine + hydrogen peroxidase.) The resulting color reaction is a measure of enzymatic activity and directly relates to the concentration of specific antibodies in the test sera. The color reaction may be read visually or spectrophotometrically. Thus by observing the color reaction of serially diluted antisera in relation to a positive and negative control, actual antibody titers can be determined for that sera.

The antigen preparation is critical for the success of the ELISA. Therefore several preparations were tested, including: a patoc formalinized antigen, a patoc erythrocyte sensitizing substance, a patoc sonicated antigen, a pomona sonicated antigen, and a grippotyphosa sonicated antigen.

The antisera used in testing the ELISA consisted of negative, low, and high titered swine sera to a wide range of leptospiral serovars.

The ELISA had to be standardized to this particular system, i.e. the optimal antigen concentration and conjugate dilution were determined by checkerboard titration.

Results

The standardized ELISA, developed in this study, consisted of utilizing the pomona sonicated antigen at a concentration of 125 ug protein/ml. In preliminary studies it was determined that the sonicated preparations of various serovars were superior to the other preparations, and specifically the pomona antigen gave the best results. Polyvinyl microtiter plates were found to be superior to polystyrene microtiter plates. The optimal conjugate dilution was 1:500. Incubation times for the various steps were antigen-one hour, antisera - one hour, conjugate - one hour, substrate thirty minutes, each separated by a ten to fifteen minute washing procedure.

The ELISA titers were correlated with the MAT titers for the same sera. A baseline MAT valve of 1:100 was selected to separate positive and negative sera. Since a linear relationship existed between the ELISA and MAT titers, a comparable ELISA baseline value was calculated statistically to be 1:25.

Discussion

The sensitivity and specificity of the ELISA for detecting leptospiral antibodies in swine sera using the pomona sonicated antigen was 100% and 85% respectively. This means the ELISA did not detect any false negatives and only 15% false positives. The predictive accuracies were 92.2% for positive sera and 100% for negative sera. Thus with an unknown serum sample, if the ELISA calls the serum sample positive the sample will be positive 92.2% of the time. If, however, the ELISA calls a serum sample negative, it will be negative 100% of the time. Thus, the ELISA demonstrates an extraordinary accuracy for a diagnostic test.

The ELISA using a pomona sonicated antigen should prove to be an effective screening test for the detection of anti-leptospiral antibodies in swine sera. The ELISA is extremely accurate, lends itself to screening large numbers of sera, and is simple enough to be used routinely by an ordinary diagnostic laboratory.

Selected References

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Terpstra, W. J., Ligthart, G. S., Schoone, G. J. Zentralbl Bakteriol A 247: 400-405, 1980.