DEVELOPMENT AND REFINEMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SWINE DYSENTERY

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The difficulty in the prevention and control of swine dysentery (SD) is the lack of suitable diagnostic tests to differentiate between swine infected with Treponema hyodysenteriae and T. hyodysenteriae-like organisms. The indirect fluorescent antibody test has been employed for the diagnosis of SD; however, its efficacy has been hampered by the difficulties encountered with cross-reactivity of T. hyodysenteriae-like organisms (Terpstra et al., 1968). One test presently employed in the detection of antibodies to T. hyodysenteriae is the microtiter agglutination test (MAT) (Joens, 1978). This test is laborious to do, requires a minimum of about 2 days due to difficulties in the settling pattern of the antigen, and is also subject to cross-reactivity of enteric organisms. Cross-reactivity has also been encountered in a hemolysis test (Jenkins et al., 1975).

Currently, the successful use of the enzyme-linked immunosorbent assay (ELISA) for immunodiagnosis of several infectious diseases has made it attractive to hypothesize that the ELISA may be useful in the diagnosis of SD (Carlsson et al., 1972; Weldkamp and Visser, 1975). The ELISA is economical and safe. The purpose of this investigation was to develop a sensitive ELISA for the rapid immunodiagnosis of SD.

In the development of the ELISA, polystyrene microtiter plates were coated with 200 l of antigen derived from T. hyodysenteriae suspended in sodium carobnate buffer (0.1 M, pH 9.5). The plates were washed 3 times with phosphate buffered saline (PBS) containing 0.5% Tween-20. A 200 µl volume of diluted serum (1:10) was added to each well, and the plates then incubated at 39°C for 3 hours. Following incubation, the plates were rewashed with PBS/Tween-20, and a 200 µl volume of antiswine IgG conjugated to alkaline phosphatase enzyme was added. Plates were then incubated for 2 hours at 37°C. To determine the presence of enzyme-linked IgG bound to the antigen-coated plates, 200 µl of p-nitrophenyl phosphate was added, and the plates were incubated for 30 minutes in a water bath (37°C). The reaction was stopped with 50 µl of 3 M NaOH. The presence of antibody to T. hyodysenteriae was established by measuring the abosrption at 450 nm on a Spectronic 20 spectrophotometer. Serum samples showing an extinction coefficient (EC) of greater than or equal to 0.21 were considered positive. Of a total of 40 sera tested from pigs known to be affected with SD using particulate antigen of T. hyodysenteriae, 21 were strongly positive (EC < 0.03), and 13 were negative. In contrast, when 340 serum samples randomly selected from herds with unknown history of SD were tested, 137 or 40.3% were positive. When 3 different antigens (TCA-extracted, phenol extract antigen, and sonicated antigen of T. hyodysenteriae) were compared, there was no significant difference ($P < 0.05$) in the number of positive reactions obtained on sera samples of known or unknown SD history.

Conclusions:

The ELISA was found to be superior to the MAT in detecting serologic activity since it was able to detect a greater number of sera containing antibody to T. hyodysenteriae. The advantage of ELISA In this respect may have been attributed to its extreme sensitivity, since the assay had the capability to detect minute quantities of antibody. However, one disadvantage of the ELISA was the lack of specificity which was indicated by numerous false positives as well as the discrepancy in the uniformity of the background reaction from one control sera to the other.

Selected References: Terpstra, J. J., et al., Neth. J. Vet. Sci., 1968, 1:5-13. Joens et al., J. Clin. Microb., 1978, 8:293-298. Jenkins, E. M. et al., Infect. Immun., 1975, 14:1106-1108. Carlsson, et al., Infect. Immun., 1972, 6:301-308. Yalken et al., J. Clin. Microb., 1977, 6: 434-444.

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