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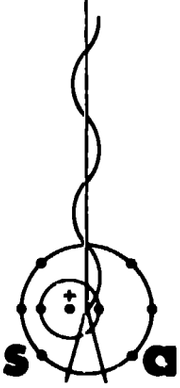

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SEROLOGICAL TESTING FOR TRICHINOSIS IN PIGS

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A test system suitable for rapid and economical screening of large numbers of swine for antibodies to Trichinella spiralis is being developed.

Several serological tests for T. spiralis have been evaluated in experimentally infected pigs during the past 10 years.[1,2] It has been demonstrated that the soluble antigen-fluorescent antibody (SAFA)[3] test can detect T. spiralis antibodies earlier and in swine fed fewer larvae than any of these tests.[4] Recently, we have been developing an enzyme-labeled antibody (ELA) test which is similar in principle to SAFA, but has been shown to be more sensitive than SAFA by comparison in a double blind test on sera from experimentally infected pigs.

Eighty-five swine sera were randomly coded by number by two people and then tested by ELA and SAFA using the second set of identifying numbers. Fourteen of these sera were pre-infection samples, the rest were taken 7 to 168 days after feeding the pigs 25, 100, 500, 2,500, or 250,000 T. spiralis larvae. The ELA and SAFA results differed for 13 of the 85 sera tested. Ten of these sera, which were positive by ELA but negative by SAFA, were taken on day 7, 14, or 17 post-infection, demonstrating the greater sensitivity of the ELA test in early infections, and presumably, to lower serum antibody levels. All sera taken from infected pigs on or after day 21 were positive by both tests except for 1 false negative by ELA and 2 by SAFA. Among the 14 pre-infection sera there were 2 false positives by ELA; none by SAFA.

ELA readouts were determined for sera from 17 gnotobiotic and 63 Specific Pathogen Free pigs. Readouts on gnotobiotic sera ranged from 0 to 20, and SPF sera ranged from 10 to 70. Sera from 205 farm-raised pigs were collected at Schwartzman's Packinghouse in Albuquerque, NM. ELA readouts on these sera ranged from 0 to well over 200. Before testing these packinghouse sera, we had regarded ELA readings of 100 or more as definitely positive. Using 100 as a positive value, 15% of the packinghouse pigs would have been called positive. Since the incidence of T. spiralis infection in farm-raised swine is estimated to be about 0.3%,[6] we suspected that most, if not all, of these were false positives.

ELA testing sera from 85 naturally infected, digestion positive pigs revealed that 33 of them had ELA readouts of less than 220, i.e., the readings overlapped with readings for some of the noninfected packinghouse pig sera. These data show that if we set the positive ELA readout value so that the false negative rate is 0, the false positive rate would be 39%; if we set the positive readout value so that the false positive rate is 0, the false negative rate would be 37%. Neither of these alternatives is acceptable for the purpose of screening swine for the presence of T. spiralis. However, selection of a positive ELA readout value such that the false negative rate is known, with a high degree of certainty, to be 0 could make the test useful, even in its present state of development, for identification of T. spiralis free swine.

On the basis of our present findings, we feel that the problem is primarily one of a high false positive rate, for the following reasons:

1. Attempts to demonstrate the presence of T. spiralis larvae in the false positive packinghouse pigs were negative.
2. Attempts to demonstrate the presence of T. spiralis specific antibodies in false positive sera by double diffusion tests in agar were negative.
3. Preliminary results of serum fractionation studied show that the false positive sera do not contain detectable 7S gamma-globulin antibodies to trichinosis antigen. These sera do contain a material which is active in the ELA test and elutes from DEAE-cellulose columns in the fractions reported to contain only 19S immunoglobins.[7]

The fact that the 7S antibodies can be separated from the false positive serum factor means that effective antigen purification may eliminate the false positives in the ELA test for trichinosis.

While the ELA test has proven to be highly sensitive for detection of antibodies to T. spiralis in experimentally infected swine, it is not suitable, in its present form, for testing swine for trichinosis in situations where a pre-infection serum sample is not available for comparison; i.e., in a field situation such as a packinghouse. For this purpose it will be necessary to purify the antigen to eliminate the reaction between the false positive serum factor(s) and the present crude antigen preparation; or, to devise a serum treatment which will specifically inactivate the false positive serum factor(s) without affecting the serological activity of the T. spiralis specific antibody in a positive serum.

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References: 1. Scholtens, R.G., Kagan, R.G., Quist, I.G., and Norman, L.G. (1966). Am. J. Epidemiol. 83: 489. 2. Ruitenberg, E.J., Kampelmacher, E.H., and Berkvens, J. (1968). Neth. J. Vet. Sci. 1:143. 3. Sadun, E.H. and Gore, R.W. (1967). Exp. Parasitol. 20: 131. 4. Clinard, E.H. (1975). Am. J. Vet. Res. 36: 615. 5. Saunders, G.C. and Clinard, E.H. Submitted to J. Clin. Microbiol. (1976). 6. Zimmerman, W.J. and Zinter, D. E. (1971). NSMHA Health Rep. 86:937. 7. Saif, L.J., Bohl, E.H., and Gupta, R.K.P. (1972). Infec. and Immunity 6:600.