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TOXOPLASMOSIS IN SHEEP

THE RELIABILITY OF A MICROTITER SYSTEM IN SABIN AND FELDMAN'S DYE TEST*

By
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WALDELAND, H.: *Toxoplasmosis in sheep. The reliability of a microtiter system in Sabin and Feldman's dye test.* Acta vet. scand. 1976, *17*, 426—431. — Sera were tested in 2 series both by the conventional technique (dilution in tubes) and by the microtiter system. In 1 series of comparative examinations the tests were performed with the same batch of accessory factor serum and antigen preparation, and in another series the tests were performed at 2 different laboratories. There was a high correlation between the 2 methods, but the titres obtained by the microtiter system were about 1 to 2 twofold dilutions lower than those obtained by the conventional dilution technique. The 95 % confidence limits by the microtiter system were plus and minus 1 twofold dilution.

toxoplasma infection; serological technique; sheep.

The *Sabin & Feldman* (1948) dye test (DT) is a useful and reliable method for the serological examination of toxoplasmosis. However, the necessity of the so-called accessory factor serum (AFS) of human origin is a disadvantage, since sera of most DT negative donors have a modifying effect on the trophozoites of Toxoplasma gondii. Dilution of the sera with physiological saline suppresses this effect (*Aagaard* 1960), and some sera that can not be used undiluted as AFS are usable after dilution with physiological saline (*Folkers* 1964). Addition of sodium citrate or Ca^{++} also has an inhibiting effect on the Toxoplasma modifying activity of serum (*Te Punga & Penrose* 1965). Sera suitable as AFS are accordingly easier to find for these modified methods, and, by using a micromodification of the DT which requires small amounts of the reagents (*Feldman & Lamb* 1966), the

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scarce availability of AFS no longer forms a limiting factor in large-scale serological surveys.

In the micromodification of the DT described by *Feldman & Lamb* the antigen preparation contained about 1/10 of the number of trophozoites of T. gondii commonly used in the conventional technique, and the microscopy was done with an inverted microscope. The purpose of the present work was to examine the reliability of a micromodification of the DT performed with antigen prepared as in the conventional technique, and in which the microscopy was done with a phase contrast microscope.

MATERIALS AND METHODS

Antigen was obtained from the peritoneal cavities of mice according to the method used by *Beverley & Beattie* (1952). Human serum giving not more than 15 % modified trophozoites in the control set up as described by the same authors was used as AFS, if necessary with the addition of sodium citrate (1 part 3 % w/v sodium citrate, 5 parts AFS). Smaller amounts of sodium citrate (1 part 3 % w/v sodium citrate, 12 parts AFS) were added even if the AFS could be used undiluted, because of the viscosity that otherwise might interfere with the thorough mixing of the reagents in the small wells in the microdilution trays.

Microtiter system

Twofold serial dilutions of test serum inactivated at 56°C for 30 min. were prepared in physiological saline with the "Takátsy microtitrator dilution set"*, using the 0.25 µl diluters. To each well was added antigen/AFS mixture in the proportions given by *Beverley & Beattie*. Suitable positive and negative controls were included. The plates were carefully tapped to mix the reagents, and covered to avoid evaporation. After incubation for 1 hr. at 37°C, 1 drop of alkaline methylene blue (*Sabin & Feldman* 1948) was added to each well.

Microscopy

Drops were placed on slides under coverslips and examined with a phase contrast microscope with neofluar objective (× 40, ocular × 8). The titres are given by the highest final serum

* Metrimpex, Budapest, Hungary.

dilution at which at least 50 % of the trophozoites were modified as described by *Lelong & Desmonts* (1951), i.e. the serum dilution after addition of AFS and antigen suspension. The addition of methylene blue is not necessary when the microscopy is performed with a phase contrast microscope (*Lelong & Desmonts*), but it was done to reduce the hazard of infection (*Kåss* 1954).

Comparative examinations

Forty-seven coded serum samples from sheep were examined both by the microtiter system and by the technique described by *Beverley & Beattie*, using antigen and AFS from the same preparation. Sixteen other samples from sheep and 13 samples from man were also tested by both methods, the one described by *Beverley & Beattie* in another laboratory* where the DT was performed routinely by this method (*Tönder* 1968).

The samples were selected to provide sera with titres in the range from negative ($< 1/8$) to strongly positive ($\geq 1/1024$).

Reproducibility

Sera from 25 sheep were selected, 8 of which on previous examinations had titres $< 1/8$, and the others ranging from 1/8 to 1/128. Aliquots of serum from each sheep were stored in each of 4 coded vials at —20°C until examined by the microtiter system on 4 different occasions, i.e. with different batches of antigen preparations.

RESULTS

There was a high correlation between the 2 methods in the comparative examinations (Fig. 1). Only 2 of the 76 samples showed a higher titre by the microtiter system than by dilution in tubes. The differences in titres obtained by the 2 methods were about the same in human sera as in sheep sera.

In the investigation of the reproducibility, 7 of the 25 sera showed the same titre at all 4 examinations. In only 4 sera there was a difference of 2 twofold dilutions between the highest and the lowest reading. In the 14 others, there was only 1 twofold difference. The standard deviation was ± 0.52 twofold dilution.

* The examination was kindly performed at the Department of Microbiology, The Gade Institute, School of Medicine, University of Bergen, Bergen, Norway.

DISCUSSION

Most of the sera showed titres 1 to 3 twofold dilutions lower by the microtiter system than by the conventional technique. *Feldman & Lamb* (1966) found a better consistency between the 2 methods, and on the few occasions when the difference was more than 1 twofold dilution, the microtiter system gave the higher titre. This discrepancy in the comparative examinations was probably due to differences in the dilution of the Toxoplas-

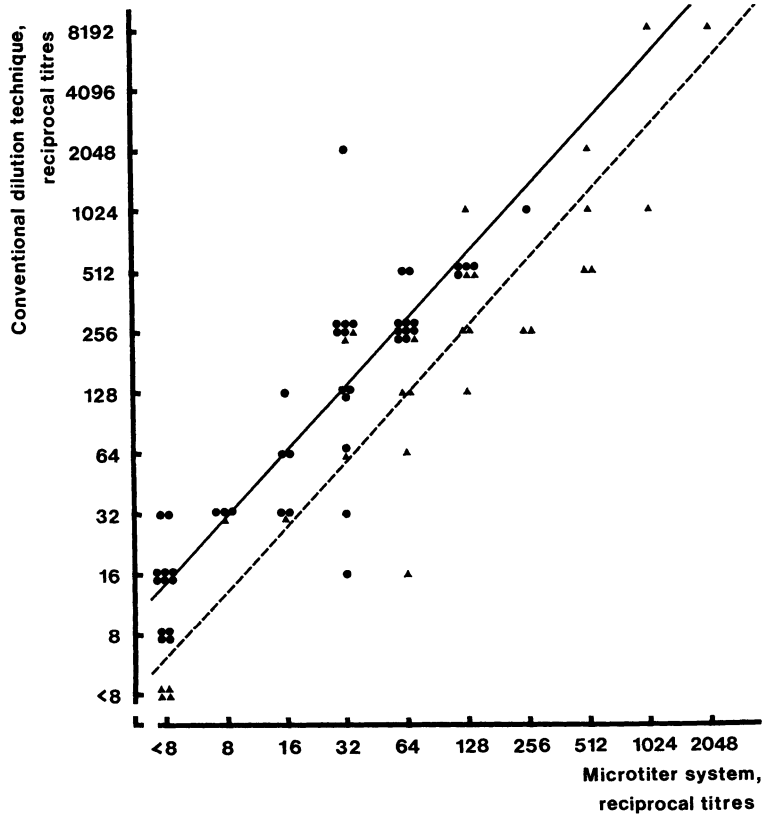

Figure 1. Comparison of titres for Toxoplasma antibodies (Sabin and Feldman's dye test) by dilution with microtiter equipment and by dilution in tubes.

● Titre readings in tests performed with the same batch of accessory factor serum and antigen preparation. The solid line gives the regression line for these observations (Y = antilog. (0.522 + 1.07 log. X)). The correlation coefficient is 0.89.

▲ Titre readings in tests performed at 2 different laboratories. The broken line gives the regression line for these observations Y = antilog. (0.154 + 1.08 log. X)). The correlation coefficient is 0.91.

ma suspension (*Beverley & Beattie* 1952, *Palm et al.* 1958). In this laboratory an inverted microscope as used by *Feldman & Lamb* was not available, and dilution of the antigen to the order recommended by these authors would make microscopic examination very time-consuming with either a phase contrast microscope or an ordinary light microscope.

According to comparative examinations of serial dilutions by *Takátsy* (1967) and to experiences by other authors (*Evans* 1973), lower titres by the microtiter system could be expected. Later examinations by the microtiter system of anti-Toxoplasma serum obtained from the International Laboratory for Biological Standards (WHO) also showed titres 1 to 2 twofold dilutions lower than the reference titre.

There was a high correlation between the methods in both examinations recorded in Fig. 1, and the gradients of the best "straight line fits" are in close agreement, but the titres obtained by the conventional technique were about 1 twofold dilution higher in this laboratory than those found in the other laboratory. This might be due to differences in the AFS'a or in the antigen preparations. The conventional technique was used only on this particular occasion in this laboratory, but routinely performed in the other laboratory. This might also have contributed to the differences between the results.

The standard deviation by the microtiter system was about $\pm$ 0.52 twofold dilution, which implies that the 95 % confidence limits are about plus and minus 1 twofold dilution. This is comparable to other tests for Toxoplasma antibodies, and less than by the conventional technique (*Engelbrecht* 1971). In long-term serological observations of the same individual the reproducibility of the test is of great importance, and in this respect the microtiter system has advantages compared with the conventional technique. In addition, it is easily and rapidly performed and requires only small amounts of the reagents.

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SAMMENDRAG

Toxoplasmose hos sau. Påliteligheten av et mikrotiter system i Sabin og Feldman's dye test.

To serier av serumprøver ble undersøkt både ved den konvensjonelle teknikken (fortynning av serum i reagensrør) og ved bruk av et mikrotiter system. I den ene serien ble det brukt antigen og accessory factor serum fra den samme tilberedning ved begge metoder. I den andre serien ble hver prøve undersøkt ved to forskjellige laboratorier som nyttet hver sin metode. Det var en god korrelasjon mellom de to metodene, men ved bruk av mikrotiter systemet lå titerverdiene 1 til 2 doble fortynninger lavere enn ved den konvensjonelle metoden. Konfidensintervallet (95 %) ved bruk av mikrotiter systemet var pluss og minus en dobbel fortynning.

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