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Simplified Hemagglutination Test as a Serologic Test for Toxoplasmosis

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For diagnosis of toxoplasmosis, isolation of the parasite is definitely the most favorable evidence of the disease; but it is rather difficult to obtain adequate materials from most cases so that the indirect methods, that is, various immunological tests have been playing an important role. It is known that the dye test is the most reliable among various serological tests, but it can be emplyed only in limited number of laboratories since it reguires living *Toxoplasma* and the accessory factor. Therefore more practical test methods are desirable. In Japan, the indirect hemagglutination (HA) tests according to the methods of Lewis and Kessel (1961) and Jacobs and Lunde (1957) are widely employed instead of the dye test. These methods have an unavoidable disadvantage that sensitized erythrocytes must be prepared each time. The authors and associates made effort to establish a more simplified HA test which would show at least the same reliability as the above HA tests and succeeded to introduce a simplified diagnostic kit, named TOXO-TEST 'Eiken', into the market. This paper describes attempts to evaluate the reliability of the HA tert using TOXO-TEST in comparison with the Lewis and Kessel HA test and dye test on human sera and to reveal the correlation between the isolation of the parasite from tissues of pigs, cats and dogs and HA test results.

Materials and Methods

Three hundred and forty seven serum samples from normal persons and patients with suspected or overt toxoplasmosis, stored in the Institute of Medical Sience, University of Tokyo, Tokyo, were tested by using the TOXO-TEST and the results were compared with those of the Lewis and Kessel HA test or the dye test. Ninety pigs, 99 cats 100 dogs were examined for correlation between HA titers with the TOXO-TEST and the presence of *Toxoplasma* in diaphragm muscle and/or brain.

The TOXO-TEST used in these studies consisted of lyophilized antigen-coated sheep erythrocytes, lyophilized sheep erythrocytes for absorption of non-specific antibodies in test sera, and diluent. Fixation of the erythrocytes was carried out with pyruvic aldehyde by the method of Jennis (1966). Sensitization of the fixed erythrocytes with antigen, which was prepared from the peritoneal exudate of mice infected with *Toxoplasma* of the RH strain by filtration, sonication and ultracentrifugation, was done repeatedly with the aid of bis-benzotized-benzidine (BDB) as a mediator. The diluent was a sterilized phosphate-buffered saline (PBS), pH 7.2, containing 0.4% inactivated bovine serum albumin. The constituents of the TOXO-TEST were stable in a refrigerator for at least two years.

The serum-antibody titration was run on serum samples that had been inactivated at 56°C for 30 minutes. Absorption was carried out in a mixture containing 0.1 ml of the serum, 0.3 ml of the diluent and 0.4 ml of 10% suspension of the fixed erythrocytes in the diluent overnight at 4–10°C or an hour at 37°C. The supernatant of the absorbed serum (1 : 8, 0.2 ml) was used to make serial four-fold dilutions in wells on a plastic plate, which contains 80 shallow U-bottom wells (diameter 16 mm). A 1.3% suspension of the sensitized, fixed erythrocytes (0.05 ml) was added into each well containing 0.6 ml of serum dilution, thoroughly agitated and incubated overnight at room temperature. The HA patterns were read according to the method of Stavitsky (1954).

The dye test was performed by the method of Kobayashi *et al.* (1968), and the Lewis and Kessel HA test by the original procedure. The dye test titers of 1 : 4 or higher and HA test titers of 1 : 32 or higher were judged as positive reactions.

Isolation of *Toxoplasma* from tissues of pigs, cats and dogs was attempted as follows ; a 10% homogenate of the tissue in saline was prepared and filtered through double-leyered gauze. The filtrate was centrifuged at 2,000 rpm for 5 minutes, and after the supernatant was discarded the sediment was resuspended in saline of the original volume of the homogenate. The suspension was then injected intraperitoneally along with appropriate doses of penicillin and dihydrostreptomycin into 5 mice (1 ml per mouse). If the inoculated mice survived without symptoms, they were bled to death 4 weeks later, and their brains were examined for the presence of *Toxoplasma* cysts under microscope. When no cyst was detected pooled homogenates of their brains were subinoculated into new mice. If the inoculated mice showed symptoms and/or died, various organs were examined for the presence of trophozoites and/or cysts in stamped preparations by Giemsa staining or the fluorescein-antibody technique. The demonstration of cysts or trophozoites in the firstly inoculated or subinoculated mice, and a positive HA test result in the sera of the mice were the criteria for the presence of *Toxoplasma* in the original tissue.

Results

Comparison of HA titers in human sera obtained by the TOXO-TESTand the Lewis and Kessel method : The results of comparison between the two HA tests, the TOXO-TEST and the Lewis and Kessel method, is shown in Figure 1. Qualitative agreement between the two tests was apparent. Among 159 tests, 146 sera (91.8%) gave qualitatively consistent results (20 negative, 126 positive in both tests). Discrepancy was observed in 13 sera, which were positive at 1 : 32 or higher in the Lewis and Kessel method but negative in the TOXO-TEST.

Ninety point four percent (149/159) of the samples gave results within one tube (one four-fold dilution) difference and 52.8% gave identical titers in both tests.

Comparison of titers in human sera determined by the TOXO-TEST and the dye test : The data showed good qualitative agreement between the HA test by the TOXO-TEST and the dye test, as presented in Figure. 2. One hundred eighty sera (95.7%) out of 188 were consistent (92 positive, 88 negative in both tests). Eight sera were positive with rather low titers in one test but negative in the other.

Qvantitatively, among 92 sera which were positive in both tests, 87 sera (94.6%) showed higher titers in the HA test than those in the dye test.

HA titers in the TOXO-TEST and isolation of Toxoplasma in pigs, cats and dogs : HA titers in the TOXO-TEST and *Toxoplasma* isolation are compared in Figure 3.

From apparently healthy pigs, the parasites were isolated from diaphragm muscle in 9 (10%) out of 90 pigs. Eight out of 9 pigs showed 1 : 4096 or higher HA titers and one pig showed a low titer of 1 : 16.

In 47 (47.5%) out of 99 cats *Toxoplasma* was isolated from diaphragm muscle and/or brain. All the 47 cats possessing *Toxoplasma*

Figure 1 Distribution of titers among 159 sera tested for Toxoplasma antibodies with the two HA tests, the TOXO-TEST and the methed of Lewis and Kessel

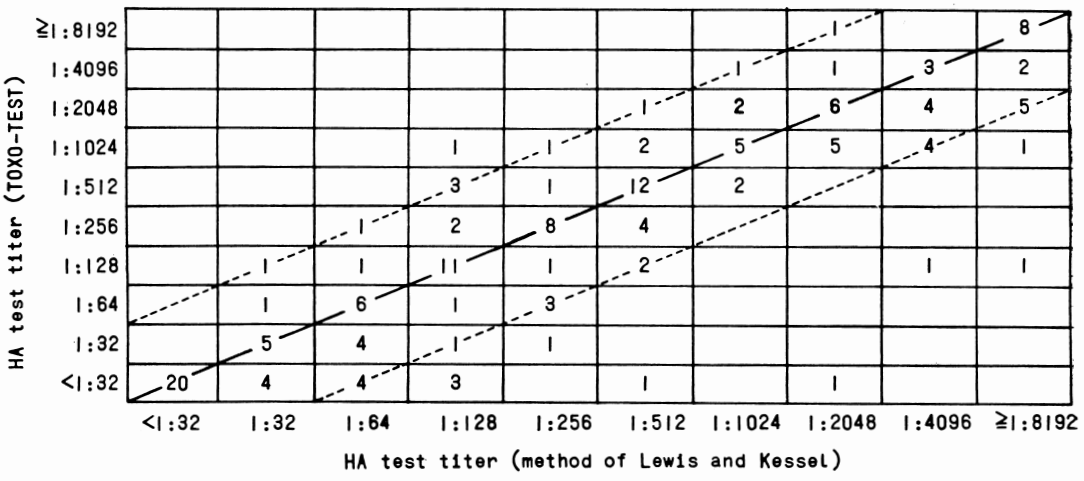
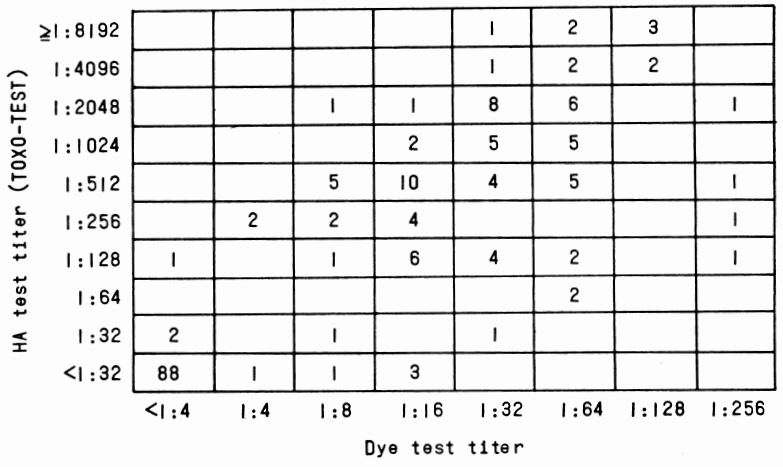


Figure 2 Comparison of titers of 188 sera examined for Toxoplasma antibodies with the HA test by the TOXO-TEST and the dye test



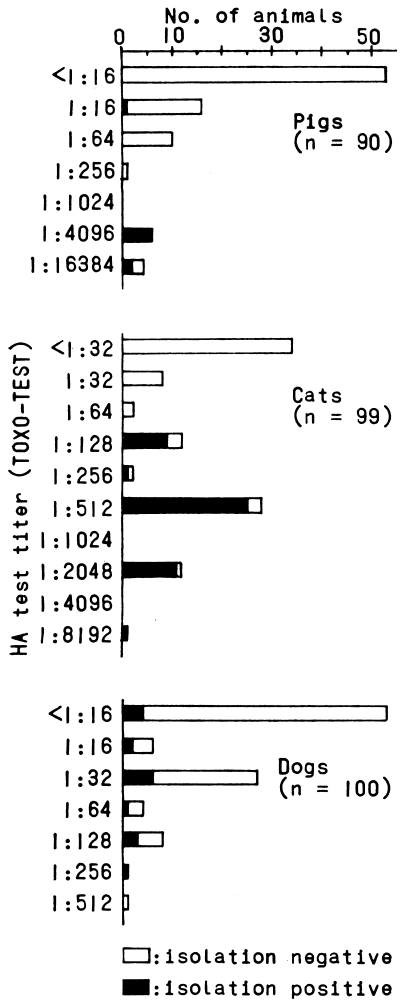
showed 1 : 128 or higher titers.

Toxoplasma was isolated from diaphragm muscle and/or brain in 17 out of 100 dogs. HA titers in the *Toxoplasma* positive dogs were lower than those in pigs and cats and correlation between the HA titers and *Toxoplasma* isolation was not apparent.

Discussion

In the present study it was found that the HA test by the TOXO-TEST, featuring a potentiated sensitivity and stability of sensitized erythrocytes showed a good qualitative agreement with the HA test by the Lewis and Kessel method and with the dye test with high agreement percentages of 91.8 and 95.7% respectively. Quantitatively, the HA titers by the TOXO-TEST were similar to those by the Lewis and Kessel method, but higher than those of the dye test. Qualitative agreement between the dye test

Figure 3 HA test titers and Toxoplasma isolation in pigs, cats and dogs



and the Lewis and Kessel method was reported in 90.0% of 232 samples of human, dog and cat serum (Kobayashi *et al.* 1971). Thus the simplified HA test using the TOXO-TEST seems to be useful in place of the Lewis and Kessel method and the dye test.

The TOXO-TEST was found to possess a good specificity to carriers of *Toxoplasma* at least in pig and cat. HA titers of the *Toxoplasma* carrier were 1 : 4096 or higher in pig and 1 : 128 or higher in cat. Thus the TOXO-TEST seems to be useful for detection of *Toxoplasma* carriers in pigs and cats, which are both considered to be infecting sources for human toxoplasmosis.

The reason for the lack of correlation between the HA titers of dog sera and the isolation of *Toxoplasma* is not clear since constituents of antibodies in dog have not precisely been investigated. It is possible that the level of γG-globulin is lowered while that of γ_1a-globulin is raised when immunization persists and the latter has little reactivity in the precipitin reaction and in the passive HA test (Fujimoto and Tada, 1968).

Summary

The reliability of the indirect hemagglutination (HA) test with a new diagnostic kit TOXO-TEST for toxoplasmosis, in which lyophilized, pyruvic aldehyde-fixed, sensitized sheep erythrocytes are employed, was evaluated by testing human serum samples in comparison with the Lewis and Kessel HA test and the dye test. The correlation between HA titers by the TOXO-TEST and the isolation of *Toxoplasm* from the tissues of pigs, cats and dogs was also examined. Qualitative consistency between the two HA tests was found in 91.8% of 159 sera and 90.4% of the samples gave titers which agreed within one four-fold dilution difference between the two HA tests. On comparison with the dye test 95.7% of 188 sera gave qualitatively consistent results. In the animal studies HA titers in the TOXO-TEST and *Toxoplasm* isolation was in good correlation in pigs (isolation positive at 1 : 1024 or more), and cats (isolation positive at 1 : 128 or more), but not in dogs. The HA test using TOXO-TEST seems to be useful because of its simplicity in place of the Lewis and Kessel method and the dye test.

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トキソプラズマ症の血清学的診断法としての簡易化赤血球凝集試験

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新たに開発されたピルヴィックフルデヒド固定感作羊赤血球凍結乾燥品を用いたトキソプラズマ症診断用試薬（トキソテスト）による間接赤血球凝集（HA）試験の信頼性を，人血清について Lewis and Kessel 法による HA 試験ならびに色素試験と比較して評価した．一方，豚，猫および犬について，トキソテストによる HA 抗体価と原虫の組織からの分離との関係を調べた．

2種の HA 試験間の定性的一致率は，159 血清中 91.8 %で，その内抗体の存在を示したものの抗体価を比較したところ，90.4 %において両試験間の抗体価の異同が1稀釈段階（4倍稀釈）以内であつた．色素試験と比較した結果は，188 血清中 95.7 %に定性的一致を見た．動物では，豚（1：1024 以上で原虫分離陽性）および猫（1：128 以上で分離陽性）にトキソテストによる HA 抗体価とトキソプラズマ分離の一致を認めたか，犬では一致しなかつた．

トキソプラズマ症の診断において，トキソテストを用いた HA 試験は，その簡易性の故に，Lewis and Kessel 法あるいは色素試験の替りとして使用に耐える信頼性を持ち，有用であると結論される．