AVAILABILITY OF ACCESSORY FACTOR FOR SABIN-FELDMAN DYE TEST AND SEROPREVALENCE OF ANTI-TOXOPLASMA ANTIBODIES IN CHIANG MAI CATS

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ABSTRACT

A micro-modification of dye test for detection of anti-Toxoplasma antibodies was performed. Screening of 39 blood donor sera revealed that 15.4% of them could be used satisfactorily as accessory factor source. Employing these suitable sera in dye test, a seroprevalence of anti-Toxoplasma antibodies in 60 cats was determined to be 18.4 %.

INTRODUCTION

Sabin and Feldman first developed a serological method for detection of anti-Toxoplasma antibodies.(1) This method was based upon the observation that *Toxoplasma gondii* tachyzoites failed to be stained with alkaline methylene blue after incubation with immune serum.(2) The test was later widely used and known as the Sabin-Feldman dye test (DT).(3-8, 11) It was claimed to be sensitive and highly specific for detection of antibody against Toxoplasma in human and animal sera.(9)

One of the drawbacks of the test was the need for human serum to serve as a source for accessory factor in the test system. Without suitable serum, DT can not be satisfactorily performed.(4) The purpose of this study, therefore, was to find out whether or not suitable human serum could be obtained locally. Furthermore, DT will be modified to reduce the amount of reagents used especially as a source of the accessory factor. Finally, the micro-version of DT will be used for detection of

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anti-Toxoplasma antibodies in cat sera.

MATERIALS AND METHODS

Parasite

Toxoplasma gondii was obtained from Research Institute, Phra Mongkut Klao Hospital, Bangkok. Since then, the parasite has been maintained in the laboratory in Swiss mice by syringe passage of infected peritoneal fluid.

Antigen for Dye Test

Mice were injected with 3×10^6 organisms each intraperitoneal (ip). Three days later, they were sacrificed by chloroform inhalation and 3-5 ml of phosphate buffer saline (PBS), ph 7.2, containing 5 units of heparin/ml, was injected, ip.[4,5] Toxoplasma-containing peritoneal exudate was collected and centrifuged at 600 x g for 6 min. The sediment was washed once with PBS before being resuspended to 2×10^7 organisms/ml. Parasite suspension was examined under the microscope and only that containing white blood cell less than 5 % was selected for further use.[10]

Immunizing antigen

Toxoplasma sediment prepared as described above was resuspended in cold distilled water so as to obtain a concentration of 5×10^8 organisms/ml.[10,11] Lysed Toxoplasma was kept in the refrigerator over night, then stored at -40°C. When needed, lysed Toxoplasma suspension was thawed and centrifuged at 10,000 x g.for 30 min. The sediment was washed with PBS for 3 times and resuspended in the same buffer. The optical density of the antigen suspension was measured at 540 nm. and adjusted to be 0.5[12] At this density, the antigen suspension contained 22.5 mg protein/ml as determined by Lowry's method.[13]

Rabbit Immune Serum

Immunizing antigen was freeze-thawed three times. A half ml of antigen suspension was injected intravenously (iv) into the marginal ear vein of a rabbit and the blood was collected 7 days after injection (12) Serum was aliquoted and stored at -70°C.

Collection of Human Serum

Twenty ml of blood was collected from each blood donor at Blood Bank Unit, Maharaj Nakorn Chiang Mai Hospital. After standing at room temperature for 1 hour and in ice bath for 2 hour, the serum was collected, centrifuged, aliquoted and stored at -40°C.

Preliminary Screening of Human Serum

The method was a modification of that described by NIH. (14) Eight tenth ml. of human serum, 0.2 ml of 0.2 M sodium citrate, and 0.25 ml of Dulbeco's phosphate buffer saline (DPBS), pH 7.4 were added to 2×10^7 organisms. A 50 µl portion of this accessory factor-parasite mixture was mixed with 50 µl of 0.85% NaCl in a round-bottom well of a microtiter plate, then 50 µl of alkaline methylene blue was added. The plate was incubated at 37°C for 15 min. A drop of mixture in each well was removed and placed onto a hemocytometer and examined under the microscope with 400 x magnification. Percent of blue-stained organisms was then determined. A yellow filter (Kodak K 2) was sometimes introduced into illumination path to increase the contrast between stained and unstained organisms. (15)

Final Screening of Human Serum for Accessory Factor Source

The test is a micro-modification of classical Sabin - Feldman dye test. (4,515) Heat-inactivated rabbit immune serum (60°C, 30 min.) was four-fold diluted with 0.85% NaCl in U-bottom microtiter plate (cook Dynatech Laborataries, Inc., Alexandra, Virginia): the final diluted

serum in each well was 50 µl. To each well, 50 µl of accessory factor-parasite mixture was added, and the plate was incubated for 1 hour at 37°C. This was followed by addition of 50 µl of alkaline methylene blue to each well. The plate was further incubated for 15 min at 37°C and percent of stained organisms was determined. Negative control was included in each test by substituting immune serum with 0.85% NaCl.

Cat Sera

Blood samples were collected from femoral veins of 60 cats. Ten of them were house cats undergoing treatment at Chiang Mai Veterinary Diagnostic Center. The other 50 cats were living in several Wats located in Sri Phum District, Chiang Mai. Age and sex were not determined in this study.

Human Antiserum

TOX-60, a pool serum containing 1,000 Internation Units of anti-Toxoplasma antibodies per ml, was a kind gift from Statens Serum Institute, Copennagen, Denmark.

Determination of Dye Test Titer of Cat Sera and Human Antiserum

The test was carried out essentially the same as described in the final screening of human serum described above, except that accessory factor source (human serum) used was previously screened and found to be suitable. Cat sera were heat-inactivated for 30 min at 60°C while TOX-60 was inactivated at 56°C before performing dilution in a microtiter plate.

RESULTS

Preliminary Screening of Human Serum

To pass the screening, human serum used for accessory factor

source must allow more than 90% or 95% organisms stained.[4,14] As can be seen in Table 1, 11 out of 39 sera tested passed this preliminary test. These 11 sera were next subjected to final screening below.

Final Screening of Human Serum for Accessory Factor Source

To be a good source for accessory factor, human serum should yield highest titer with known positive serum.[14] When sera selected from the preliminary test were screened with the use of rabbit immune sera, 6 of them gave the immune serum titer between 1 : 5, 120 to 1 : 10,240 (Table 2). Thus human sera suitable for accessory factor could be obtained from 6 out of total 39 blood donors, or about 15.4%.

Determination of Dye Test Titer of Cat sera and TOX-60

Cat sera and TOX-60 were examined for anti-Toxoplasma antibodies using dye test. The accessory factor used was human serum which passed the final screening above. TOX-60 gave a dye test titer of 1:5, 120. The dye test titers of cat sera were summarized in Table 3. As can be seen, ten of them had a titer of 1:16, and the remainders had a titer being less than 1:16, If a titer of 1:16 or more was used to indicate past infection, 13.4% of cats would be positive in this study. This demonstrates the presence of naturally infected definitive host of Toxoplasma in Chiang Mai.

DISCUSSION

Heat-labile accessory factor in human serum is the important component in demonstrating dye test phenomenon. It was later shown to be classical complement components.[2] Human serum, to be suitable for dye test, must not contain anti-Toxoplasma factor and/or has low complement activity.[15] In our study, the purpose of preliminary screening was to exclude sera which contained anti-Toxoplasma factors, and of the

TABLE 1. Preliminary Screening of 39 Human Sera for Accessory Factor Source

Percent Toxoplasma stained	No. of Sera
More than 95	11 (29.1)*
90 - 94	9(23.1)
Less than 90	19(47.8)

*Number of sera giving degree of staining of *Toxoplasma* as specified in the left-handed column. The number in parenthesis represents percent of total sera examined.

TABLE 2. Final Screening of 11 Human Sera for Accessory Factor Source

Titer of Rabbit Immune Serum	No. of Sera
10,240	4*
5,120	2
2,560	3
1,280	2

*Number of sera giving specified immune serum titer.

TABLE 3. Dye Test Titers of Sixty Cat Sera

Titer	No. of Serum Positive
More than 1:64	0(0)*
1:64	4(6.7)
1:16	7(11.7)
Less than 1:16	49(81.6)

* Number in parenthesis represents percent of total cat sera examined.

final screening, was to select those sera exhibiting good complement activity. Six out of 39 blood donor sera (15.4%) passed the screening tests (Table 2). Thus suitable accessory factor source for dye test could be obtained and used when needed.

When the micro-modified dye test was carried out to study prevalence of anti-Toxoplasma antibodies in cats, 18.4% were positive (Table 3). The percent positivity could have been higher if a titer of 1:2 or more was used.[6] However, we feel that a titer of 1:16 or greater appeared to be more reasonable.[16] The data demonstrated the presence of natural Toxoplasma transmission among cats in Chiang Mai. Seropositivity in cats in other countries had been shown to range from 5 % in London, England, to 58.0% in Italy.[7] In central Thailand, the seropositivity of cats had been estimated to be 20.8%.[17]

Feldman and Lamb described the micro-dye test in details,[18] however, the technic was not practical in our laboratory since it requires special microtiter plate and inverted microscope with 400 x mag-

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nification. Our micro-version was similar to their method except that counting the stained organisms was done under the ordinary light microscope. This, although takes more time, avoids the use of the expensive inverted microscope. In studying seroprevalence in animals, the dye test has an advantage over indirect fluorescent antibody test in that it does not require specific anti-immunoglobulin, and over indirect hemagglutination in that heterophile antibodies need not to be absorbed prior to testing; indeed, the dye test is still popular and used by several investigators.[3,5,7,11,16] The micro-version of the dye test allowed us to spend less amount of human serum which is accessory factor source. Indeed, 4 ml of serum was sufficient to perform screening for anti-Toxoplasma antibodies of 96 sera. The modified test was standardized with TOX-60 and gave a titer of 1:5, 120, which is an expected titer. This is comparable to antibody concentration of 31.2 Internation Units/ml in reaction well.

In conclusion, the Sabin-Feldman dye test can be performed in the laboratory and will be a valuble tool in studying Toxoplasma transmission among animals in future.

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การตรวจเซรุ่มจากผู้ขายโลหิต เพื่อใช้เป็น accessory factor ใน sabin- feldman dye test และอัตราการตรวจพบแอนติบอดี้ในแมวในเชียงใหม่

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บทคัดย่อ

ได้ทำการตรวจเซรุ่มจากกลุ่มผู้ขายโลหิต เพื่อหาเซรุ่มที่เหมาะสมที่จะใช้เป็นaccessory factor สำหรับ micro Sabin-Feldman dye test พบว่าร้อยละ 15.4 ของตัวอย่างเซรุ่มทั้งหมด 39 รายได้ใช้ เมื่อนำเซรุ่มที่ได้ไปใช้ใน dye test เพื่อตรวจหาแอนติบอดี้ต่อเชื้อ *Toxoplasma* ในเซรุ่มแมว จำนวน 60 ตัว พบว่าร้อยละ 18.4 ให้ผลบวก แสดงว่ามีการติดเชื้อ *Toxoplasma* ในแมวในจังหวัดเชียงใหม่ จากผลการทดลองแสดงให้เห็นว่า วิธี micro dye test ทำได้ในห้องปฏิบัติการและจะมีประโยชน์ในการศึกษาอุบัติการ การติดเชื้อ *Toxoplasma* ในสัตว์ต่อไป

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