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COMPARISON BETWEEN ELISA AND DYE TEST FOR DETECTION OF NATURALLY ACQUIRED TOXOPLASMA GONDII ANTIBODIES IN THE GOAT

By

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BERDAL, BJØRN P., ØRJAN OLSVIK, TORBJØRN ALMLID, HANS JØRGEN LARSEN and ANNE-MARIE LORENTZEN-STYR: *Comparison between ELISA and dye test for detection of naturally acquired Toxoplasma gondii antibodies in the goat.* Acta vet. scand. 1983, 24, 65—73. — Toxoplasma gondii IgG antibodies were measured in 212 goat sera, comparing the Sabin-Feldman dye-test and a three-layer sandwich enzyme-linked immunosorbent assay (ELISA). With 98 % concordance obtained between these 2 tests, the results are at the same paragon as for human sera. Accordingly, the ELISA sandwich procedure appears to be suitable for large-scale analysis of goat sera. The discordant 2 % were ELISA positive and dye-test negative. One possible explanation of the divergent titres is given using an immunized goat model.

Toxoplasma gondii; goat; antibodies; dye-test; ELISA.

Toxoplasma gondii, a parasite giving rise to abortions in e.g. sheep, goats and humans can be diagnosed serologically by the Sabin-Feldman dye test (DT) (*Sabin & Feldman* 1948). Being considered the reference test for toxoplasmosis (*Denmark & Chessum* 1978), this test is, however, very time-consuming and expensive.

For serological diagnosis of some parasitic infections, enzyme-linked immunosorbent assay (ELISA) procedures appear well suited (*Ruitenberg & von Knapen* 1977, *Ambroise-Thomas et al.* 1978). This has convincingly been demonstrated for human toxo-

plasmosis, where several authors have recommended ELISA pro-
cedures for demonstration of IgG — (*Voller et al.* 1976, *Walls et
al.* 1977, *Lin et al.* 1980) as well as IgM — (*Naot & Remington*
1980) T. gondii antibodies. When the number of sera to analyse
is large the ELISA procedure inherently have an advantage, being
relatively inexpensive and easy to perform.

In connection with a number of T. gondii — caused abortions
in a goat herd, the large number of serum samples emphasized
the limit of the available DT capacity. Hence, a T. gondii ELISA
sandwich procedure was adapted for use with goat sera, and
compared with the standard DT.

MATERIAL AND METHODS

Serum samples

Blood samples were drawn from 212 goats of different ages
in a Norwegian goat herd. The sera were stored at —20°C before
analysis. Several cases of abortions due to toxoplasmosis were
observed during the last year.

One goat was immunized with a saline suspension of for-
malinized parasites of the T. gondii RH strain, once every 2
weeks. One ml of suspension containing 5×10^6 parasites was in-
jected intravenously 5 times, over a total of 10 weeks. The goat
was bled 2 weeks after the last injection.

Dye test

The DT was performed using a micro-modification (*Stray-
Pedersen & Lorentzen-Styr* 1979) of the classical Sabin-Feldman
method. The titres recorded corresponded to the terminal serum
dilution in which 50 % of 100 counted parasites (LD 50) were
unstained or modified as revealed by phase contrast microscopy.

ELISA test

The ELISA test followed closely the procedure described by
Lorentzen-Styr (1980).

C o a t i n g   o f   E L I S A   m i c r o t i t r e   p l a t e s. Flat bot-
tom polystyrene microtiter plates (cat. no. 2-69787, NUNC,
Copenhagen, Denmark) were coated with T. gondii antigen (RH-
strain) according to the following procedure. Live parasites were
harvested from the peritoneal fluid of mice infected intraperi-
toneally 3 days prior with 0.2×10^6 parasites in saline. The para-

site-containing peritoneal fluid was centrifuged, and the para-
sites washed 3 times in saline, before adjustment in distilled
water to 5.8×10^6 parasites per ml. This solution was freezed/
thawed 3 times before centrifugation. The supernatants were
stored at —20°C in 2 ml aliquots. The antigen was diluted 1:50
in a 0.06 mol/l carbonate buffer pH 9.8. To each well of the
microtitre plates was added 100 µl of this antigen solution. The
plates were stored at 4°C for at least 3 days before use. They
should preferably be used before 2 weeks storage under these
conditions.

P r e p a r a t i o n   o f   E L I S A   c o n j u g a t e. Rabbit anti
goat IgG was purified from whole serum (RAG/IgG H+L, Nordic
Immunology, Tilburg, Holland) on protein A-Sepharose (Phar-
macia Fine Chemicals, Uppsala, Sweden), in principle following
the manufacturer's recommendations. One ml serum was passed
through 0.8 ml gel at a speed of 0.2 ml/min. After the serum
passage, the gel was washed with 50 volumes of saline, before
elution with a 0.1 mol/l glycerine-HCl buffer pH 2.8. The eluate
was immediately neutralized in 0.1 mol/l borate-NaOH pH 7.65,
1 volume of eluate to 2 volumes of the borate buffer. The IgG-
containing fractions were re-concentrated on a Diaflo PM 10 filter
(Amicon Corporation, Lexington, Massachusetts, USA) in a
stirred cell (Amicon), giving a final protein concentration of 4
mg/ml as measured by optical density (OD) at 280 nm. The
yield from 1 ml of serum was 8.5 mg/ml of purified IgG. All
column and concentration procedures were performed at room
temperature.

Two mg IgG was conjugated to 5 mg alcaline phosphatase
(AP) (cat. no. P-5521, Sigma, St. Louis, Missouri, USA) follow-
ing a one-step glutaraldehyde procedure (*Voller et al.* 1980). The
IgG and the AP was dialyzed for 3 days in phosphatase-buffered
saline (PBS) pH 7.2, at 4°C. Thereafter, glutaraldehyde (cat. no.
G-5582, Sigma) was added to a concentration of 0.2 %. After
stirring, this mixture was allowed to react in the dark for 1½ h
at room temperature, before further dialyzing for 3 days in PBS
at 4°C. A last, dialysis of the IgG-AP conjugate was performed
in 0.01 mol/l Tris-HCl pH 8.0 for 3 days. Diluted 1:4 in the Tris-
HCl with 10 mg/ml bovine serum albumin and 0.1 % sodium
azide added, the conjugate was stored at 4°C for up to 6 months
until use.

Performance of the three-layer sandwich
ELISA procedure. The excess antigen in the microtitre
plates was removed by 4 washings using PBS with 0.05 % Tween
(Technicon, Tarrytown, New York, USA) and 0.02 % sodium
azide added (PBS-Tween).

Ten DT negative goat sera and the immunized serum were
diluted 1:10, 1:50, 1:100, 1:150 and 1:200 in PBS-Tween, in
order to determine the level of non-specific activity in the ELISA
procedure. All the 212 test sera were diluted 1:100 for the
ordinary ELISA run.

The serum dilutions were added to the microtitre plates, 0.1
ml to each of 2 wells (the test was run in duplicate). After in-
cubation overnight at 4°C in a humid chamber, the plates were
washed 4 times with PBS-Tween. 0.1 ml of the AP-anti goat IgG
conjugate, diluted 1:200 in PBS-Tween containing 1:100 or nor-
mal rabbit serum, was then added to each well. After 2 h incuba-
tion at 37°C in the humid chamber, the plates were washed 8
times with PBS-Tween. Finally, 0.1 ml of a 0.5 mg/ml solution
of p-nitrophenyl phosphate (Sigma) in diethanolamine buffer pH
9.8 (*Voller et al.* 1980) was added to each well. The yellow colour
reaction following the AP hydrolysis of the p-nitrophenyl phos-
phate substrate at room temperature, was continually observed.
After 20 min, the yellow colour development of the control was
satisfactory, while the negative background remained clear. The
colour reaction was then arrested with 0.025 ml of a 4 mol/l
NaOH solution added to each well, whereafter the OD values of
the colour reaction were recorded photometrically at 410 nm in
a Minireader MR 590 (Dynatech, London, England). Zero adjust-
ment was done against PBS instead of test serum in the ELISA.

RESULTS

The ELISA response of the dilutions of the 10 DT negative
sera (in this work defined as DT titres of 16 or less) and the
immunized goat serum, used to determine the suitable dilution
of the sera to be tested in the ELISA, are shown in Fig. 1. ELISA
reactions at lower dilutions were considered to be non-specific
as they disappeared at 1:50 or higher, while the immunized goat
control serum maintained a high ELISA response with increasing
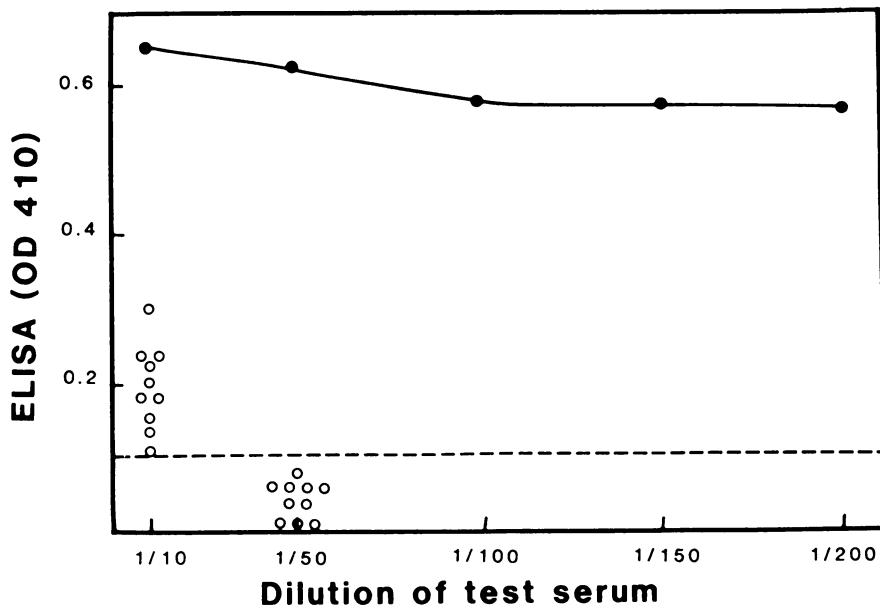dilutions.

F i g u r e 1. The ELISA response of different dilutions of test sera. The positive control (filled spots) are not much affected by dilutions up to 1:200, while all 10 DT negative sera (open spots) show negative ELISA response (OD < 0.10) upon 1:50 dilution.

Out of the 212 sera tested, 36 were recorded as positive in both assays, 3 were negative in DT and positive in ELISA, whereas 172 were negative in both DT and ELISA (Fig. 2). None were positive in DT and negative in ELISA. One should notice that the 172 DT negative sera (with a titre of 16 or less) separated distinctly from the DT positive sera, which demonstrated DT titres of 512 or more.

` With an ELISA positivity cut off value of OD > 0.10, the difference between positivity and negativity in the ELISA results was less accentuated, although 89 of the negative samples showed an ELISA OD of 0.00. Four DT negative sera had an ELISA OD of more than 0.07. There was no direct linear correlation between the titre in DT and the OD in ELISA for the positive serum samples.

Compared with the high ELISA OD response of 0.58, the DT titre of 32 in the experimentally immunized goat was low.
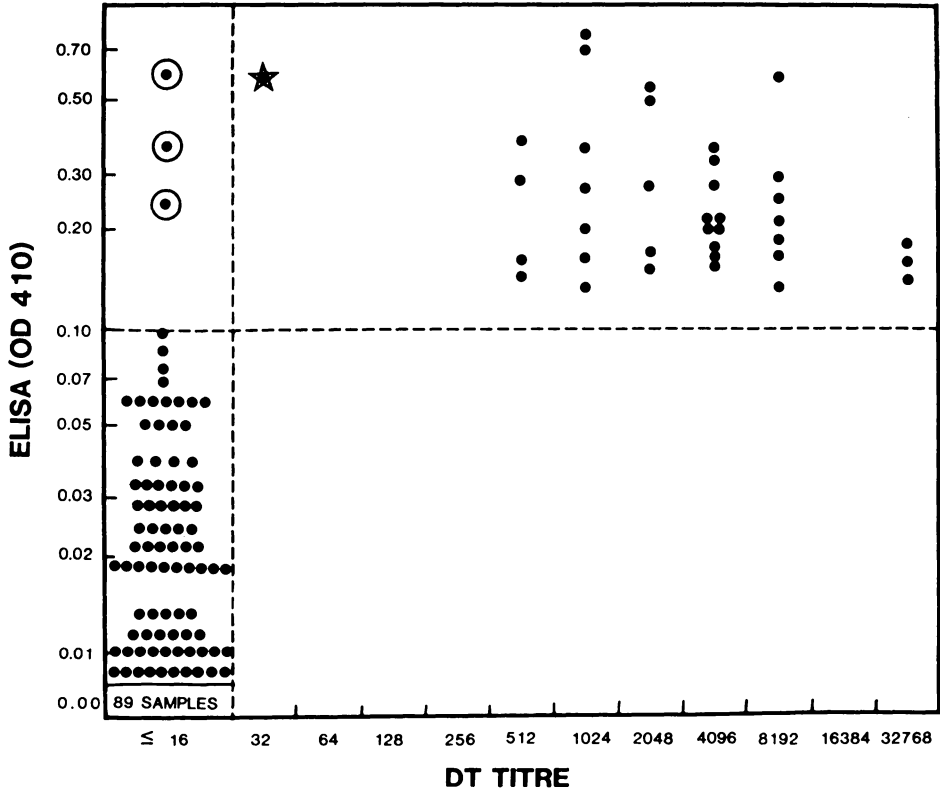
Figure 2. Comparison of the ELISA response (OD) and the DT (titre) for Toxoplasma gondii antibodies of sera from 212 goats (filled spots) and 1 experimental immunized goat (spot with star). Positive sera were considered to give an ELISA response higher than OD 0.10, and a DT titre higher than 16. Three sera were positive in ELISA and negative in DT (spots with circles), these were considered to be false positive.

DISCUSSION

Discrepancies between the DT titre and the ELISA OD values were first noted by *Voller et al.* (1976). Their ELISA sandwich procedure did not distinguish between sera with medium DT titres 256—612 and those with high titres, over 1024. *Pettersen* (1981) used a rabbit model with live parasites to study immune response of toxoplasma infection. In the early stages of disease, there was a rapid antibody rise measured by DT, including both IgM and IgG antibodies. The ELISA OD rose slowly, probably because a sandwich ELISA detects better "late" antibodies, with

a high proportion of IgG and low proportion of IgM. This discrepancy between DT and ELISA was important the first 5 weeks of infection, then levelling out (*Pettersen* 1981).

Discrepancies in the opposite direction, with low DT titres and high ELISA OD, may be related to the nature of the antigen used in DT and ELISA. Surface antigens in the DT are to a certain extent replaced by cytoplasmic antigens in the ELISA (*Camargo et al.* 1978). The whole parasite antigen preparation which had been frozen and thawed repeatedly, corresponded roughly to the mixture of the cytoplasmic soluble- and membrane pellet antigen described by *Naot & Remington* (1981). According to their work with monoclonal antibodies, the membrane antigen gave both high ELISA and high DT response while the cytoplasmic antigen gave moderately high ELISA and low DT response. When the experimentally immunized goat which received formalinized parasites developed a high OD response but a low DT titre (of 32), the mode of ELISA antigen preparation could not be incriminated as cause of this discrepancy, since the majority of the positive sera showed concordance in the 2 tests. Conceivably, the formalinization procedure of the T. gondii parasites used for immunization might have denatured these to an extent where the surface antigenicity suffered. In view of this observation, the 3 sera with high ELISA OD and low DT titre, are probably not reflecting a natural Toxoplasma infection. On the contrary, the possibility of cross-reactivity with another agent should be considered.

The positivity/negativity correlation between the DT and the ELISA of 98 % is at the same level as the results obtained for human sera (*Walls et al.* 1977). Accordingly, ELISA procedures applied to the serological diagnosis of goat toxoplasmosis should be potentially useful.

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SAMMENDRAG

Sammenligning mellom ELISA og vitalfarging for påvisning av anti-stoffer mot Toxoplasma gondii hos naturlig infiserte geit.

IgG antistoffer mot Toxoplasma gondii ble målt i 212 geit ved hjelp av Sabin-Feldman's dye test, og en tre-lags sandwich ELISA ("enzyme-linked immunosorbent assay"). Med 98 % sammenfallende resultater i disse 2 testene, var overensstemmelsen på lik linje med hva man finner for humane sera. Derav følger at ELISA prosedyren også bør egne seg for masseundersøkelser av geitesera.

De 2 % avvik var ELISA positive og dye-test negative. En mulig forklaring på denne divergensen er beskrevet ved hjelp av en immuni-sert geit-modell.