

## Identification of a *Theileria mutans*-specific antigen for use in an antibody and antigen detection ELISA

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**Summary** Purified piroplasm of *Theileria mutans* were used to immunize BALB/c mice to generate monoclonal antibodies (MoAbs). The MoAbs recognized an antigen of a relative molecular mass of 32 kDa in Western blots. This antigen was also recognized by sera from cattle which had recovered naturally from experimental tick-transmission or infections induced by the blood stages of *T. mutans*. The MoAbs did not react, in indirect immunofluorescence or enzyme-linked immunosorbent assays (ELISA), with the common haemoparasites of cattle, namely, *T. parva*, *T. annulata*, *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma congolense*, *T. vivax* or *T. brucei*. An antigen capture ELISA was established with two of the MoAbs which recognized different epitopes on the 32 kDa molecule. Using this test it was possible to detect circulating antigens or immune complexes in sera collected from cattle during the acute or chronic phases of infection. When the purified 32 kDa protein was used as antigen in a micro-ELISA to detect circulating antibodies in both experimental and field cattle sera, it was found that the titres of antibodies ranged between 1:20 and 1:10240. Results of this study indicate that the antigen and immune complex capture assays and the antibody detection ELISA can be complementary in the immunodiagnosis of acute and chronic *T. mutans* infections. Moreover, the tests are useful in the differential diagnosis of the disease and for epidemiological studies.

**Keywords:** *Theileria mutans*, identification, antigen, monoclonal antibody, immune complexes, ELISA

### Introduction

*Theileria mutans*, a protozoan parasite of cattle, occurs in most African countries south of the Sahara. The parasite, which is transmitted by several species of *Amblyomma* ticks, multiplies in leucocytes and erythrocytes of the mammalian host. The African buffalo is an important reservoir of the parasite (Young *et al.* 1978a, Uilenberg 1981). *T. mutans* is

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an opportunistic and benign parasite which, in conjunction with other tick-borne haemoprotozoan parasites, causes anaemia and decreased production (Paling, Grootenhuis & Young 1981, Saidu 1981). Also, outbreaks of *T. mutans* causing severe disease with fatalities have been reported from East Africa (Irvin *et al.* 1972, Uilenberg, Robson & Pedersen 1974, Uilenberg *et al.* 1976, Uilenberg *et al.* 1977, Purnell 1977, Young *et al.* 1978b, Perie, Uilenberg & Schreuder 1979).

Diagnosis of *T. mutans* is based on either the identification of the piroplasm stages in Giemsa-stained blood smears or the detection of antibodies against *T. mutans* in the indirect immunofluorescent antibody test (IFAT) (Lohr & Ross 1969, BurrIDGE 1971). Microscopic examination of stained blood and lymph node smears for piroplasms and schizonts, respectively, has its limitations in that the schizont stage is transient, and the piroplasms are difficult to differentiate from those of other *Theileria* spp.

Other serological tests which have been developed include a complement fixation test (Schindler & Mehlitz 1969) and a capillary tube agglutination test (Ross & Lohr 1972). These tests, as well as the IFAT, are limited in sensitivity and are difficult to perform and interpret.

Outbreaks of bovine theileriosis in the field often involve mixed *Theileria* infections. In many instances, these mixed infections are due to either *T. parva* and *T. mutans* or due to *T. annulata* and *T. mutans*. Under these circumstances, an assay capable of discriminating between these *Theileria* parasites would permit accurate diagnosis of the disease outbreak. Additionally, such an assay would be useful in epidemiological studies, allowing precise definition of the prevalence and incidence of the parasite in an area. In this paper we report on the identification of a *T. mutans*-specific antigen and the development of assays to detect antigens, immune complexes and antibodies in infected cattle.

## Materials and methods

### PARASITES

The *T. mutans* stocks used in this study were isolated from cattle at Intona, in the Transmara District, Kenya [stabilate 3008] (J.J.Mutugi and A.S.Young, unpublished observations) and from cattle at Unguja island of Zanzibar [stabilate 3263] (E.Flach, S.P.Morzaria and T.T.Dolan, unpublished observations).

A stock of *Babesia bigemina* was supplied through the courtesy of Dr S.Kelly, National Veterinary Laboratories, Kabete, Kenya. *T. parva* schizonts and piroplasms originated from the Muguga stock [stabilate 3087] while *Anaplasma marginale* was obtained from the Veterinary Research Laboratories, Kabete, Kenya. *Trypanosoma congolense* (ILNat 1.2), *T. vivax* (ILDat 1.3), and *T. brucei* (MITat 1.2), are held as frozen stabilates at ILRAD.

### CATTLE

Friesian (*Bos taurus*) steers between 4 and 12 months of age and Boran (*B. indicus*) steers of 2 years of age were screened for antibodies to *Theileria parva*, *T. mutans*, *B. bigemina*, *A. marginale*, *T. brucei*, *T. congolense* and *T. vivax* by the IFAT (BurrIDGE 1971,

Goddeeris *et al.* 1982, Katende *et al.* 1987) and enzyme-linked immunosorbent assay (ELISA) (Voller 1977). Animals which were negative for *T. mutans* were kept in a tick-free environment at ILRAD.

#### INFECTION OF ANIMALS AND COLLECTION OF SERA

*A. variegatum* adults infected with *T. mutans* (Zanzibar) were fed on two Friesian cattle, E284 and E281. Approximately 1000 ticks were applied on E284, while 800 ticks were applied on E281. The animals were screened daily for *T. mutans* schizonts and piroplasms by examination of Giemsa-stained blood and lymph node smears. Sera were collected every 3 days and stored at  $-20^{\circ}\text{C}$  until tested.

Field sera were collected from cattle in *T. mutans*-endemic areas, namely Transmara, Kenya and Unguja island, Zanzibar, Tanzania.

#### PREPARATION OF *T. MUTANS* SOLUBLE CRUDE ANTIGEN

*T. mutans* (Transmara)-infected blood (4 l), with a piroplasm parasitaemia of 28%, was collected in Alsever's solution. The erythrocytes were depleted of leucocytes by washing  $\times 3$  in phosphate-buffered saline glucose (PSG) pH 8.0 (Lanham 1968). The erythrocytes were resuspended in 2.5 l of PSG and lysed by sonication for 15 min (relative output 50) on ice using a 3/8 probe (Virsonic 16-850, Vitris company, Gardiner, NY, USA). The lysate was centrifuged at 400 g for 30 min at  $4^{\circ}\text{C}$  to pellet intact erythrocytes and leucocytes. The supernatant, rich in *T. mutans* piroplasms, was collected and centrifuged at 7000 g for 20 min. The pellet which contained mostly *T. mutans* piroplasms was washed  $\times 3$  with PSG and the deposit was finally resuspended in 30 ml PSG containing protease inhibitors (5 mM ethylenediamine tetra-acetic acid, 5 mM iodoacetamide, 1 mM phenylmethylsulphonyl fluoride, 0.1 mM N-alpha-p-tosyl-L-lysine chloromethyl ketone and 1 mM leupeptin). The piroplasm suspension was disrupted by sonication (relative output 80) as described above, followed by centrifugation at 45 000 g for 1 h and the pellet discarded. The protein concentration of the supernatant was determined as described (Lowry *et al.* 1951). The supernatant was tested in a micro-ELISA (Voller 1977) at concentrations of 5, 2, 1 and 0.5  $\mu\text{g}$  of antigen per microtitre well against sera collected from animals recovered from *T. mutans* infection to determine the optimal antigen concentration for use in ELISA.

#### PREPARATION OF HYPERIMMUNE BOVINE SERUM TO *T. MUTANS*

A steer (D721) was infected with *T. mutans*-Transmara infected blood, and 60 days later a booster dose of 1 mg of *T. mutans* lysate in incomplete Freund's adjuvant was given intramuscularly. Serum (100 ml) was collected 2 weeks after the booster dose and the IgG immunoglobulins were purified as described (McGuire, Musoke & Kurtti 1979).

#### GENERATION OF MONOCLONAL ANTIBODIES (MOABS)

Monoclonal antibodies were raised by immunizing BALB/c mice with the *T. mutans* lysate in Freund's adjuvant and fusing spleen cells, harvested from the immunized mice, with  $\times 63$ -Ag8 myeloma cells according to the method described by Pearson *et al.* (1980).

Culture fluids of hybrids were screened for specific antibodies using the IFAT on acetone-fixed *T. mutans*-infected erythrocytes (Burridge 1971) and by indirect ELISA (Voller 1977) using the antigens of the *T. mutans* lysate which had been trapped in the microtitre plates by IgG antibodies isolated from the hyperimmune serum from D721.

The cloning and ascitic fluid production were performed as described (Pearson *et al.* 1980). The immunoglobulins were purified from the ascitic fluid (Musoke & Williams 1975) and tested by ELISA against crude *T. mutans* antigen and lysates of bovine platelets, erythrocytes and leucocytes to determine their specificity.

#### PREPARATION OF CONJUGATES

Antisera to mouse (Musoke & Williams 1975) and bovine (McGuire, Musoke & Kurtti 1979) immunoglobulins were prepared in goats and were conjugated to fluorescein isothiocyanate (FITC, isomer 1) (Clark & Shepard 1963) or horseradish peroxidase [HRP, Serva-Heidelberg, West Germany] (Wilson & Nakane 1978). One of the MoAbs raised against *T. mutans* (TM41.8) was conjugated to HRP similarly.

A chequerboard titration was used to determine the optimal dilution of the conjugates that could be used to discriminate between a negative and positive reaction.

#### SELECTION AND DETERMINATION OF THE OPTIMAL CONCENTRATION OF THE MOAB FOR USE IN THE ANTIGEN-CAPTURE ASSAY

A flat-bottomed micro-ELISA plate (Dynatech, Plochingen, West Germany) was coated with MoAbs in 0.01 M Dulbecco's phosphate-buffered saline (DPBS) pH 7.4 for 2 h at 37°C, at protein concentrations ranging from 1000 ng to 125 ng per well. Uncoated reactive sites were blocked with 0.2% sheep serum albumin in DPBS. The plates were washed  $\times 3$  with the washing buffer, DPBS containing 0.01% Tween 20. Aliquots of 100  $\mu$ l per well of crude *T. mutans* antigen, adjusted to 1 mg/ml of protein, were added to the coated wells. The plates were incubated for 30 min at 37°C then washed  $\times 3$  with DPBS. The TM41.8-HRP conjugate was added at a dilution of 1:2000. The plate was incubated for 30 min and washed as before. The substrate, 1% hydrogen peroxide and the chromogen, 40 mM 2,2-azinobis 3-ethyl-benzylthiazoline 6-sulphonic acid, ammonium salt (ABTS), were added and the colour allowed to develop in the dark for 1 h. The optical density was determined in a Titertec reader (Flow Laboratories) at 414 nm.

#### PURIFICATION OF THE MOLECULE RECOGNIZED BY THE MOAB

An affinity column containing MoAb TM41.1 was prepared by coupling the MoAb to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described by the manufacturer. The slurry was suspended in DPBS and distributed into minicolumns in aliquots of 2 ml (Pierce, Rockford, Illinois, USA). A purified *T. mutans* piroplasm pellet was lysed in 5 ml of DPBS containing 0.1% NP40. The lysate (10 mg) was loaded onto the minicolumn and mixed for 2 h at room temperature. The eluted fraction was checked for purity by SDS-PAGE (Laemmli 1970) and silver staining.

#### NATURE OF EPITOPES RECOGNIZED BY THE MOABS

The method described by Woodward, Young & Bloodgood (1985) was used to determine whether the MoAbs recognized carbohydrate or non-carbohydrate epitopes.

#### WESTERN BLOTTING

Western blotting was performed following the method described by Towbin, Staehelin & Gordon (1979).

#### ESTABLISHMENT OF ASSAYS FOR DETECTION OF ANTIGEN, ANTIBODIES AND IMMUNE COMPLEXES

##### (a) Antigen detection

Micro-ELISA plates were coated with MoAb TM41.1 at 500 ng per well in DPBS at 4°C overnight. The plates were kept at 4°C until used. The coated plates were washed × 3 with the washing buffer and the uncoated sites were blocked with 0.2% sheep serum albumin. Aliquots of 100 µl of serum, diluted 1:2 in diluting buffer (DPBS, 0.1% Tween-20, 0.2% serum albumin), were added to the microtitre wells, the plates sealed and incubated for 30 min at 37°C. A lysate of *T. mutans* piroplasms (1 mg/ml) and normal bovine serum diluted to 1:2 served as positive and negative controls, respectively. The rest of the assay was performed as described above using TM41.8-HRP as conjugate.

##### (b) Immune complexes detection

The micro-ELISA plate was coated with MoAb TM41.1 and uncoated sites were blocked as described above. Aliquots of 100 µl of test sera diluted 1:20 in diluting buffer were added to the microtitre plates; the plates were sealed and incubated at 37°C for 30 min. A lysate of *T. mutans* piroplasms (1 mg/ml in diluting buffer) sensitized with *T. mutans*-specific bovine IgG (from D721), together with normal bovine serum at a dilution of 1:20 in diluting buffer were included as controls. The rest of the assay was carried out as described above except that sheep anti-bovine Ig-HRP was used as a conjugate (dilution 1:5000).

##### (c) Antibody detection

Micro-ELISA plates were coated with 20 ng/well of the purified *T. mutans* antigen (32 kDa) in a 50 µl volume overnight at 4°C. The test sera and positive and negative sera diluted 1:20 in diluting buffer were tested as described above.

## Results

#### THEILERIA MUTANS-SPECIFIC MOABS

Culture fluids from the hybridomas were tested by ELISA on *T. mutans* antigens captured on a micro-ELISA plate by bovine IgG purified from sera of cattle that had recovered

from *T. mutans* infection. Of the 96 wells, 31 were found to secrete antibodies to proteins recognized by such sera. These results were confirmed by IFAT on acetone-fixed *T. mutans* piroplasms. Eight clones (Table 1), all secreting IgG<sub>1</sub> immunoglobulins, were expanded and ascitic fluid was produced in BALB/c mice. The 8 MoAbs, when tested by IFAT and ELISA against acetone-fixed parasites and lysates of *T. parva* (schizonts and piroplasms), *T. lawrencei* (schizonts), *T. annulata* (schizonts), *T. taurotragi* (schizonts), *B. bigemina*, *B. bovis*, *A. marginale*, *T. brucei*, *T. congolense* and *T. vivax*, were found not to be reactive. Similar results were found with lysates of erythrocytes leucocytes and platelets from uninfected cattle. These MoAbs were not tested against the schizont stage of *T. mutans* and the piroplasm stage of *T. lawrencei* and *T. taurotragi* because these stages are present in very low numbers *in vivo* and also cannot be cultured *in vitro*. The *T. annulata* piroplasms were not available.

#### IDENTIFICATION OF THE ANTIGEN RECOGNIZED BY THE MOAB

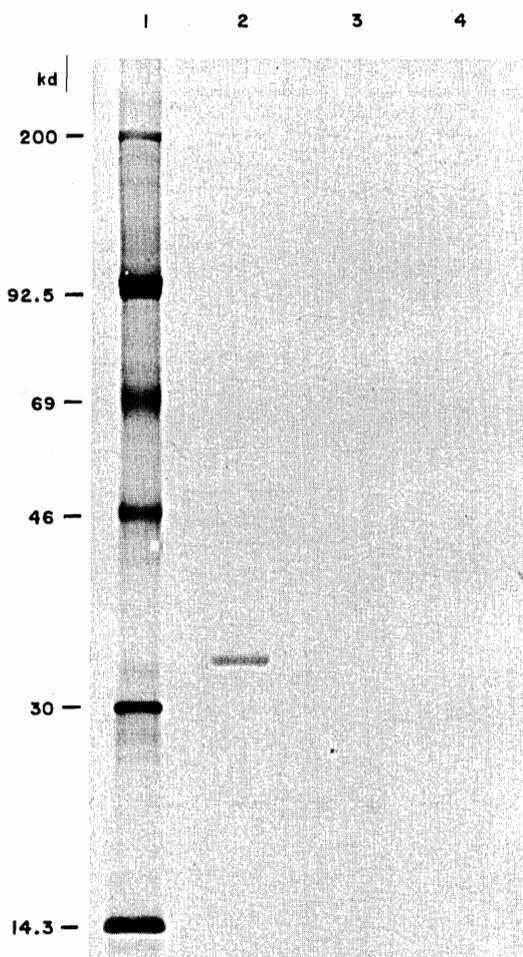
Purified immunoglobulins from the 8 MoAbs identified were tested by Western blotting using a *T. mutans* lysate. All the 8 MoAbs identified a protein with a relative molecular mass ( $M_r$ ) of 32 kDa (Figure 1).

The antigen was purified on an immunoabsorbent column. When the eluate was checked for purity by SDS-PAGE and silver staining, only the 32 kDa protein was detected (Figure 2). The isolated protein was reactive in ELISA and Western blots with the MoAbs and with recovered bovine serum. Treatment of the 32 kDa antigen with sodium periodate abolished its reactivity with MoAbs TM41.2, TM41.6, TM41.8 and TM41.14 but not with MoAbs TM41.1 and TM41.3 (Table 1), indicating that the former group of MoAbs identified carbohydrate moieties while the latter group recognized protein determinants on the 32 kDa molecule. Two MoAbs, namely TM41.1 (protein reactive) and TM41.8 (carbohydrate reactive) were selected for further study. In order to

**Table 1.** The isotypes, the chemical nature of determinants recognized by, and reactivities of the 8 monoclonal antibodies specific for *Theileria mutans*

MoAb	Isotype	Nature of determinant	Titre against crude Ag
TM41.1	IgG <sub>1</sub>	Protein	78, 125
TM41.2	IgG <sub>1</sub>	Carbohydrate	3, 125
TM41.3	IgG <sub>1</sub>	Protein	78, 125
TM41.6	IgG <sub>1</sub>	Carbohydrate	15, 645
TM41.8	IgG <sub>1</sub>	Carbohydrate	15, 645
TM41.10	IgG <sub>1</sub>	ND	15, 645
TM41.14	IgG <sub>1</sub>	Carbohydrate	15, 645
TM41.16	IgG <sub>1</sub>	ND	78, 125

ND: Not determined.

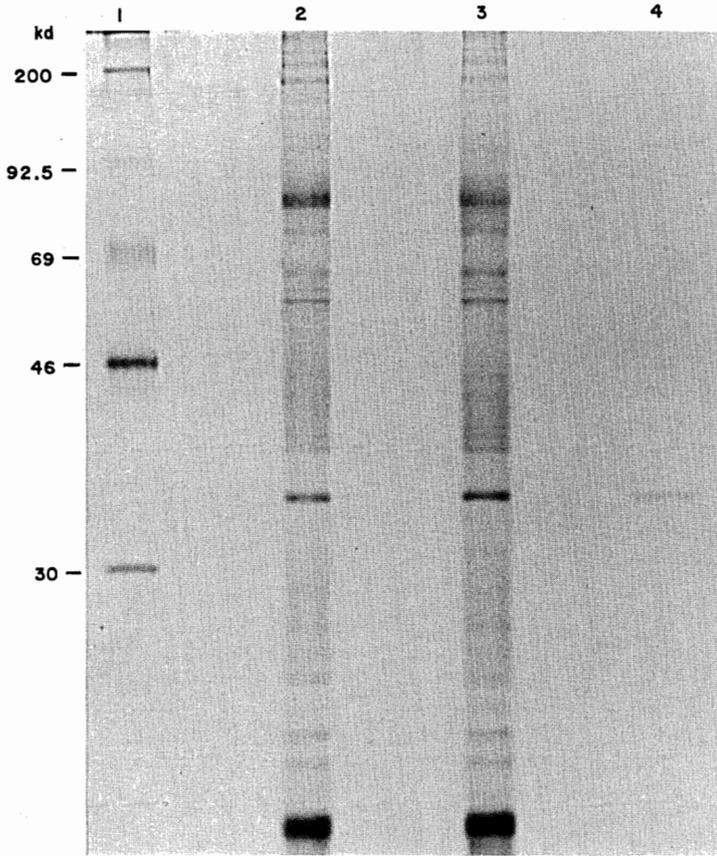


**Figure 1.** A Western blot gel showing Lane 1: Molecular weight markers; Lane 2: *T. mutans* lysate (Intona); Lane 3: Red blood cell lysate; Lane 4: White blood cell lysate. The blot was developed with MoAb TM41.1 as first antibody. The gel (gradient 7.5–17.5% acrylamide) was run under reducing conditions.

confirm that MoAbs TM41.3 and TM41.8 identified different epitopes on the 32 kDa molecule, a competition assay was performed. The results showed that MoAb TM41.1 inhibited itself completely but failed to inhibit the binding of MoAb TM41.8 to the 32 kDa antigen, confirming that the 2 MoAbs recognized different determinants (data not shown).

#### ESTABLISHMENT OF THE ASSAYS FOR DETECTION OF ANTIGENS, ANTIBODIES AND IMMUNE COMPLEXES

When the MoAbs were used in ELISA to trap antigen from a crude *T. mutans* lysate, and MoAb TM41.8-HRP was used as conjugate, it was evident that all the MoAbs were



**Figure 2.** SDS-PAGE of the affinity purified *T. mutans* antigen. Lane 1: Molecular weight markers; Lane 2: *T. mutans* lysate (Itona); Lane 3: *T. mutans* lysate (Zanzibar); Lane 4: Purified *T. mutans* antigen. The gel (gradient 7.5–17.5% acrylamide) was run under reducing conditions and stained with silver nitrate.

capable of capturing the 32 kDa antigen (Table 2). However, wells coated with MoAbs TM41.1 and TM41.3 gave higher optical densities compared to the rest of the MoAbs (Table 2). Furthermore, a combination of MoAb TM41.1 (as the trapping MoAb) and MoAb TM41.8 (as a conjugate) gave a higher reading than any other combinations (Table 2) and therefore was selected for use in developing the antigen capture assay. A concentration of 500 ng of MoAb TM41.1 was selected as the optimal concentration for coating microtitre wells for detection of both antigen and immune complexes.

In order to determine the lowest concentration of antigen that could be detected in the antigen capture ELISA, doubling dilutions of the purified 32 kDa protein (20 µg/ml) in DBPS containing 0.05% Tween-20 were made. Aliquots of 100 µl of each dilution were added to each well of a microtitre plate which had been coated with 500 µg per well of MoAb TM41.4. The plate was developed with MoAb TM41.8-HRP conjugate. It was found that the minimum amount of antigen that could be detected was 30 ng/ml.



ANALYSIS OF SERA FROM EXPERIMENTALLY INFECTED CATTLE

E281 and E284 which were experimentally infected with *T. mutans* (Zanzibar) showed parasitaemia on day 15 after infection and reached a peak between days 20 and 30 (Figures 3 & 4). Thereafter both animals maintained a low parasitaemia. E281 was splenectomized on day 48 to check whether the levels of antigen and/or immune complexes would rise. Splenectomy was followed by an immediate rise in the parasitaemia which reached a peak of 28% 3 weeks later (Figure 4).

Using the antigen capture ELISA, the *T. mutans* antigen was detected in sera of both animals by day 10, before parasites were detected (Figures 3 & 4). The antigenaemia persisted in E281 until day 48. In E284 antigens remained detectable up to day 57 (Figure 3).

The immune complexes were detectable in both animals from day 21 (Figures 4 & 5) and persisted until day 72 in E281. Following splenectomy there was an increase in immune complexes but not in antigen or antibody (Figure 4).

Antibodies to the 32 kDa antigen in the sera of E281 and E284 were detectable between days 10 and 12, with titres ranging from 1:80 to 1:2560 (Figures 4 & 5).

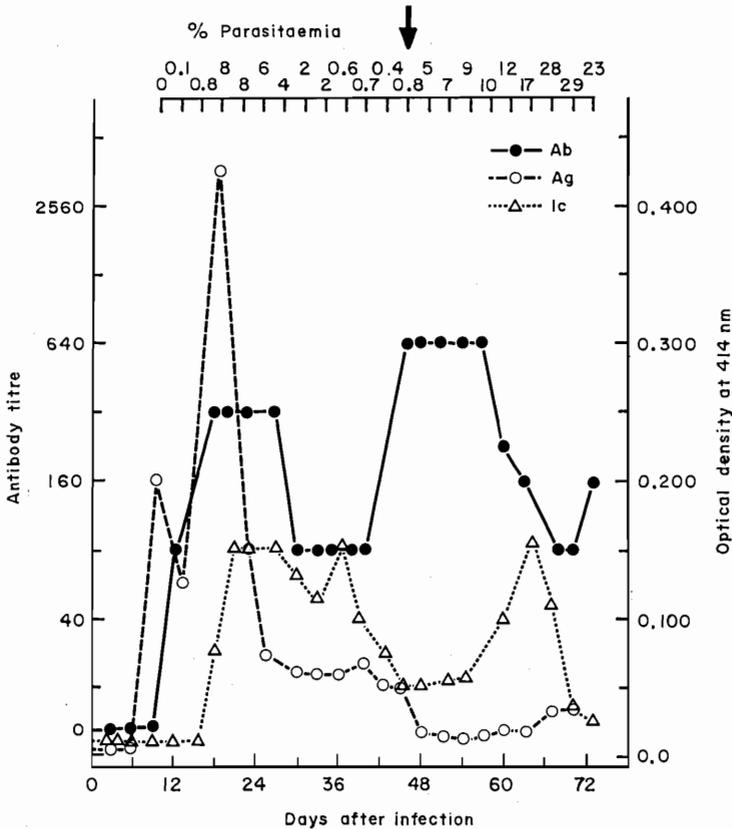


Figure 4. Titres of antigen, immune complexes, and antibodies in animal E281 which was experimentally infected with *T. mutans* (Zanzibar). Arrow indicates day of splenectomy.

Antibodies were detectable at titres above 1:160 until the termination of the experiment (day 84).

ANALYSIS OF FIELD SERA

The sera from the 20 cattle from Zanzibar were screened for antibodies to *T. mutans* prior to immunization against *T. parva* and found to be negative. After immunization, the animals grazed in an area where both *T. parva* and *T. mutans* infections were endemic. The animals developed piroplasm parasitaemias which ranged between 0 and 75%. When the three tests were used to analyse the sera from these animals, it was found that the majority of the animals with *T. mutans* parasitaemia had antigens and/or immune complexes in their sera (Figure 5). One animal, 5801, which had a piroplasm parasitaemia of 40%, had no detectable *T. mutans* antigens or immune complexes (Figure 5). Later, it was demonstrated by parasitological and serological examinations that this animal had only a *T. parva* infection. Fourteen animals were found to have high titres of antibodies to the 32 kDa antigen.

The 59 random sera from animals in a *T. mutans* endemic area (Transmara, Kenya) were initially tested for antibodies to *T. mutans* using a crude *T. mutans* antigen in an

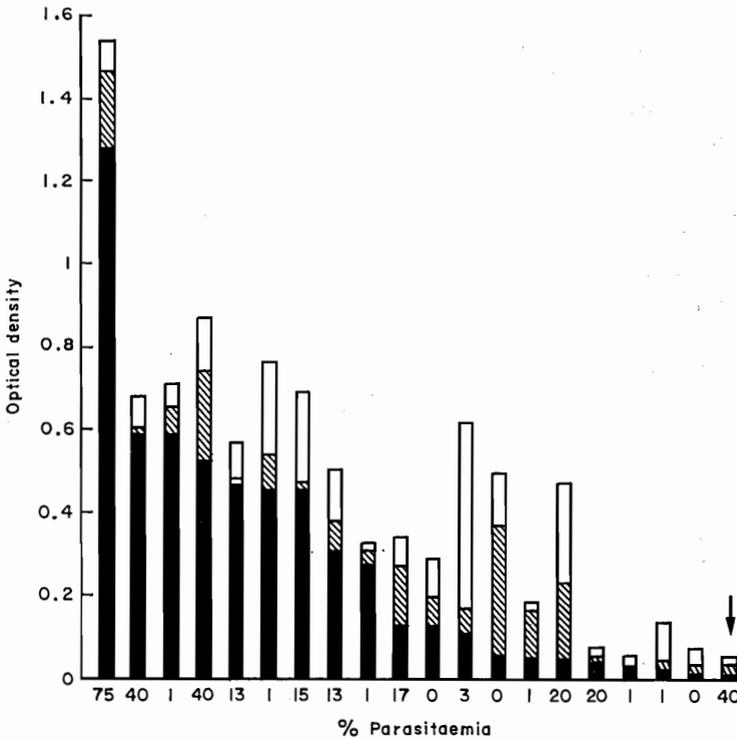


Figure 5. Detection of antigen, immune complexes and antibodies in sera collected from animals in a *T. mutans* endemic area (Zanzibar). The sera were diluted 1:2 for antigen detection and 1:20 for both immune complexes and antibody. Arrow denotes animal number 5801. ■, Antigen; ▨, immune complex; □, antibody.

indirect ELISA: 42 sera were positive, indicating that the animals had been exposed to *T. mutans* infection. However, when the purified 32 kDa was used for antibody detection, 12 more samples were found to be positive, indicating that the antibody test using the purified antigen was more sensitive. Out of these 54 positive sera, 18 had significant levels of circulating antigens and immune complexes, 7 had significant levels of circulating antigens only, 11 had immune complexes while 18 were negative for circulating antigens and immune complexes.

## Discussion

In this study we have used *T. mutans* MoAbs and a 32 kDa antigen recognized by the MoAbs to develop an ELISA for the detection of antigens, immune complexes and antibodies in sera of cattle undergoing acute or chronic infections. The assays were specific for *T. mutans* since there was no cross-reactivity with other *Theileria* parasites, namely, *T. taurotragi*, *T. bovis*, *T. annulata* and *T. parva* or with other common cattle haemoparasites. Moreover, in a preliminary study the determinants recognized by our MoAbs were not detected on *T. sergenti* and *T. buffeli* by Western blotting (A.J.Musoke and K.Fujisaki, unpublished observations). The antigen assay detected circulating antigens in early *T. mutans* infection as well as in carrier animals.

It has been demonstrated that *T. mutans* recovered animals with either very low or undetectable parasitaemia and showing high antibody titres, can transmit infections by blood passage (S.P.Morzaria, unpublished observations). Since the antibody titres remained high in the experimental cattle despite undetectable or low parasitaemia, the antibody assay could be utilized to identify *T. mutans* carrier animals. This observation is important because *T. mutans* is usually an intercurrent infection and could undermine immunization against haemoparasites such as *Anaplasma* and *Babesia* (Ilemobade 1981). Also, when animals immunized against *T. parva* are exposed to field challenge, mixed infections with different *Theileria* spp. may occur (Uilenberg *et al.* 1976). Under these circumstances the antigen and antibody assays would be useful in confirming the presence or absence of *T. mutans* infection.

It is interesting that the splenectomized animal showed a transient increase in immune complexes with a resultant fall in the antibody level. Despite the increase in parasitaemia, there was very little release of antigen, which probably accounted for the rise in immune complexes. The analysis of sera from an endemic area (Zanzibar) showed that a large number of the sera contained high titres of antigen, indicating that the donor animals had recently been infected. The situation was different with the sera from Transmara as more than 90% of the sera were positive for antibodies while only 32% showed antigen. The latter finding suggests that the animals were carriers of *T. mutans* infections.

Two MoAbs rather than one were used in developing the antigen detection assay. This approach has the advantage that the determinant of interest need not be repeated on the target antigen. In such circumstances, the use of antibodies recognizing different epitopes for capture and conjugate would allow the epitopes reacting with the conjugated MoAb to interact freely, thereby increasing the sensitivity of the assay. In developing the assays we have observed that the MoAbs which are directed against protein determinants are better at capturing antigen than those against carbohydrate moieties. The reason for this is not clear but it may be related to the avidity of the antibody.

The 32 kDa antigen is glycosylated and most of the MoAbs generated identified carbohydrate moieties. Moreover, this antigen seems to be the major glycoprotein of *T. mutans* recognized by the murine immune system. Other unpublished data suggest that the antigen is also immunodominant in cattle. The epitopes recognized by the MoAbs on the 32 kDa antigen may be conserved in many *T. mutans* stocks since the parasite stocks used in the present study were isolated from locations which were geographically widely separated. The latter suggestion is strengthened by the fact that using this assay system, similar results were obtained with sera from cattle in Kivu, Zaïre (unpublished data).

The assay is currently being evaluated using sera from animals with known haemoprotozoan infections. In order to develop this assay for widescale use the 32 kDa antigen gene is being cloned and characterized for the production of a synthetic or recombinant antigen.

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