The specific immunoglobulin response in cattle immunized with *Theileria parva* (Muguga) stabilate

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**SUMMARY**

Immunoglobulin synthesis in response to experimental infection of cattle with *Theileria parva* (Muguga) was studied by Sephadex G 200 chromatography. Serum samples were collected for 88 days following inoculation. Indirect fluorescent antibody (IFA), complement fixation (CF), indirect haemagglutination (IHA) and immunodiffusion (ID) tests were used to detect activity against *T. parva* piroplasm antigen. There was a small and transient IgM response in the IFA test, thereafter all IFA activity was 7S Ig. A sequential production of IgM and 7S Ig CF antibodies was demonstrated. In all sera tested only IgM activity was demonstrated by the IHA test. These results are compared with those from similar studies with *T. parva* and other protozoan diseases, especially in the differential immunoglobulin response shown by the contrasting results from the three serological tests.

**INTRODUCTION**

East Coast fever (ECF) continues to be one of the most economically damaging diseases of cattle within East Africa. It is caused by the protozoan parasite *Theileria parva* and is primarily transmitted by the vector tick *Rhipicephalus appendiculatus*. The disease is characterized by two intracellular forms of the parasite, a schizont stage within cells of lymphoid origin and a piroplasm stage within erythrocytes.

Cattle recovering from clinical ECF almost invariably demonstrate a specific immunity lasting at least 43 months (Burridge, Morzaia, Cunningham & Brown, 1972) and when challenged at various periods either show no reaction or react mildly. However, associated with these mild reactions is an increase in the incidence of febrile responses correlated with the length of time since last exposure to *T. parva* (Burridge et al. 1972). This indicates that there is a gradual loss of immunity in the absence of reinfection, which verifies the conclusions of Neitz (1957) and Barnett (1963).

In contrast, duration of the serological response in recovered cattle after one exposure to *T. parva* has been found to be much shorter (Burridge & Kimber, 1973). Utilizing the indirect fluorescent antibody (IFA) test, it was found that a significant antibody titre to piroplasm antigen persisted for an average of 13.8
weeks after infection, while significant titres to schizont lasted about 30 weeks. Although experience suggests that a substantial IFA response is a good indication of specific immunity (Burridge, personal communication), there is no evidence that this antibody as measured in the IFA test represents protective antibody. These results are simply interpreted as induction of antibody response to parasite specific antigens (which may not be protective).

Very little work has been done to determine the mechanism of immunity to ECF, and whether cellular or humoral factors or both are involved. The first part of such an investigation should be the elucidation of the immunoglobulin responses of cattle to *T. parva* infection where there is a spectrum of reactions including no reaction, mild to severe reactions with recovery, and finally severe reactions leading to death.

Much of the earlier work on immunity to ECF was hindered by the lack of a suitable method of inducing uniform infections or administering uniform challenges to cattle. The use of stabilates (Lumsden & Hardy, 1965; Cunningham, Brown, Burridge & Purnell, 1973), prepared from ground-up ticks containing infective particles of *T. parva* (Purnell, Brown, Cunningham, Burridge, Kirimi & Ledger, 1974), has greatly improved the standardization of laboratory-induced ECF. Although inoculation of such stabilates is usually lethal, concurrent treatment with an antibiotic such as oxytetracycline usually results in mild reactions with recovery and subsequent immunity (Brown & Radley, personal communication).

During a continuing programme of research on the isolation of *T. parva* antigens, we have developed complement fixation (CF), indirect haemagglutination (IHA) and agar gel immunodiffusion (ID) tests for ECF (Duffus & Wagner, 1973; Wagner, Brown, Duffus, Kimber, Crawford & Lule, 1973; Wagner, Duffus, Kimber & Lule, 1974). These tests, together with the IFA test which has been widely applied to ECF (Schindler & Wokatsch, 1965; Lohr & Ross, 1969; Burridge, 1971), provide a variety of techniques with which to demonstrate specific theilerial immunoglobulins.

This report describes the first in a series of investigations on the immunoglobulin responses of cattle to *T. parva*. The initial study examines the response during infection and concurrent antibiotic treatment. Subsequent reports will describe the reactions of cattle inoculated with the isolated *T. parva* schizont and piroplasm antigens and the responses during infection without concurrent antibiotic prophylaxis.

**MATERIALS AND METHODS**

**Infection of experimental animals**

Cattle of Bos taurus type, aged between 9 and 12 months, were used. They were inoculated with 1-0 ml of a stablate (Lumsden & Hardy, 1965; Cunningham *et al.* 1974) containing infective particles (Purnell *et al.* 1974) of *T. parva* (Muguga) (Brocklesby, Barnett & Scott, 1961). The injection was given subcutaneously behind the left ear. Thirty minutes beforehand each animal received an intramuscular injection of oxytetracycline (Pfizer Corporation) at a dose of 5 mg per kg body weight. Three similar doses of antibiotic were subsequently given at 24 h
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Daily rectal temperatures were taken from all cattle after inoculation and, upon enlargement of the local drainage lymph node, Giemsa-stained lymph node biopsy and daily blood smears were prepared and examined for the presence of *T. parva* parasites.

Preinoculation serum samples were collected from all cattle, and after inoculation samples were collected twice weekly. All sera were inactivated at 56 °C for 30 min and stored at −30 °C until required.

**Serum fractions**

Sera were fractionated through Sephadex G 200 (Pharmacia, Uppsala, Sweden) in columns measuring 100 × 2.5 cm. Upward flow separation was used, the diluent being 0.1 M phosphate buffered saline solution (PBS), pH 7.2, containing sodium azide at a concentration of 0.04%. The flow rate was 16.0 ml/h and each fraction was collected in 8.0 ml aliquots, the optical density being monitored at 280 nm.

**Immunoelectrophoresis**

Fractions from peaks 1 and 2 of the Sephadex serum profiles were tested for the presence of bovine IgM and 7S Ig by immunoelectrophoresis (IEP) (Scheidegger, 1955). For development of the slides, a pool of rabbit antisera specific for bovine serum proteins was used. The preparation of the developing antisera was as follows: several rabbits received repeated inoculations of aliquots of various diethyl-amino-ethyl cellulose (DEAE) and Sephadex G200 chromatography fractions of serum from non-infected cattle. The antigenic materials were emulsified in oil adjuvant and administered in several sites on each occasion. The rabbits were bled periodically and the sera pooled and stored when IEP showed that 16–20 precipitin arcs were being developed.

Rabbit antisera specific for bovine IgG2 and IgM were prepared according to Butler & Maxwell (1972).

**Serological tests**

For the IFA test antigen, smears of bovine erythrocytes containing *T. parva* piroplasms were prepared on microscope slides as previously described (Löhr & Ross, 1969; Burridge, 1971). The smears were wrapped in foil and stored at −70 °C until required. The antibovine γ-globulin was prepared by inoculating rabbits with 5.0 mg of bovine IgG (purified through DEAE) in Freund’s Complete Adjuvant. A similar inoculation was given after 4 weeks and the rabbits exsanguinated 10 days later. The γ-globulin was precipitated from the rabbit antisera with 50% ammonium sulphate, purified through DEAE using 0.01 M Tris-HCl, pH 8.0, and 0.02 M NaCl and labelled with fluorescein isocyanate Isomer I (BDH Chemicals Ltd., Poole, England). In the IFA test this preparation was used at a concentration of 0.1 mg protein/ml. The IFA technique was similar to that described by Burridge (1971). Sera were tested at doubling dilutions starting at a dilution of 1:2. Antibody titres were expressed in terms of the highest dilution of serum or serum fraction which gave specific staining of *T. parva* piroplasms.

Complement fixation tests, using the *T. parva* piroplasm antigen, were per-
formed essentially as described by Cowan & Trautman (1967), and were based on
the fixation of four out of five 50% haemolytic units of guinea-pig complement.
Sheep blood cells, Haemolysin, guinea-pig complement and diluent were pre-
pared as described by Mayer (1964). All reactions were incubated for 30 min at
37 °C prior to adding sensitized cells.

Immunodiffusion tests were carried out as previously described (Wagner,

Indirect haemagglutination tests were performed as described by Duffus &
Wagner (1973) using sheep erythrocytes sensitized with \textit{T. parva} piroplasm
antigen.

**RESULTS**

\textbf{Cattle reactions}

A thermal response, as determined by a rise in rectal temperature above 39.5 °C,
was recorded as early as 10 days after inoculation, and 6 of 9 cattle had reacted
with temperatures by 18 days. No piroplasms were seen in the blood smears of
any of the cattle under study. Small numbers of schizonts were seen in 4 of the 9
as early as 13 days.

\textit{Immunoelectrophoresis}

Immunoelectrophoresis analyses of the fractions from peaks 1 and 2 of the
Sephadex serum profiles showed bovine IgM to be the exclusive immunoglobulin
in peak 1 and 7S Ig to be predominant in peak 2.

\textit{IFA results}

IFA activity against \textit{T. parva} piroplasms was first detected 22 days after
inoculation and peak activity was reached on day 39. Detailed results are shown
in Fig. 1.

On fractionation of the sera a small and transient IgM response was detected

![Fig. 1. Antibody response to \textit{Theileria parva} piroplasm antigen as shown by the IFA
test. Vertical lines indicate the standard deviation.](https://www.cambridge.org/core/terms)

![Days post inoculation](https://www.cambridge.org/core/terms).
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Fig. 2. Antibody response to *Theileria parva* piroplasm antigen as shown by the CF test. Vertical lines indicate the standard deviation.

Fig. 3. Antibody response to *Theileria parva* piroplasm antigen as shown by the IHA test. Vertical lines indicate the standard deviation.

on day 22 (Fig. 4B). Thereafter in all sera fractionated up to the last day of sampling, day 88, IFA activity was confined to the second peak (Figs. 5, 6).

**CF results**

CF activity against *T. parva* piroplasm activity was first detected 14 days after inoculation and peak activity was found between days 22 and 27 as shown in Fig. 2. The CF antibodies remained until day 52 and then no further activity was detected up to the last sample on day 88.
Fig. 4. Results of serological tests on fractions obtained by Sephadex G 200 separation of sera collected 17 days (A) and 22 days (B) following inoculation with *Theileria parva* stabilate.

On the first appearance of CF antibody all activity was found in the first peak (IgM), an example being shown in Fig. 4A. However, CF activity was detected in the second (7S Ig) peak 3–5 days after the initial appearance of IgM activity; thereafter CF activity was equally divided between the first and second peaks (Figs. 4, 5) until day 48, when in some fractionated sera activity was only found in the second peak (Fig. 6A).

**IHA results**

IHA antibodies were first detected 14 days after inoculation, rose to a peak after 22 days and then gradually fell, activity still being detected at 88 days (Fig. 3).

In all the fractionated sera tested for IHA antibody, activity was only demonstrated in the first peak, no activity ever being detected in the second peak. This is conclusively shown in Figs. 4, 5 and 6 and is in marked contrast to the IFA results.
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Fig. 6. Results of serological tests on fractions obtained by Sephadex G 200 separation of sera collected 48 days (A) and 88 days (B) following inoculation with Theileria parva stabilate.

ID results

Specific precipitin reactions were first detected 22 days after inoculation, and were maintained throughout the duration of the experiment. Precipitins could be detected in the first peaks of sera 22 and 30 days after inoculation, but only in the second peak of those thereafter.

DISCUSSION

A few important points should be noted before the results of the present study can be applied to an interpretation of the immune response in ECF. The method of inoculating T. parva stabilate plus concurrent oxytetracycline leads to mild, often asymptomatic, reactions with only a small proliferation of schizonts and an even smaller or absent proliferation of piroplasms (Brown & Radley, personal communication); as a result the amount of schizont and/or piroplasm antigen available to the host’s immune system is very much smaller than that which is available in animals undergoing clinical ECF. No piroplasms were observed in any of the nine cattle under study while schizonts were detected in 4 of 9. However, the remarkable uniformity of the antibody responses in the 9 cattle (sera from all 9 first showed IFA reactivity on day 22) indicates considerable homogeneity of the stabilate preparation from an antigen standpoint. The observations on the schizont and piroplasm development further suggest that parasitism of only a few cells is necessary for induction of an antibody response. The question whether this humoral response in any way contributes to a resultant ECF immunity will be discussed in another publication.

It is also well documented that IgM antibodies are more efficient at CF and agglutination than IgG (Grey, 1964; Robbins, Kenny & Suter, 1965). For example, Greenbury, Moore & Nunn (1963) found that specific rabbit IgM will agglutinate human erythrocytes 750 times more effectively than IgG antibody. The IgG classes, on the other hand, have been found to be more efficient precipitins (Pike, 1967).

In their study on the sequential production of serum IFA against T. parva,
Löhr & Ross (1969) found that antibodies were first detected 8–14 days after the application of infected ticks, and peak titres were found after 30 days. When it is taken into consideration that ticks will only transmit *T. parva* to the host an average of 5 days after attachment (Wilde, Brown, Hullinger, Gall & MacLeod, 1968), then Löhr & Ross first detected IFA activity 3–9 days after antigen became available to the host. This result differs from our data where IFA activity first appeared after 22 days. A possible explanation for this difference could be the variation in the severity of the induced disease: Löhr & Ross showed that with their experimental cattle recovering from clinical ECF, 6 of 9 cattle had severe reactions and 7 of 9 had piroplasms; while in the present study no clinical signs were apparent and no piroplasms were detected. Also Burridge & Kimber (1973) found that piroplasm-specific IFA antibodies were first detected 28 days after experimental infection with ECF, with a peak at about 35 days, and although the levels of parasitaemia in the animals used were not reported, the results correspond closely to those of the present experiment. That a difference in the levels of parasitaemia achieved can radically alter the humoral immune response has been reported for *Plasmodium* infection (Cox & Turner, 1970a).

In a subsequent publication Ross & Löhr (1972) describe similar experiments with the capillary tube agglutination test, in which antibodies were first detected 13–23 days after infection and reached a peak on the 30th day. In our results IHA antibodies were first detected after 14 days and reached a peak on day 22. Schindler & Mehlitz (1968), using the CF test, found antibodies in an experimental bovine 13 days after infection with *T. parva*, with a peak titre from day 27; our CF results agree closely.

The results after chromatography of the antisera clearly reveal contrasts between the serological tests used. Except for a very transient IgM response, antibodies in the IFA test were exclusively 7S Ig; while in all sera examined, IHA antibodies were exclusively IgM. Both the ID and the CF tests detected an initial production of IgM, after which time both 7S Ig and IgM were detected to similar titres.

In studies on *Babesia microti* infection in mice, Cox & Turner (1970b), using an IFA test, were able to detect both IgM and IgG antibody, but were unable to demonstrate a sequential production of IgM and IgG, the latter occurring early on in the antibody response and always to a much higher titre than the IgM antibodies. Similar results have been described for *Plasmodium* infection in mice (Cox, Crandall & Turner, 1969; Cox & Turner, 1970a) and human malaria (Collins & Skinner, 1968).

It has been suggested that the acceptance of the usual sequential production of IgM and IgG during an immune response should be re-evaluated (Freeman & Stavitsky, 1965), especially in the light of results in *Babesia* piroplasmosis mentioned above (Cox & Turner, 1970b). However, these latter results and other papers on piroplasmosis have utilized the IFA test in examining the immunoglobulin response, and the possibility exists that the IFA technique, as employed in this study, is heavily biased towards the detection of IgG antibodies. We are engaged on further experiments to test this hypothesis. It has been astutely observed
Immunoglobulin response to Theileria parva (Abele, Tobie, Hill, Contacos & Evans, 1965) that the different methods of assay for malarial antibodies could lead to variable results when studying specific immunoglobulins. As shown above, the immunoglobulin response as detected by the CF and ID tests demonstrated a normal sequential production of IgM and 7S Ig. Murphy, Osebold & Aalund (1966) published similar results with Anaplasma marginale infection in cattle, with an early increase in IgM CF activity followed by a similar increase in IgG activity.

In contrast, only IgM antibodies were detected with the IHA test. As mentioned earlier, one explanation for this fact is that IgM is reported as being more efficient in an agglutinating reaction than IgG. Since 7S Ig antibodies were demonstrated by both the CF and IFA tests, it seems unusual that none was detected by IHA. The exclusive production of IgM antibodies in an immune response has been well documented for lipopolysaccharide antigens (Weidanz, Jackson & Landy, 1964; Britton & Möller, 1965; Britton, 1969). Also Cerný & Iványi (1967) have shown that small doses of antigen will produce a purely IgM response, whilst larger doses result in the initiation of an IgG response. Although the polysaccharide content of T. parva has been demonstrated (Dasgupta, 1960), the influence of a polysaccharide component on the immune response reported here must remain hypothetical until further work is completed.

The present results suggest considerable heterogeneity in the immunoglobulin response to T. parva. That this heterogeneity may be a manifestation of the antigens used is currently under study (Wagner, Duffus & Burridge, 1974) but preliminary results indicate that with different conditions of antigenic stimulus, IHA antibodies are often detected within the 7S Ig class and that sometimes IHA antibodies are exclusively 7S Ig.

These results will be presented in other papers in this series.

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