MONOCLONAL ANTIBODY RESPONSE AND *SfiI* AND *EcoRI* FRAGMENTS OF GENOME DNA OF *THEILERIA PARVA* MELELA OF EASTERN TANZANIA

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SUMMARY

Genomic DNA and monoclonal antibody response of *Theileria parva* Melela, of Morogoro, Tanzania were compared with those of *T.parva* Muguga. Using indirect fluorescent antibody test positive reactions at 1:50 and 1:200 dilutions were observed with MAb 1, 2, 3, 4, 10, 12 and 22, non specific fluorescence in < 0.1% of cells for MAb 7 and 21 and negative for MAb 15, 20 and 23 with very close response to *T. parva* Muguga. There were restriction fragment length polymorphisms with Muguga stock on digestion of genomic DNA by *Eco*RI and *Sfi*I endonucleases, separated by agarose and pulsed field gel electrophoresis respectively. *T. parva* Melela DNA digested with *Eco*RI resolved multiple restriction fragment bands 603-2400 kb. *Sfi*I digestion and pulsed field gel electrophoresis resolved 24 discrete fragments 65-1800 kb. There were more fragments above 800 kb than in *T. parva* Muguga, indicating a large genome of 1200-1400 megabase pairs (mb) for Melela *T. parva* compared to 10 mb for Muguga. Variations in hybridization signals for *T. parva* Melela and Muguga were observed when southern blots were probed with small subunit ribosomal RNA gene probe pRIBSSI, 1.4 kb repetitive *T. parva* sequence TprI PMB3 probe cloned in plasmid pUC8, for *Eco*RI digested DNA and 8.1 kb synthetic telomeric oligonucleotide DNA probe for *Sfi*I DNA digests.

INTRODUCTION

East Coast fever is a highly infectious fatal disease of cattle caused by the protozoan parasite *Theileria parva*, transmitted by the widely distributed tick *Rhipicephalus appendiculatus*. The only available method of immunization is by infection with *T. parva* sporozoites and simultaneous treatment with a long acting oxytetracycline (Radley, 1981). This method was originally reported to protect over 90% of immunized cattle and viable economically (Mukhebi *et al.*, 1990, Mukhebi *et al.*, 1992; Mutugi *et al.*, 1991; Young *et al.*, 1990). This technique of immunization was introduced for wide scale application in Tanzania by the Food and Agricultural Organization (FAO) of the United Nations in 1991. So far more than 8,000 cattle have been immunized.

Two major problems of this method are the limitation of protection to immunizing stocks (Dolan *et al.*, 1980) and that some animals become (Dolan, 1986; Maritim *et al.*, 1989). Following immunization in Tanzania some immunized cattle have been observed to develop disease (Unpublished observations). This may result from stock specificity of the vaccine or a short duration of immunity which is so far incompletely investigated. These problems can be eliminated by use of local *T. parva* stocks. Thus *in vivo* and *in vitro* characterization of *T. parva* field isolates is necessary because they cannot be distinguished by morphology, vector specificity or serology.

In vivo methods identify different immunological *T. parva* stocks. *In vitro* methods such as protein analysis (Allsopp

et al., 1985), monoclonal antibody profile (Minami *et al.*, 1983, Conrad *et al.*, 1987a; Maritim *et al.*, 1989), genomic DNA restriction fragment length polymorphisms and DNA probes (Conrad *et al.*, 1987b, Allsopp *et al.*, 1988; 1989, Bishop *et al,* 1993), contour clamped homogenous electric field, field inversion and pulsed field gel electrophoresis (Morzaria *et al.*, 1990, Young and Morzaria, 1991; Morzaria and Young, 1992) and two dimensional polyacrylamide gel electrophoresis and immunoblotting (Sugimoto *et al.*, 1992) have been found to detect phenotypic and genotypic differences. DNA probes are further useful in detection of carrier animals (Bishop *et al.*, 1992).

The very little information on the *T. parva* stocks of Tanzania, where ECF poses most serious threat appears to greatly affect the success of immunization. The variations in stocks was earlier not perceived to be a problem but because of the vast areas involved different stocks of varying pathogenicity and infectivity exist. The success of large scale immunization by infection and treatment was based on the assumption of uniform stocks in all areas. Since ECF occurs even in immunized cattle it is apparent that there are many stocks with antigenic properties differing from those of the Muguga cocktail vaccine. It is definite that immunization could be more effective if local stocks of *T.parva* are used.

There are four *T. parva* stocks isolated in Tanzania; *T. parva* Pugu and Idogoro (Dolan *et al.*, 1980), *T. parva* Pemba Mnarani (Morzaria *et al.*, 1990) causing cattle derived ECF and *T.parva* Serengeti (transformed) causing buffalo derived infections (Young *et al.*, 1973). The incidence rates of ECF are high, some cases with abnormally severe signs of subcutaneous haemorrhages (P. Mtei and M. Yongolo, Ministry of Agric, unpublished) others chronic and debilitating especially in calves (Mbassa *et al.*, 1993), makes isolation of stocks with unusual characteristics essential for improvement of the vaccine. This paper presents *in vitro* characteristics of a very pathogenic *T. parva* stock associated with severe pancytopaenia (Mbassa *et al.*, 1994), recently isolated from zebu cattle in a traditional Maasai herd that suffered greater than 90% mortality rate at Melela village, Morogoro eastern Tanzania.

MATERIALS AND METHODS

T.parva Melela isolation

A Tanganyika short horn zebu steer with clinical signs of East Coast fever (ECF) was obtained from an outbreak of the disease in a traditional Maasai herd at Melela village Morogoro, Tanzania. The piroplasm parasitaemia was above 20%, and few schizonts were found in parotid and prescapular lymph nodes. Blood analysis revealed anaemia of 3.64×10^{12}/1 erythrocyte counts, 0.15 1/1 packed cell volume 1.15 mmol/1 haemoglobin concentration and leukopaenia of 4.5×10^{9}/1 leukocyte counts. The infection was picked by 10,000 clean *Rhipicephalus appendiculatus* (Muguga) nymphs applied on ears (22.8 1992 - 27.8 1992). The ticks were incubated at 20°C and 80% relative humidity for 6 weeks to moult as adults, which were prefed on rabbits for 4 days and dissected to determine *Theileria parva* infection rates in salivary glands.

Tick dissections indicated 139.7 infected salivary gland acini per female and 24.5 infected acini per male (mean 82.1 infected salivary gland acini per tick). A stabilate (ILRI 3969) of 17.3 ticks/ml of 7.5 % glycerol in minimum essential medium (MEM) was prepared. The concentration was 1420.3 infected acini/ml, dispensed in straws and immediately frozen to -196°C.

Each of the four Boran steers BJ 371, BJ 379, BK 141 and BK 200 were inoculated with 1 ml of *T. parva* Melela stabilate (3969) subcutaneously in front of the right ear, above the right parotid lymph node and monitored for development of disease. Rectal temperatures were monitored daily and lymph node biopsies taken from day 5 after infection for schizont examination. Biopsies from left prescapular lymph gland were taken when the right ear gland was positive for schizonts and blood smears were prepared for piroplasms examination. Lymph biopsies were taken for *in vitro* isolation from animal BK 141. Clean *Rhipicephalus appendiculatus* ticks were applied on the animals for infection pick up. Animal BK 141 was treated with oxytetracycline to prolong the infection for completion of tick pick up, whereas BJ 371 with high piroplasm parasitaemia was bled to obtain piroplasms for DNA purification for *in vitro* characterization.

Monoclonal antibody profile of *T. parva* Melela

Testing on reactions to monoclonal antibodies (MAb) was done by indirect fluorescent antibody test (IFA). *T. parva* Melela and *T. parva* Muguga antigens coated on slides were tested each by addition of 20 μl of MAb 1, 2, 3, 4, 7, 10, 12, 15, 20, 21, 22, and 23 at 1:50 and 1:200 dilutions in the standard indirect fluorescent antibody technique.

Purification of *T. parva* Melela schizont DNA

Lymphocytes infected with *T. parva* Melela obtained from lymph node biopsies of infected animals (0.5 ml cell pellets) were lysed by incubation at 50°C for 4 h in 5 ml 1 M TNE buffer pH 8.0 (12.1 g 19 mM TRIS, 3.72 g 1 mM EDTA, 58.44 g 100 mM NaCl dissolved/l) with 250 μl 10% sodium dodecyl sulphate (SDS) pH 7.2 and 2 mg proteinase K. The DNA was extracted by mixing and spinning for 30 m once in an equal volume of phenol equilibrated with Tris-HCl pH 7.4, twice with a 1:1 phenol/chilling chloroform solution, the chloroform contained drops of iso amylalcohol (24:1), and once in equal volume of chloroform/iso amylalcohol. DNA was precipitated from the viscous supernatant with 3M sodium acetate pH 5.2 in the ratio of 10:1, and chilling absolute ethanol 6 times the volume of DNA. This was kept at -20°C for 16 h and centrifuged at 3000 rpm for 20 m. The DNA pellets were suspended in 70% ethanol 10 times its volume and centrifuged at 3000 rpm for 25 m, the ethanol supernatant decanted and the DNA dried under vacuum pump. The dry DNA pellets were dissolved in sterile deionized water (1:10) or 1 x TE buffer (TE = 10 mM Tris-HCl, 1mM EDTA, pH 8.0) and incubated at 50°C for 1-2 h. The concentration of the DNA was determined spectrophotometrically at 260 nm wavelength under UV light. The optical density of *T.parva* Melela was 0.58 and concentration of 0.29 μg/μI.

Preparation of piroplasm DNA

Blood samples were collected in Alsevers solution pH 6.0 (0.55 g citric acid, 20.5 g glucose/dextrose, 4.2 g NaCl, 8.0 g sodium citrate per L) with 50 IU/ml heparin, centrifuged at 3,500 rpm for 30 m (Sorval) or 3000 rpm (Heraeus minifuge). The supernatant and buffy coat were discarded, while the erythrocyte pellets were suspended in Alsevers solution, washed in this way four times and once in 1x TEN buffer (20 mM Tris-HCl 2.424 g, 10 mM EDTA 3.72 g, 100 mM NaCl 5.844 g per L, pH 8.0). The packed cells and an equal volume of saponin 1 mg/ml in distilled water were pre-warmed at 37°C for 30 m separately, mixed for 10 s to lyse erythrocytes. Lysis was stopped by addition of TEN buffer

four times the volume of packed cells and centrifuged at 3,500 rpm for 20 m (Sorval) or 3,000 rpm (Heraeus minifuge). The cell membrane pellet was discarded while the supernatant spanned at 10,000 rpm for 30 m (Beckman), discarding the supernatant. The soft pellet was resuspended in TEN buffer and repeatedly span at 10,000 rpm for 30 m to wash off haemoglobin. The volume of the pellet was estimated and piroplasm quantity assessed from smears. The piroplasm pellets were stored at -70°C in aliquotes or used for preparation of DNA in solution and or embedded blocks.

To obtain a solution of DNA, piroplasm pellets were suspended in equal volumes of lysing buffer (250 mM EDTA, 0.5% SDS, 25 mM Tris-HCl, 500 μg/ml proteinase K) and incubated at 50°C for 14 - 16 h. DNA was precipitated with salt and ethanol after phenol/chloroform extraction as for schizonts DNA.

Piroplasm DNA was embedded in blocks in 1.5% (w/v) In Cert grade low melting point agarose (FMC Bio Products, Redeland, USA) in RPM1 1640 medium. Equal volumes of piroplasm pellet and agarose were mixed in moulds, and placed on ice for 30 m to solidify into blocks. The blocks were transferred into lysing buffer at 50°C for 48 h to lyse the proteins and release the DNA, then washed in 10 mM EDTA for 2 hours, in 10 mM EDTA containing 1 mM phenylmethyl sulphoryl fluoride (PMSF) for 2 hours and in 10 mM EDTA six times, then stored at 4°C.

Enzyme digestion of Schizont and Piroplasm DNA

Six mg each of *T. parva* Melela and Muguga DNA were digested with *Eco*RI by incubation with 30 units of the enzyme for 6 hours at 37°C. Embedded DNA blocks of *T. parva* Melela and Muguga were digested by *Sfi*I for 1 h at 37°C.

Agarose gel electrophoresis

*Sfi*I digested DNA fragments were separated by electrophoresis in 1.5% agarose gel (w/v) in 0.38x TBE (1xTBE = 89 mM Tris-Borate,pH 8.3, 2 mM Na_2 EDTA) under pulsed field at 400 volts (v) and 10 s pulses for 16 h and 200 v, 40 s pulses for 5 h. *Eco*RI digested DNA fragments were separated in 1.0% w/v agarose gel in 1x TAE buffer (50x TAE = 242 g Tris, 57.1 ml glacial acetic acid, 10 ml 0.5 M EDTA pH 8.0). Gels were run at 40 v for 16 h at room temperature. DNA markers were lambda DNA digested by *Hind*III to produce 8 fragments 130, 125 - 23 base pairs (bp), and øX174 DNA digested by *Hae*III to produce fragments of 72 - 1353 bp.

Gels were stained with 1.0 μg/ml ethidium bromide in water for 30 m, washed in water for 30 m and photographed in a polaroid camera under UV illumination (Chromato-Vue Trans illuminator Ultra Violet Products San Gabriel CA, USA).

Southern blots

Gels were acid depurinated in 0.25 N HCl for 20 m, rinsed in water, denatured with two changes in 1.5 M NaCl + 0.5 M NaOH each of 30 m, neutralized in 1.5 M NaCl + 0.5 M Tris-HCl) 2 changes of 30 m each and washed in water.

Restriction fragments were transferred onto hybond nylon filters (Hybond, Amsherm International Buckinghamshire, UK) by Southern blotting for 16 h. The filters were washed in 2x SSC and cross linked by exposure to UV light in a UV Stråta linker (Stratagene, USA) at 0.12 Joules for 2-4 m, then prehybridized in 6x SSC, 5x Denhardt solution, 0.1% SDS and 0.1% sodium pyrophosphate at 65°C for 6 h.

Radio-labelling and Hybridization with DNA probes

The DNA probes used for hybridization to southern blots were (a) small subunit ribosomal RNA (pRIBSS1)

(b) repetitive *T. parva* sequence (*Tprl*-PMB3) probe

(c) telomeric oligonucleotide 8.1 kilobase pair (kb) DNA probe for *Sfi*I digested DNA fragments.

The *T. parva* repetitive DNA probe PMB3 is a 623 bp *Sau* 961 restriction fragment isolated from *T. parva* Muguga genomic DNA and cloned in plasmid pUC8 forming a 1.4 kb repetitive sequence in *T. parva* Muguga genome *(Tprl)* (Allsopp and Allsopp, 1988).

The small subunit ribosomal RNA (SSUrRNA) gene probe pRIBSS1 was isolated by cloning a 1.1 kb segment of *T. parva* SSUrRNA gene into plasmid pUC19 from ribosomal sequences of *T. parva* Muguga by polymerase chain reaction (Bishop *et al.*, 1993).

The telomeric probe was a 28 mer synthetic oligonucleotide (5'.. CCCTGAACCCTGAA..3')$_2$ corresponding to a repeat sequence of *Plasmodium berghei* telomeric clone (Bishop *et al.*, 1993).

DNA probes were radio-labelled to specific activity of 1.3, 1.4 and 1.9 x10^9 counts per minute (cpm/μg) DNA of *Tprl*, telomeric oligonucleotide and SSUrRNA probes respectively, with ^{32}P alpha dCTP (3000 Ci/mmol) by random priming with a mega-prime it kit (Amersham, UK). Two μl of probe DNA, 5 μl of primer were dissolved in 26.5 μl water and denatured by incubation at 95°C in a polymerase chain reaction thermocycler for 10 m and cooled at room temperature. To this solution 10 μl of labelling buffer (mixture of alpha dATP^{32}, dGTP and dTTP), 2 μl DNA ligase Klenow fragment of DNA polymerase I and 5 μl ^{32}P alpha dCTP, were added and incubated at 37°C for 1 h. Radioisotope probes were filtered through NucTrap push columns which separate unincorporated nucleotides from radiolabelled DNA or RNA probes as small as 17 bp and large as 50000 bp (Stratagene, La Jolla CA, USA), then denatured by boiling for 10 minutes. Labelled denatured probes at concentrations of 1.3, 1.4 and 1.9 x 10^9 cpm/μg for PMB3, telomeric oligonucleotide and SSUrRNA probes respectively were added to the hybridization solution (6x SSC, 5x Denhardt solution, 0.1% SDS and 0.1% sodium pyrophosphate containing the southern blot filters) and hybridized at 65°C for 16 h. Filters were then washed under low stringent; once in 2x SSC, 0.1% SDS, 0.1 sodium pyrophosphate for 1 h, dried and exposed to Fuji RX 100 film with intensifying screens at - 70°C (Fuji Photo Co. Japan) for 12 - 15 h.

RESULTS

Salivary glands of the ticks fed on the animal from the field in Melela, dissected and stained with Fulgen reaction were found to have 139.7 infected acini per female, 167.6 infected acini per infected female with an infection rate of 83.3%. The infection rates in male ticks were lower than in females. There were 24.5 infected acini per male, 40.8 infected acini per infected male and an infection rate of 60.0%. The second passage ticks that were fed on animals used for stabilate testing were also found to have equally high infections.

Infectivity of T. parva Melela

This *T. parva* stock was observed to be very pathogenic to the four boran cattle infected with the stabilate, with short disease course. Schizonts in lymph biopsies at site of inoculation were detected on day 6 post infection. The mean

duration of schizonts was 12.75 days (10 - 18). Animals took 7 - 8 days to develop fever (mean 7.25) which persisted for 9 - 12 days (mean 9.5). Piroplasms were detected on day 10 in all experimental animals and persisted for 7 - 13 days (average 8.75). The maximum temperatures reached were 41.3, 40.2, 40.8 and 41.0°C for animals BK 141, BK 200, BJ 371 and BJ 379 with death after 23, 16, 17 and 16 days postinfection respectively. Tick pick up of infection indicated high infection rates.

All infected cattle showed enlarged lymph nodes on day 5. Few schizonts in ear lymph glands from inoculation side appeared on day 6. The schizonts increased and were detected in contralateral prescapular lymph glands on days 8 and 9, until death. Parasitaemia was observed in all animals from day 11. The mean parasitaemia in the four animals were 0.15, 0.95, 3.2, 9.1, 12.5, 16.2% on days 11, 12, 13, 14, 15 and 16 respectively. Three animals died by day 17. Parasitaemia in the remaining animal continued to rise from 16.4 to 26.2% at the time of death on day 23.

Monoclonal antibody profile

Monoclonal antibody testing was performed by using the indirect fluorescent antibody test. Positive reactions of *T. parva* Melela at 1:50 and 1:200 dilutions were observed with MAb 1, 2, 3, 4, 10, 12 and 22 (Table 1). Schizont fluorescence was observed in very few cells (< 0.1 %) for MAb 7 and 21, thus practically negative. *T. parva* Melela was negative for MAb 15, 20 and 23. These agreed closely with results of *T. parva* Muguga and an originally buffalo derived stock (Young *et al.*, 1973), except for MAb 7 where Muguga and Melela were negative. *T. parva* Muguga was further negative to MAb 10 and 12.

Genomic DNA restriction fragment length polymorphisms

The markers for *Eco*RI digests were a mixture of lambda DNA *Hind*III digest producing 8 fragments of 23 - 125, 130 bp, øX174 DNA *Hae*III digest of 72 - 1,353 bp. Digestion of *T. parva* Melela piroplasm DNA with *Eco*RI resolved multiple restriction fragment bands on ethidium bromide stained gels 603-2400 kb (Fig. 1A, B, C) and was similar to Muguga stock.

Hybridization of *Eco*RI digested piroplasms and schizonts DNA to the *T. parva* repetitive DNA probe PMB3 indicated hybridization signals with fragments of 2.0, 4.3, 6.5, 9.4 and 9.5 - 23. 1 kb for *T. parva* Muguga (Fig. 1A). The only signals with PMB3 repetitive probe with *T. parva* Melela were fragments 9.4 and 23.1 kb. Hybridization signals for Schizont DNA was apparent and appeared to be similar for *T. parva* Melela and Muguga stocks at 23.1 kb.

The SSUrRNA gene clone pRIBSSI DNA probe hybridized to blots of *Eco*RI digested *T. parva* Melela and Muguga piroplasm DNA signalled to a single *Eco*RI fragment of 4.36 kb (Fig. 1C). In schizont DNA this signal was observed in addition to others of 9.42 kb, a faint one of 2.0 kb for *T. parva* Melela, and 9.42, 6.56 and 23.1 kb for *T. parva* Muguga. These additional ones were probably of bovine origin (Fig. 1C lane 2 and 4).

The marker for *Sfi*I digested DNA was *Saccharomyces cerevisae* yeast chromosome PFG (Biolabs, New England) with 12 fragments 225 to 1900 kb. In *Sfi*I digested pulsed field electrophoresis gels there were distinct and discrete fragments. The *Sfi*I digested *T. parva* Melela genomic DNA resolved into 24 fragments between 65 and 1800 kb (Fig. 2A, B), with initial faint band 1800 kb, then, three intense bands of 1350, 800 and 750 kb.

Table 1: Monoclonal antibody response to *T. parva* schizonts.

MAb titre	Kiambu5 50	Kiambu5 200	Serengeti 50	Serengeti 200	Melela 50	Melela 200	Muguga 50	Muguga 200	Specificity origin
1	+	+	+	+	+	+	+	+	Muguga
2	-	-	+	+	+	+	+	+	
3	-	-	+	+	+	+	+	+	
4	+	+	+	+	+	+	+	+	
7	+	+	+	+	±	±	±	±	
10	+	+	+	+	+	+	±	±	Kiambu5
12	+	+	+	+	+	+	±	±	
15	-	-	-	-	-	-	-	-	Marikebuni
20	-	-	-	-	-	-	-	-	*lawrencei*
21	±	±	-	-	-	-	-	-	
22	-	-	+	+	+	+	+	+	
23	-	-	-	-	-	-	-	-	*taurotragi*

These were followed by an intense region of three resolvable very close bands 740, 715 and 700 kb and a series of distinct discrete bands 500, 370, 250, 190, 150 down to 65 kb (Fig. 2B). This indicates more regular *Sfi*I restriction sites in this parasite stock. There were more large fragments than those observed in *T. parva* Muguga indicating that the genome of this stock is larger (1200 - 1400) megabase pairs (mb) compared to 10 mb for *T. parva* Muguga.

Hybridization of a telomeric oligonucleotide probe which detects telomeric sequences in *T. parva* to *Sfi*I digested blots identified one fragment of 80 kb for *T. parva* Melela and Muguga (Fig. 2B).

DISCUSSION

The objective of this investigation was to compare the characteristics of *T. parva* isolated from eastern Tanzania to those of known stocks, particularly the reference one *T. parva* Muguga. The eastern Tanzania stock was observed to be very lethal, causing massive piroplasm parasitaemia with very severe pancytopaenia (Mbassa *et al.*, 1994). There were very high infection rates in ticks feeding on the sick animals. The rates in female and male ticks fed on the field steer were 83.3 and 60.0% respectively. Infection rate quantification has been found to correlate well with DNA probes for *Theileria parva* (Chen *et al.*, 1991).

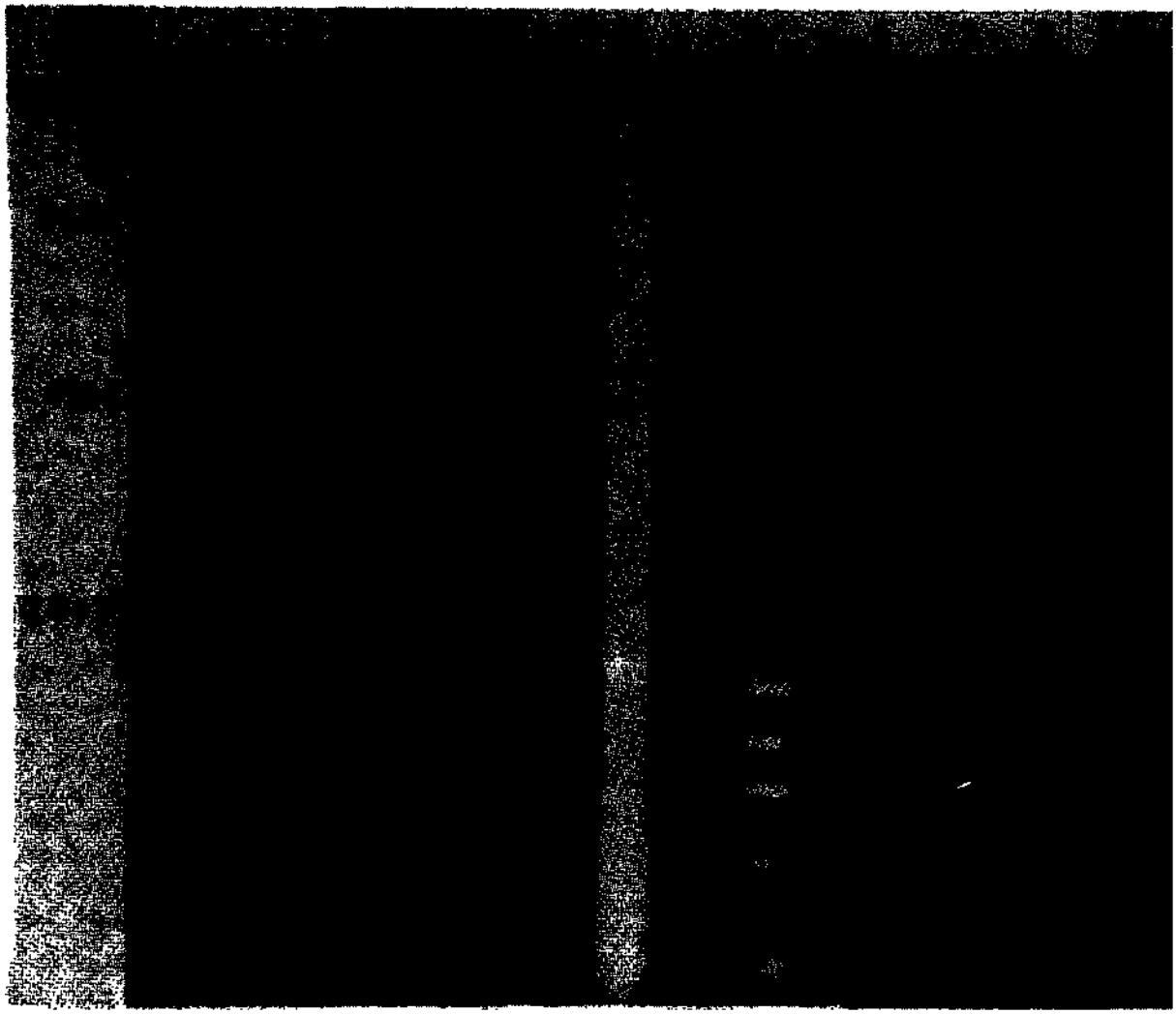

Fig. 1A: An Autoradiograph of Southern blots of *Eco*RI digested DNA from *T. parva* Melela and Muguga piroplasms and schizonts, hybridized with cloned *T. parva* repetitive sequence (*Tprl*) DNA probe PMB3. Lanes contain 6 μg of schizont DNA or purified piroplasm DNA digested with 30 units *Eco*RI, electrophoresed through a 1% agarose gel in 1x TAE buffer and transferred onto a nylon filter. The filters were hybridized with radiolabelled PMB3 probe at 65°C for 16 h washed in 2x SSC, 0.1% SDS, 0.1% sodium pyrophosphate for 1 h. Fragments of *T. parva* piroplasm DNA are seen for Melela (lane 1) Muguga (lane 2), schizont DNA of Melela (lane 3) and Muguga (lane 4). **Fig. 1B**: Agarose electrophoretic separation of *Eco*RI digested piroplasm DNA fragments of *T. parva* Melela and Muguga through 1% agarose gel and visualized by staining with ethidium bromide *T. parva* Melela piroplasm (lane 1), *T. parva* Muguga schizont DNA (lane 2) and marker DNA, a mixture of *Hind*III digested lambda DNA and *Hind*III digested ϕX174 DNA (lane 3). **Fig. 1C**: An autoradiograph of Southern blots of *Eco*RI digested *T. parva* Melela and Muguga piroplasm and schizont DNA, hybridized with SSUrRNA gene clone pRIBSS1 probe. Lanes contain 6 μg schizont or purified piroplasm DNA digested with 30 units *Eco*RI, electrophoresed through 1% agarose gel in 1x TAE buffer and transferred onto nylon filter. The filters were hybridized with radiolabelled pRIBSS1 at 65°C for 16 h, washed in 2x SSC, 0.1% SDS, 0.1% sodium pyrophosphate (1 h). Hybridized fragments of *T. parva* piroplasm DNA for Melela (lane 1) and Muguga (lane 3), schizont DNA for Melela (lane 2) and Muguga (lane 4) are observed.

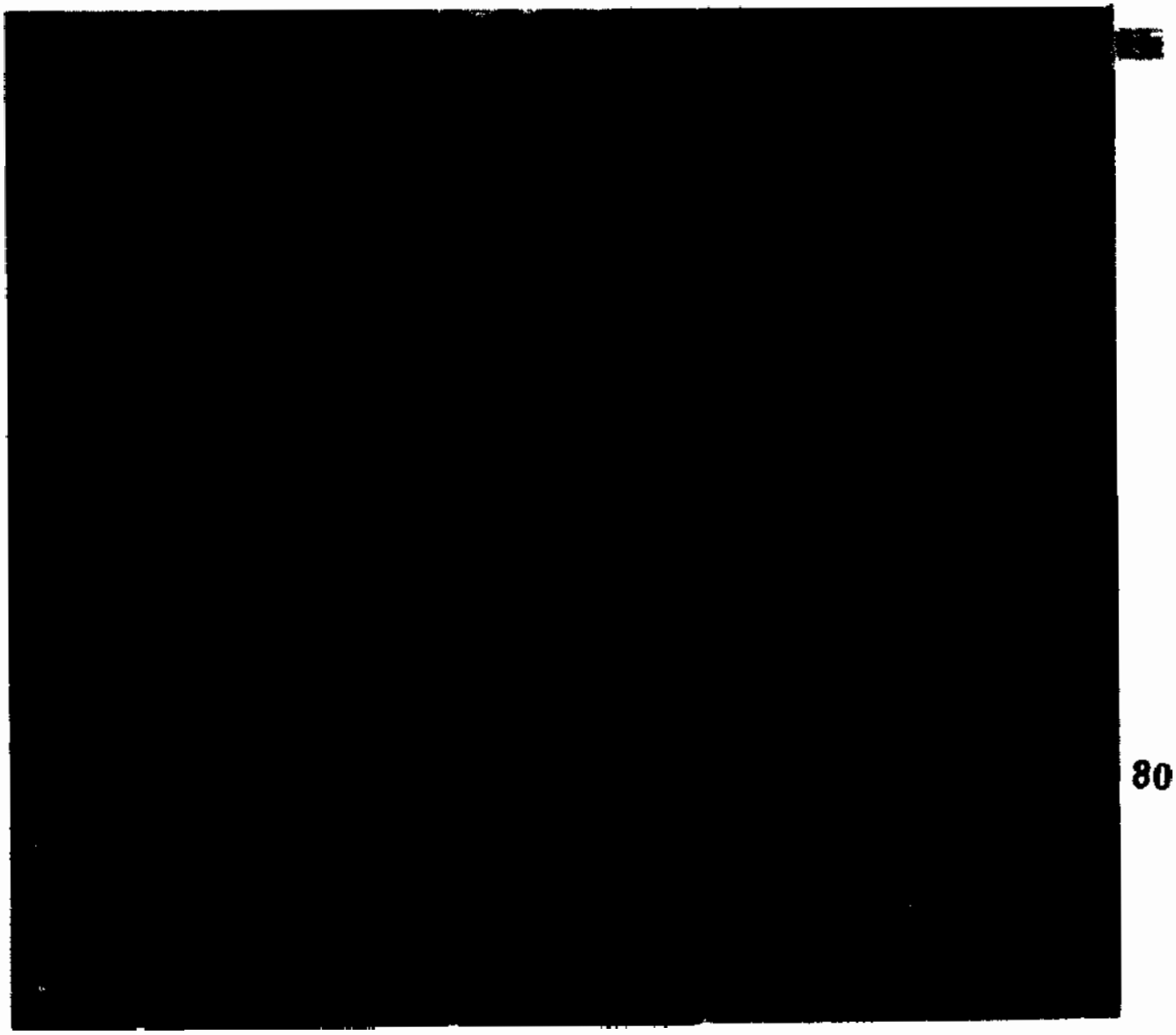

Figure 2A: *Sfi*I digested *T. parva* Melela DNA (lanes 1-4), separated by 1.5% agarose pulsed field gel electrophoresis at pulses of 10s, 400 volts for 16 h followed by 40s pulses, 200 volts for 5 h. Fragments range from 30 to 1800 kb, more numerous and intense than those reported for *T. parva* Muguga.

Figure 2B: Hybridization of *Sfi*I fragment of *T. parva* Melela and Muguga piroplasm genomic DNA with telomeric repeat probe. Lanes contain 6 μg DNA digested with 40 units *Sfi*I, electrophoresis by 1.5% agarose in 0.38x TBE under pulse field at 400v 10s pulses for 16h and 200v 40s pulses for 5h, transferred onto nylon filters which were hybridized with radiolabelled telomeric oligonucleotide DNA probe at 65°C for 16h, washed in 2x SSC, 0.2% SDS and 0.1% sodium pyrophosphate for 1h. Identical hybridization signals of *T. parva* piroplasm DNA are seen for Melela (lane 1 and 6) and Muguga (lane 2 - 5).

This indicates that the higher the infection rates in ticks, the higher the infectivity in cattle. The clinical syndrome caused by this parasite combines signs of *T. parva* cattle derived and *T. mutans* causing anaemia. In experimental cattle the parasite caused cattle derived ECF signs, with short course and massive piroplasm parasitaemia.

This stock was positive for schizont monoclonal antibodies (MAb) 1, 2, 3, 4, 10, 12 and 22, negative for 7, 15, 20, 21 and 23. Similar reactions were observed for *T. parva* Muguga and buffalo derived stock *T. parva lawrencei* (Serengeti transformed) (Young *et al.*, 1973). The resemblance of *T. parva* Melela to buffalo derived parasites suggest its possibility of being derived from buffaloes. This possibility exists because Melela is within reach of Mikumi National Park where thousands of buffaloes (*Syncerus caffer*) live.

The numerous piroplasms in the infected animals make its appropriate position into cattle rather than buffalo derived infection because the latter has fewer schizonts and piroplasms (Uilenberg, 1981).

There was a difference from *T. parva* Muguga on MAb 10 and 12 where the latter was negative. MAb 1-7 were raised against *T. parva* Muguga, 8-13 against *T. parva* Kiambu, 14-16 against *T. parva* Marikebuni 17-20 against *T. parva* Lawrencei and 21 (IL-S27) and 22 (IL-S23) against *T. parva lawrencei* (buffalo derived) (Pinder and Hewett, 1980; Irvin *et al.*, 1983; Newson *et al.*, 1986; Conrad *et al.*, 1987a). MAb 23 was raised against *T. taurotragi* (Kearney *et al.*, 1979).

Antigenic differences, however, occur between isolates as has been observed for buffaloes and cattle calves (Conrad et al., 1987a). The behaviour of isolates differ from animal to animal and even those from the same animal taken at different times. It is also known that there are always mixed populations of theilerial parasites. MAb 8,11, 12, 13 were reported to detected antigen on schizonts in isolates from buffaloes. This stock has a broad range of reactivity to different MAb suggestive of broad antigenic variations. It is however apparent that *T. parva* Melela has also a broad range of overlap with buffalo and cattle derived stocks.

Genomic DNA restriction fragment length polymorphisms

*Eco*RI digests of piroplasm DNA (Fig. 1A, 1B) do not differ very much from those of *T. parva* Muguga although polymorphisms are apparent (Fig. 1 B), with multiple restriction fragment bands. *T. parva* Melela appears to have more fragments that are small in size than the latter.

Tprl a repetitive element from *T. parva* genome detected by PMB 3 probe enables stock characterization (Baylis *et al.*, 1991; Bishop *et al.*, 1992; 1993). It appears in tandem arrays with an 8.1 kb sequence from one end. It is a 1.44 kb with open reading frames from 5' to 3' end. The region contains 4 large open reading frames, 2 with only *Tprl*, the third with *Tprl* and a 0.55 bp part (*Tpr2*) and the largest fourth with *Tprl* and a 422 bp element *(Tpr3)* located 5' of *Tpr2*. The final arrangement is a continuous element of *5'-Tpr3-Tpr2-Tprl-3'*. *Tprl* repetitive PMB3 probe produced hybridization signals at 2.0, 4.2, 4.3, 6.5, 9.4 and 9.5-23 on *Eco*RI digested piroplasm DNA for Muguga stock, 1 kb (Fig. 1A) and 9.4, 23.1 kb for *T. parva* Melela. This probably makes an important difference The *Tprl* hybridization pattern is identical to those of *T. parva* Muguga and some clones of buffalo derived *T. parva* DNA probed with another probe for *T. parva* Muguga (*pgTpM*-23) (Conrad *et al.*, 1989). Conrad and colleagues (1989) also

observed that antigenically distinct theilerial parasites in different lymphoblastoid cell clones show restriction fragment length polymorphisms but similar subclones derived from the same cloned cell lines have identical hybridization patterns to each. Further that there are phenotypic and genotypic differences between buffalo parasites because hybridization patterns produced with DNA from buffalo and cattle derived parasites produce considerable genomic diversities.

On hybridization of SSUrRNA gene clone pRIBSS1 to *Eco*RI *T. parva* Melela and Muguga digests indicated main signals at 4.3 kb (Fig. 1C) showing close similarities.

In pulsed field gel electrophoresis after digestion of low melting point agarose embedded DNA by *Sfi*I and separation by clamped homogenous electric field electrophoresis restriction fragment length polymorphisms were observed between *T. parva* Melela and *T. parva* Muguga. Digests of the latter are described by Morzaria and Young (1992). *T. parva* Melela contains two additional larger fragments of 1800 kb and 700 kb (Fig. 2A) than of *T. parva* Muguga shown by Morzaria and Young (1992). This indicates a larger genome (12-14 mb) than that of Muguga stock which has a 10 mb genome (Morzaria and Young, 1992).

Hybridization of telomeric repeat oligonucleotide DNA probe which detects telomeric sequences on *T. parva* to *Sfi*I blots identified one 80 kb fragment in Muguga and Melela stocks (Fig. 2B). Seven fragments were observed to produce hybridization signals in *T. parva* Muguga (Morzaria and Young, 1992).

This results of this investigation provides further evidence of the large variations in *T. parva* stocks. There are similarities of this parasite stock to *T. parva* Muguga on reactions to monoclonal antibodies. A wide overlap exists to both the reference Muguga stock and buffalo derived parasites. Some phenotypic and genomic differences, however, exist between them. The genome of *T. parva* Melela appears to be slightly larger than that of *T. parva* Muguga based on the fact that there are more large fragments than in the latter. The wide overlap with other stocks on monoclonal antibody profile makes this isolate a potential candidate for a vaccine in Tanzania especially when numerous breakthroughs are being reported after immunization with the Muguga stock (Mbassa and Silayo, 1995).

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