Characterization of Some Theileria parva Stocks from Zambia Using Monoclonal Antibodies

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\textbf{ABSTRACT.} Theileria parva parasites have been isolated from different locations in Zambia where malignant theileriosis has been recorded. A total of 16 bovine lymphocytic cell lines infected with T. parva schizonts were characterized using a panel of anti-schizont monoclonal antibodies (MAbs). Comparison of the Theileria stocks isolated before (old) and after (new) the Muguga cocktail of T. parva from Kenya was used to vaccinate cattle against theileriosis in Zambia revealed differences in their reactivity against MAbs. The new isolates are showing MAb profiles similar to that exhibited by the Muguga cocktail which was used to vaccinate cattle in these areas between 1983 and 1989. These results suggest that the use of the Muguga cocktail to vaccinate animals against theileriosis in Zambia may have introduced Theileria stocks of different antigenic properties. — \textbf{KEY WORDS:} immunization, monoclonal antibody, Muguga cocktail, Theileria parva.


Two Theileria parasites, \textit{T. annulata} and \textit{T. parva} cause severe clinical disease in cattle in Africa, Asia and the Middle East. The most economically important species in Africa is \textit{Theileria parva}. \textit{T. parva} parasites cause diseases that are of considerable economic importance in Zambia and other countries in East, Central and Southern Africa. In Zambia, theileriosis manifests itself in two forms namely Corridor disease (CD) and East Coast fever (ECF). CD appears in Southern, Central, Lusaka and Copperbelt provinces while ECF is found in the Northern and Eastern provinces (Fig. 1). Cattle can be immunized against ECF and CD by an infection and treatment method [9, 19]. However, immunity induced using a particular \textit{T. parva} stock may not necessarily confer protection against challenge with other \textit{T. parva} stocks [6, 19, 20]. This fact makes it necessary to characterize the existing \textit{Theileria} parasites from areas where this disease is prevalent before choosing a particular stock for use in the infection and treatment method. Several researchers have attempted to characterize \textit{Theileria} parva stocks using \textit{in vitro} methods such as monoclonal antibody (MAb) profiles [15] and the polymerase chain reaction (PCR) amplification of Tpr 1 repetitive DNA sequences [1]. Here we used \textit{T. parva} antischizont MAbs obtained from the International Livestock Research Institute (ILRI) to characterize some \textit{T. parva} stocks obtained from different locations in Zambia after the "Muguga cocktail" was used to vaccinate cattle against theileriosis in Southern and Central provinces of Zambia. The Muguga cocktail is a concoction of 2 Kenyan bovine

\textit{Theileria} stocks and a buffalo-derived \textit{Theileria} stock from Tanzania passaged in cattle several times in Kenya. The old Zambian stocks were isolated by the Food and Agricultural Organization (FAO) between 1982 and 1986 and by the Belgian Animal Disease Control Project (ASVEZA) between 1985 and 1989. We then compared the MAb profiles of the new isolates obtained after 1994 with those of the old Zambian \textit{Theileria} stocks which were isolated before the cocktail was used. All the old Zambian

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**Theileria** isolates were previously characterized by ILRI.

**MATERIALS AND METHODS**

**Cattle:** The cattle used were either experimentally infected in the laboratory or were field clinical cases. The animals which were used for experimental infections were bought from farms with no history of theileriosis. They were in addition screened for *Theileria* antibodies and only those found negative were used.

**Origin of *Theileria parva* cultures:** Old *T. parva* stocks were obtained from the Belgian project designated Lundazi, Katete, Langa, Genda, Zambia 2, Zambia 3, Zambia 5, Zambia 22 and Zambia 23 (Fig. 2). The Zambia 2, 3, 5, 22, and 23 were provided in the form of cell lines in culture while the Katete, Lundazi, Langa and Genda stocks were supplied as stabitate forms and therefore had to be passaged through clean calves from which cell lines were established [3]. Some of the new *T. parva* stocks were isolated from infected cattle in the field and were named according to their place of origin (nearest town, village or farm) while others were isolated from experimentally infected cattle in the laboratory as described by Cunningham et al. [5]. *Theileria* infected lymphoblastoid cell cultures were then established with infected lymphoid cells from the blood and lymph node samples from the infected cattle by the method of Brown [3]. Table 1 shows the old and new Zambian *Theileria* stocks used in this study.

**Source of monoclonal antibodies:** MAb s were obtained from ILRI, Nairobi, Kenya. They were macroschizont-based. MAb s 1, 2, 3, 4 and 7 were derived from mice inoculated with *T. parva* (Muguga)-infected bovine lymphocytes [18]. MAb s 10 and 12 were raised against *T. parva* (Kiambu 5), while 15 was raised against *T. parva* Marikebuni [15]. MAb 20 was raised from mice inoculated with lymphocytes infected with a buffalo-derived *Theileria* parasite [17, 18].

**Preparation of antigen:** Antigen slides were prepared from cultures infected with schizonts by a method similar to that described by Goddeeris et al. [10]. Briefly, two hundred milliliters of cell culture containing approximately 2 x 10^6 cells were centrifuged at 200 g for 10 min, and the cells were washed twice, and cell counts and viability were determined in 1% trypan blue solution. The percentage of schizont-infected cells was determined at the time when the antigen smears were being prepared. It was found that 75–85% of the cells were infected with *Theileria* schizonts as determined by microscopic observation of Giemsa stained smears. An equal volume of fixative (3.7% formaldehyde in phosphate-buffered saline, PBS at pH 7.2) was added drop by drop with gentle stirring. Cells were then centrifuged at 200 g for 5 min, washed 3 times, resuspended in 2 ml of PBS, and adjusted to give 1 x 10^6 cells per ml. Cells were distributed onto Wellcome PTFE Multispot slides (C. A. Hendley, Essex, UK) by adding one drop to each well and immediately sucking off the liquid and used as antigens for indirect fluorescent antibody (IFA) test.

**Test procedure:** The IFA test was carried out based on the procedure described by Goddeeris et al. [10]. Multispot antigen slides prepared from the *T. parva* infected cells as described above were tested. The first two wells on each slide were used as positive and negative controls. *T. parva* Lusaka-infected lymphoblasts were served as positive control. MAb s were diluted in PBS to 1:200, and further two-fold serial dilutions were made. Rabbit anti-mouse IgG conjugated with fluorescein isothiocyanate (Miles Laboratories, Slough) was diluted at a concentration of 1:1000 in PBS containing 0.01% Evans blue. Finally, slides were examined for fluorescence using a fluorescence microscope.

The reaction was regarded as positive case when fluorescence was observed at dilutions greater than 1: 200 of MAb s. In this paper, when MAb s gave fluorescence at dilutions of 1: 200 to 1: 400, 1: 400 to 1: 800, or greater than 1: 800, the reactions were recorded as mild, medium, or strong positive reaction, respectively.

**RESULTS**

The MAb profiles of the 7 newly isolated *Theileria* -infected cell lines plus those of the previously established cell lines are shown in Fig. 2. They also differed markedly with the MAb profiles of the old stocks from Eastern and Southern provinces which were previously characterized by ILRI (Fig. 2). The old stocks still gave negative reactions with MAb s 2, 3, 15, and 20. Although the MAb profiles for the newly isolated stocks from Southern, Central and Lusaka...
provinces were similar in that they all gave positive reactions with MAb 2, 3, 15, and 20, only 60–70% of the schizont-infected cells reacted with MAb 2, 3, 15, and 20. Even though the new stocks from Southern, Central and Lusaka provinces showed similar MAb profiles reacting with MAb 2, 3, 15, and 20 in addition to those reacting with the old ones, the intensity of their reactions differed remarkably. The Mumbwa (Central province) and the Lusaka (Lusaka province) isolates showed strong reactions with almost all the MAb they reacted with in general. The Kabwe East & Chisamba (Central province) isolates showed medium reactions while the Ufwenuka, Sikabenga and Magoye group showed mild positive reactions.

**DISCUSSION**

The results obtained from this study using *Theileria* anti-schizont MAb reveals antigenic diversity among Zambian *Theileria* stocks. The results also revealed that the stocks newly isolated in the Southern province reacted differently from old stocks, and that *Theileria* stocks from the Central and Lusaka provinces exhibited similar reacting profiles as those of stocks from the Southern province.

The observation that certain Zambian isolates exhibited different reactions with MAb (mild, medium, and strong reactions) and that specific MAb reacted with only a portion of the schizont-infected cells indicate that these isolates may contain mixed populations which differ in their expression of antigenic determinants on schizonts. Similar reactions with anti-schizont MAb were observed in some Zimbabwe *Theileria* isolates [13] and in some *Theileria* isolates from Kenyan buffalo which were found to consist of a number of distinctly different populations of *Theileria* parasites after cloning in vitro [4]. One of the major reasons for this change in character of the Southern stocks could be the characteristic carrier state of *Theileria* parasites in their mammalian hosts as described by Maritim et al. [14]. *T. parva* induces carrier state in cattle [8, 15]. Recovery from *T. parva* infection either naturally [2, 21] or following immunization [7] or treatment [8], may result in a persistent transmissible infection. Maritim et al. [14] also reported that after the infection and treatment method the MAb profiles of parasites from persistent infected animals appeared to be similar to the original immunizing stock. This finding is being confirmed by our results in that the isolates from the Southern and Central provinces are showing a binding profile similar to that of the original immunizing stock (Muguga cocktail). Since the cocktail was used in these provinces, the disease has spread rapidly and has even gone beyond its original borders into the Lusaka and Copperbelt provinces [16]. As already stated in this paper the disease in the Southern and Central provinces was originally diagnosed as CD, but one wonders the validity of this term. All the Zambian isolates were put in group B of *T. parva* together with *Theileria* stocks from other countries which did not react with MAb 2, 3, 15 and 20 [11, 12]. In the advent of this change in character there may be need of testing the MAb profiles of *T. parva* parasites in the other countries especially in those countries where foreign stocks were used in the immunization programs. Dolan [9] stated that although the cocktail provides protection against most isolates its use in other
areas other than those from which the isolates were made carries with it the potential danger of introducing parasites of an antigenic type against which local cattle may have no immunity. For this reason the use of locally isolated stocks in the immunization programs is strongly advocated.

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REFERENCES


