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Isolation and Morphological Study of *Trypanosoma (Megatrypanum) theileri* from Bovine Peripheral Blood and Spleen in Japan

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Abstract

In Aomori Prefecture, the northern district of Japan, *Trypanosoma* (*Megatrypanum*) *theileri* was detected from the primary culture of peripheral blood and splenic cells in the bovine animals at 42.9% and 31.5%, respectively. Upon Giemsa's staining the growth types of tripomastigote, epimastigote, promastigote, amastigote and fission forms, were recognized. Under the scanning electron microscope, well-defined the flagellum and the undulating membrane were observed. Under the transmission electron microscope it was noted the flagellum consisted of the axial filament with 2 central fibers and 9 pairs of peripheral fibers. Growth of flagellum was closely related to the kinetoplast and the basal body. Further, deficiency of the subpellicullar fiber, the characteristic structure of *Trypanosoma*, was clearly observed.

Key words: *Trypanosoma* (*Megatrypanum*) *theileri*, bovine, peripheral blood, spleen

Introduction

Trypanosoma (*Megatrypanum*) *theileri* (*T.*(*M.*) *theileri*) is a protozoon which was discovered in 1902 by Southern African Theiler from peripheral blood of healthy cattle (Theiler, 1903). The distribution of this protozoon is extremely wide, and it has been reported in various parts of the world such as in United States (Schlsfer, 1979; Woo *et al.*, 1970), Canada (Cross *et al.*, 1971), England (Wells *et al.*, 1968) and Africa (Schlsfer, 1979).

We investigated its morphology of *T.*(*M.*) *theileri* and distribution of infecting states in Aomori Prefecture, Japan, and happened to find this protozoon in the lymphocyte culture of persistent lymphocytosis.

Materials and Methods

Peripheral blood samples (20 cases) and spleens (114 cases) were collected from adult cattle (4–9 years old) separately and one of persistent lymphocytosis in Towada meat center of Aomori Prefecture in June–September, 1994.

Cell culture: Leucocyte layer obtained from the blood in a test tube containing EDTA and spleen cells collected with aseptic conditions were cultured in the MEM solution (Mcholland-Raymond *et al.*, 1978), and cultured for 3 days.

Optical microscopy: Cultured cells were observed by Giemsa staining after a thick layer smear.

Scanning electron microscopy: Cultured cells centrifuged at 1,800 rpm for 5 minutes at 4°C, and were prefixed with a mixture of 1.5% paraformaldehyde/0.5% glutalaldehyde in phosphate buffer. Further, they were placed on a glass slide that had been treated by poly-L-lysine (SIGMA Co., Ltd.) and postfixed in 1% osmic acid. Then, dehydration by alcohol/isoamyl acetate replacement and critical point desiccation were carried out, and the specimens were processed for platinum shadow casting, and observed with a Hitachi HS-450 type scanning electron microscope.

Transmission electron microscopy: After the prefixation mentioned above, cultured cells were postfixed in buffered 0.1 M cacodylic acid solution (pH 7.4), and they were embedded in the epoxy resin after dehydration by alcohol. Ultrathin sections were double stained by uranyl acetate and lead citrate and

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observed by Hitachi HS-8 type electron microscope.

Results

The infection rate of *T.(M.)theileri* was 42.9% (9/21) including of one persistent lymphocytosis cattle in the peripheral blood and 31.6% (36/114) in the spleen. The isolation rate of the protozoon showed a tendency to be higher within 24 hours of culture, but though quite rarely *T.(M.) theileri* was isolated on the first day and after the fifth day of culture. Once *T.(M.)theileri* was isolated, it continued a vigorous proliferation by MEM exchange. Moreover, no difference was observed in the monthly isolation rate from June to September.

Optical microscopical findings: The following five stages were observed (Figs. 1 and 2).

a) Trypanosoma stage (trypomastigote form): The size was large, and the anterior end and the posterior end were sharp, and one nucleus located near the center of the body had a circular or oval form. Kinetoplast (KP) was located at the posterior side of the nucleus, and flagella which grew from KP formed the undulating membrane along the side of the body. Further, free flagella were observed at the

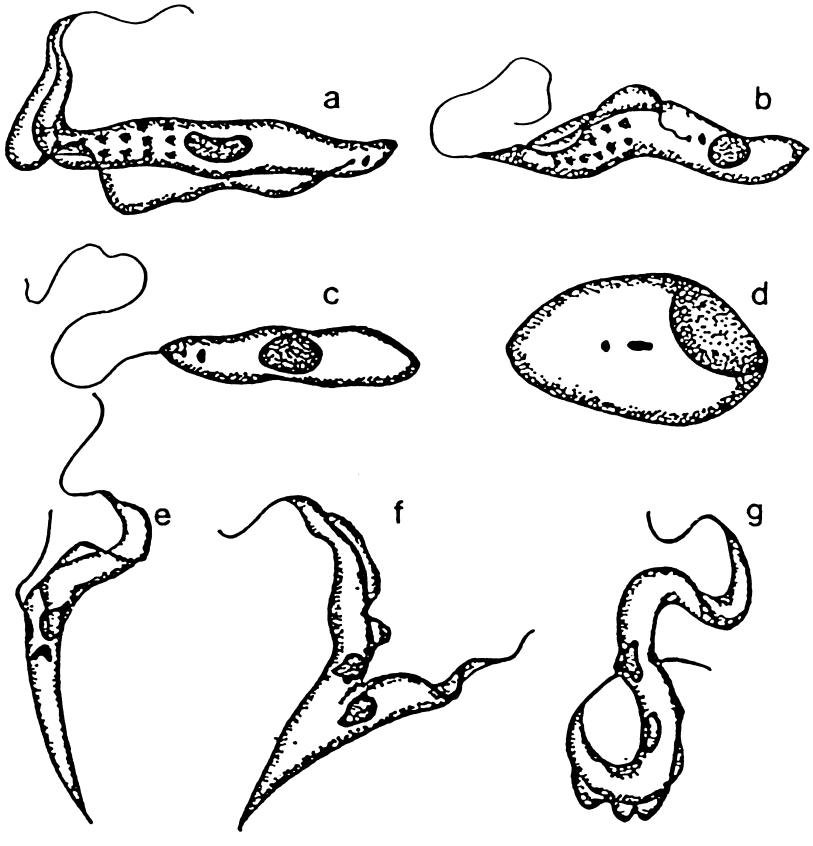

Fig. 1 Schematic diagram of *Trypanosoma* (*Megatrypanum*) *theileri*.
a–d: developmental stage of *T.(M.) theileri*
a: Trypanosoma stage (trypomastigote form)
b: Crithidial stage (epimastigote form)
c: Leptomonad stage (promastigote form)
d: Leishmanial stage (amastigote form)
e–g: Fission form

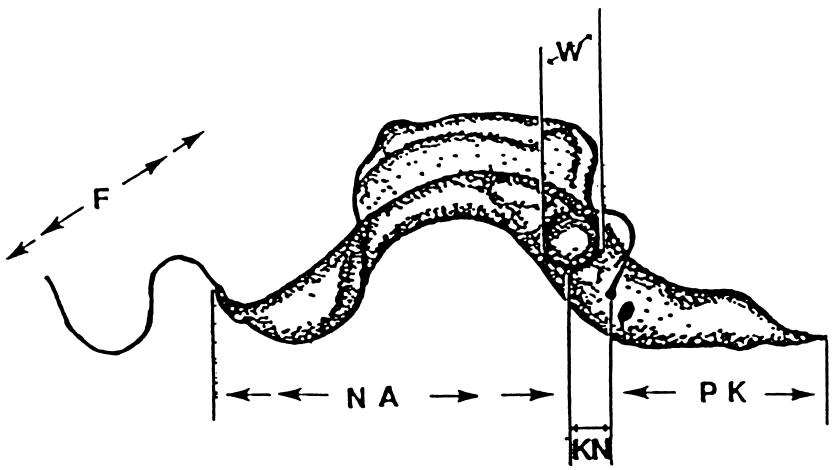

Fig. 2 Morphometric parameters of *T.(M.) theileri* from culture.
Total length: 43.9±12.9 (22.7–84.0) μm
Body length: 24.6 (14.4–45.9) μm
PK: distance from the posterior end to the kinetoplast = 7.6±3.7 (2.2–16.3) μm
KN: distance from the kinetoplast to the middle of nucleus = 5.1±2.4 (2.3–12.5) μm
NA: distance from the nucleus to the anterior end = 12.0±4.7 (4.6–26.4) μm
F: length of the free flagellum = 19.4±6.7 (8.3–33.9) μm
W: Width = measured through the center of the nucleus, perpendicular to the long axies = 2.0±0.7 (0.9–3.4) μm
PN: PK+KN = 12.7±4.7 (5.0–25.3) μm
Nuclear index: PN/NA = 1.2±0.6 (0.57–2.69) μm
Kinetoplastic index: PN/KN = 2.8±1.0 (1.25–5.78) μm

anterior end.

b) Crithidial stage (epimastigote form): This form was recognized most widely in the culture period. The development of nucleus and free flagella was the same as that of the trypanosoma stage, but KP moved to the vicinity of the nucleus, and flagella which grew from this site formed the undulating membrane.

c) Leptomonad stage (promastigote form): This resembled the crithidial stage, but the formation of undulating membrane was not recognized at this stage.

d) Leishmanial stage (amastigote form): This form showed a tendency that it was recognized under inferior culture conditions. KP was located around the nucleus, and it lacked flagella and undulating membrane.

e) Fission form: This was frequently observed in the trypanosoma and the crithidial stages. Binary fission first began at the anterior end of the body, and progressed to flagella as well as KP, further to the division of nucleus.

Scanning electron microscopical findings: The size of *T.(M.) theileri* showed a rather decreasing tendency from that of a smear specimen (20–55 μ). The inner structure of nucleus and of KP was unclear, but the anterior and posterior ends were sharp, and developed flagella, undulating membrane, and fission form were clearly observed (Fig. 3).

Transmission electron microscopical findings: The observation was made to the crithidial stage. There was a well developed cell membrane, and the periplast (PP) existed underneath. The PP was composed of tubular subpellicular fiber that had a constant interval in between. The nucleus was covered with a doublefold membrane, and equipped with one to several nuclear bodies. Moreover, KP, lamellar body (LB), and mitochondria (M) were recognized. Further, flagella (F) which was one of the most characteristic forms of *Trypanosoma* was com-

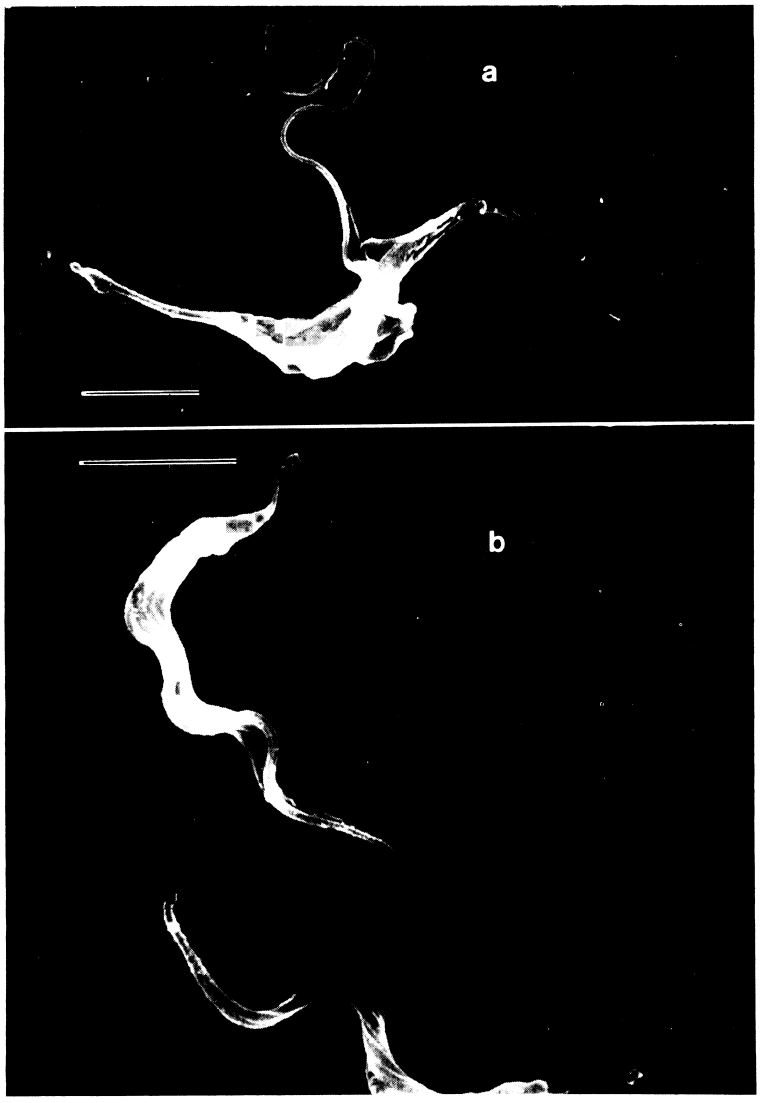

Fig. 3 Scanning electron micrograph of fission form (a) and trypomastigote form (b). Bar = 5 μm.

posed of axial filaments (AF) and formed from two central fibers to nine pairs of peripheral fibers. At the outside of cells, an undulating membrane that was enclosed with trilamellar membrane was observed as a flagellar sheath. On the other hand, we also often encountered an image in which flagella grew from flagellar pockets (FP). In this case, KP and basal body (BB) were found. Moreover, subpellicular fibers were not recognized under periplast of FP. Besides that, extremely large numbers of ribosome and well developed Golgi apparatus, rER and sER were observed in the cytoplasm. Further, figure of division was also recognized (Fig. 4).

Discussion

The infection rate of *T.(M.) theileri* in bovine in Aomori Prefecture in Japan was 42.9% in the peripheral blood, and 31.6% in spleen. The morphology of protozoa detected from the culture of periph-

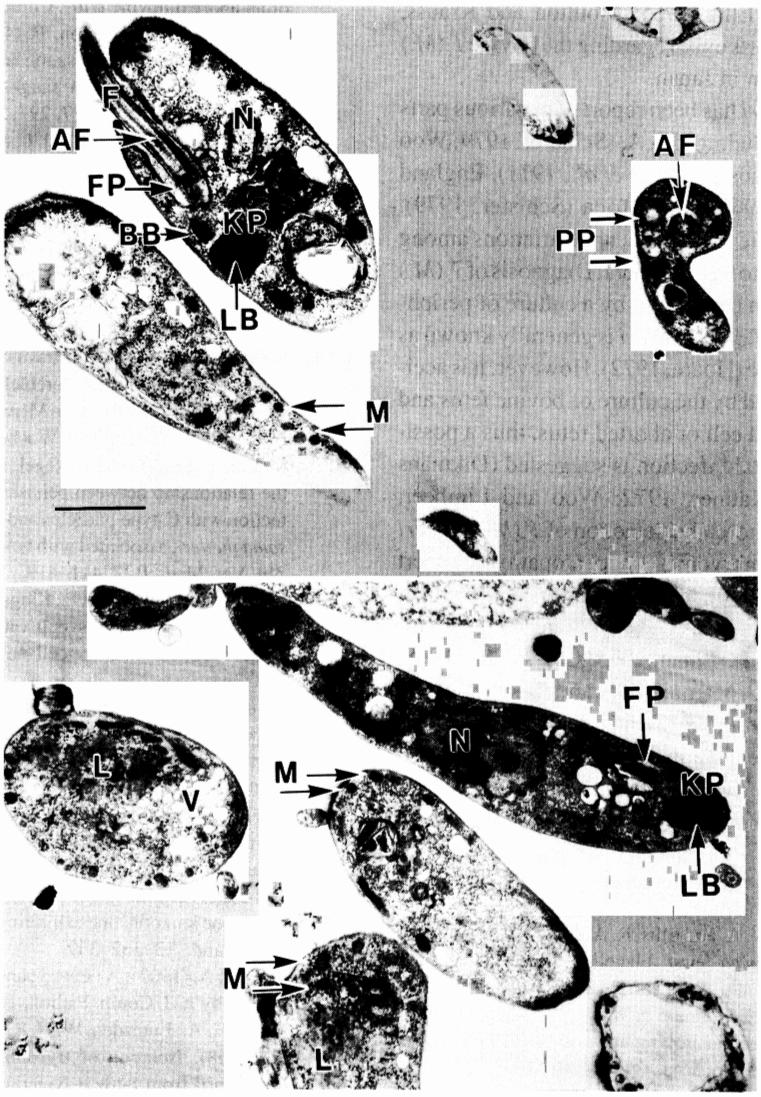

Fig. 4 Transmission electron micrograph of crithidia. PP: periplast, KP: kinetoplast, LB: lamellar body, N: nucleus, M: mitochondria, F; flagella, AF: axial filament, FP: flagellar pocket, BB: basal body, L: lipid, V; vesicle. Bar = 1 μm.

eral blood and spleen was similar to that of *T.(M.) theileri* in many aspects (Hoare and Wallare, 1966; Mcholland-Raymond *et al.*, 1978; Schlsfer, 1979). The frequency of protozoa in each developmental stage was mostly composed of that of trypomastigote and epimastigote form, but due to subculture, other stages showed a tendency to be more easily found along with the frequent divisions (Mcholland-Raymond *et al.*, 1978). This suggests the vital growth ability of this organism. Reports regarding the fine structure of *T.(M.) theileri* are few. In this examination, the growth of flagellum was observed with an extremely close relationship with KP and basal body (Moulton and Krauss, 1972), and the lack of subpellicular fibers that is the characteristic structure of *T.(M.) theileri* was clearly recognized

(Anderson and Ellis, 1965; Moulton and Krauss, 1972). Few reports exist regarding the bovine *T.(M.) theileri* infection in Japan.

T.(M.) theileri has been reported in various parts of the world including U.S.A. (Schlsfer, 1979; Woo *et al.*, 1970), Canada (Cross *et al.*, 1971), England (Wells *et al.*, 1968), and Africa (Schlsfer, 1979). The infection rate is 10–90%, and variations among territories seem to be prominent. Diagnosis of *T.(M.) theileri* has been carried out by a culture of peripheral blood, and *T.(M.) theileri* is generally known as nonpathogenetic (Hoare, 1972). However, it is accidentally detected by the culture of bovine fetus and embryonic renal cell of aborted fetus, thus a possibility of placental infection is suggested (Dikmans *et al.*, 1957; Kaliner, 1972; Woo and Limbeer, 1971). Furthermore, the detection of *T.(M.) theileri* from bovine embryonic encephalopathy (Grunert and Anderson, 1970), meningoencephalitis (Kaliner, 1972), bovine leukosis and persistent lymphocytosis (Chander and Gilman, 1975; Cross *et al.*, 1968; Hare *et al.*, 1970; Mammerickx and Dekegel, 1975; Strandstrom *et al.*, 1972) has been reported. Although the present study reported the incidence and morphology of *T.(M.) theileri*, further detailed investigations are necessary regarding pathogenicity.

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