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SHORT REPORTS

A PRELIMINARY OBSERVATION ON THE PATHOGENIC PROTOZOA *PIROPLASMA BIGEMINA* IN THE CATTLE FROM TAIWAN[1]

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Yien-Shing Chow, Yu-Hua Chen, Pen-Ching Lin and Shen-Chang Wung (1983) A preliminary observation on the pathogenic protozoa *Piroplasma bigemina* in the cattle from Taiwan. *Bull. Inst. Zool., Academia Sinica* **22**(2): 255-259 An endoparasitic protozoan *Piroplasma bigemina* was identified from the red blood corpuscles of cattles infected with red water fever. The protozoa probably caused the normal red corpuscles with irregular shape under the scanning electron microscopic observation. The irregularity probably was a protrusion of the corpuscle membrane. More than 2 protozoans within a red blood cell were observed. Similar microorganisms were identified in the salivary glands of the tropical cattle tick *Boophilus microplus*, which probably is the vector of the parasite.

In Taiwan, protozoan diseases, such as trypanosomiasis, piroplasmosis and anaplasmosis of cattle have been reported (Chang, 1975; Cheng, *et al.*, 1982; Liu, 1981; Su, *et al.*, 1950). Under the light microscopic studies, large protozoa such as *Piroplasma bigemina* (2 $\mu \times 1$ μ) and small protozoan *Theileria mutans* (1.5 $\mu \times$ 0.4 μ) could be differentiated by their dimensions. Although *Babesia bovis* from European origin are similar to *P. bigemina* in size, *B. bovis* do not cause pear-shaped protozoa in infected blood smear sample (Lery and Ristic, 1980). Here we would like to report our studies of the external morphological features of the parasite within the red blood cells from the cattle infected with red-water fever.

MATERIALS AND METHODS

The adults of tropical cattle tick *Boophilus microplus* were fed in a lace-like cloth fastened on a cattle ear for 10 days. (Hsieh, *et al.*, 1982) When the body temperature (40.5°C) of the cattle went higher than the normal level (39°C), blood samples were drown from ear vessels, and

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blood smears were prepared. Slides were stained with Wright's or Giemsa's stain at PH 6.4–6.7 and methylene blue (Chow, *et al.*, 1972; Chow, 1981). The slides were then examined under a light microscope. If the protozoa suspected was found, the sample was further placed on a large specimen stubs by means of double cellotape and coated with gold in a vacuum evaporator. Micrographs were taken with a Hitachi 450 scanning electron microscope at an acceleration voltage of 15 K. V. Micro-techniques for transmission electron microscopy of the salivary gland of tick were similar to the previous report (Chow, 1981).

RESULTS AND DISCUSSION

Under light microscope, the blood smears prepared with methylene blue were better that Wright's or Giemsa's stain. One of the pear-shape protozoan obtained with methylene blue is presented in Fig. 1a (arrow). Since this protozoa is a divergent, club-shaped organism, measuring about 3 μ long and 1 to 2 μ wide, it was thus identified as the large protozoa *P. bigemina*. Under scanning electron microscope, this protozoa lies either superficially in the blood corpuscle or on the surface, causing the irregular shape of the red blood cells as shown in Fig. 1b. The irregularity could be best viewed by higher magnification (Fig. 2a and b) as a protrusion of the corpuscle membrane. The geometric configuration should be taken into account when one views the measurement of the size of the parasite in the electron-micrograph. From this point of view, the observers feel that the longitudinal axis of the parasite is shorter than the red blood cell, but actually it is not. This is what happens when a 3 dimensional configuration was observed from a 2 dimensional view. Besides, more than one microorganisms were found in our electron-micrograph (Fig. 2b). Due to the possibility of finding a multiform was greater than finding a pear-shape protozoa, we propose that this protozoa multiples inside the cattle red blood cells with binary fission is quite common. So, with 2 binary fissions before the breakage of the host cell, a four organisms would be resulted (Fig. 2b). Four merozoites arranged in the pattern of a Maltess cross have been reported in *Babesia equi* (Arthur, 1962). But it's role in the asexual reproduction of protozoa is not yet known (Wilson, 1982). Usually, normal red corpuscles are larger than those being parasitized (Fig. 2b). This phenomenon will give good advantage for diagnosis of the disease for the suspect cattle.

After careful examination of the ultra-structure of the tick tissues, only the salivary gland had protozoan-like organisms. In Fig. 2c, one arrow near the right bottom indicates a suspect protozoa. The organism measured about 3 μ long and 1 μ wide, resembling that of observed by light microscope (Fig. 1a). In the electron-micrograph of a glandular tissue (Fig. 2c), large nuclei and many microvilli could be found easily. Besides, many small spherical bodies existed in the intracellular space as pointed out by arrow in the left-hand side (Fig. 2c). The organism probably was the cross sections of a protozoa, since their size was smaller than those of tick haemocytes (Chow, 1981). The organism shown here also resembles the rickettsia casual agent of *Anaplasma marginale* in appearance. Due to the protozoa *P. bigemina* has many reproductive stages within the vector's tissues (Fig. 2c) which probably is a developmental stage of such protozoa. Because this is a first scanning electron micrograph of the protozoa *P. bigemina*, the quality of our micrograph is still to be improved, but tick as the vector of piroplasmosis is certained from the present observation.

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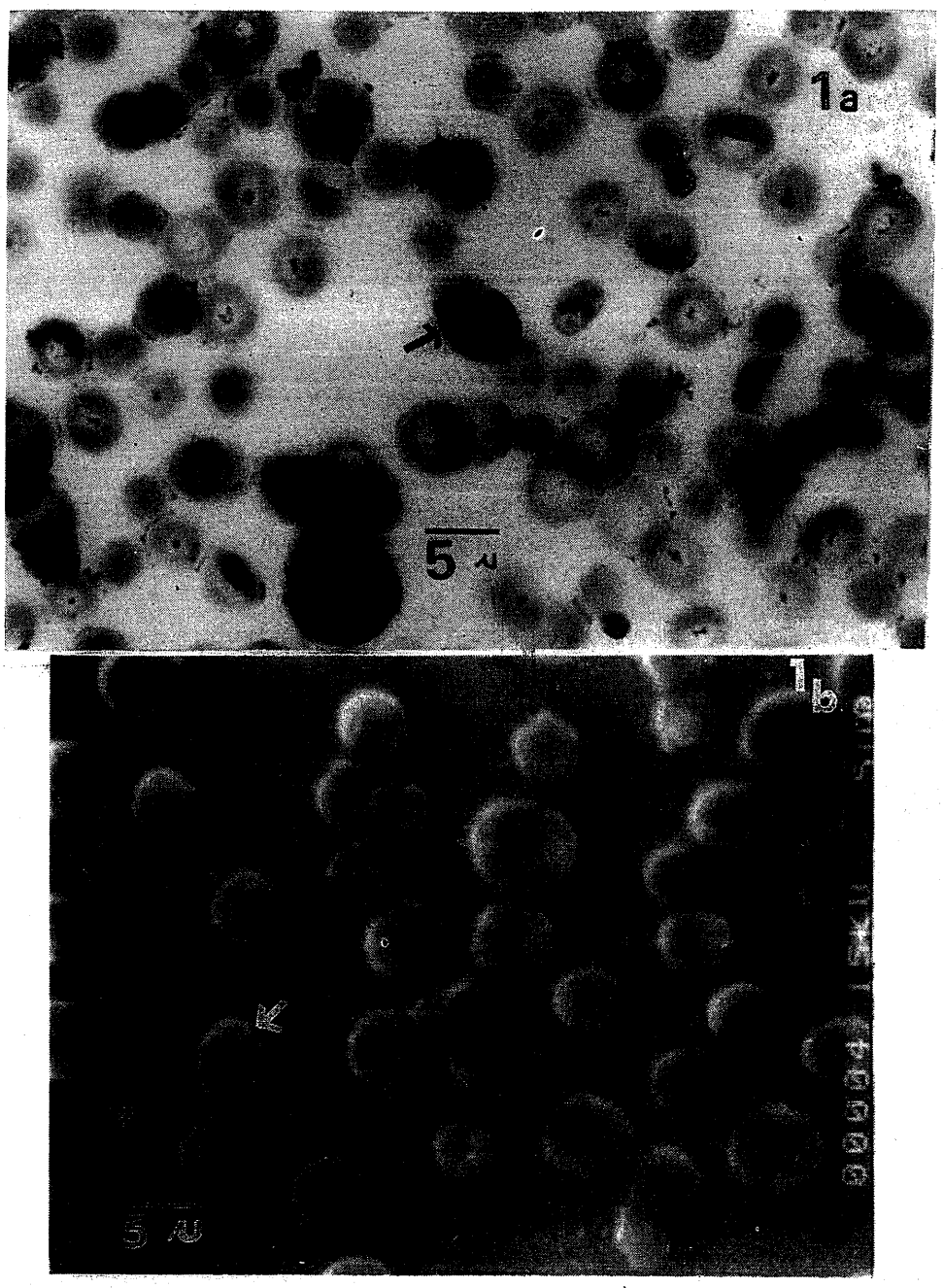

Fig. 1. Micrographs of the parasitic protozoan in the red blood cell of cattle. a, photomicrograph of a blood smear showing a pear-shape *Piroplasma bigemina* (arrow); b, scanning electron micrograph of the above slide, arrow showing the *P. bigemina*.


2b
N
1.0 μ
2c

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臺灣牛隻焦蟲病的初步觀察

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自本省飼養之荷蘭乳牛耳動脈取血做紅血球塗片時，有時可發現原蟲寄生，此原蟲是大型焦蟲 *Piroplasma bigemina*。如用掃描電子鏡觀察時，此焦蟲可使紅血球變形，使紅血球的細胞膜向外突出，被多於兩個焦蟲寄生的血球亦曾被觀察到；而此焦蟲的類似個體，在牛壁蝨的唾液細胞中亦發現甚多。

Fig. 2. Enlarged view of the protozoa *P. bigemina.* a, scanning electron micrographs of the pear-shape protozoan; b, scanning electron micrographs of a parasitic cell with 4 trophozoites and a normal red blood cell; c, ultrastructure of a tick salivary gland showing the suspect protozoan (arrow) and many intracellular inclusion bodies within the tissues.