Morphology of *Babesia gibsoni* in Canine Erythrocytes

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The causative agents of canine babesiosis are *Babesia gibsoni* and *B. canis* in Japan [8]. The former species has been reported polymorphic [1, 2, 3, 4, 7], but no precise morphological study on the species has been made.

We examined polymorphic *B. gibsoni* organisms in the peripheral blood by light microscopy to classify them into forms. If necessary, some forms of the organisms were additionally observed by transmission electron microscopy (TEM). The *B. gibsoni* strain used was originated from a naturally infected dog at Beppu in Ohita Prefecture and has been passaged through splenectomized dogs in our laboratory. The parasites were intravenously inoculated into 10 dogs at a dose of 10^9 – 10^{10} parasitized erythrocytes. After inoculation, smear specimens were prepared with peripheral blood from day 1 to day 10–22 after inoculation, and the day before the death of dogs. The specimens were stained with Giemsa and examined for *B. gibsoni* organisms. The blood samples for TEM were fixed with a mixture of 1.25% glutaraldehyde and 1% osmic acid in an ice bath at pH 7.2 for 2 hours and embedded with Epon 812 after dehydrated in a series of ascending concentrations of ethanol. *B. gibsoni* organisms could be classified into six forms: the ring form, paired pyriform, petaloid form, amoeboid form, reticulate form and dot or comma form, but some of them could not be classified into any of these forms. The ring form, $2.71 \pm 0.55 \times 1.61 \pm 0.51 \mu\text{m}$ in size ($n=30$), had the nearly round protoplasm (Fig. 1) and was mostly detected in the initial stage of infection. The paired pyriform (Fig. 2) was composed of two ring-form organisms, $2.10 \pm 0.47 \times 0.94 \pm 0.25 \mu\text{m}$ in size ($n=30$), which were attached to each other at the apical end, and was seen in small numbers from day 14 after inoculation to the time of death of dogs. The petaloid form (Fig. 3) consisted of more than three ring-form organisms, $2.42 \pm 0.25 \times 0.92 \pm 0.22 \mu\text{m}$ in size ($n=30$),

which were attached to each other at the apical end, and was detected in smaller numbers than the paired pyriforms after day 14 after inoculation. The amoeboid form (Fig. 4), 3.79 (range 2.46 – 7.24) (*) $\times 2.66 \pm 0.79 \mu\text{m}$ in size ($n=30$; * normality can be denied with $P < 0.05$, in this case), was detected after day 12 after inoculation together with the paired pyriform and petaloid form with higher frequencies than these two forms. The reticulate form (Fig. 5) consisted of 2–8 organisms which had a single or a few spherical or kidney-shaped chromatin dots and were connected to each other by the net-like parts. The reticulate form was hardly detected except in the terminal stage of infection and occupied most part of the cytoplasm of infected erythrocytes, the component organisms measuring $2.34 \pm 0.56 \times 1.71 \pm 0.40 \mu\text{m}$ in size ($n=30$) excluding the net-like part. By TEM, this form had many ribosomes, a single nucleus, rhoptry-like and microneme-like structures, a single rough-surfaced endoplasmic reticulum and a cavity of low electron density in addition to the constricted cytoplasm to take an elongated form (Fig. 8). The reticulate form was detected in 7 of 10 days with serious parasitemia, and increased in number in the course of infection. Many ring forms were also recognized with the reticulate form. The dot form, $0.83 \pm 0.14 \times 0.64 \pm 0.13 \mu\text{m}$ in size ($n=30$), (Fig. 6), or comma form, 1.69 (range 0.97 – 3.54) (*) $\times 0.53 \pm 0.16 \mu\text{m}$ in size ($n=30$), (Fig. 7), had the reddish chromatin dots and were detected on day 10 or so after inoculation. A few organisms, however, could not be classified into any of the above forms (Fig. 1).

Fowler *et al.* [1] classified *B. gibsoni* organisms into five forms: the young ring form, signet ring form, band or rod form, paired pyriform and multiple form. Compared with the present results, these five forms corresponded to the dot or comma form, ring form, paired pyriform, petaloid form and amoeboid form of the present study respectively, but no form corresponded to the reticulate form. Mimori *et al.* [5] observed the binary fission stage of *B. gibsoni* in the erythro-

Fig. 1-7. Endoerythrocytic forms of *B. gibsoni*.
 A: amoeboid form, C: comma form, D: dot form,
 PA: paired pyriform, PE: petaroid form, R: ring
 form, RE: reticulate form, U: un-classified form,
 Giemsa stain. $\times 2000$.

Fig. 8. Reticulate form $\times 12,900$. TEM.

C: cavity of low electron density, E: erythrocyte, ER: rough-surfaced endoplasmic reticulum, M: microneme-like structure, N: nucleus, R: rhoptry-like structure.

cytes and erythroblasts collected from the spleen, liver and bone marrow, but this stage of organisms seems to correspond to the paired pyriform in this study. The reticulate form will be in the early stage of development judging from the report of Potgieter [6] describing that the rhoptry and microneme disappear after the organism has matured. Consequently, *B. gibsoni* organisms in the peripheral blood are classified into six forms including the reticulate form which is first described in this report.

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要 約

犬赤血球内における *Babesia gibsoni* の形態(短報)：並河和彦・須永藤子・菅野康則(麻布大学獣医学部伝染病学数室)——*Babesia gibsoni* 人工感染犬の末梢血で、従来報告のない2～8個の虫体が網状物で連なる像(網状型原虫)が認められた。電顕観察では、核、多数のリボソーム、ロプトリー様構造物、ミクロネーム様構造物および粗面小胞体が認められた。