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Electron microscopic investigations on stages of dog piroplasms cultured in vitro: Asian isolates of *Babesia gibsoni* and strains of *B. canis* from France and Hungary

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**Abstract** Stages obtained from two Asian *Babesia gibsoni*-isolates cultured in vitro were studied by means of transmission electron microscopy and compared to strains of *B. canis* cultured in vitro. While the developmental stages of the latter preserved their shape in culture, many of the initially small stages of the *B. gibsoni* strains grew considerably and often looked rather similar to *B. canis.*

**Introduction**

Piroplasmosis of dogs and other canids is caused by two *Babesia* species: *B. canis*, a so-called large-sized species, and *B. gibsoni*, a small-sized species. While *B. canis* is known in regions worldwide, where its vector ticks (*Dermacentor marginatus, D. reticulatus, D. variabilis, Rhipicephalus sanguineus*) occur, *B. gibsoni* has been described in some regions of Bangladesh, India, Sri Lanka, Central Asia, China, Japan, South Saharan countries of Africa (e.g. Mali), some regions of the United States and in Southern Europe, apparently being transmitted by ticks such as *Haemaphysalis bispinosa* or *R. sanguineus* (Mehlhorn and Schein 1984; Yamane et al. 1993; Kakoma and Mehlhorn 1994). Originally, it was thought that in dogs and other canids only one large and one small *Babesia* species existed. However, different strains/subspecies were described in the case of *B. canis* and at least three small babesian species were detected in canids by means of DNA/RNA comparisons (Conrad et al. 1992; Zahler et al. 2000a, b, c). One of them (looking like *B. microti; Zahler et al. 2000b*) was already described as *Theileria annae*, while some of the American strains of *B. gibsoni* were proposed for transfer into the genus *Theileria* (Zahler et al. 2000c). While light microscopic pictures of blood smear preparations derived from dogs allow the differentiation between the large *B. canis* forms (mainly appearing as pyriform stages, 4–5 μm long, and dividing by binary fission) and the small group (named until now as *B. gibsoni*), such microscopic determination among the small forms is not possible. The latter appear in at least 5–6 different shapes, although the ovoid stages (or ring forms), 1.6–2.4 μm in size, are rather common in dogs (Patton 1919; Rao 1926; Büttner 1968; Fowler et al. 1970; Namikawa et al. 1988; Yamane et al. 1993).

When grown under in vitro culture conditions, the large *B. canis* stages mainly kept this pyriform shape and only a few developed into typical babesian gamonts (Mehlhorn and Schein 1984). However, with respect to the small forms, a great change in size may occur. Thus, Murase et al. (1991), Onishi et al. (1993) and Zweygarth and Lopez-Debollar (2000) found in their cultures not only a few (rather small) typical *B. gibsoni* stages, but also “giant” ones, often nearly filling the parasitized erythrocyte. This phenomenon was even more significant in the experiments of Fujisaki et al. (2000), who transferred canine red blood cells into SCID mice and infected them later with a Japanese strain of *B. gibsoni.* They noted very large spherical stages, large pyriform stages (closely resembling *B. canis*) and even “giant Maltese-cross”-stages. Although there were enormous changes in shape, these stages did not lose their infectivity for dogs, which was also retained in the experiments of Zweygarth and Lopez-Debollar (2000), who were the first to establish a continuous culture for more than 100 days. Thus, the shape of (at least) the small *B. gibsoni* stages is apparently dependent on the immune system of the canine host. This fact makes the
discrimination between *B. canis* and *B. gibsoni*/*T. gibsoni* cases in the field even more difficult, since *B. canis*-like stages might be *B. gibsoni*, or some stages of *B. gibsoni* might be hidden among true *B. canis* stages. Therefore, an exact species determination is needed, since *B. gibsoni* requires a different drug regimen from *B. canis*. Both species introduce similar clinical symptoms, such as fever, anaemia, icterus and haemoglobinuria (in severe cases; Mehlhorn et al. 1993; Casapulla et al. 1998; Boch and Supperer 2000), and are not distinguishable by means of conventional serology (e.g. immunofluorescent antibody tests). The present paper examines in vitro cultivated stages of *B. canis* (from France and Hungary) and *B. gibsoni* (from Asia) by means of electron microscopy in order to detect significant morphological features (since size alone is not sufficient for species discrimination), as needed for effective treatment (e.g. imidocarb against *B. canis*, parvaquine against *B. gibsoni*).

**Materials and methods**

**Origin of parasites**

*Babesia canis*

The parasites originated from experimentally infected dogs (French strain, Schein, in Berlin 1981; Hungarian strain, Schein 1998) and were cultured at the Düsseldorf Institute, as described elsewhere (Mehlhorn et al. 1981).

**B. gibsoni**

The parasites and their host cells were taken from continuous in vitro cultures of Zweygarth and Lopez-Rebollar (2000), originating from naturally infected dogs from Sri Lanka and Bangladesh.

**Light and electron microscopy**

**Smears**

Blood smears taken from the cultures were conventionally treated and coloured by means of the typical Giemsa stain method (see Mehlhorn et al. 1993).

**Transmission electron microscopy**

Samples (1 ml) of *B. canis* or *B. gibsoni*-infected (cultured) erythrocytes were filled into 10 ml of a cold (4 °C) fixation fluid (consisting of 5% glutaraldehyde in 0.1 M sodium cacodylate (v/v) buffer at pH 7.2) and were mixed by shaking the two fluids for 1 min. After this procedure, the samples were kept for at least 24 h in the refrigerator at 4 °C before airmail shipment (in the case of the *B. gibsoni* material) or before starting further procedures.

The glutaraldehyde-fixed samples were centrifuged and washed three times in fresh cacodylate buffer and then post-fixed for at

**Figs. 1–4** Light micrographs of blood smears

**Fig. 1** *Babesia canis* – cultured forms. Note the typical pyriform appearance. *E* Erythrocyte. ×1,400

**Fig. 2** *B. gibsoni* – natural infection of a dog. Note the small ovoid forms. ×1,400

**Fig. 3** *B. gibsoni* – cultured forms. Note the existence of small and large intracellular forms and free forms. ×2,500

**Fig. 4** *B. gibsoni* – cultured forms. ×1,300