(or food) required for flight by dragonflies can be calculated from knowledge of the mass of the animal and the speed of flight. If we assume that *Hagenius* (mass = 1.2 g) flies up to 9 m/s (20 mph), the cost of flight would be about 0.179 W. This translates to approximately 0.155 g of food (this assumes that there are 20.92 J/(g dry mass) in the food and that the food contains 80% water) necessary to fuel 1 h of flight, and this rate of intake would net the dragonfly about 0.124 g of water per h. Thus, *Hagenius* could conceivably consume the equivalent of 60% of its body mass in food in about 4.6 h. However, it is still unknown at what rate water would be lost from dragonflies in flight, and thus, it cannot yet be determined if flying animals can obtain enough water from the food they must eat to account for their needs throughout the day. It can be said with considerable certainty, however, that without access to food or water, *Hagenius* would continually lose water at a rate that could be stressful and could cause these dragonflies to exhibit hydroregulatory behavior directed at retarding desiccation.

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The invasion and growth of *Babesia bovis* in tick tissue culture

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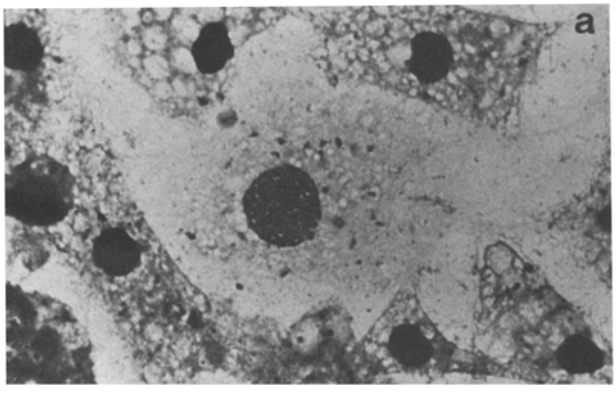
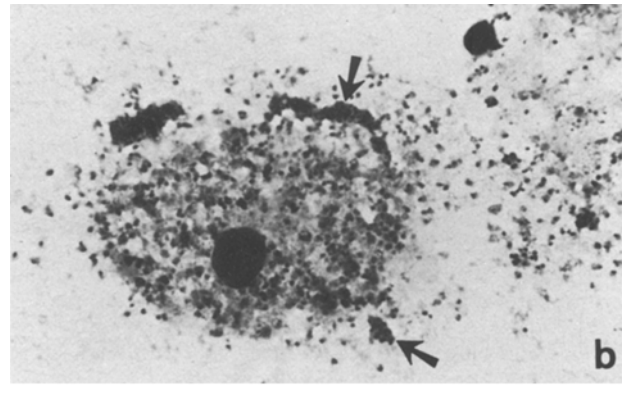
Summary. Erythrocytic forms of *Babesia bovis* inoculated into cell cultures of the tick *Boophilus microplus* invaded the tick cells and showed multiplication for up to 48 h after inoculation.

Babesia bovis is a tickborne intra-erythrocytic parasite of cattle that causes economic loss in many countries of the world. A common vector is the one-host tick, *Boophilus microplus.* Although an attenuated vaccine prepared from the blood of infected cattle is available for the control of the disease[2], there are risks involved in its use such as the accidental transmission of other diseases and the production of haemolytic anaemia in new born calves[3]. *B. bovis,* grown in tissue culture, would be ideal as the basis of an improved vaccine and also as a source of antigens for immunological studies. However, the conditions for the invasion of tissue culture cells by a *Babesia* and its subsequent multiplication in vitro have not yet been established[4]. The invasion and multiplication of *B. bovis* in the cultured cells of the tick *Boophilus microplus* are reported in this paper and constitute the first successful attempt to grow a member of the genus *Babesia* in such a system.

Materials and methods. B. bovis was grown in splenectomized calves[5] and infected blood was drawn using EDTA as anticoagulant. The infected erythrocytes were concentrated by differential lysis in hypotonic saline[6]. Suspensions





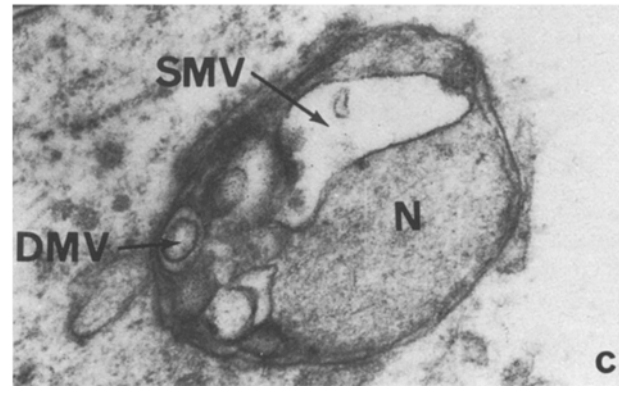


Fig. 1. *a* The invasion of the tick cells by *B. bovis* 8 h after inoculation of the culture with infected blood. ×730. *b* Showing the increase in the number of *B. bovis* in the cells after 48 h. Arrows indicate the large multinucleate bodies. ×730. *c* Electron micrograph of *B. bovis* inside a cultured tick cell, 24 hPI. N: nucleus, DMV: double-membraned vacuole, SMV: single-membraned vacuole. ×20,000.

containing 98-100% infected intact erythrocytes were then resuspended in enriched Leibovitz's (L-15) medium[7] to which 10% fresh bovine serum was also added. Embryonic cells of the tick *B. microplus* were cultured in enriched L-15 medium[7] essentially following the techniques of Pudney et al.[8]. Cells from primary, 1st and 2nd subcultures were grown on coverslips in Leighton tubes. Tick cells growing on coverslips consisted of different morphological forms. The majority resembled epithelial and fibroblast cell types. 8-10-day-old Leighton tube cultures were inoculated with 1.5 ml of the infected erythrocyte suspension to give approximately 2×10^8 erythrocytes per culture. Control culture tubes received a similar number of non-infected erythrocytes. Infected and control culture tubes were incubated stationary at 28±1 °C.

After 8 h post inoculation (hPI) all erythrocytes and free parasites in the medium were separated from the culture by rinsing the coverslips in fresh L-15 medium and transfering them to new tubes. The original medium was centrifuged at 2000× g for 10 min to sediment the parasitized erythrocytes and free parasites. The supernatant fluid was then added to the cultures. At 8 hPI and subsequently at different hPI the coverslips from 2 tubes selected at random were removed, washed by rinsing in 2 changes of L-15 medium, air dried, fixed in methanol and stained with Giemsa. The total number of parasites within or associated with tick cells on each coverslip was calculated by counting the parasites in 10 oil immersion fields under a light microscope at a magnification of × 1000.

Results and discussion. At 8 hPI approximately 3% of the parasites in the inoculum had invaded the tick cells and these organisms then became the only source from which the *Babesia,* observed after 8 hPI, were derived. 8 experiments all gave similar results. There was an increase in the number of infected tick cells as well as the number of parasites in each cell, reaching a maximum between 40-48 hPI (figures 1a and 1b). The total number of parasites contained by the cultures increased approximately 20-fold between 8 and 40 hPI (figure 2). This rate of multiplication was comparable to that observed in the bovine host[9]. After 48 hPI, the number of parasites declined. A cytopathic effect on the tick cells, manifest by vacuolation of the cytoplasm and rounding of shape, was evident as early as 24 hPI. No cytopathic effect was observed in control cultures. The heavily parasitized cells developed larger vacuoles in their cytoplasm and subsequently disintegrated. Lightly infected cells appeared to recover after babesial multiplication.

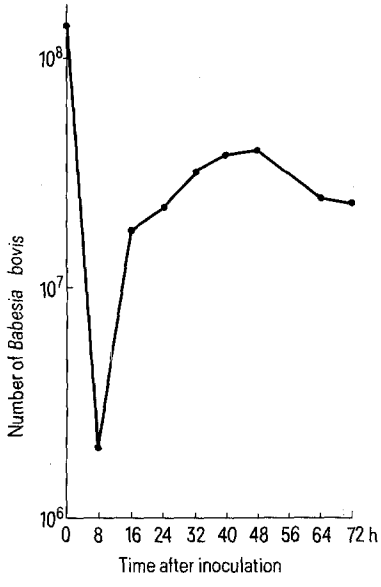


Fig. 2. The growth curve of *B. bovis* in tick tissue culture. Taken from 1 typical experiment. The maximum number of parasites produced by multiplication was less than the original number inoculated because 97% of the inoculum was removed at 8 hPI. The small residual population (3%) inside the cells were the only parasites left to multiply after 8 h.

It was difficult to determine the method(s) of babesial multiplication in the tissue cultures. Characteristic paired bodies were observed in the cultures although no 'intermediate' budding forms were seen. Many single forms appeared to have double nuclei and perhaps these were in a stage of binary fission. At about 16 hPI, compact bodies, containing up to 10 nuclei, were observed in the cytoplasm of the culture cells. The size, number and nucleus count of such bodies increased up to 48 hPI (figure 1b). They appeared to be similar to the schizonts that have been described during the development of *B. bovis* in living ticks[10] and perhaps multiple fission occurred in the tissue cultures. Only a small proportion of the inoculum (3%) invaded the tick cells. This may have been a density-dependent phenomenon associated with high number of parasites used. However, it has also been observed that many *B. bovis* blood-forms ingested by engorging ticks degenerate in the gut and suggested that relatively few are capable of initiating the invertebrate life cycle[10]. A similar situation may have occurred in tissue culture.

A preliminary ultrastructural study of *B. bovis*-infected tick cells at 24 hPI indicated distinct intracellular localization of the parasites (figure 1c). Mostly they were encased in single-membraned, parasitophorous vacuoles. The cytoplasm of the parasite contained 1-2 single- or double-membraned vacuoles adjacent to the nucleus. The nucleus was large and occupied about half the body of the parasite. Fine ribosomes formed the remainder of the cytoplasm. Unlike blood forms, *B. bovis* from cultured tick cells did not have a double-layered inner membrane, polar ring, rhoptries and endoplasmic reticulum[11]. However, the ultrastructure of *B. bovis* from cultured tick cells resembled the blood forms more closely than that of the gut or salivary gland forms described in ticks[12].

This is the first occasion that a haemoprotozoan parasite has been grown in the cultured cells of its vector and in addition to its obvious application in the study of babesiosis, the system should serve as a useful model for the development of new techniques for culturing a variety of haemoparasites in the absence of red cells including *Plasmodium, Anaplasma* and *Theileria.*

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