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Brief Communication

CONTINUOUS IN VITRO CULTIVATION OF BABESIA DIVERGENS

Methods for short term and continuous in vitro cultivation of Babesia bovis (*Erp et al.* 1978, 1980, *Levy & Ristic* 1980) and some other babesias (*Bautista & Kreier 1979, Timms* 1980) have been reported recently. The development has given new scope to the investigation of these parasites. For example, cell culture derived antigens can be used in various analytical and immunogenicity studies. This paper presents the preliminary results of in vitro cultivation of Babesia divergens and describes some characteristics of its growth.

The cultivated B. divergens strain was isolated in eastern Finland in 1976. It had been passaged several times in splenectomised calves and stored at — 70°C in the intervals between passages. Blood for in vitro cultivation was collected when an infected splenectomised Ayshire calf showed parasitemia of 10—20 % of infected red cells. Both infected and normal blood was defibrinated by shaking with glass beads and centrifuged to remove the buffy coat. The serum was used immediately or stored at — 20°C. Suspension of normal erythrocytes was stored at + 4°C for up to 1 week.

The technique used was the microaerophilous stationary phase (MASP) culture system of *Levy & Ristic*. The pH of the babesia tissue culture medium (Medium 199 (Gibco) and normal bovine serum 60:40) was adjusted to 7.2—7.4. To start the culture, infected defibrinated blood was diluted with the culture medium to a PCV of 8—12 %. Adding of normal erythrocyte suspension reduced the initial proportion of parasitised red cells to approximately 2 %. Cultures in multichambered containers (Lab-Tek) were incubated at 38°C in a humidified atmosphere by the candle jar system (*Trager & Jensen* 1976). The overlying medium was removed and replaced with fresh medium every day and a new subculture was started after 48—96 h incubation, diluting the old culture by 1:2 or 1:3 with a fresh suspension of normal erythrocytes.

The parasite can be maintained continuously in cell culture. So far, B. divergens has kept multiplying through 27 subcultures during a period of 85 days. After 35 days in culture, Babesia caused a severe clinical disease in a splenectomised calf. The parasite frequency during the study period remained between 5—10 % of infected red cells. The maximum parasitemia for MASP culture of B. bovis has been reported to be as high as 38 % (*Levy & Ristic*).

At the end of each subculture the amount of extraerythrocytic parasites increased and they were seen singly or clumped in stained smears. The agglutinated parasites showed a tendency to degenerate.

The darkening of sedimented erythrocytes noticed by *Levy & Ristic* (1980) was also apparent under the present culture conditions and provided a simple means of measuring the growth of the parasite.

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