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EPERYTHROZOON - A LESSON FOR ARBOVIROLOGISTS

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Summary. - *Eperythrozoon* was detected in suckling mice inoculated with a suspension of nymphal *Ixodes ricinus* ticks. The organism was filterable through 220 nm Millipore membranes, moderately sensitive to diethyl ether, pathogenic to suckling but not to adult mice when given intracerebrally, intraperitoneally or subcutaneously, and mimicked an arbovirus. *Eperythrozoon* should be considered as an agent potentially interfering with experiments performed on laboratory mice.

Key words: arboviruses; Eperythrozoon; isolation assays; mouse

Between 1976 and 1992, we examined virologically nearly 30 000 ixodid ticks of eight species in the Czech and Slovak Republics. A total of 15 arbovirus strains were isolated and identified (Hubálek *et al.*, 1991): Central European tick-borne encephalitis virus (2 strains, from *I. ricinus*), Uukuniemi virus (9 strains, from *I. ricinus*), Tribeč virus-Brezová subtype (3 strains, from *I. ricinus*) and Bhanja virus (1 strain, from *Dermacentor marginatus*). Moreover, a filterable pathogenic agent (PV57) was detected that has long remained unidentified. The objective of this paper is to describe our effort to identify the agent.

The homogenate PV57 of 14 partially engorged nymphal *I. ricinus* parasitizing two *Lacerta agilis* lizards was prepared in phosphate buffered saline (PBS) pH 7.2 with 10 % foetal calf serum, clarified by centrifugation and inoculated intracerebrally (ic) into 2-3 day-old suckling SPF outbred ICR mice (SM) (purchased from Velaz, Prague). Out of 12 inoculated 4 SM died on days 8 to 10 post inoculation (p. i.). Serial ic passages in SM of 10 % brain homogenates, prepared in Eagle's MEM with 10 % calf serum, penicillin 200 IU/ml and streptomycin 100 μg/ml, centrifuged at 1000 x g for 15 mins, resulted in a 100 % mortality rate and in a reduction of the average survival time (AST) from 9.0 to 2.2 days. The log LD_{50}/ml was 9.4 at the 7th passage, and no bacterial growth was detected in the suspension when tested in meat-peptone broth, thioglycollate broth or on blood agar at either 37 °C or 28 °C. The high-passaged agent killed all SM when given intraperitoneally (ip) or subcutaneously (sc); AST were 4.0 and 4.9 days, respectively. However, no mortality or signs of illness were observed in adult mice when inoculated ic, ip or sc. PV57 did not produce any readily visible morphological changes in Vero cell monolayers, whereas a marked cytopathic effect (CPE) was observed in PS and SPEV (embryonic pig kidney) cells 2-3 days p. i., and a lower-degree CPE in

CV-1 (monkey kidney) cells 3 days p. i.; log $TCID_{50}$/ml titers in PS, SPEV and CV-1 cells were 8.5, 7.8 and 6.3, respectively.

The pathogenic agent was further characterized by standard procedures for arboviruses and rickettsiae (Lennette and Schmidt, 1969). PV57 passed through 220 nm Millipore membranes (though the infectious titer decreased by 1.8 log), but not through 100 nm membranes. A moderate sensitivity to diethyl ether and a resistance to 0.1 % sodium deoxycholate were observed in that the infectious titer decreased by 2.0 and 0.6 log, respectively, after the exposure. The infectivity persisted in diluents containing antibiotics penicillin (200 IU/ml), streptomycin (200 μg/ml) or gentamicin (100 μg/ml), and also during freeze-drying a 10 % brain suspension. A rapid fall in the infectivity was observed when PV57 suspensions in PBS without serum were frozen and thawed repeatedly.

The filterability of the agent indicated that it could be an arbovirus. However, PV57 antigens prepared from infected SM brains in a borate buffer pH 9.0 and by the sucrose-acetone extraction (Clarke and Casals, 1958) did not react in complement fixation test (CFT) with immune sera or ascitic fluids to known European arboviruses, nor with mouse hepatitis virus (MHV) and *Chlamydia*. A partial CF reaction (1:16) of PV57 antigen was observed only against *Coxiella burnetii* antiserum, but no specific antibodies agglutinating the *C. burnetii* phase II corpuscular antigen (Fiset *et al.*, 1969) were detected in sera gained by ip inoculation of PV57 into mice, guinea pigs or a rabbit. The antigen (sucrose-acetone preparation) did not agglutinate goose erythrocytes. In addition to MHV, other rodent viruses were then excluded by testing PV57 immune mouse serum (3 ip doses given at weekly intervals) in CFT, haemagglutination-inhibition test (HIT) or enzymatic immunoassay (EIA): murine cytomegalovirus (EIA), Sendai virus (HIT), pneumonia virus of mice (HIT), reovirus-3 (HIT), Theiler's mouse encephalomyelitis virus GDVII (HIT), minute virus of mice (EIA), MHV-1 and MHV-3 (EIA), lymphocytic choriomeningitis virus (CFT), and mouse adenovirus (CFR). Moreover, quality status of the purchased ICR mice assures that this mouse strain is free of infection with coccidiae, intestinal protozoa, *Toxoplasma, Mycoplasma pulmonis*, and specific bacterial murine pathogens.

CFT indicated rickettsial nature of the agent. When PV57 was given ip to three adult male guinea pigs (450 g) in a dose of 8.2 log LD_{50}, the animals remained symptomless, their rectal temperature was normal at daily recording and no scrotal reaction was present. No rickettsiae were observed in the Giemsa or Gimenez stained smears of *tunica vaginalis* and spleen 33 days p. i. PV57 was then inoculated ip into 12 SM as a 1 % SM brain suspension in MEM with antibiotics, and the animals were sacrificed when they all showed signs of illness 5–7 days p. i. The blood smears stained by Giemsa-Romanowski exhibited numerous very small (0.2–2.0, mostly 0.4–0.5 μm), light violet to pink spherical or subspherical organisms situated on/in erythrocytes or free in the blood plasma. Nearly a half of the red blood cells were found to be infected, each with 1–15 microorganisms. Their morphological pattern was characteristic of the

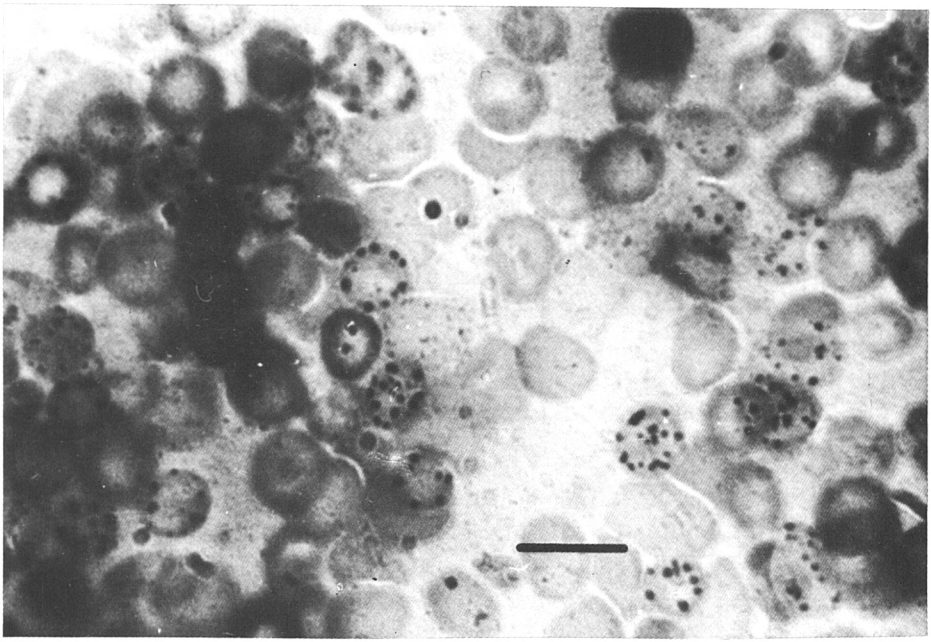

Fig. 1
Eperythrozoon on red blood cells of a suckling mouse 6 days p. i.
Stained by Giemsa-Romanowski. Bar = 10 μm.

genus *Eperythrozoon (Rickettsiales, Anaplasmataceae;* Ristic and Kreier, 1984). *E. coccoides* Schilling is a filterable murine parasite that can be transmitted by lice (*Polyplax serrata)* and is only pathogenic to the immunocompromised (e. g. splenectomized) adult mouse host (Baker *et al.*, 1971). We have found that SM are more susceptible to *Eperythrozoon,* obviously due to their incompetent immune response at that age.

The agent PV57 had probably been infecting some of the SM that had been used in the isolation assay. A re-isolation attempt on SM with the original tick homogenate stored for 3 years at -60 °C remained unsuccessful. Virologists, microbiologists and immunologists should be aware of *Eperythrozoon* as an agent potentially interfering with experiments performed on laboratory mice. The infection can be monitored and detected by microscopy of Giemsa's stained blood smears of the animals.

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