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Attenuation of *Ehrlichia canis* by multiple passages in two different cultures

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INTRODUCTION

Canine monocytic ehrlichiosis (CME) is a tick-borne disease with global importance. While trying to determine the minimal time-lag needed for transmission of *Ehrlichia canis* by *Rhipicephalus sanguineus*, trials to infect ticks by feeding on infected dogs were carried out. The results of the current study suggest that the organism was attenuated during multiple passages in two different cell cultures.

MATERIALS AND METHODS

Two healthy 4-year-old beagle dogs (Nos. 10 and 11) sero- and PCR-negative for *E. canis* were used. The dogs were intravenously inoculated with 10^6 DH82 cells heavily infected (>80%) with viable *E. canis* (Israeli strain No. 611) to serve as reservoirs [1]. The rickettsia used in this study had undergone multiple passages over 8 years in two different cell lines, the DH82 and J774.A1. Frequent physical and haematological examinations were performed for both dogs during acclimatisation and post-infection (PI) periods. The dogs were not treated against CME during the course of the study. The study was approved by and met all criteria set by the Animal Care and Use Committee.

Naïve laboratory reared *R. sanguineus* ticks were used in this study. The pre-imaginal stages were fed on gerbils and the adult ticks on rabbits. The non-feeding stages were maintained in an incubator at 27°C and 80% relative humidity. Two hundred and ten nymphs from both sexes were placed in ear bags on the experimentally *E. canis*-infected dogs (one bag/dog) on day 23 PI when the dogs were rickettsemic as determined by PCR. The ear bags were checked daily for a period of 10 days until detached. Ticks that were not attached to the ears during the first 72 h were discarded (30 nymphs). PCR was performed on DNA extracted from the ticks after moulting in order to detect tick infection with *E. canis* [2].

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RESULTS

Both dogs became infected with *E. canis* as confirmed by seroconversion and positive PCR on day 12 PI. Neither of the two dogs developed clinical signs during the course of the study; however, they developed thrombocytopenia (Fig. 1). No other haematological alterations were recorded. Both dogs seroconverted on day 12 after primary infection. Previous inoculations of dogs with preliminary passages of the same cultured *E. canis*, with a similar inoculum size and infection route, evoked severe clinical signs [1]. Trials to infect ticks by feeding on infected dogs resulted in no detectable tick infection, as manifested by the negative PCR. The results of this study suggested further investigation.

Therefore, 119 days after primary infection, the dogs were challenged intravenously with 5 mL heparinised blood, drawn from a sick, PCR-confirmed *E. canis*-naturally infected dog. Simultaneously, five additional dogs were inoculated in the same manner with the same infection source in a different experiment and were used as controls for the current infection challenge [3].

Dog 11 showed mild petechiation and splenomegaly for one day only (day 42 post-challenge); however, dog 10 showed no clinical illness post-challenge infection. Both dogs had no fever and their platelet counts remained mildly thrombocytopenic post-challenge for a period of 42 days (Fig. 1). No other haematological abnormalities were detected. Both dogs were PCR-negative on the challenge day and PCR-positive on days 7, 11, 28 and 42 post-challenge. All five dogs from the concurrent experiment that were used as controls for the current study developed severe clinical and haematological signs consistent with *E. canis* infection [3]. *E. canis*-DNA was detected in blood samples of all five dogs on days 7–10 PI, and they all seroconverted by day 12 PI [3].

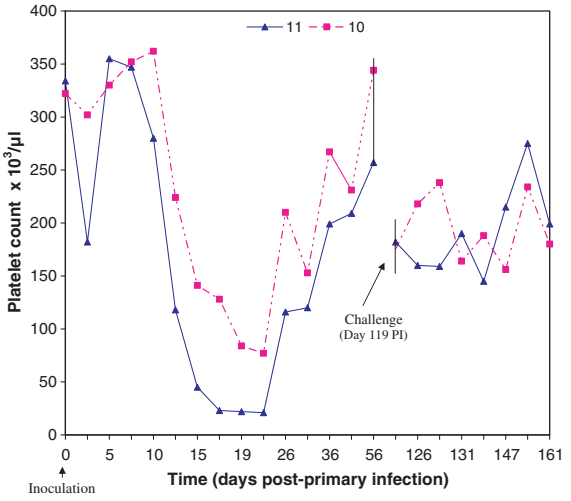

Fig. 1. Mean platelet counts of two dogs experimentally infected with *Ehrlichia canis*. Day 0 is the inoculation day with *E. canis*-infected DH82 cells (multiple passage culture). Day 119 is the challenge day (with a wild strain of *E. canis*).

DISCUSSION

Failure to transmit cultured *E. canis* to ticks by feeding unengorged nymphs on infected dogs was previously described in one report [4]. However, in the latter, the agent maintained its infectivity and pathogenicity for dogs as opposed to our canine experimental infection results. The difference between the latter results and our study could be related to the greater number of passages of the organism in culture, and to the use of different non-canine culture cells (J774.A1) as additional transfer media. The findings of both studies suggest that the rickettsia may have lost its infectivity for *R. sanguineus* during the repeated passages in culture cells.

While a previous study showed that *E. canis* may persist in the blood of infected dogs for years post-infection [5], the blood of dogs 10 and 11 was found to be PCR-negative for *E. canis* on the day of infection challenge, suggesting the spontaneous elimination of the organisms. The latter finding, together with the mild thrombocytopenia, the absence of clinical signs in one dog and the mild clinical signs in the other dog post-challenge infection with a virulent strain of *E. canis*, suggests that these dogs were immunised by the primary inoculation.

The results of this study suggest that *E. canis* might have undergone attenuation after multiple passages in cell cultures and might serve as a potential vaccine candidate for CME. However, further studies in a larger group of dogs are required to ascertain our findings.

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