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Transmission of *Ehrlichia canis* by *Rhipicephalus sanguineus* ticks feeding on dogs and on artificial membranes

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ABSTRACT

A South African strain of *Ehrlichia canis* was isolated and used to infect a laboratory-bred Beagle dog. *Rhipicephalus sanguineus* nymphs, which fed on this dog, moulted to adult ticks which carried infection rates of *E. canis* between 12% and 19% and were used in a series of *in vivo* and *in vitro* experiments.

Five groups of 6 dogs were challenged with the infected *R. sanguineus* ticks, which were removed 24 h, 12 h, 6 h or 3 h after the ticks had been released onto the dogs. The animals were monitored for fever and thrombocytopenia and were considered infected if they became serologically positive for *E. canis* antibodies as well as PCR positive for *E. canis* DNA. Seven dogs became infected with *E. canis* in the following groups: Group 1 (24 h tick challenge) 1 out of 6; Group 2 (12 h) 1 of 6; Group 3 (6 h) 2 of 6; Group 4 (6 h) 2 of 6 and Group 5 (3 h) 1 out of 6. Six of those 7 infected dogs developed fever and a significant thrombocytopenia. One dog did not show any symptoms, but seroconverted and was found PCR positive on several occasions. Five additional dogs were PCR positive on one test sample only but were not considered infected because they did not develop any specific *E. canis* antibodies.

In vitro, *R. sanguineus* ticks attached and fed on bovine blood through silicone membranes with attachment rates up to 72.5% after 24 h increasing to 84.2% at 72 h. The ticks transmitted *E. canis* as soon as 8 h post application as demonstrated by *E. canis* DNA found in the nutritive blood medium.

In conclusion, transmission of *E. canis* by *R. sanguineus* ticks starts within a few hours after attachment, which is earlier than previously thought. These findings underpin the need for acaricides to provide either a repellent, an anti-attachment and/or a rapid killing effect against ticks in order to decrease the risk of transmission of *E. canis*.

1. Introduction

Worldwide, there is a growing recognition of the importance of rickettsial pathogens in dogs and humans, which are transmitted by a number of different ixodid tick

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species (Nicholson et al., 2010). *Rhipicephalus sanguineus*, the brown dog tick, is the most widespread tick in the world and is a well-recognized vector of tick-borne pathogens affecting dogs and occasionally humans (Dantas-Torres, 2010). *Rhipicephalus sanguineus* transmits a broad range of pathogens to dogs such as *Babesia vogeli*, *Babesia gibsoni*, *Hepatozoon canis*, *Rickettsia conorii*, *Rickettsia rickettsii*, *Ehrlichia canis* and *Anaplasma platys* (Jongejan and Uilenberg, 2004; Beugnet and Marié, 2009).

Rhipicephalus sanguineus is the primary vector of *E. canis*, which is the cause of monocytic ehrlichiosis (Groves et al., 1975). *Ehrlichia canis* has a worldwide distribution (Asia, Africa, Europe and the Americas), where the majority of clinical cases occur in sub-tropical and Mediterranean regions where the vector is abundant. Canine monocytic ehrlichiosis is characterized by thrombocytopenia, leukopenia, fever, depression and bleeding tendencies (epistaxis) (Harrus et al., 1999). *Ehrlichia canis* is transmitted transstadially and intrastadially by *R. sanguineus* ticks (Bremer et al., 2005). In dogs, *E. canis* develops in monocytes and macrophages, whereas in ticks the infection is localized in midgut and salivary glands.

Effective control of ticks on dogs is a necessity in many parts of the world. In addition to the killing effect on ticks, acaricides which can prevent transmission of tick borne-pathogens bring an important added value. The decrease of the transmission rate can be explained by several properties of the acaricidal molecule: a repellent/irritant effect inhibiting tick infestation and attachment; a neurohormonal disruption of tick attachment and intake of the blood meal; and/or a quick speed of kill before transmission can occur (Halos et al., 2012). These properties can be combined together depending on the acaricidal molecules and their concentrations. Several studies have demonstrated the capability of anti-tick products based on either amitraz, fipronil and/or permethrin to help in preventing pathogen transmission. Field studies conducted thus far suggested that topically applied acaricides can assist in the prevention of the transmission of specific tick-borne pathogens (Davoust et al., 2003; Otranto et al., 2008). Controlled laboratory studies have also focussed on these prophylactic efficacies (Jongejan et al., 2011; Fourie et al., 2013a,b). A transmission blocking model was recently used to evaluate the efficacy of two topical products, CERTIFECT® spot on (fipronil 6.26% w/v, amitraz 7.48% w/v, (S)-methoprene 5.63% w/v) and SERESTO® collar (imidacloprid, flumethrin) in preventing transmission of *Babesia canis* by *Dermacentor reticulatus* to dogs (Jongejan et al., 2011; Fourie et al., 2013a). Another transmission blocking model was used to demonstrate the efficacy of (CERTIFECT®) in preventing the transmission of *E. canis* by *R. sanguineus* to dogs (Fourie et al., 2013b).

In order to successful prevent pathogen transmission, the speed of kill asserted by a particular acaricidal compound is of prime importance. The time elapsed from tick attachment to pathogen transmission varies according to the nature of the pathogen. For instance, in one study on the transmission of *Borrelia burgdorferi*, *Ixodes ricinus* nymphs were allowed to feed on gerbils during limited time intervals. It was found that 50% of gerbils became infected within 17 h of tick attachment, whereas all gerbils developed *Borrelia* infections after the ticks were allowed to feed for 48 h (Kahl et al., 1998). The speed of *E. canis* transmission by infected *R. sanguineus* ticks has not been determined in any laboratory study thus far. Importantly, there is no report published wherein *R. sanguineus* has been adapted to feed on artificial membranes in order to study the pathogen transmission dynamics *in vitro*.

Here, the speed of *E. canis* transmission by infected *R. sanguineus* ticks to dogs was determined in a controlled laboratory study. In addition, *R. sanguineus* ticks were successfully adapted to feed *in vitro* by modifying the methods originally developed for *in vitro* feeding of *I. ricinus* ticks (Kröber and Guerin, 2007a). The speed of transmission of *E. canis* by infected *R. sanguineus* ticks attached for defined periods on dogs was compared with the transmission of *E. canis* by *R. sanguineus* ticks attached and feeding through artificial membranes.

2. Materials and methods

2.1. Dog study design

The *in vivo* component of the study was conducted with five groups of six dogs, which were adult males (19) and females (11) of mixed breed. Prior to the experiments the dogs were all negative for *E. canis* as demonstrated by the absence of specific antibodies in the indirect fluorescent antibody test (IFA). They had not been treated with any acaricidal spot-on or spray for 12 weeks prior to the first tick challenge. There were two study phases with an assessment time point for the second study phase (Groups 4 and 5) based on the outcome of the first study phase (Groups 1–3). The study employed a randomized block design with 18 dogs allocated to Groups 1–3 and a further 12 dogs allocated to Groups 4 and 5. The dogs were sedated and infested with 100 adult ticks each. The infection rate of the ticks ranged between 12% and 19%. In the first phase of the study, dogs were challenged with ticks followed by removal at 24 h post challenge (Group 1), at 12 h post challenge (Group 2) and at 6 h post challenge (Group 3). In the second study phase dogs were challenged with ticks, which were removed at 6 h post challenge (Group 4) and at 3 h post challenge (Group 5). The study was in compliance with the South African animal welfare regulations and carried out according to International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products Guideline 9: Good Clinical Practice (EMA, 2000).

2.2. Ehrlichia canis strain

A laboratory-bred Beagle dog, inoculated with blood derived from a local clinical case of canine monocytic ehrlichiosis, identified in Bloemfontein, South Africa served as the source of the infectious material. EDTA blood collected from this animal was frozen at −80 °C with 10% DMSO as a cryoprotectant and thereafter stored in liquid nitrogen. Gp36, an immunodominant glycoprotein-encoding gene, was PCR amplified from this blood and used as a tool for the classification of *E. canis* isolates as previously published (Doyle et al., 2005; Hsieh et al., 2010).