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## RED AND GRAY FOXES — POTENTIAL RESERVOIR HOSTS FOR *Ehrlichia canis*<sup>□</sup>

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**Abstract:** Infection with *Ehrlichia canis* was successfully established in the red fox, *Vulpes fulva*, and the gray fox, *Urocyon cinereoargenteus*. Transtadial transmission of *Ehrlichia canis* from an infected fox to a laboratory Beagle dog by the tick, *Rhipicephalus sanguineus*, was demonstrated.

### INTRODUCTION

*Ehrlichia canis* infection in various breeds of dogs has been described.<sup>8,10,11</sup> The agent was first identified in the United States in dogs in Oklahoma in 1963.<sup>5,6</sup> Its widespread distribution in the United States is now evident from recent reports of isolations of *E. canis* from dogs in Florida,<sup>10</sup> Texas,<sup>15</sup> and Arkansas.<sup>9</sup>

While basic pathogenicity and chemotherapy studies have been done, the epidemiology of this disease remains for the most part, speculative. The host range and natural cycles of transmission have not been established. Unsuccessful attempts have been made to infect mice, rats, guinea pigs, hamsters, rabbits, sheep, cattle and cats.<sup>4,8</sup> Neitz and Thomas<sup>14</sup> suggested that the wild dog, *Lycaon pictus*, served as a reservoir for *Ehrlichia* in the Kruger National Park in Africa, and reported that the jackal can be infected. The only evidence for a susceptible host, other than the dog, in the United States was supplied by Ewing and co-workers<sup>7</sup> when they experimentally infected a coyote, *Canis latrans*. It is apparent from these reports that the host range may be limited to canines.

The most numerous and widespread wild canines in the United States are the red and gray foxes.<sup>2,3</sup> Both are common

in the southern United States, especially the gray fox in Florida and Texas. In these areas *Rhipicephalus sanguineus*, the natural arthropod vector of *Ehrlichia*, can overwinter out of doors<sup>10</sup> and would be more likely to attack wildlife. This is also the area where *Ehrlichia* is endemic. The present report describes experimental infections with *E. canis* in red and gray foxes, and transmission of the agent from foxes to dogs via the natural arthropod vector.

### MATERIALS AND METHODS

The isolate of *Ehrlichia canis* used in this study was recovered from a German Shepherd dog. The severity of the experimental disease produced by this strain in dogs has been described previously.<sup>1,10,12</sup> Six young adult foxes, four red and two gray, were used in the experiment. One red and one gray fox were held as uninoculated controls. The others were inoculated intravenously with 5 ml of blood collected in ethylenediaminetetraacetic acid (EDTA) from a laboratory Beagle in the acute stage of infection with *E. canis*. Each fox was examined daily thereafter for clinical signs of ehrlichiosis. Following inoculation and 2 weeks prior to inoculation, 3 ml of blood were collected twice a week in sealed

□ In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences — National Research Council, U.S.A.

vacuum tubes<sup>[2]</sup> containing EDTA. These samples were used for determination of packed cell volume, erythrocyte sedimentation rate and erythrocyte, leukocyte, and thrombocyte counts. A standard microhematocrit centrifuge was used for all packed cell volume determinations. The Wintrobe tube was used for determination of erythrocyte sedimentation rates. Leukocyte and erythrocyte counts were determined with an electronic cell counter.<sup>[3]</sup> Thrombocyte counts were made with a phase contrast microscope. Blood films were made twice per week, stained by the Giemsa method, and examined for morulae of *E. canis*.

One month following experimental infection, 5 ml of blood were collected in EDTA from the infected gray fox and inoculated into the control gray fox. When morulae of *E. canis* were demonstrated in the peripheral blood during the acute stage of infection, approximately 6,000 larvae of *R. sanguineus* were fed on the fox. The procedures for feeding the ticks closely followed those described by Groves et al.<sup>[4]</sup> The larvae were from a colony of ticks which had been maintained in the laboratory over three generations by feeding on normal healthy dogs and had never transmitted disease. The engorged larvae were recovered, allowed to molt, and the resultant nymphal ticks allowed to feed on a normal laboratory Beagle. The Beagle was then examined and specimens collected as described for the foxes.

## RESULTS

Nasal or ocular discharges, anorexia, and weight loss, which characterize *E. canis* infection in dogs, were not observed in the experimentally infected foxes. Clinical laboratory tests demonstrated a mild anemia, thrombocytopenia, and leukopenia in the acute stages of the disease, along with a rise in erythrocyte sedimentation rate which varied greatly with the

individual animal. Figure 1 summarizes the findings in the first 2 months following inoculation. Typical morulae of *E. canis* were easily demonstrated in Giemsa stained venous blood smears from the 2nd week through the 5th week post inoculation. Three months following inoculation blood was collected from the foxes and inoculated into laboratory dogs. The dogs developed signs of disease, and the organism was recovered. This confirms persistence of the infection in the fox as in the dog.

*R. sanguineus* ticks fed on the infected gray fox as larvae, transmitted *E. canis* to the Beagle as nymphs. Eight days after the ticks were fed, the Beagle began to show the early signs of infection, and 11 days after feeding, morulae of *E. canis* were demonstrated in the blood.

## DISCUSSION

These studies confirm the susceptibility of foxes to infection with *E. canis*. The mild disease observed in the fox is in contrast to the severe, often fatal, disease described in the German Shepherd dog and referred to as tropical canine pancytopenia.<sup>11,12</sup> This study provides further evidence that disease manifestations of infection with *E. canis* are host dependent. The strain of *E. canis* used in this study has been used in pathogenicity studies in German Shepherd and Beagle dogs.<sup>1,10,12</sup> The strain produced severe disease with high mortality in German Shepherd dogs, clinical disease with no mortality in Beagle dogs and mild disease with no clinical signs in foxes.

Transmission of the disease from infected foxes to dogs by the tick, *R. sanguineus* is important epidemiologically. *R. sanguineus*, which is the only reported vector of *E. canis*,<sup>8</sup> is a three-host tick which feeds in all stages on canines.<sup>13</sup> In the southern part of the United States where foxes are numerous, *R. sanguineus*

[2] Vacutainer, Becton-Dickinson and Company, Rutherford, New Jersey, U.S.A.

[3] Coulter Electronics, Inc., Hialeah, Florida, U.S.A.

[4] Groves, M. G., G. L. Dennis, and D. L. Huxsoll: Transmission of *Ehrlichia canis* by *Rhipicephalus sanguineus*. Presented at the 52nd Annual Meeting of the Conference of Research Workers in Animal Disease. Chicago, Illinois. Nov. 29 and 30, 1971.

may overwinter out of doors.<sup>16</sup> Tick sharing by foxes and dogs may occur where dog and fox populations intermingle in rural and suburban areas.

In areas where the disease occurs in domestic dogs, wild foxes and other wild canines may serve as reservoir hosts for *E. canis*.

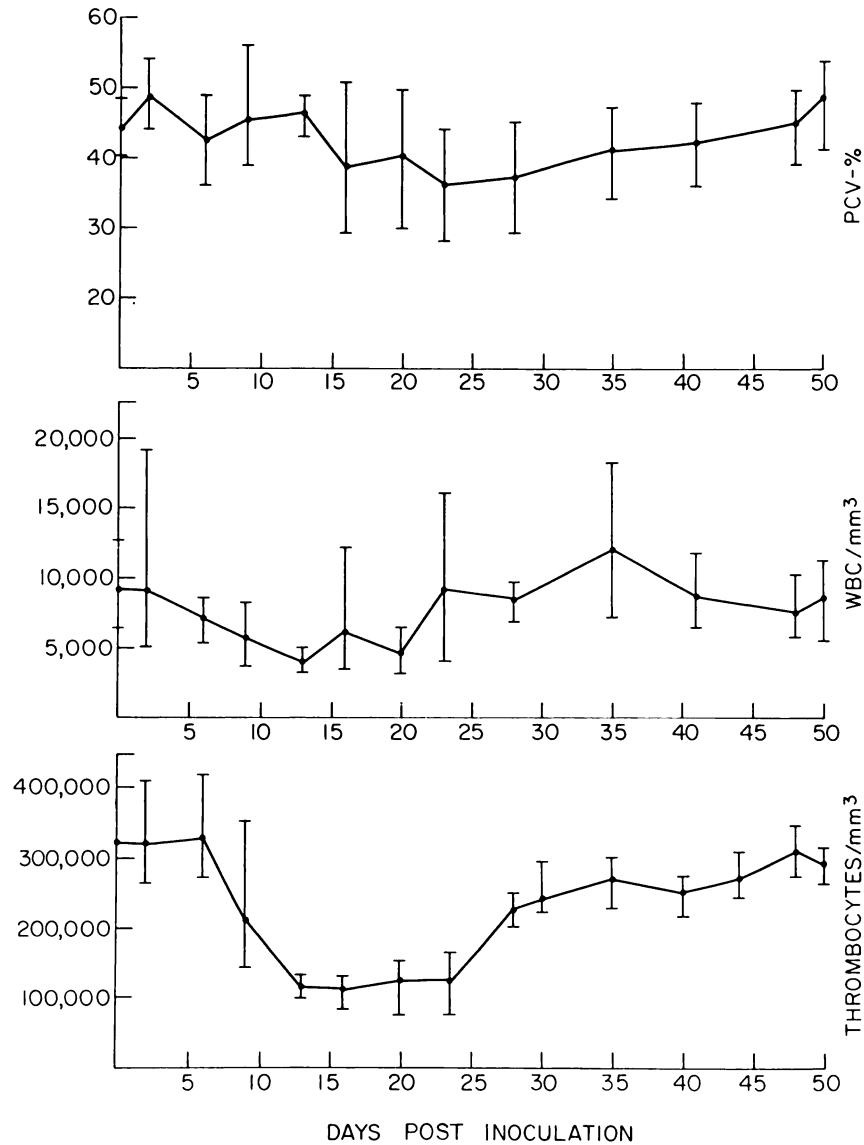


FIG. 1. Mean and range of packed cell volume, white blood cell count, and thrombocyte count of foxes following intravenous inoculation with *Ehrlichia canis*.

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