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ILIA TSACHEV

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Detection of Antibodies Reactive with *Ehrlichia canis* in a Kennel in Bulgaria

Iliia TSACHEV*

Department of Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora,
BULGARIA

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Abstract: A seroepidemiological study on *Ehrlichia canis* infection was performed in 16 dogs in a kennel in the region of Plovdiv in Bulgaria. For this purpose, anti-*E. canis* antibodies were detected by the indirect immunofluorescence antibody test.

The results showed that 75% of the dogs examined were positive to *E. canis*. The antibody titres 1:100, 1:200 and 1:400 were detected.

Key Words: *Ehrlichia canis*, seroprevalence, Bulgaria, IFA test, dog

Canine monocytic ehrlichiosis (CME) is caused by rickettsia *Ehrlichia canis* and is transmitted by the vector tick *Rhipicephalus sanguineus*. CME is detected in many countries in Europe, Asia, the Middle East, Africa, and North and South America. The geographical distribution and the seroprevalence of *E. canis* are as follows: Israel 30%, Egypt 33%, Zimbabwe 42%, and Spain 3% to 67% (1-4). In Greece, *E. canis* is the most common tick-borne infection in dogs (5). In the Mediterranean region of Adana in Turkey, Batmaz et al. (6) reported that 65% of dogs were seropositive to *E. canis*.

There are no reports on the incidence of this infection in Bulgaria. The serological study of CME was motivated by the following circumstances: 1) the observation of clinical symptoms similar to those of CME in a considerable number of dogs (16 dogs) during the summer months of 2002 in a kennel in the region of Plovdiv; 2) the unclear epidemiology of the disease in Bulgaria and the lack of studies on animal ehrlichiosis as a whole; 3) the presence of the vectors of the disease in this country, and 4) the detection of CME in adjacent countries.

Blood sera were collected from the 16 animals mentioned above, and were stored at -20 °C until analysis. The samples were taken during January 2003 from all dogs aged between 1 and 12 years.

All sera were assayed by the indirect fluorescent antibody (IFA) test for the detection of IgG. The antigen used was a formol-inactivated suspension of cells (2.10^6 cells/ml) infected with *E. canis* (Synbiotics Europe, France), which was fixed in 18-well special immunofluorescence slides. The sera were added to the slides after serial dilutions (1:100, 1:200, 1:400 and 1:800) in PBS (pH 7.2). Positive and negative control sera were also tested. Slides were incubated at 37 °C for 30 min and washed twice in PBS for 5 min each time. The monospecific rabbit anti-canine IgG labelled with fluorescein (Sigma) was added at a dilution of 1/25 in PBS, and further incubated for 30 min at 37 °C. After washing as before, slides were air-dried, mounted with Fluoprep (Bio-Merieux) and observed under a fluorescence microscope (Olympus) at 40x. The serum samples at titres of 1:100 and higher were considered positive (7).

* E-mail: ilia_tsachev@abv.bg

Of the 16 dogs examined, 12 (75%) were positive to *E. canis* and 4 (25%) were negative. The lowest titre (1:100) was detected in 8% of the dogs. Those with titres of 1:200 and 1:400 made up 42% and 50%, respectively. The IFA test for the detection of antibodies to anti-*E. canis* has been proven to be a specific and accurate means for detecting dogs with clinical and subclinical ehrlichiosis (8).

The results obtained represent a seroprevalence of 75%, which indicates high possible exposure to infection with *E. canis*. Some of these dogs had probably suffered clinical CME, had recovered from the disease, and had *E. canis* antibodies at the time of testing, while others were probably subclinical carriers of the rickettsia (9).

However, some seropositive dogs might have a cross-reactive response to other *Ehrlichia* species such as *E. chaffensis*, *E. equi*, *E. risticii* and *E. ewingii*. (7) To the best of the author's knowledge, none of the above organisms have been reported to occur in Bulgaria.

The history records showed data for tick infestation in 8 (50%) of the dogs studied. *Ehrlichia* antibody production was proved in all of them. The high percentage obtained in our study is easily explained, because the brown dog tick (*Rhipicephalus sanguineus*) is widespread in Bulgaria (10).

It is concluded that antibodies reactive with *E. canis* were detected in a kennel in Bulgaria for the first time, suggesting possible exposure of dogs to *E. canis*.

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