Survey of antibodies reactive with *Ehrlichia canis* among dogs in South Bulgaria

I. TSACHEV*, V. KONTOS², I. ZARKOV¹ and S. KRASTEV¹

**SUMMARY**

A serological survey was conducted in dogs from five geographical regions of South Bulgaria (Plovdiv, Yambol, Bourgas, Stara Zagora and Blagoevgrad) for determining the prevalence of anti-*Ehrlichia canis* antibodies. Serum samples from 100 dogs were assayed by an indirect fluorescent antibody test and serum reactive at a titre of 1:100 or greater was considered positive. The overall seroprevalence in South Bulgaria was 30%, and a marked heterogeneity of the results was observed according to the geographical region: Plovdiv 50%, Yambol 40%, Bourgas 30%, Stara Zagora 15% and Blagoevgrad 15%. The distributions of serologic titres were 1:100 (40%); 1:200 (33%); 1:400 (23%); 1:800 (4%). Moreover the sensitivity of dogs to *E.canis* exposure was independent from age and sex. The results demonstrate that dogs in South Bulgaria are extensively exposed to *E. canis*.

**Keywords**: *Ehrlichia canis*, seroprevalence, Bulgaria, immunofluorescence, dog.

**Introduction**

*Ehrlichia canis* is the aetiological agent of the canine monocytic ehrlichiosis (CME) that was first described in dogs from Algeria in 1935 [12]. *Ehrlichia canis* is a Gram-negative bacterium. The organism is transmitted by *Rhipicephalus sanguineus* and, in the acute stage of infection, there is often fever, anorexia, lymphadenomegaly, splenomegaly and thrombocytopenia [15]. Most dogs survive to the acute stage and enter in the sub clinical phase of the disease, which might last for years. During this phase, animals are apparently healthy although thrombocytopenia is common. Dogs may spontaneously eliminate *E. canis* during the sub clinical phase [7, 16] or go on to develop the chronic phase of the disease in which there is marked weight loss and signs resulting from pancytopenia.

The disease has been reported in Asia, Africa, Europe and America. In neighbour Greece, *E. canis* is the commonest tick-borne infection in dogs [18] and in the Mediterranean region of Turkey (Adana), BATMAZ *et al.* [5] reported that 65% of dogs were seropositive to *E. canis*.

Worldwide, *E. canis* is the most important species of *Ehrlichia* in dogs. At this time, however, there have been no epidemiological data collected regarding the prevalence of *Ehrlichia in* Bulgaria. This paper presents the results of a serological survey of the prevalence of antibodies reactive with *Ehrlichia canis* among dogs in South Bulgaria. The objective of the study was to assess the regional exposure of dogs to *E.canis*, by determining the seroprevalence in privately owned pet dogs examined at veterinary clinics in five geographical areas.

**Material and methods**

**STUDY AREA AND ANIMALS**

The survey was carried out on the south part of Bulgaria (approximately 41°34’ and 42°38’ N latitude, 22°40’ and 27°29’ W longitude). The climate is typically continental.

Veterinarians from five private clinics were requested to collect serum samples from privately owned pet dogs visiting their clinics for examination or routine health care. In total 100 dogs of different breeds were examined. The geographical location of the dogs was classified into the following areas: Plovdiv: n=20, Yambol: n=20, Bourgas: n=20, Stara Zagora: n=20 and Blagoevgrad: n=20. A total of 51 males and 49 females were used in this study and the proportions of males and females sampled in each region were roughly similar (from 45% to 55%) excepted in the Yambol region (40% of males) and in Stara Zagora region (65% of males). Ages ranged from 1 to 11 years with a mean ± standard deviation (S.D.) of 3.81 ± 0.39 years. Three classes of age were defined: 54 dogs were 1 to 3 years old, 35 dogs...
were 3 to 7 years old and 11 were 7 to 11 years old. The outcome variable was seropositivity to *E. canis* and the independent variables were: sex (male, female) and age.

**BLOOD SAMPLING**

Five ml of venous blood was collected from all 100 dogs by brachial vein puncture using vacuum tubes. Blood was allowed to coagulate at room temperature. Sera were separated by centrifugation at 1700 g for 10 minutes, transported (4-7°C) to the laboratory and kept at -20°C until assayed.

**ANTIBODY DETECTION**

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 *Ehrlichia canis* (Synbiotics Europe, France), which was fixed in 15-well special immunofluorescence slides. Sera were added to the slides after serial dilution (1:100, 1:200, 1:400 and 1:1600) 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 times in PBS (5 min each time). The monospecific rabbit anti-canine IgG labelled with fluorescein isothiocyanate (Sigma, Germany) 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 and mounted with Fluoprep (Bio-Merieux) and observed under a fluorescence microscope (Olympus) at 400x magnification. Serological sera at titres of 1:100 and higher were considered as positive [22].

**Results**

Dogs with antibodies reactive against *E. canis* were found in all the regions and seroprevalences varied from 60% to 15% (Table I). The highest prevalences of *E. canis* antibodies in dogs were noticed in Plovdiv (50%) and in Yambol (40%). The lowest prevalence (15%) was detected in both Stara Zagora and Blagoevgrad.

Thirty of the 100 dogs were found to be seropositive by the IFA test and the overall seroprevalence was 30%. Twenty-two seropositive dogs (73%) presented serum antibody titres ranged between 1:100 to 1:200, whereas only 27% of dogs have shown high antibody titres (1:400 or 1:800) (Table I).

Table II shows the comparisons of the observed numbers of seropositive dogs according to their age and their sex with the theoretical numbers of infected dogs (calculated by the following formula: numbers of animals x prevalence of the infection, overall or specific for each region). Except in the Yambol region where the numbers of positive 3 to 7 years old dogs were less than expected, all other regions showed significant deviations.

### Table I

<table>
<thead>
<tr>
<th>Region</th>
<th>Plovdiv</th>
<th>Yambol</th>
<th>Bourgas</th>
<th>Stara Zagora</th>
<th>Blagoevgrad</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tested dogs</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Number of positive dogs</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Seroprevalence (%)</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Titre                1:100</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>1:200</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>1:400</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>1:800</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
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</table>

**Overall population**

<table>
<thead>
<tr>
<th>Classes of age (year)</th>
<th>Total</th>
<th>Positive</th>
<th>Plovdiv</th>
<th>Positive</th>
<th>Yambol</th>
<th>Positive</th>
<th>Bourgas</th>
<th>Positive</th>
<th>Stara Zagora</th>
<th>Positive</th>
<th>Blagoevgrad</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1; 3]</td>
<td>54</td>
<td>17</td>
<td>16</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>15</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>[3; 7]</td>
<td>35</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>[7; 11]</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

**Sex**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>OV</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>TV</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Overall**

<table>
<thead>
<tr>
<th>Region</th>
<th>Total</th>
<th>Positive</th>
<th>Plovdiv</th>
<th>Positive</th>
<th>Yambol</th>
<th>Positive</th>
<th>Bourgas</th>
<th>Positive</th>
<th>Stara Zagora</th>
<th>Positive</th>
<th>Blagoevgrad</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>100</td>
<td>30</td>
<td>22</td>
<td></td>
<td>15</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td>14</td>
<td>12</td>
<td></td>
<td>48</td>
</tr>
</tbody>
</table>

**Notes**

- The numbers of positive dogs are marked with OV (Observation Value).
- The numbers of positive dogs are marked with TV (Theoretical Value).
- The formula used for calculating the theoretical values is: number of animals x prevalence of the infection.
dogs weakly differed from the expected values, the other observed values whatever the age of dogs were very closely related to the theoretical values for the total population studied and for each specific region. Consequently, *E. canis* infection equally affected young and old dogs. In the same way, the observed numbers of total positive males and positive females or from each specific region did not significantly differ from calculated values. Nevertheless, in Stara Zagora region, *E. canis* infection seemed to be more often detected in males. But, globally, males were not preferentially affected.

**Discussion**

The prevalence of *E. canis* is largely dependent on the distribution of the vector, *R. sanguineus*, which occurs mainly in tropical and subtropical regions. The brown dog tick (*Rhipicephalus sanguineus*) is widely spread, notably in Bulgaria [28], especially in the South part [1]. Surveys conducted in Spain, Mexico, Egypt, Israel, Germany and France have revealed that *E. canis* antibody seroprevalence were 3.5-3.3, 3.2, 3.0, 2.9, 3.1 and 3.5% respectively [5, 6, 11, 12, 13, 26, 27]. In the present study, the total seroprevalence of *E. canis* in South Bulgaria was 30% but was ranged from 15% to 50% according to the region. These variations may be due to some epidemiological factors, such as the distribution of the vector, animal behaviour, and the average age of the study population [26]. Some dogs of the present study probably suffered from a clinical CME, and partially recovered from the disease, but presented *E. canis* antibodies as markers of the disease, while others were probably sub clinical carriers of the rickettsia [16, 30, 31]. However, some seropositive results may be related to cross-reactive responses with other *Ehrlichia* spp [20]. Indeed, serological cross-reactivity between *E. canis* and *E. chaffeensis was already signalled [23]. *E. chaffeensis* is the agent of human monocytic ehrlichiosis, which was first described in the United States of America (USA) in 1987 [2]. Subsequent studies have indicated that human infections also occur in Europe [21] and Africa in [8, 29]. There are now over 400 studies have indicated that human infections also occur in United States of America (USA) in 1987 [2]. Subsequent already signalled [23]. In this study, the observed sex values were also very closely related to theoretical values except for the Stara Zagora region. Therefore, the *E. canis* infection does not preferably affect the males. The data are in agreement with the report of BANETH et al. [4] and BOTROS et al. [6]. However, our finding is in disagreement with BATMAZ et al. [5], CHANDRASEKAR et al. [9] and LEIB and MONROE [19]. In order to overcome this discrepancy and to identify a potential predilection for a specific gender, the future surveys should be conducted on higher numbers of dogs [5].

In conclusion, these results indicate a high exposure of the dogs in Bulgaria to the rickettsia *E. canis* or closely related species. This finding suggests that clinicians and dog owners in Bulgaria, and those travelling into Bulgaria, should increase their awareness towards this potentially fatal canine disease.

**Acknowledgements**

We thank Athens Animal Hospital, Athens, Greece for their technical assistance and collaboration. Funding was provided by the Trakia University, Bulgaria.

**References**


