

Seroepidemiological Research of Canine Monocytic Ehrlichiosis in the Autonomous Province of Vojvodina, Serbia*

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ABSTRACT

Background: Monocytic ehrlichiosis is an important disease in canine pathology and is present worldwide and has a potentially lethal outcome. This infection as a 'new' infective disease of dogs and people and it has not been sufficiently researched, especially from the aspect of clinical picture, pathogenesis, diagnostics and differential diagnostics. The aims of this seroepidemiological research of the *E. canis* infection with dogs are to determine the values of G class antibodies prevalence to *E. canis* antigens, also to determine a agreement of positive serological findings between two immunodiagnostic tests (iELISA and IFAT), and to make an overview of the epidemiological characteristics of this canine infection.

Materials, Methods & Results: One hundred dogs were involved in the seroepidemiological research of the *E. canis* infection. These dogs were from the City of Novi Sad area and its outskirts, Autonomous Province of Vojvodina, Serbia. The epidemiological survey of the closed type has been used in the research. The survey has been conducted with all of the dogs involved in this research. Apart from identifying veterinary practices and the patients (breed, sex, age), the survey was designed to collect data about the tick bites (first bite or a repeated one); the number of the removed ticks; the dogs' lifestyles and information on whether the dogs had been abroad. An overall of 62 ticks were collected from the bodies of these dogs. By applying the screening iELISA test, the seroprevalence of class G specific antibodies was determined with dogs to *E. canis* antigens in 25%, while 75% of dogs were negative to the presence of specific antibodies of IgG class to *E. canis*. Out of 25 seropositive dogs, with antibodies to *E. canis* discovered through iELISA test, 12 (48%) dogs were in contact with ticks for the first time, while the repeated contact with ticks was experienced by 9 (36%) dogs. For 4 (16%) dogs, the owners negated that their dog had been bitten by a tick. Upon the application of a confirming IFAT, a seroprevalence was determined of specific class G antibodies with dogs to *E. canis* antigens in 16%, while 84% were negative to the presence of specific antibodies of IgG class antibodies to *E. canis*. Out of 16 seropositive dogs, in which antibodies were discovered with IFAT to *E. canis*, 9 (56%) dogs had been in contact with ticks for the first time, while 4 dogs (25%) had repeated contacts with ticks. For 3 dogs (19%), the owners negated that ticks had bitten their dogs. Through a statistical processing of the results gained from the applied immunodiagnostic tests, a moderate agreement (Kappa value of 0.48) was determined. In the group of dogs that had been bitten by a tick, three types were determined: *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Ixodes ricinus*. Overall 35 ticks of *Dermacentor marginatus* ticks were replaced from the dogs' bodies just as were 15 *Rhipicephalus sanguineus* ticks and 12 *Ixodes ricinus* ticks.

Discussion: The determined values of the seroprevalence in our country (iELISA - 25% and IFAT - 16%) are in harmony with the acquired values in other countries with warm climate. The values of seroprevalence determined in this research point to the fact that this disease must be taken into consideration in everyday health protection of dogs, especially after a registered tick bite within a differential diagnostics procedure.

Keywords: canine monocytic ehrlichiosis, *Ehrlichia canis*, dogs, ticks.

INTRODUCTION

In the past few decades, infectious diseases that are transmitted by ticks as vectors have become a growing problem in the public health around the world. Multiple occurrences of new causes of infections transmitted by ticks and discovering their etiology have been cause for a rise in the awareness about these zoonoses [17].

Monocytic ehrlichiosis is an important disease in canine pathology. It is present around the world and is potentially lethal. The agent of this infection is bacteria *Ehrlichia canis*, which is transmitted by *Rhipicephalus sanguineus* ticks [8]. The disease is, for the most part, present in tropical and subtropical countries with warm climate. In Europe, the canine infection with *Ehrlichia canis* is generally present in Mediterranean countries (Spain, France, Italy and Turkey) and rarely there have been reports of infection in Central Europe. Canine infection with *E. canis* is endemically present in certain regions of Italy and Spain, while a group of autochthonous cases of infection has been identified in the north of France [20].

The Autonomous Province of Vojvodina has similar geographical and climatic features to the other countries that have confirmed the presence of the vectors and causes of monocytic ehrlichiosis within the canine population. So far no data has been reported that are in connection to a seroepidemiological research of canine infection with *E. canis* in the Autonomous Province of Vojvodina.

The aims of this seroepidemiological research of the *Ehrlichia canis* infection with dogs are to determine seroprevalence IgG antibodies to *E. canis* antigens; to determine a agreement of positive serological findings between two immunodiagnostic tests (iELISA and IFAT) and to make an overview of the epidemiological characteristics of this canine infection.

MATERIALS AND METHODS

Study Design

One hundred dogs have been involved in the seroepidemiological research of *Ehrlichia canis* infection. These dogs were from the City of Novi Sad area and its outskirts. The epidemiological survey of the closed type has been used in the research. The survey has been conducted with all of the dogs involved in this research. Apart from identifying veterinary practices

and the patients (breed, sex, age), the survey was designed to collect data about the tick bites (first bite or a repeated one); the number of the removed ticks; the dogs' lifestyles and information on whether the dogs had been abroad.

Samples

Full vein blood samples drawn using aseptic venepuncture *v. cephalica antebrachii*, and stored in sterile vacutainers with coagulation activators. One hour after blood samples had been taken, blood serums were being separated for ten minutes using a centrifuge at 865 x g. Removing the ticks from the dogs was performed at veterinary practices, and full vein blood samples had been taken from the same dogs. A total of 62 ticks were removed from the dogs' bodies.

Serological methods

In order to determine the presence of specific G class antibodies to *Ehrlichia canis* antigens, two immunodiagnostic tests were used. As a screening test, an indirect immunoenzymatic test (iELISA)¹, intended for a qualitative detection of IgG antibodies in a dog serum to *E. canis* antigens. Both iELISA test and test results interpretation were done according to the producer's protocol. To read the optical density (OD) a filter of 450 nm was used. The test was considered to be positive when the OD values were higher than 0.5, and negative when they were lesser than 0.2, while the cut-off values were between 0.2 and 0.5. titer of the specific antibodies IgG in the dogs' serum to *E. canis* antigens 1:100 and higher was considered to be positive. As a confirmation test, an indirect immunofluorescent test was used (IFAT)². As a source of antigens in the test, DH82 *E. canis* infected culture of cells was used. The positive test was characterised with the appearance of clearly fluorescing cytoplasmic inclusive elementary corpuscles (morules), groups of elementary corpuscles (initial corpuscles) and free elementary corpuscles. A negative finding was characterised by the absence of the described fluorescence. The titer of specific antibodies of IgG in the dog serum to *E. canis* antigens of 1:50 and higher was considered to be positive.

Ticks determination

The species of the collected ticks were determined according to Estrada-Pena key [6].

Statistical methods

The results of seroepidemiological findings of research on *Ehrlichia canis* infection with dogs were statistically processed using the Kappa test, i.e. the testing took place of agreement in the determined positive serological findings between two immunodiagnostic tests (iELISA and IFAT) used in this research.

RESULTS

By applying the screening iELISA test, the seroprevalence of class G specific antibodies was determined with dogs to *Ehrlichia canis* antigens in 25%, while 75% of dogs were negative to the presence of specific antibodies of IgG class to *E. canis*. Out of 25 seropositive dogs, with antibodies to *E. canis* discovered through iELISA test, 12 (48%) dogs were in contact with ticks for the first time, while the repeated contact with ticks was experienced by 9 (36%) dogs. For 4 (16%) dogs, the owners denied that their dog had been bitten by a tick. Within the group of seropositive dogs, generally just 1 tick was found on the dog's body (9 dogs), while three dogs had a large number of ticks (2, 5, and 30) on them. The largest number of seropositive dogs, detected after iELISA test to *E. canis* did not live exclusively in an enclosed space (16 or 64%) whilst 7 (28%) of the dogs mainly spent their time within an enclosed space, and 2 (8%) lived both inside and outside the owner's house. Only two dogs from this group had been taken abroad (Australia and Hungary). Dogs in this group were of different breeds (Table 1).

Upon the application of a confirming IFAT, a seroprevalence was determined of specific class G antibodies with dogs to *E. canis* antigens in 16% (Figure 1), while 84% were negative to the presence of specific antibodies of IgG class antibodies to *E. canis*. Out of 16 seropositive dogs, in which antibodies were discovered with IFAT to *E. canis*, 9 (56%) dogs had been in contact with ticks for the first time, while 4 dogs (25%) had repeated contacts with ticks. For 3 dogs (19%), the owners denied that ticks had bitten their dogs. Within the group of seropositive dogs one tick was found on most of the dogs (6 dogs), while four of the dogs had more than one tick (2; 2; 2 and 5). The largest number of seropositive dogs (11 or 68%), which showed antibodies to *E. canis* through IFAT testing had lived outside, while 3 (19%) had lived in a closed space and 2 (13%) had lived both ways. Only one dog in this group had been taken abroad (Hungary). Dogs in this group were of different breeds (Table 2).

Within a group of dogs that had been bitten by a tick, three sorts of ticks were determined *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Ixodes ricinus*. Overall 35 ticks of *Dermacentor marginatus* ticks were replaced from the dogs' bodies just as were 15 *Rhipicephalus sanguineus* ticks and 12 *Ixodes ricinus* ticks.

Kappa value of 0.48 was achieved after statistical data processing and cross-examination of agreement of the determined positive serological findings between the two applied immunodiagnostic tests in this research.

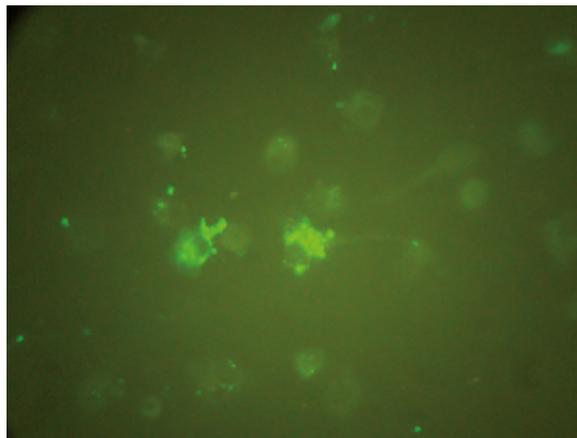


Figure 1. Positive result of IFAT to *Ehrlichia canis* antigens.

Table 1. Epidemiological data on seropositive dogs to *Ehrlichia canis* (iELISA).

Nº. record	First contact with ticks	Repeated contact with ticks	Nº. removed ticks from dogs	Dog's lifestyle	Stayed abroad	Dog's breeds
2	Yes	No	1	inside	No	Poodle
19	Yes	No	30	outside	No	German shepherd
21	Yes	No	0	inside	No	Labrador Retriever
30	Yes	No	1	outside	No	German Shorthaired Pointer
34	No	Yes	1	outside	No	German Shorthaired Pointer
35	No	Yes	0	inside	No	German shepherd
45	No	Yes	0	outside	No	German Shorthaired Pointer
48	No	Yes	0	inside	No	Out of breed
63	No	No	0	both ways	No	German hunting terrier
68	Yes	No	0	outside	No	Out of breed
70	Yes	No	0	outside	No	Puli
75	Yes	No	5	outside	No	English Cocker Spaniel
77	Yes	No	1	outside	No	Rottweiler
81	Yes	No	1	outside	No	German boxer
95	Yes	No	1	outside	No	Out of breed
96	No	Yes	1	outside	No	Out of breed
97	No	Yes	2	outside	No	Out of breed
102	Yes	No	0	inside	No	English Cocker Spaniel
234	No	Yes	1	outside	No	Serbian Hound
235	No	Yes	2	inside	No	English Setter
276	No	No	0	inside	Australia	Out of breed
277	No	No	0	outside	Hungary	American Staffordshire Terrier
279	No	No	0	outside	No	American Staffordshire Terrier
280	Yes	No	0	both ways	No	Small Munsterlander
282	No	Yes	1	outside	No	Flat Coated Retriever

Table 2. Epidemiological data on seropositive dogs to *Ehrlichia canis* (IFAT).

Nº. record	First contact with ticks	Repeated contact with ticks	Nº. removed ticks from dogs	Dog's livestyle	Stayed abroad	Dog's breeds
2	Yes	No	1	inside	No	Poodle
43	No	No	0	outside	No	German Shorthaired Pointer
48	No	Yes	0	inside	No	Out of breed
54	Yes	No	2	outside	No	Out of breed
55	No	No	0	both ways	No	Out of breed
65	Yes	No	1	outside	No	Rottweiler
68	Yes	No	0	outside	No	Out of breed
75	Yes	No	5	outside	No	English Cocker Spaniel
81	Yes	No	1	outside	No	German boxer
95	Yes	No	1	outside	No	Out of breed
97	No	Yes	2	outside	No	Out of breed
217	Yes	No	1	outside	No	Dobermann
235	No	Yes	2	inside	No	English Setter
277	No	No	0	outside	Hungary	American Staffordshire Terrier
280	Yes	No	0	both ways	No	Small Munsterlander
282	No	Yes	1	outside	No	Flat Coated Retriever

DISCUSSION

This infection can be appropriately described as one of the best ‘copycats’ among the infective diseases, due to the fact that its clinical picture can resemble metabolic, neurological or urogenital diseases. This is exactly why it reports come that it is very difficult to separate canine ehrlichiosis from other diseases. Another reason for difficulties in determining a quick and precise diagnosis of canine ehrlichiosis regards a lack of standardised diagnostic procedures, diagnosticum for routine research, as well as the source of adequate data.

The prevalence of the infection with *Ehrlichia canis* depends to a great extent on the distribution of the *Rhipicephalus sanguineus* vector, which is for the most part present in tropics and subtropics. This tick is present in the countries of Southern Europe and in Serbia [4,12]. The determined values of the seroprevalence in Serbia (iELISA - 25% and IFAT - 16%) are in harmony with the acquired values in other countries with warm climate [5,8,15]. The values of prevalence antibodies/pathogens to *E. canis* determined by IFAT was reported in Germany to be 24.8% [11]. Out of 44 dogs with no owners in Portugal (Setúbal), that have been serologically examined and a seroprevalence of 54.5% to *E. canis* antigens was determined, by ap-

plying the test of indirect immunofluorescence [2]. In Italy (Imola, Emilia-Romagna), out of 154 examined dogs with no owners and using the test of indirect immunofluorescence a seroprevalence to *E. canis* was discovered in 72% [3]. Within the period from 1986 to 1991, 118 cases of monocyte canine ehrlichiosis were diagnosed in the laboratory of Messina University in Italy [13]. In Spain, seroprevalence to *E. canis* of 66.7% was determined by applying the test of indirect immunofluorescence, with 36 hunting dogs in a Madrid kennel, while seroprevalence of 2.3% was determined with 131 examined police dogs from nearby kennels [16]. Seroepidemiological research of whether military dogs had been infected with *E. canis* was conducted in a number of areas in France and it has been established that the value of seroprevalence was from 0% in certain areas to 87% (out of 1989 examined dogs) in highly endemic regions like Bastia, Corsica [20]. With dogs from North-east Spain, seroprevalence of 16.7% was found to *E. canis* antigens [18] and successful isolation of this agent was reported from naturally infected dogs of this region [1]. In a study in Northern Sardinia, 50% of positive serums from dogs were found to *E. canis* antigens upon the application of the test of indirect immunofluorescence, with the biggest seroprevalence of 62.5% among the dogs aged from 13 to 60 months [5]. Upon the application of PCR test, presence of *E. canis* was identified in 3 out of 14 blood samples collected from seropositive dogs in Italy, and similarity of sequences of two amplicons of 99.2% with the Oklahoma strain of *E. canis* [10]. In the molecular survey of *Ehrlichia canis* and *Anaplasma phagocytophilum* from blood of dogs in Italy, the values of prevalence to *E. canis* reported were as follows in Northern Italy of (2.9%), Central Italy (8%) and Southern Italy (9.7%) [19]. In Turkey, a proved infection with *E. canis*, upon application of PCR test with three dogs has been reported in Ankara, Turkey, and confirmed that 16S rRNA sequence of the genome with this strain (marked as Hutahya) identical to *E. ovina* and with the strain isolated from dogs in Venezuela (marked as VDE), and a very similar (99.9%) to the Oklahoma *E. canis* strain [21]. Through a seroepidemiologic research of canine infection with *E. canis* in Switzerland, a presence of specific antibodies has been determined with 2.2% dogs [14] and authors believe that these dogs had most probably been infected abroad, considering that the vector of this infection is present south of Alps.

The results of the research point out to a fact that there is no significant difference in the seroprevalence of class G antibodies to *E. canis* antigens related to a presence of a tick bite. This can be explained with the fact that a chronic state of this infection with dogs is often present, and also that apart from the referent IFAT for the diagnostics of this infection, there are other serological tests like iELISA that have not been standardised. In our research work a moderate agreement between iELISA and IFAT has been confirmed through a statistical processing of the results gained through immunodiagnostic tests, so that iELISA test can be applied as reliable a screening test in the serological research of the presence of this infection with dogs [1,7,9,22].

CONCLUSION

The determined values of G class antibodies prevalence by iELISA is 25% and by IFAT is 16% to *Ehrlichia canis* antigens. Through a statistical processing of the results gained from the applied immunodiagnostic tests (iELISA and IFAT), a moderate agreement (Kappa value of 0.48) was determined. In our study dogs of different breeds, mostly had contact with ticks for the first time. Three types of ticks were determined: *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Ixodes ricinus*. The largest number of seropositive dogs to *E. canis* antigens had been lived outside.

The values of seroprevalence determined in this research point to the fact that this disease must be taken into consideration in everyday health protection of dogs, especially after a registered tick bite within a differential diagnostics procedure.

SOURCES AND MANUFACTURERS

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Acknowledgements. This research was supported by Provincial Secretariat for Science and Technological Development, Autonomous Province of Vojvodina, Republic of Serbia (grant title: Research of Lyme disease and other vector-borne zoonoses in Vojvodina, grant number 114-451-1892/2011).

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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