

VETERINARSKI ARHIV 85 (4), 385-394, 2015

The seroprevalence of *Anaplasma phagocytophilum* infection in dogs in the Autonomous Province of Vojvodina, Serbia

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POTKONJAK, A., V. VRAČAR, S. SAVIĆ, B. LAKO, V. RADOSAVLJEVIĆ, M. CINCOVIĆ, LJ. SUVAJDŽIĆ, A. JURISIĆ, A. PETROVIĆ: The seroprevalence of *Anaplasma phagocytophilum* infection in dogs in the Autonomous Province of Vojvodina, Serbia. Vet. arhiv 85, 385-394, 2015.

ABSTRACT

Granulocytic anaplasmosis in dogs is a disease that is distributed worldwide, caused by the pathogen *Anaplasma phagocytophilum*. This disease is transmitted by ticks of the *Ixodes* genus. So far, no data have been published about the presence and prevalence of dog infections with *A. phagocytophilum* in the Autonomous Province of Vojvodina and the Republic of Serbia proper. The aim of this seroepidemiological research was to determine the seroprevalence of IgG antibodies to the agent *A. phagocytophilum* in the population of dogs in Vojvodina, Serbia. This seroepidemiological research involved 84 randomly selected dogs from the area of the Autonomous Province of Vojvodina. Aiming to determine the presence of antibodies of class G to the agent *Anaplasma phagocytophilum*, we used the indirect immunofluorescence antibody test (IFAT). By applying IFAT, in the area of the Autonomous Province of Vojvodina, Serbia, specific antibodies of class G to agent *A. phagocytophilum* were found in the serums of 13/84 dogs, which points to a seroprevalence of 15.5 %. The detection of antibodies to the agent *A. phagocytophilum* in the dog population in the area of the Autonomous Province Vojvodina is the basis for further epidemiological, clinical and diagnostic research of this infection.

Key words: canine granulocytic anaplasmosis, *Anaplasma phagocytophilum*, seroprevalence, dogs, indirect immunofluorescence antibody test, Serbia

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Introduction

The cause of granulocytic anaplasmosis in people and animals (until recently known as granulocytic ehrlichiosis) is *Anaplasma phagocytophilum*. *A. phagocytophilum* is transmitted by vectors - ticks from the *Ixodes* genus (CARRADE et al., 2009; LILLINI et al., 2006).

Infections caused by *A. phagocytophilum* have been reported worldwide in areas in the northern hemisphere, with endemic occurrence of *Ixodes* ticks (STUEN et al., 2013). The disease has been sporadically reported in several European countries, thus SOLANO-GALLEGO et al. (2006) reported seroprevalence of 11 % in dogs in Spain. In Germany antibodies to *A. phagocytophilum* have been confirmed in 43.2 % of the examined dogs (JENSEN et al., 2007). By applying the IFAT, PUSTERLA et al. (1998) have shown the value seroprevalence of the infection caused by *A. phagocytophilum* in dogs in Switzerland of 7.5 %. In a seroepidemiological study conducted in Slovenia from 1999 to 2006, infection with *A. phagocytophilum* was confirmed by serology in 70.5 % (481/682) of dogs considered to have an infection, and by PCR in 2.5 % (25/682) respectively (RAVNIK et al., 2009).

Considering that chronic anaplasmosis in dogs has not been documented, the clinical symptoms almost exclusively appear in the acute phase of the disease, during the bacteriemic phase (GREIG and ARMSTRONG, 2006). The symptoms of granulocytic ehrlichiosis are non-typical and include apathy, febrile episodes, inappetence, diarrhea, vomiting, splenomegalia, arthritis and lymphadenopathy (COCKWILL et al., 2009; EGENVALL et al., 1996; EGENVALL et al., 1998; JENSEN et al., 2007; RAVNIK et al., 2009). The most common laboratory findings noted in sick dogs are leukopenia with lymphopenia and neutropenia, followed by leukocytosis with lymphocytosis thrombocytopenia, anemia and increased liver enzymes (RAVNIK et al., 2009; RAVNIK et al., 2011). However, according to Foley, dogs that are naturally infected with *Anaplasma phagocytophilum* usually remain healthy and become asymptomatic carriers of the disease (FOLEY et al., 2001).

The (IFAT) test is the most sensitive method for detection of specific antibodies to *A. phagocytophilum*. The sensitivity of detection is greater at 2 - 4 weeks after the onset of the disease compared to the PCR method, microscopy of blood smears and isolation of the agents in the cell culture (BAKKEN and DUMLER, 2006).

Even though *A. phagocytophilum* has been detected in a wide range of wild animals, the reservoir species in Europe has not yet been established (STUEN et al., 2013). Dogs could be considered as potential reservoirs for this pathogen. So far in the Autonomous Province of Vojvodina and the Republic of Serbia there have been no data published on the presence and scope of dog infections with the *A. phagocytophilum*.

The aim of this seroepidemiological research was to determine the seroprevalence of IgG antibodies to the agent *A. phagocytophilum* in the population of dogs in Vojvodina, Serbia.

Materials and methods

Study design. This seroepidemiological research included 84 randomly selected dogs from the area of the Autonomous Province of Vojvodina. A closed-type epidemiologic survey was used in the research, and was conducted for all the dogs involved in the research. The data in this survey refer to the patient's basic identification data, tick bites (first or repeated bites), the dogs' lifestyles and information about whether the dogs had spent any time abroad.

Samples. Samples of full vein blood were collected into sterile vacutainers, with coagulation activators, by aseptic venipuncture from the v. cephalica antebrachii. One hour after collecting the blood samples, the blood serum was separated following ten-minute centrifuge at 3000 rpm.

Indirect immunofluorescence antibody test. In order to determine the presence of class G antibodies to the agent *Anaplasma phagocytophilum* we used the indirect IFAT produced by Fuller Laboratories, USA. As the source of antigens in the IFAT, we used semi-purified elementary corpuscles and a morulae agent multiplied on cell culture. The positive reaction is characterized by the appearance of clearly defined, small cocci of apple-green fluorescent color on a red field. The negative results are characterized by the absence of the green fluorescence described. A titer of 1:80 and higher of specific IgG antibodies against *A. phagocytophilum* in the canine serum is considered to be positive.

Results

By applying the IFAT test in the area of the Autonomous Province of Vojvodina, Serbia, specific class G antibodies to the vector-borne pathogen *A. phagocytophilum* were noted in blood serums of 13/84 dogs (Fig. 1), which represents a prevalence of 15.5 %.

Seropositivity was noted in individuals of both sexes, 7 females and 6 males. This seroepidemiological research showed that the youngest seropositive dog was one year old, and the oldest seropositive dog was 13 years old. At the moment of blood sampling, in the group of seropositive dogs ticks were registered on 7/13 of them (53.84 %). This research showed that 12 out of altogether 13 seropositive dogs (92.3 %), in which antibodies to agent *A. phagocytophilum* were found, lived outside, while one seropositive dog (7.7 %) lived indoors. In this research none of the dogs that were seropositive to antigens of agent *A. phagocytophilum* had been abroad (Table 1). From Fig. 2 it can be seen that the highest seroprevalence was registered in the South Bačka district followed by the Central Banat, Sirmia and North Bačka districts, while all samples from West Bačka, North Banat and South Banat districts were negative.

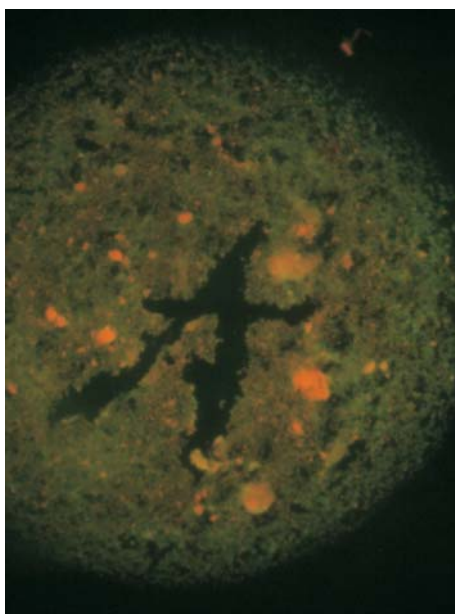


Fig. 1. Positive result of IFAT to *A. phagocytophilum* antigens

Table 1. Epidemiological data of the dogs seropositive to *A. phagocytophilum*

Area	Sample	Sex	Age	Dog's keeping	Tick bite
Zrenjanin	Zr1	male	10 years	outdoors	/
	Zr2	male	4.5 years	outdoors	/
	Zr3	female	7.5 years	outdoors	/
	Zr4	male	7 years	outdoors	/
Novi Sad	NS4	female	3 years	outdoors	repeated
	NS8	female	10 years	outdoors	/
	NS12	female	4 years	outdoors	repeated
	NS20	male	1 year	outdoors	repeated
	NS50	female	11 years	indoors	repeated
Subotica	Su6	male	2.5 yeras	outdoors	/
Rumenka	R4	female	6 years	outdoors	repeated
	R22	male	9 years	outdoors	repeated
	R24	female	2 years	outdoors	repeated



Fig. 2. Distribution of the positive and total samples by districts of the Autonomous Province of Vojvodina

Discussion

The first case of granulocytic anaplasmosis in dogs caused by the agent *A. phagocytophilum* was registered in 1982 in California, USA. However, researchers became increasingly interested in this pathogen from 1994, when the first case of human granulocyte ehrlichiosis caused by this agent was described (BAKKEN et al., 1994; MADEWELL and GRIBBLE, 1982). In Europe, cases of dogs infected with the agent *A. phagocytophilum* have been sporadically reported (BARUTZKI et al., 2006; EBANI et al., 2008; EKERSTAD et al., 1996; SCHAW et al., 2005; SKOTARCZAK et al., 2004; TOZON et al., 2003; TSACHEV et al., 2008).

In Serbia, so far there have been no data published about the seroprevalence of antibodies to the agent *A. phagocytophilum* among the canine population. However, in 2008 MILUTINOVIĆ et al. (2008) for the first time reported data about the confirmed presence of *A. phagocytophilum* in a population of *Ixodes ricinus* ticks in the territory of Serbia. According to the same authors, the prevalence of *A. phagocytophilum* in ticks from two locations in the region of Vojvodina was 17.6 %. Experimental studies by TOMANOVIĆ et al. (2008) proved the presence of *A. phagocytophilum* in two other species of tick in the area of Serbia, that is the *Haemophysalis concinna* and *Dermacentor reticulatus*.

There is significant regional variability in the seroprevalence of *A. phagocytophilum* in dogs. As AMUSATEGUI et al. (2008) report the seroprevalence of antigens to antibodies of the agent *A. phagocytophilum* on a sample of 479 dogs in north-western Spain, confirmed by the IFAT at 5 %, while BEALL et al. (2008) show a prevalence of 55.4 %, confirmed also by the IFAT, on the sample of 731 dogs in Minnesota. The seroprevalence of antibodies of 15.5 % to antigens of the agent *A. phagocytophilum* in dogs on the territory of the Autonomous Province of Vojvodina obtained in this research corresponds to the results of other authors from dogs in Sweden and North Arizona (DINIZ et al., 2010; EGENVALL et al., 2000). The seroprevalence of canine granulocytic anaplasmosis in dogs recently reported in Latvia (11.5 %) also corresponds to our results (BERZINA et al., 2013). However, the seroprevalence determined in our study is noticeably lower compared to the seroprevalence of 43 % reported by KOHN et al. (2011) in a population of 522 dogs in Germany.

This research, as previous research has indicated, registers no gender predisposition to the canine infection caused by *A. phagocytophilum* (BERZINA et al., 2013; JENSEN et al., 2006; M'GHIRBI et al., 2009; SOLANO-GALLEGO et al., 2006). As Amusatogui reports, seropositivity is more frequent among dogs who live outdoors, which is also confirmed by this study, considering that only 1 of 13 seropositive dogs was kept indoors (AMUSATEGUI et al., 2008). As in the results of other researchers, it has been noted that the frequency of the infection grows with age (BERZINA et al., 2013; EGENVALL et al., 2000; KOHN et al., 2008; SANTOS et al., 2011). As none of the seropositive dogs stayed abroad we cannot consider any of the cases as imported infection.

Conclusion

Due to the non-specific clinical picture of the canine disease caused by *A. phagocytophilum*, as well as the close connections between dogs and people, dogs can be a significant factor in maintenance and transmission of this vector-born, natural-focal zoonosis.

The presence of *A. phagocytophilum* infection has been registered in the population of the examined dogs in the area of the Autonomous Province of Vojvodina, Serbia. The value of the prevalence of the specific IgG antibodies determined by using IFAT is 15.5 %.

The detection of antibodies to *A. phagocytophilum* in the dog population in the area of the Autonomous Province Vojvodina is the basis for further epidemiological, clinical and diagnostic research into this infection.

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).

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Received: 29 May 2014

Accepted: 30 January 2015

POTKONJAK, A., V. VRAČAR, S. SAVIĆ, B. LAKO, V. RADOSAVLJEVIĆ, M. CINCOVIĆ, LJ. SUVAJDŽIĆ, A. JURISIĆ, A. PETROVIĆ: Seroprevalencija infekcije izazvane uzročnikom *Anaplasma phagocytophilum* kod pasa u Autonomnoj Pokrajini Vojvodini, Srbija. Vet. arhiv 85, 385-394, 2015.

SAŽETAK

Granulocitna anaplazmoza pasa proširena je diljem svijeta, a prouzročena je vrstom *Anaplasma phagocytophilum*. Prenosi se krpeljima roda *Ixodes*. Do sada u Autonomnoj Pokrajini Vojvodini i Republici

Srbiji nema objavljenih podataka o prisutnosti i raširenosti infekcije pasa uzročnikom *A. phagocytophilum*. Cilj je ovoga seroepidemiološkog istraživanja da se utvrdi seroprevalencija IgG protutijela za *A. phagocytophilum* u populaciji pasa u Vojvodini, Srbija. Ovim seroepidemiološkim istraživanjem, obuhvaćena su 84 nasumično izabrana psa s područja Autonomne Pokrajine Vojvodine. U cilju utvrđivanja prisutnosti protutijela razreda IgG za *A. phagocytophilum* rabljen je test indirektno imunofluorescencije (IFAT). Primjenom IFATa, na području Autonomne Pokrajine Vojvodine, Srbija, utvrđena su specifična protutijela IgG na uzročnika *A. phagocytophilum* u serumu 13 pasa, što predstavlja seroprevalenciju od 15,5 %. Dokaz protutijela za *A. phagocytophilum* u populaciji pasa na području Autonomne Pokrajine Vojvodine osnova je za daljnja epidemiološka, klinička i dijagnostička istraživanja ove infekcije.

Ključne riječi: granulocitna anaplazmoza pasa, *Anaplasma phagocytophilum*, seroprevalencija, psi, indirektna imunofluorescencija, Srbija
