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PCR detection of *Anaplasma phagocytophilum* in stray dogs in Batman, Turkey

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ABSTRACT *Anaplasma phagocytophilum* causes high-grade fever in humans, ruminants, horses, cats, and dogs. *Anaplasma phagocytophilum*, which causes granulocytic anaplasmosis in dogs, has a wide geographical distribution in the world, but there are not many studies on the Anaplasma species in dogs in Turkey. *Anaplasma phagocytophilum*, which infects leukocytes in dog, might also be zoonotic. This study aimed to investigate the *A. phagocytophilum* prevalence in stray dogs in Batman province of Turkey using Polymerase Chain Reaction (PCR) technique. Blood samples were drawn into EDTA tubes from the venae cephalica antebrachii of randomly selected 97 asymptomatic dogs in total. Identification of *A. phagocytophilum* was carried out using the conventional PCR method that was conducted by using the DNAs obtained from the samples. *Anaplasma phagocytophilum* prevalence was found to be 3.1% in the examined blood samples. In conclusion, infection of dogs in Batman province with *A. phagocytophilum* has been revealed molecularly for the first time with this study. It is considered that detection of this species, which can cause disease (Human granulocytic anaplasmosis) in humans as well, has contributed epidemiologically to the data on the spread of the disease throughout Turkey. Moreover, investigating the tick species that transmit the disease in the region where the study was conducted is important for developing effective control strategies against the disease.

Keywords: *Anaplasma phagocytophilum, Dog, Pathogen, PCR*

ÖZ

Batman İli Sokak Köpeklerinde *Anaplasma phagocytophilum*'un PCR ile Belirlenmesi

Anaplasma phagocytophilum insan, ruminant, at, kedi ve köpeklerde şiddetli ateşli hastalıklara neden olmaktadır. Köpeklerde granülositik anaplasmosise neden olan *A. phagocytophilum* dünyada geniş bir coğrafik dağılım göstermektedir. Ancak Türkiye'de köpeklerde *Anaplasma* türleri üzerine çok fazla çalışma bulunmamaktadır. Bu çalışma, Batman ili sokak köpeklerinde *A .phagocytophilum* yaygınlığının Polimeraz Zincir Reaksiyonu (PZR) yöntemi ile araştırılması amacıyla yapılmıştır. Rastgele seçilen toplam 97 asemptomatik köpeğin vena cephalica antebrachii'lerinden EDTA'lı tüplere kan örnekleri alınmıştır. Alınan örneklerden elde edilen DNA'lar kullanılarak yapılan konvensiyonel PCR yöntemi ile A. *phagocytophilum* identifikasyonu gerçekleştirilmiştir. İncelenen kan örneklerinde *A. phagocytophilum* prevalansı %3.1 olarak bulunmuştur. Sonuç olarak, bu çalışma ile Batman ili sokak köpeklerinin *A. phagocytophilum* ile enfeksiyonu moleküler olarak ilk kez ortaya konmuştur. İnsanlarda da hastalık oluşturabilen (Human granulocytic anaplasmosis) bu türün tespit edilmesinin, hastalığın Türkiye genelinde yayılışı konusundaki verilere epidemiyolojik anlamda katkı sağladığı düşünülmektedir. Ayrıca, çalışmanın yapıldığı bölgede enfeksiyonu nakleden kene türlerinin araştırılması hastalığa karşı etkili kontrol stratejilerinin geliştirilmesi bakımından önem taşımaktadır.

Anahtar Kelimeler: *Anaplasma phagocytophilum, Köpek, Pathojen, PCR*

INTRODUCTION

Anaplasma phagocytophilum is an obligate intracellular bacterium which causes tick-borne zoonotic disease and can infect primarily neutrophils and also eosinophils (Carrade et al. 2009). This species, which causes granulocytic anaplasmosis in dogs, has been identified also in cats (Bjo¨ersdorff et al. 1990), horses (Passamonti et al. 2010), dogs (Bjo¨ersdorff et al. 1990, Jennifer et al. 2009), rodents (Liz et al. 2000, Christova and Gladnishka 2005), deer (Jenkins et al. 2001) and humans (Petrovec et al. 1997) besides domestic ruminants. It is considered that dogs, horses, and humans are accidental hosts. Granulocytic anaplasmosis in dogs was first reported in California in 1982 (Madewell and Gribble 1982), later its existence has been revealed at increasing rates year by

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year (Carrade et al. 2009). The vector transmitting the disease is the tick species of *Ixodes ricinus* (*I. ricinus*) in Europe, *Ixodes persulcatus* (*I. persulcatus*) in Asia, and *Ixodes pacificus* (*I. pasificus*) and *Ixodes scapularis* (*I. scapularis*) in North America (Woldehiwet 2010). In the regions where the disease is endemic, any clinical findings were not observed in many of the dogs that were detected to be 60% seropositive. However, lethargy, inappetence, and fever are observed in the dogs infected with *A. phagocytophilum* (Beall et al. 2008; Eberts et al. 2011). Splenomegaly and lymphadenopathy have been reported in anaplasmosis and it was concluded that they were caused by lymphoid hyperplasia. In a great portion (90%) of anaplasmosis cases that are caused by *A. phagocytophilum*, thrombocytopenia develops depending on the destruction of thrombocytes (Eberts et al. 2011). Diagnosis of *A. phagocytophilum* infections in dogs is usually made by the serological methods such as immunofluorescence antibody technique (IFAT) and rapid diagnostic ELISA test. However, cross-reactivities may be observed in the dogs infected with *A. phagocytophilum* and *A. platys*. It has been reported that this creates a serious disadvantage in regions where both two species progress together (Carrade et al. 2009). In recent years, PCR method, which has a higher specificity and sensitivity than microscopic and serological diagnosis methods, has become a reason for preference in the diagnosis of anaplasmosis in dogs. By means of this technique, the infection can be detected even in the animals having very low parasitemia and thus reservoir animals can be determined (Massung and Slater 2003; Santos et al. 2011; Ebani et al. 2013; Vargas-Hernandez et al. 2016; Huber et al. 2017). In Turkey, the number of studies on the *Anaplasma* species in dogs is limited. Blood samples of 371 dogs in the Aegean Region were examined by nested PCR in terms of *A. platys* and *A. phagocytophilum*, and *A. platys* was detected in 146 dogs (39.4%), and *A. phagocytophilum* was detected in 193 (52%) of them (Karagenc et al. 2005). Moreover, a case related to *A. platys* was reported (Ulutas et al. 2007). However, the existence of *A. phagocytophilum* was shown both in possible vectors and in cattle, sheep, and wild mice using both serological and molecular methods (Aktas et al. 2010, 2011; Sen et al. 2011).

This study aimed to investigate the *A. phagocytophilum* prevalence in the peripheral whole blood samples of stray dogs in Batman province by using the PCR technique.

MATERIALS and METHODS

In the Veterinary Technical Health Center of the Municipality of Batman in various periods in 2018, blood samples were drawn into the EDTA tubes from the venae cephalica antebrachii of 97 asymptomatic dogs in total. The animals from which the blood samples were collected were the stray dogs that were brought to the Technical Health Center for sterilization or treatment. Animal ethics, method and sampling approval was obtained from the University of Van Yuzuncu Yil Animal Ethics Committee (approval number: 05/04/2019-27762). All samples were stored at -20ºC until the laboratory studies were conducted. Genomic DNA was obtained from the blood samples collected from the stray dogs by using commercial blood kit (GeneAll® ExgeneTM Tissue SV (plus!), Korea). The obtained DNA samples were stored at -20ºC until the PCR examinations were carried out. In analyzing the obtained DNA samples in terms of A. phagocytophilum, they were processed by KO97 numbered commercial PCR kit (Genekam Biotechnology, Germany) in compliance with the procedure that the producing company had reported. The amplification products were electrophoresed on 1.5% agarose gel and were evaluated at the gel documentation system (Avegene, Taiwan).

RESULTS

In the examinations conducted using PCR method, 3 (3.1%) of the 97 asymptomatic dogs were found infected with *A. phagocytophilum*. The information on sex and gender of the examined animals were summarized in Table 1.

Table 1. Relation to age and gender of the canine study population

		Study population No. = 97	PCR positive dogs No. = 3.1 (%)
Gender			
	Female	50	2
	Male	47	1
Age (year)			
	<1	14	0
	1-2	50	2
	>2	33	1

As a result of the amplification and the agarose gel electrophoresis of the DNA that was extracted from the blood samples in the PCR, positive bands of about 444 bp long were obtained (Figure 1).

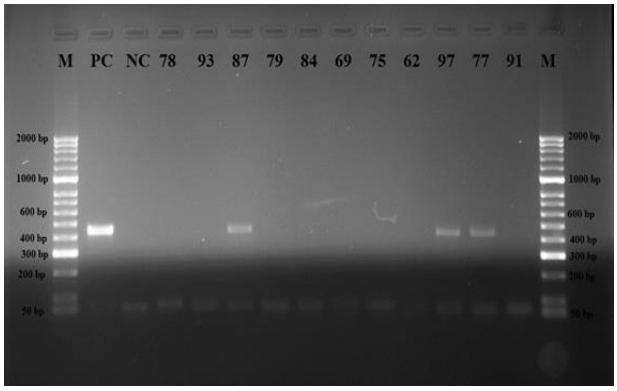

Figure 1. Appearance on agarose gel electrophoresis (1.5%) stained with ethidium bromide of *A. phagocytophilum* DNA extracted from the blood samples. M: 50 bp DNA ladder, 87, 97, 77: Positive samples, NC: Negative control, PC: *A. phagocytophilum* Positive control.

DISCUSSION

Currently, it is important to know the presence and spread of the tick-borne parasites in dogs, and to reveal their molecular epizootiologies for necessary treatment, control and protection measures. *Anaplasma phagocytophilum*, one of these blood parasites, is a zoonotic species which is transtadially transmitted to humans, dogs, and other animals through ticks of family Ixodidae. Several methods were used in the diagnosis of this parasite in dogs. Although it is the oldest among these models, microscopic examination is still a reliable method (Sainz et al. 2015). This method facilitates diagnosis of the disease mostly in its acute periods. Besides, serological methods, which are

based on the detection of antibodies that develop against the parasite, are also used (Potkonjak et al. 2015). False positives due to the cross-reactivities that emerge during serological diagnosis (especially positives that develop against *A. platys* species) are tried to be removed by recombinant antigens. Molecular diagnostic methods that are based on the detection of the parasite DNA, which has been widely used in recent years, are also used in the diagnosis of the *Anaplasma* species, as in the diagnosis of several infectious factors. PCR is used as a sensitive and specific method for detecting the *Anaplasma* spp. DNA from the clinical samples taken from ticks and animals. Ebani et al. (2013) detected the parasite with the specific primers that they designed from *A. phagocytophilum* 16S rRNA gene by using Nested-PCR method. Besides, Santos et al. (2011) conducted the identification of *A. phagocytophilum* in dogs with Real-Time PCR using different gene region and primers. The same researchers revealed partial sequences of the isolates they detected according to msp2 gene region. Melter et al. (2007) conducted the identification of A*. phagocytophilum* with a PCR in a dog with tick infestation, acute onset of fever, ataxia, and lethargy. The commercial PCR kit protocols which were prepared specifically for *A. phagocytophilum* were used in this study. The bands with 444 bp long were obtained as a result of the amplification of the positive control and positive samples in the kit protocol. Three (3.1%) of 97 stray dogs in total were found to be infective. In Turkey, a few molecular studies, which were conducted on *A. phagocytophilum*in dogs by using the conventional and Real-Time PCR techniques, were reported. Pasa et al. (2009) reported that 4 of 10 dogs had been infected with *A. phagocytophilum* in a study conducted using conventional PCR in the Aegean Region. Duzlu et al. (2014) detected *A. phagocytophilum* at 7.8% in a molecular study conducted on dogs in the Kayseri region by using Real Time PCR technique. In this study, the molecular prevalence of *A. phagocytophilum* in the dogs of Batman province was detectedto be 3.1%. This result was found to be in parallel with the prevalence rates reported from the Aegean Region and the Kayseri region.

CONCLUSION

Granulocytic anaplasmosis has been investigated for the first time in the stray dogs living in Batman province and found to be low. However, it is considered that the study has contributed epidemiologically to the data on the spread of the disease throughout Turkey. More extensive studies, in which vectors are investigated and serological methods are included, are needed in order to reveal this parasite, which has a zoonotic potential for humans, in more detail.

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