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RESEARCH ARTICLE

Survey of Anaplasma Infections in Small Ruminants from East Part of Turkey

Kürşat ALTAY [1,2] Nazir DUMANLI [3] Münir AKTAŞ [3] Sezai ÖZÜBEK [3]

[1] Department of Parasitology, Faculty of Veterinary Medicine, University of Cumhuriyet, TR-58140 Sivas - TURKEY
[2] Department of Parasitology, Faculty of Veterinary Medicine, Kyrgyzstan - Turkey Manas University, KG-720044 Bishkek - KYRGYZSTAN
[3] Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, TR-23119 Elazığ - TURKEY

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Summary

This study was carried out to determine the presence and frequency of *Anaplasma ovis* and *Anaplasma phagocytophilum* in small ruminants from Bingol, Elazig, Malatya and Mus provinces. A total of 422 (291 sheep and 131 goats) blood samples were collected from apparently healthy animals. To determine of *A. ovis* and *A. phagocytophilum* in small ruminants, species-specific PCRs were set up using 60 kDA chaperonin gene (cpn60, also known as hsp60 or groEL) and 16S SSU rRNA gene primer sets, respectively. A total of 301 (71.32%) animals were found infected with *A. ovis* and/or *A. phagocytophilum*. The percentages of positive animals for *A. ovis* and *A. phagocytophilum* were 67.06% (283/422) and 19.66% (83/422), respectively. The rate of concurrent infections was 15.40% (65/422). Four PCR products from positive samples were purified from agarose gel and sequenced. These sequences were identical to the reported nucleotide sequences of *A. ovis* and *A. phagocytophilum*. This is the first molecular based study on the detection of *A. phagocytophylum* and *A. ovis* in small ruminants from East Anatolia Region. Further studies are needed on the determination of the genotypes and vectors of the species.

Keywords: *Anaplasma ovis, Anaplasma phagocytophilum, Sheep, Goat, East Anatolia Region*

Doğu Anadolu Bölgesinde Koyun ve Keçilerde *Anaplasma* Enfeksiyonlarının Araştırılması

Özet

Bu çalışma Bingöl, Elazığ, Malatya ve Muş yöresindeki koyun ve keçilerde *Anaplasma ovis* ve *Anaplasma phagocytophilum*'un araştırılması amacıyla yapılmıştır. Rastgele seçilen 422 sağlıklı (291 koyun 131 keçi) hayvandan kan örneği alınmıştır. *A. ovis* için 60 kDA chaperonin gen (cpn60, hsp60 veya gro EL olarak da bilinir) ve *Anaplasma phagocytophilum* için 16S SSU rRNA genlerine spesifik primerler kullanılarak tür spesifik PCR yapılmıştır. PCR sonucunda toplam 301 (%71.32) hayvan *A. ovis* ve/veya *A. phagocytophilum* ile enfekte bulunmuştur. Örneklerin %67.06 (283/422)'sı *A. ovis*, %19.66 (83/422)'sı *A. phagocytophilum* ve %15.40 (65/422)'ı her iki tür yönünden pozitif olarak tespit edilmiştir. *A. ovis* ve *A. phagocytophilum* yönünden pozitif olan PCR ürünleri agaroz jelden purifiye edilerek sekanslanmıştır. Elde edilen DNA dizilimlerin daha önce bildirilen *A. ovis* ve *A. phagocytophilum*'a ait dizilimlerle aynı olduğu görülmüştür. Bu çalışma Doğu Anadolu Bölgesinde koyun ve keçilerde *A. ovis* ve *A. phagocytophilum*'un moleküler yöntemlerle teşhisi üzerine yapılan ilk araştırmadır. Türlerin genotipleri ve vektörlerinin belirlenmesine yönelik çalışmaların gerekliği düşünülmektedir.

Anahtar sözcükler: *Anaplasma ovis, Anaplasma phagocytophilum, Koyun, Keçi, Doğu Anadolu Bölgesi*

INTRODUCTION

Anaplasma species are known to be important tick-borne pathogens of humans and animals. The genus *Anaplasma* comprises *A. phagocytophilum* (previously recognised as *Ehrlichia equi* and *E. phagocytophila*), *A. centrale, A. marginale, A. bovis, A. ovis* and *A. platys*. The species are mainly transmitted by ixodid ticks [1-4].

İletişim (Correspondence)
+90 346 2191010
altayvet@hotmail.com

Anaplasma phagocytophilum has a wide range hosts including domesticated ruminats, equids, cats, dogs, wild animals and humans. The pathogen causes tick-borne fever in ruminants and granulocytic anaplasmosis in humans, equines and canines [1]. *A. ovis* infects sheep, goats and wild ruminants [5]. It is known as a non-pathogenic species, but the agent has been detected to cause severe disease in bighorn sheep [6]. On the other hand, like with other *Anaplasma* infections, *A. ovis* may predispose hosts to other pathogens [4].

The main tick-borne diseases in Turkey are babesiosis and theileriosis. They are endemic in almost all regions of Turkey [7,8]. In small ruminants, clinical and subclinical *A. ovis* infections were detected in a few studies by microscopic examination [9,10]. Until 2005, in Turkey there was no report that shows the presence of *A. phagocytophilum* in sheep. In parallel with the emergence of tick-borne diseases in humans in recent years, this agent that is thought to be zoonotic was determined in domestic animals and ticks [11-14]. Further studies are needed for epidemiological information on the anaplasmosis.

There are a few molecular studies on *Anaplasma* sp. in the country. Recently, the presence of *A. phagocytophilum* was documented in cattle while *A. ovis* was reported in sheep and some tick species [11-14].

Molecular diagnostic methods as polymerase chain reaction (PCR) have become widely used as sensitive and specific tools for detection and discrimination of tick-borne parasites such as *Theileria* sp., *Babesia* sp. and *Anaplasma* sp. in their vectors and hosts [7,8]. This study was carried out to determine the presence and frequency of *A. ovis* and *A. phagocytophilum* in small ruminants using species-specific polymerase chain reaction (PCR) and sequence analyse methods in the eastern region of Turkey.

MATERIAL and METHODS

The blood samples from sheep and goats were collected from Bingol, Elazig, Malatya, and Mus located in East Anatolia Region of Turkey. A total of 422 (291 sheep and 131 goats) blood samples were collected from apparently healthy animals *(Table 1)*. All of the animals were older than one year.

Total genomic DNA extraction from the blood samples was performed according to previously described method [15]. Briefly, 125 µl of blood was added to 250 µl of lysis mixture (0.32 M sucrose, 0.01 M Tris, 0.005 M $MgCl_2$, 1% Triton X-100, pH 7.5) and the mixture was centrifuged at 11.600 x g for 1 min. The pellet was washed three times by centrifugation with 250 µl lysis buffer. The supernatants were discarded and the final pellets were resuspended in 100 µl of PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8), 0.1% TritonX-100, pH 8.3). Proteinase K (50 µg/ml) was added to the pellet suspension and the mixture was then incubated at 56°C for one h. At last, the samples were boiled at 95°C for 10 min.

To determine the presence and frequency of *A. ovis* and *A. phagocytophilum* in small ruminants, species-specific PCRs were set up using 60 kDA chaperonin gene (cpn60, hsp60 or groEL) and 16S SSU rRNA gene primer sets, respectively. Primers SSAP2f (5'-GCTGAATGT GGGGATAATTTAT - 3') and SSAP2r (5'-ATGGCTGCTTCCTTT CGGTTA - 3') are specific for *A. phagocytophilum* [16]; JH0011 (5'- TAAAAGCCAAGGAGGCTGTG - 3') and JH0012 (5'- TTGCTCTCCTCGACCGTTAT - 3') are specific for *A. ovis* [17]. The PCRs were performed according to previously described methods [16,17].

Four positive PCR products, 2 for each species were purified from agarose gel using a commercial PCR purification kit (Wizard SV gel and PCR clean-up system, Promega, Madison, WI, USA). The partial sequences correponding to the 60 kDA chaperonin gene of *A. ovis* and 16S SSU rRNA gene of *A. phagocytophilum* were obtained. The sequences were compared to sequence databases using the BLAST algorithm. The new sequences were submitted to EMBL/GenBank database.

A chi-squared test was used to evaluate the differences between different parameters. $P<0.05$ was accepted to be statistically significant.

RESULTS

The infection prevalences were determined as the percentage of positive animals for the pathogen DNA detected by species-specific PCR. Four hundred and twenty two blood samples, 291 from sheep and 131 from goats were investigated for presence of *A. phagocytophilum* and *A. ovis* in four provinces (Bingol, Elazig, Malatya, Mus) of East Anatolia Region. As summarized in *Table 1*, 301 (representing 71.32% of analyzed small ruminats) animals were found infected with *A. ovis* and/or *A. phagocytophilum* (data not shown). The percentages of positive animals for *A. ovis* and *A. phagocytophilum* were 67.06% (283/422) and 19.66% (83/422), respectively *(Table 1)*. 65 (15.40%) of analyzed animals was concurrently infected with *A. ovis* and *A. phagocytophilum* (data not shown). The number of *A. ovis* infected sheep and goats were 196 (67.35%) and 87 (66.41%) respectively, whereas the number of *A. phagocytophilum* infected sheep and goats were 55 (18.90%) and 28 (21.37%), respectively *(Table 1)*.

There was not statistically significant diffrences in the prevalence of *Anaplasma* spp. infections between sheep and goats ($P>0.05$). On the other hand, *A. ovis* infection rate was higher than *A. phagocytophilum* in analyzed animals ($P<0.05$).

Table 1. *Anaplasma phagocytophilum and Anaplasma ovis species - specific PCR results by location and animal species in East Anatolia Region*
Tablo 1. *Doğu Anadolu Bölgesinde yerleşim yeri ve hayvan türlerine göre Anaplasma phagocytophilum ve Anaplasma ovis tür spesifik PCR sonuçları*

Locations	*Anaplasma ovis* Percentage (Number) of Positivity						*Anaplasma phagocytophilum* Percentage (Number) of Positivity					
	Sheep		Goats		Total		Sheep		Goats		Total	
	%	n	%	n	%	n	%	n	%	n	%	n
Bingöl	91.30	42/46	45.00	18/40	69.76	60/86	34.78	16/46	10.00	4/40	23.25	20/86
Elazığ	57.55	80/139	46.42	13/28	55.68	93/167	12.94	18/139	35.71	10/28	16.76	28/167
Malatya	62.71	37/59	95.23	20/21	71.25	57/80	16.94	10/59	14.28	3/21	16.25	13/80
Muş	78.72	37/47	85.71	36/42	82.02	73/89	23.40	11/47	26.19	11/42	24.71	22/89
Total	67.35	196/291	66.41	87/131	67.06	283/422	18.90	55/291	21.37	28/131	19.66	83/422

PCR products positive for *A. ovis* and *A. phagocytophilum* were purified and sequenced. The partial sequences of 16S SSU rRNA of *A. phagocytophilum* (GenBank accession nos. JF807995 and JF807994) and 60 kDA chaperonin gene of *A. ovis* (EMBL accession nos. HE580282 and HE580283) were identical to the reported nucleotide sequences of the *A. phagocytophilum* and *A. ovis*.

DISCUSSION

Anaplasmosis is a worldwide tick-borne disease caused by *Anaplasma* species [3]. A paucity of information exists concerning the current actual size of anaplasmosis in Turkey. Recently, *A. phagocytophilum* has been reported in cattle, sheep and ticks [12-14] and *A. ovis* in *R. bursa* [11]. this study was planned to investigate the presence and frequency of *A. phagocytophilum* and *A. ovis* in sheep and goats from the provinces of East Anatolia Region (Bingol, Elazig, Malatya, Mus). We used species-specific PCRs to amplify 60 kDA chaperonin gene (cpn60 or hsp60) [17] and 16S SSU rRNA gene [16] of *A. ovis* and *A. phagocytophilum*, respectively. We confirmed specificity of the PCRs with sequences analysis. The sequences of DNA fragments obtained in this study showed 100% identity to the recently reported sequences of *A. phagocytophilum* and *A. ovis*.

From the 422 animals, 301 (71.32%) were infected with *A. phagocytophilum* and/or *A. ovis* (data not show). The concurrent infection rate in analyzed animals was 15.40% (65/422) (data not show). Prevalence of *A. phagocytophilum* and *A. ovis* were 18.90% (55/291) and 67.35% (196/291) in sheep, 21.37% (28/131) and 66.41% (87/131) in goats, respectively. Prevalence of *A. ovis* was higher than *A. phagocytophilum* in both sheep and goats ($P<0.05$). There is not any report on ovine anaplasmosis based on molecular diagnostic tools in East Anatolia Region. This study is first molecular survey on the ovine anaplasmosis in the region.

The presence and frequence of *Anaplasma* infections might be associated with many factors including geographical and climatic diversity, tick species and reservoir hosts. *Ixodes ricinus* known to be main vector of *A. phagocytophilum* and the prevalence of the pathogen is closely related with the ratio of *Ixodes* spp. in the same enviroment [16,18]. *A. ovis* is transmitted by *Rhipicephalus. bursa* and other ticks [3]. Several Ixodid tick species are distributed in Turkey [19]. While *I. ricinus* is dominant tick species in the Black Sea region of Turkey, *Hyalomma* and *Rhipicephalus* species are dominant in the East of Turkey [10,18]. According to the literatures [9,10], *A. phagocytophilum* was detected in *I. ricinus* in the Black Sea region and *A. ovis* was detected in *R. bursa* in the East of Turkey. Tick infestation status of the examined animals was not investigated in this study. We reported that *A. ovis* and *A. phagocytophilum* infections are frequent in the East of Turkey *(Table 1)*. Further studies are needed to determine the tick vectors, reservoirs, hosts and genotypes of *Anaplasma* species.

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