Kafkas Univ Vet Fak Derg
22 (1): 133-138, 2016
DOI: 10.9775/kvfd.2015.14075

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

# Molecular Detection and Typing of Anaplasma Species in Small Ruminants in Thrace Region of Turkey [1]

Kerem ÖTER [1] Handan ÇETİNKAYA [1] Cem VURUŞANER [1]
Müfit TOPARLAK [1] Koray ERGÜNAY [2]

[1] *This research was funded by Istanbul University Scientific Research Projects Coordination Unit. Project no: 41143*
[1] Istanbul University, Faculty of Veterinary Medicine, Department of Parasitology, TR-34320 Avcilar, Istanbul - TURKEY
[2] Hacettepe Univ. Faculty of Medicine, Dept. of Medical Microbiology, Virology Unit, TR-06100 Sıhhiye, Ankara - TURKEY

**Article Code: KVFD-2015-14075 Received: 27.07.2015 Accepted: 25.08.2015 Published Online: 26.08.2015**

## Abstract

This study was conducted to determine the presence and distribution of *Anaplasma ovis* and *Anaplasma phagocytophilum* in small ruminants in Istanbul, Tekirdag, Edirne and Kirklareli provinces in Thrace region of northwestern Turkey during May-September in 2014. A total of 423 blood samples (216 sheep and 207 goats) were collected randomly from small ruminants regardless of the clinical symptoms. Species-specific polymerase chain reaction (PCR) assays, targeting the major surface protein 4 (msp4), were employed for identification of *A. ovis* and *A. phagocytophilum* and selected products were confirmed via sequencing. A total of 230 small ruminants (54.37%) were found to be infected with *A. ovis* and/or *A. phagocytophilum*. The rates of infected animals for *A. ovis* and *A. phagocytophilum* were 50.83% (215/423) and 8.51% (36/423) respectively. Coinfection rate in small ruminants was determined as 4.96% (21/423). Sequence diversity rates of 0-0.94% for *A. ovis* and 0.41-2.49% for *A. phagocytophilum* have been observed. This is the first detection of *A. ovis* and *A. phagocytophilum* in sheep and goats in Thrace region of northwestern Turkey via polymerase chain reaction and sequence characterization. Further researches are needed to determine the vectors, vector-host interactions and genotypic variants that may affect the presence and distribution of Anaplasma species in the region.

***Keywords:*** *Anaplasma ovis, Anaplasma phagocytophilum, Sheep, Goat, msp4, Thrace, Turkey*

# Türkiye'nin Trakya Bölgesindeki Küçük Ruminantlarda Görülen Anaplasma Türlerinin Moleküler Yöntemlerle Tespiti ve Tiplendirmesi

## Özet

Bu çalışma, Türkiye'nin Trakya bölgesindeki küçük ruminantlarda *Anaplasma ovis* ve *Anaplasma phagocytophilum*'un varlığı ve dağılımını belirlemek amacı ile Mayıs-Eylül 2014 tarihleri arasında İstanbul, Tekirdağ, Edirne ve Kırklareli illerinde yürütülmüştür. Klinik semptom göstermelerine bakılmaksızın rastgele seçilen küçük ruminantlardan toplam 423 kan örneği (216 koyun ve 207 keçi) toplanmıştır. *A. ovis* ve *A. phagocytophilum* türlerinin identifikasyonu için major surface protein 4 (msp4) genini hedef alan tür-spesifik polimeraz zincir reaksiyonu (PZR) kullanılmış olup seçilen ürünler sekanslanarak doğrulanmıştır. Toplam 230 (%54.37) küçük ruminantın *A. ovis* ve/veya *A. phagocytophilum* ile enfekte olduğu bulunmuştur. *A. ovis* ve *A. phagocytophilum* yönünden pozitif hayvanların yüzdesi sırasıyla %50.83 (215/423) ve %8.51 (36/423) bulunmuştur. Her iki tür için pozitif hayvanların yüzdesi %4.96 (21/423) olarak tespit edilmiştir. Sekans farklılıklıkları *A. ovis* için 0-0.94% ve *A. phagocytophilum* için 0.41-2.49% oranlarında izlenmiştir. Bu çalışma, *A. ovis* ve *A. phagocytophilum*'un Türkiye'nin Trakya bölgesindeki koyun ve keçilerde varlığı ve dağılımı üzerine polimeraz zincir reaksiyonu ve sekans karakterizasyonu ile yapılan ilk araştırmadır. Bölgedeki Anaplasma türlerinin varlık ve dağılımını etkileyebilecek vektör, vektör-konak ilişkileri ve genotipik varyantlar konusunda yeni araştırmalara ihtiyaç duyulmaktadır.

***Anahtar sözcükler:*** *Anaplasma ovis, Anaplasma phagocytophilum, Koyun, Keçi, msp4, Trakya, Türkiye*

## INTRODUCTION

The family Anaplasmataceae belongs to order Rickettsiales of class α-Proteobacteria. The genus *Anaplasma* comprises six species; *Anaplasma centrale*, *A. marginale*, *A. bovis* (formerly *Ehrlichia bovis)*, *A. ovis*, *A. phagocytophilum* (formerly *Ehrlichia equi*, *E. phagocytophila* and Human Granulocytic Ehrlichiosis [HGE] agent) and *A. platys* [1]. *Anaplasma* species are Gram negative bacteria parasitizing in the blood cells of mammals. The life cycle of *Anaplasma* include the

**İletişim (Correspondence)**
+90 464 532 6300702
oter@istanbul.edu.tr

reproduction stages taking place in both vector ixodid ticks and vertebrate animals [2]. Ticks belonging to the genera *Ixodes, Dermacentor, Rhipicephalus* and *Amblyomma* are the main biological vectors of *Anaplasma* species [3].

*Anaplasma ovis* and *A. phagocytophilum* are medically-important species, pathogenic for small ruminants [3]. *Anaplasma ovis* causes ovine anaplasmosis in small ruminants, which is associated with significant morbidity and mortality, especially in goats [4]. Moreover, *A. ovis* can cause severe clinical disease in bighorn sheep as well as predisposing animals to other pathogens [5-7]. *A. ovis* infections have been reported to be endemic world-wide including Europe [8], China [9] and United States of America [10]. *A. ovis* has been reported from Turkey's neighboring countries Greece [11], Cyprus [12] and Iran [13].

*Anaplasma phagocytophilum* is the causative agent of tick borne fever in ruminants and granulocytic anaplasmosis in humans, equines and canines [1,14]. *A. phagocytophilum* can cause subclinical or severe infection in sheep and it is seldom fatal unless complicated by other infections. In addition to crippling, direct and production losses, *A. phagocytophilum* can cause abortion and impaired spermatogenesis in sheep [15-17]. *A. phagocytophilum* has been reported in China [9], United States of America, Europe, Asian part of Russia and north Africa [18]. *A. phagocytophilum* has also been reported from Turkey's neighboring countries Bulgaria [19], Greece [20] and Iran [21]. In Turkey, early records of *A. ovis* have been reported in small ruminants by using direct microscopy [22,23]. *A. phagocytophilum* in sheep [24,25], in goats [25], in ixodid ticks [26-28], in cattle [24,29,30] and in dogs [31] as well as *A. ovis* in sheep [25], in goats [25] and in ixodid ticks [28,32] have been reported by nucleic acid detection in various regions of Turkey.

Morphological and serological techniques are not reliable to differentiate *Anaplasma* and *Ehrlichia* species due to morphological similarities and antigenic cross reactions between species [33]. Detection of the bacterial nucleic acids via polymerase chain reaction (PCR) provide tools with high sensitivity and specificity and thus, are widely used in definitive diagnosis of *Anaplasma* species. These techniques also have the advantages of detecting the positive hosts in the early acute phase of the infection as well as the carrier stages [34,35].

This study was undertaken to investigate the presence and the distribution of *Anaplasma ovis* and *A. phagocytophilum* in sheep and goats in Thrace region by species-specific PCRs, where no previous information on *Anaplasma* is available.

# MATERIAL and METHODS

### *Research Area and Sample Collection*

The study was conducted between May and September 2014 in four representative provinces (Istanbul, Tekirdag, Edirne and Kirklareli) in Thrace region of northwestern Turkey.

Totally 423 blood samples (216 sheep and 207 goats) were collected randomly from 2-4 aged small ruminants regardless of showing any clinical symptoms. Ten ml blood sample was collected in tubes containing ethylene diamine tetra acetic acid (EDTA) in (K2E BD Vacutainer®) from each individual and transferred to laboratory in cold chain. Blood samples were stored in -20°C until DNA extraction.

### *PCR and Sequencing*

Total genomic DNA extraction was performed by using a commercial kit (High Pure® PCR Template Preparation Kit Roche Diagnostics GMBH) according to the manufacturer's instructions.

For the identification of *A. ovis*, species-specific primer sets AovisMSP4Fw (5′-TGAAGGGAGCGGGGTCATGGG-3′) forward and AovisMSP4Rev (5′-GAGTAATTGCAGCCAGGG ACTCT-3′) reverse were used for amplification of the *A. ovis* major surface protein (msp4) gene 347-bp coding region [36]. For the identification of *A. phagocytophilum*, species-specific primer sets MAP4AP5 (5′-ATGAATTACA GAGAATTGCTTGTAGG-3′) forward and MSP4AP3 (5′-TTAAT TGAAAGCAAATCTTGCTCCTATG-3′) reverse were used for amplification of the *A. phagocytophilum* msp4 gene 849-bp coding region [37].

Protocols described by Torina et al.[36] and de la Fuente et al.[37] were optimized for PCR amplifications. The final PCR conditions were established as: reaction buffer 1x, 0.4 µM of each primer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, 1.25 U of Taq DNA Polymerase (ThermoScientific, Waltham, M.A.). PCR reactions were performed in an automated PCR thermal cycler (Axygen, Corning, N.Y.). For *A. ovis*, the thermal profiles for PCR were optimized as: 2 min at 94°C for denaturation followed by 35 cycles with denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 30 sec. The final extension step was 5 min at 72°C. For *A. phagocytophilum*, 15 min at 95°C for denaturation followed by 40 cycles with denaturation at 94°C for 30 sec, annealing at 54°C for 45 sec and extension at 72°C for 1 min. The final extension step was 7 min at 72°C. The programs were terminated by storing the reaction mixtures at 4°C. PCR products were visualized via observtaion under UV light in a 1.5% agarose gel containing 0.1 µg/ml ethidium bromide.

For the confirmation of positive PCR results, randomly-selected PCR products for *A. ovis* and *A. phagocytophilum*, were cleaned up using High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany), and were sequenced via sense and antisense primers. employing an ABI Prism 310 Genetic Analyzer (Applied Biosystems, CA, USA). Obtained sequences were handled using CLC

Main Workbench v7.5.2 (CLCBio, Aarhus, Denmark) and by MEGA software v5.2 [38].

### *Statistical Analysis*

Chi square test was used for statistical analysis by SPSS v13 and P<0.05 was accepted statistically significant.

## RESULTS

Among 423 small ruminants examined, 230 (54.37%) were found infected with *A. ovis* and/or *A. phagocytophilum*. The percentages of positive animals for *A. ovis* and *A. phagocytophilum* were 50.83% (215/423) and 8.51% (36/423) respectively. Coinfection rate of *A. ovis* and *A. phagocytophilum* in small ruminants was 4.96% (21/423).

The distribution of *A. ovis* and *A.phagocytophilum* in sheep and goats according to the sampling provinces in Thrace region of northwestern Turkey and the significance level of differences among sampling provinces were presented in *Table 1*.

According to *Table 1*, the percentages of *A. ovis* in sheep and goats were 58.8% (127/216) and 42.51% (88/207) respectively, whereas the percentages of *A. phagocytophilum* in sheep and goats were 11.11% (24/216) and 5.8% (12/207) respectively. There was a statistically-significant difference among the provinces in Thrace region of Turkey for prevalence of *A. ovis* in sheep (P<0.001), *A. ovis* in goats (P=0.028), *A.phagocytophilum* in goats (P=0.015) and *A. phagocytophilum* in goats (P=0.008) *(Table 1).*

Agarose gel electrophoresis of *A. ovis* and *A. phagocytophilum* PCR products extracted from sheep and goat blood samples were demonstrated in *Fig. 1*.

Representative sequences of the msp4 gene were obtained for *A. ovis* and *A. phagocytophilum* and submitted to GenBank (accession no. KT251211 for *A. ovis* and

***Table 1.*** *The distribution of A. ovis and A. phagocytophilum in sheep and goats by sampling provinces in Thrace region of northwestern Turkey and the significance level of differences among sampling provinces*

***Tablo 1.*** *Türkiye'nin Trakya bölgesindeki koyun ve keçilerde A. ovis ve A. phagocytophilum'un örneklem illerine göre dağılımı ve örneklem illeri arasındaki farklılık dereceleri*

| Parasite (Host) | Total | | Sampling Provinces | | | | | | | | P Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Istanbul | | Tekirdag | | Edirne | | Kirklareli | | |
| | n | % | n | % | n | % | n | % | n | % | |
| *A. ovis*, (Sheep) | 127/216 | 58.8 | 14/52 | 26.92$^{c}$ | 28/50 | 56.0$^{b}$ | 47/62 | 75.81$^{a}$ | 38/52 | 73.08$^{ab}$ | <0.001 |
| *A. ovis* (Goat) | 88/207 | 42.51 | 14/52 | 26.92$^{b}$ | 21/50 | 42.0$^{ab}$ | 24/53 | 45.28$^{ab}$ | 29/52 | 55.77$^{a}$ | 0.028 |
| *A. phagocytophilum* (Sheep) | 24/216 | 11.11 | 3/52 | 5.77$^{b}$ | 5/50 | 10.0$^{ab}$ | 4/62 | 6.45$^{b}$ | 12/52 | 23.08$^{a}$ | 0.015 |
| *A. phagocytophilum* (Goat) | 12/207 | 5.8 | 1/52 | 1.92$^{b}$ | 1/50 | 2.0$^{b}$ | 2/53 | 3.77$^{b}$ | 8/52 | 15.38$^{a}$ | 0.008 |

***a, b, c,*** *Differences among percentages represented with different letters in the same line is significant (P<0.05)*

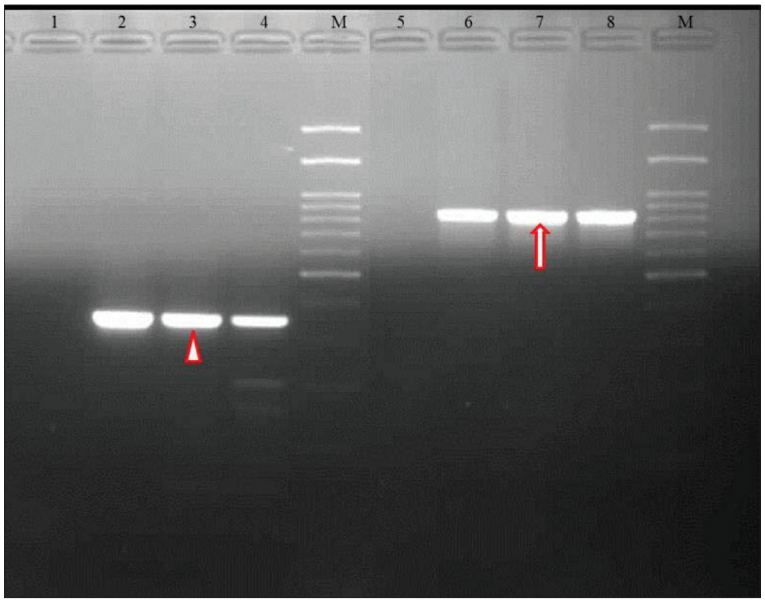


**Fig 1.** Agarose gel electrophoresis of *A. ovis* and *A. phagocytophilum* PCR products extracted from sheep and goat blood samples. Arrowhead indicates the position of 347-bp and arrow indicates the position of 849-bp PCR products. Lines: M: 100-bp DNA marker; 1: *A. ovis* negative control (PCR-grade water); 2: *A. ovis* DNA extracted from sheep; 3: *A. ovis* DNA extracted from goat; 4: *A. ovis* positive control; 5: *A. phagocytophilum* negative control (PCR-grade water); 6: *A. phagocytophilum* DNA extracted from sheep; 7: *A. phagocytophilum* DNA extracted from goat; 8: *A. phagocytophilum* positive control

**Şekil 1.** Koyun ve keçi kan örneklerinden elde edilen *A. ovis* ve *A. phagocytophilum*'a ait PCR ürünlerinin agaroz jel elektroforezi. Ok ucu 347-bp ve ok 849-bp PCR ürünlerini göstermektedir. Sıralar: M: 100-bp DNA işaretleyicisi; 1: *A. ovis* negatif kontrol (PCR-kalite su); 2: Koyun kan örneği *A. ovis* DNAsı; 3: Keçi kan örneği *A. ovis* DNAsı; 4: *A. ovis* pozitif kontrol; 5: *A. phagocytophilum* negatif kontrol (PCR-kalite su); 6: Koyun kanörneği *A. phagocytophilum* DNAsı; 7: Keçi kan örneği *A. phagocytophilum* DNAsı; 8: *A. phagocytophilum* pozitif kontrol

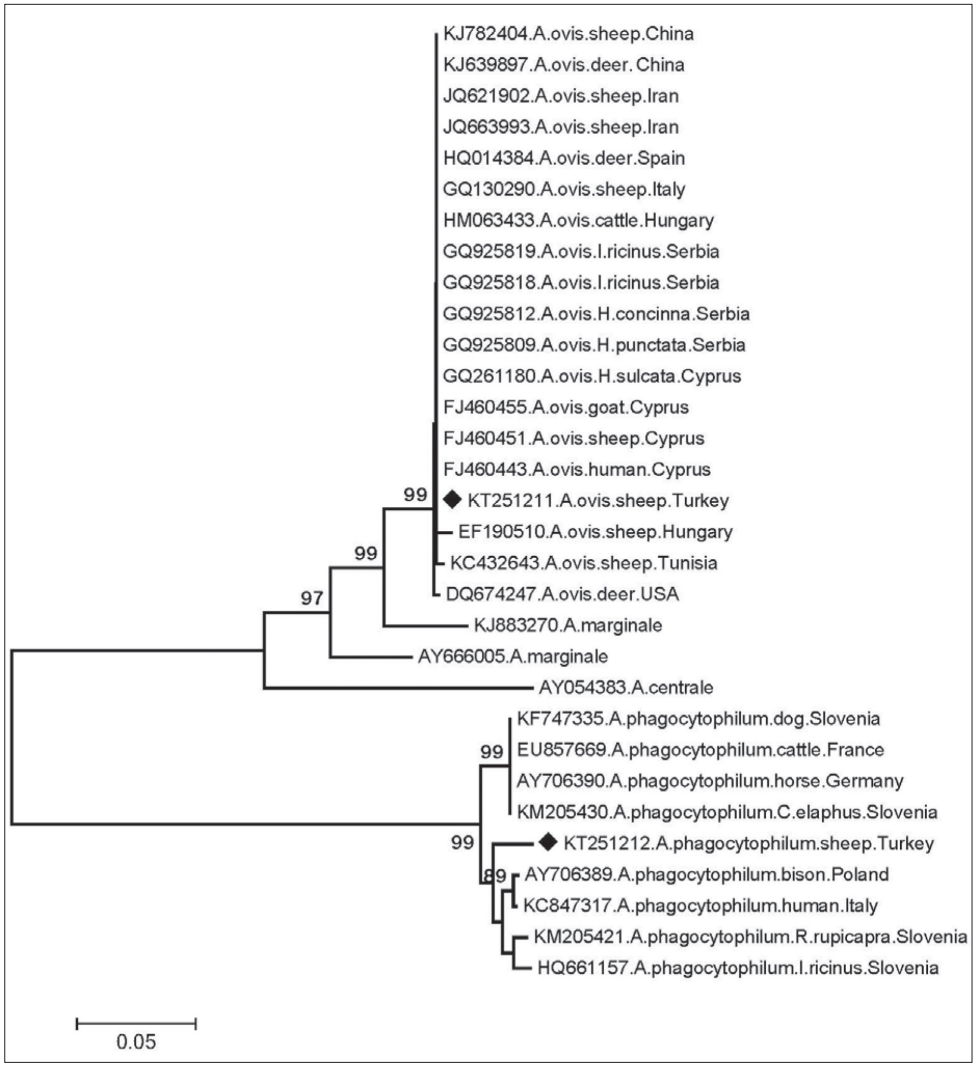


**Fig 2.** Neighbour-joining phylogenetic tree constructed via p-distance model, based on *Anaplasma* partial msp4 nucleotide sequences. Sequences included in the analysis are indicated with GenBank accession number, species, host and country of detection. Sequences characterized in this study are marked (black diamond). For the major branches, bootstrap values are indicated for 1.000 replicates

**Şekil 2.** Çalışmada elde edilen msp4 dizilerinin "p-distance" modeli ile oluşturulmuş "neighbour-joining" filogenetik ağacı. Analizde yer alan sekanslar GenBank erişim numarası, tür, konak ve tespit edilen ülkeler ile gösterilmiştir. Bu çalışmada karakterize edilen sekanslar işaretlenmiştir (siyah elmas). Ana dallanmalar için bootstrap değerleri 1.000 olarak alınmıştır

KT251212 for *A. phagocytophilum*). Comparison of *A. ovis* sequences with several selected homolog regions from various sources revealed very limited diversity, with 99.06-100% nucleotide similarities. However, *A. phagocytophilum* sequences demonstrated 0.41-2.49% divergence, and observed to constitute a distinct cluster, separated from sequences from Poland, Slovenia and Italy *(Fig. 2)*.

## DISCUSSION

Anaplasmosis is a tick borne disease caused by various species of *Anaplasma* with a significant impact on animal breeding due to the economic burden resulting from morbidity and mortiality associated with the disease. Thus, the epidemiology, diagnosis and regional prevalence of Anaplasmosis remain as an important issue for mitigating the impact of the disease in the current practice of veterinary parasitology and microbiology [3].

The circulation of various *Anaplasma* species have been investigated in Turkey. Recent studies reported the presence of *A. phagocytophilum* nucleic acids in sheep [24,25], in goats [25] in ixodid ticks [26-28], in cattle [24,29,30] and in dogs [31], as well as *A. ovis* in sheep [25], in goats [25] and in ixodid ticks [28,32] in discrete regions. In East Black Sea region of Turkey, *A. phagocytophilum* seroprevalence by IFAT has been observed 14.86% (107/720) and specific DNA ratio by nested PCR has been found 12.35% (22/178) in sheep [24]. In East Anatolia region of Turkey, 71.32% (301/422) small ruminants have been reported to be infected by *A. ovis* and/or *A. phagocytophilum*. The percentages of positive animals for *A. ovis* and *A. phagocytophilum* have been reported 67.06% (283/422) and 19.66% (83/422) respectively. Coinfections of *A. ovis* and *A. phagocytophilum* have been reported in 15.40% (65/422) of analysed small animals. The percentages of *A. ovis* in sheep and goats were 67.35% (196/291) and 66.41% (87/131) respectively, whereas the number of *A. phagocytophilum* in sheep and goats were 18.90% (55/291) and 21.37% (28/131) respectively [25].

In this study, *A. ovis* and/or *A. phagocytophilum* infections were revealed in a total of 230 (54.37%) small ruminants investigated. The detection rates of *A. ovis*

and *A. phagocytophilum* in small ruminants were 50.83% (215/423) and 8.51% (36/423) respectively. Moreover, *A. ovis* and *A. phagocytophilum* coinfection frequency was noted as 4.96% (21/423). The prevalences of *A. ovis* in sheep and goats were 58.8% (127/216) and 42.51% (88/207) respectively, whereas the prevalences of *A. phagocytophilum* in sheep and goats were 11.11% (24/216) and 5.8% (12/207) respectively *(Table 1)*.

The results according to the study location demonstrated *A. ovis* to be the most abundant in Edirne (75.81%) and Kirklareli (55.77%) in sheep and goats respectively. *A. phagocytophilum* detection frequencies were highest in Kirklareli with 23.08% and 15.38% observed for sheep and goats respectively *(Table 1)*. The prevalence of *A. ovis* in sheep in Edirne was significantly higher compared to Istanbul and Tekirdag provinces, whereas it was significantly higher in Kirklareli than Istanbul. No statistically-significant difference was noted of *A. ovis* detection rates in sheep among Kisklareli, Edirne and Tekirdag provinces (P<0.05) *(Table 1)*. In Istanbul, prevalence of *A. ovis* in sheep was statistically lower than the other provinces while the prevalence of *A. ovis* in goats was only statistically lower than Kirklareli (P<0.05) *(Table 1)*.

A comparison of *A. phagocytophilum* detection rates revealed a significantly higher the prevalence of *A. phagocytophilum* in goats in Kirklareli province than the remaining provinces, while the prevalence of *A. phagocytophilum* in sheep was statistically higher than Istanbul and Edirne. *A. phagocytophilum* prevalence in sheep in Tekirdag was statistically similar to other provinces in the study (P<0.05) *(Table 1)*. In Europe, *Ixodes ricinus* (European sheep tick) acts as the main vector of *A. phagocytophilum* [39]. It has been reported that the *Ixodes ricinus* in Istanbul metropolitan area and in Kirklareli were infected with *A. phagocytophilum* at a rate of 2.7% and 17.5% respectively [27]. In our study, the prevalence of *A. phagocytophilum* in Istanbul in sheep and goats were 5.77% and 1.92% respectively while the prevalence of *A. phagocytophilum* in Kirklareli in sheep and goats were 23.08% and 15.38%, respectively.

The identities of the PCR products for *A. ovis* and *A. phagocytophilum* were verified by sequencing of the amplicons obtained from selected samples in the study. Despite the high level of similarity observed for *A. ovis, A. phagocytophilum* sequences demonstrated divergence up to 2.49% *(Fig. 2)*. Several genotypes and variant clusters, some of which are associated with the host species have been characterized for *A. phagocytophilum* [40,41]. Moreover, phylogenetic analyses of the msp4 region were reported to differentiate strains of *A. phagocytophilum* obtained from ruminants from those obtained from humans, dogs, and horses [37]. However, sequence data from several regions have been utilized for a more precise interpretation of phylogenetic relations among *A. phagocytophilum* isolates. Given that sequence data was available only from selected samples and employed for confirmatiory purposes, a thorough analysis of *A. phagocytophilum* sequence variations was not possible. Limited divergence was reported from various targets such as 16S rRNA and ankA sequences from Turkey [25,31].

So far, *Anaplasma* infections in small ruminants have not been documented in Thrace region of Turkey. Herein, we reported the presence and the distribution of *Anaplasma ovis* and *A. phagocytophilum* in sheep and goats in Thrace region of northwestern Turkey for the first time by using species-specific PCRs *(Table 1)*. Potential vectors of Anaplasmosis are known to be endemic in Thrace region of Turkey [42-44]. Therefore further researches are needed to determine the vectors, vector-host interactions and genotypic variants that may affect the presence and distribution of *Anaplasma* species in Thrace region of northwestern Turkey.

## Acknowledgements

Authors are grateful to Prof.Dr. Munir AKTAS and Dr. Sezayi OZUBEK for technical assistance and providing positive control DNAs for *A. ovis* and *A. phagocytophilum*. Koray Ergunay is a recipient of the Georg Forster Research Fellowship (HERMES) for Experienced Researchers award of the Alexander Von Humboldt Foundation, 2015.

## REFERENCES

**1. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR:** Reorganisation of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*, 51, 2145-2165, 2001.

**2. Rikihisa Y:** The tribe Ehrlichieae and ehrlichial diseases. *Clin Microbiol Rev*, 4, 286-308, 1991.

**3. Rymaszewska A, Grenda S:** Bacteria of the genus *Anaplasma* - characteristics of *Anaplasma* and their vectors: A review. *Veterinarni Medicina*, 53 (11): 573-584, 2008.

**4. Friedhoff KT:** Tick-borne diseases of sheep and goats caused by *Babesia*, *Theileria* or *Anaplasma* spp. *Parassitologia*, 39, 99-109, 1997.

**5. Tibbitts T, Goff W, Foreyt W, Stiller D:** Susceptibility of two rocky mountain bighorn sheep to experimental infection with *Anaplasma ovis*. *J Wildl Dis*, 28 (1): 125-129, 1992. DOI: 10.7589/0090-3558-28.1.125

**6. Kocan KM, de la Fuente J, Blouin EF, Garcia-Garcia JC:** *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): Recent advances in defining host-pathogen adaptations of a tick-borne Rickettsia. *Parasitology*, 129, 285-300, 2004. DOI: 10.1017/S0031182003004700

**7. Uilenberg G:** International collaborative research: Significance of tick-borne hemoparasitic diseases to world animal health. *Vet Parasitol*, 57, 19-41, 1995. DOI: 10.1016/0304-4017(94)03107-8

**8. Ioannou I, Sandalakis V, Kassinis N, Chochlakis D, Papadopoulos B, Loukaides F, Tselentis Y, Psaroulaki A:** Tick-borne bacteria in mouflons and their ectoparasites in Cyprus. *J Wildl Dis*, 47, 300-306, 2011. DOI: 10.7589/0090-3558-47.2.300

**9. Liu Z, Ma M, Wang Z, Wang J, Peng Y, Li Y, Guan G, Luo J, Yin H:** Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. *Appl Environ Microbiol*, 78, 464-

470, 2012. DOI: 10.1128/AEM.06848-11

**10. de la Fuente J, Atkinson MW, Hogg JT, Miller DS, Naranjo V, Almazan C, Anderson N, Kocan KM:** Genetic characterization of *Anaplasma ovis* strains from bighorn sheep in Montana. *J Wildl Dis*, 42, 381-385, 2006. DOI: 10.7589/0090-3558-42.2.381

**11. Papadopoulos B:** Cattle and small ruminant piroplasmosis in Macedonia, Greece. *Parassitologia*, 41 (Suppl 1): 81-84, 1999.

**12. Psaroulaki A, Chochlakis D, Sandalakis V, Vranakis I, Ioannou I, Tselentis Y:** Phylogentic analysis of *Anaplasma ovis* strains isolated from sheep and goats using groEL and mps4 genes. *Vet Microbiol*, 138, 394-400, 2009. DOI: 10.1016/j.vetmic.2009.04.018

**13. Hosseini-Vasoukolaei N, Oshaghi MA, Shayan P, Vatandoost H, Babamahmoudi F, Yaghoobi-Ershadi MR, Telmadarraiy Z, Mohtarami F:** *Anaplasma* infection in ticks, livestock and human in Ghaemshahr, Mazandaran province, Iran. *J Arthropod Borne Dis,* 8 (2): 204-211, 2014.

**14. Carrade DD, Foley JE, Borjesson DL, Sykes JE:** Canine granulocytic Anaplasmosis: A review. *J Vet Intern Med*, 23, 1129-1141, 2009. DOI: 10.1111/j.1939-1676.2009.0384.x

**15. Stuen S, Bergstrom K, Palmer E:** Reduced weight gain due to subclinical *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) infection. *Exp Appl Acarol*, 28, 209-215, 2002. DOI: 10.1023/A:1025350517733

**16. Woldehiwet Z, Scott GR:** Tick-borne (pasture) fever. **In,** Woldehiwet Z, Ristic M (Eds): Rickettsial and Chlamydial Diseases of Domestic Animals. 233-254, Pergamon Press, Oxford, 1993.

**17. Giudice E, Giannetto C, Torina A, Gianesella M:** *Anaplasma phagocytophilum* ıntragranulocytic morulae in aborting sheep: A herd case in Sicily. *Transbound Emerg Dis,*58, 263-267, 2011. DOI: 10.1111/j.1865-1682.2010.01190.x

**18. Yu XJ, Walker DH:** The Order Rickettsiales. **In,** Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (Eds): The Prokaryotes. 493-528, Springer, New York, 2006.

**19. Christova IS, Dumler JS:** Human granulocytic ehrlichiosis in Bulgaria. *Am J Trop Med Hyg,* 60 (1): 58-61, 1999.

**20. Daniel SA, Manika K, Arvanitidou M, Diza E, Symeonidis N, Antoniadis A:** Serologic evidence of human granulocytic ehrlichiosis, Greece. *Emerg Infect Dis,* 8 (6): 643-644, 2002.

**21. Bashiribod H, Kazemi B, Eslami G, Bigdeli S, Bandehpour M, Rahbarian N, Ramezani Z:** First molecular detection of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in Iran. *J Med Sci*, 4, 282-286, 2004. DOI: 10.3923/jms.2004.282.286

**22. Ozcan HC:** Ankara ve civarında evcil hayvanlarda görülen Piroplasmose vakaları ve tedavileri üzerinde araştırmalar. *Doçentlik Tezi.* Ankara Üniv Vet Fak Yay, 143, 83, 1961.

**23. Sayın F, Dinçer Ş, Karaer Z, Çakmak A, Yukarı BA, Eren H, Değer S, Nalbantoğlu S:** Status of the tick-borne diseases in sheep and goats in Turkey. *Parasitologia,* 39 (2): 153-156, 1997.

**24. Gokce HI, Genc O, Akca A, Vatansever Z, Unver A, Erdogan HM:** Molecular and serological evidence of *Anaplasma phagocytophilum* infection of farm animals in the Black Sea region of Turkey. *Acta Vet Hung*, 56, 281-292, 2008. DOI: 10.1556/AVet.56.2008.3.2

**25. Altay K, Dumanli N, Aktas M, Ozubek S:** Survey of *Anaplasma* infections in small ruminants from East part of Turkey. *Kafkas Unv Vet Fak Derg*, 20, 1-4, 2014. DOI: 10.9775/kvfd.2013.9189

**26. Aktas M, Vatansever Z, Altay K, Aydin MF, Dumanli N:** Molecular evidence for *Anaplasma phagocytophilum* in *Ixodes ricinus* from Turkey. *T Roy Soc Trop Med H,* 104, 10-15, 2010. DOI: 10.1016/j.trstmh.2009.07.025

**27. Sen E, Uchishima Y, Okamoto Y, Fukui T, Kadosaka T, Ohashi N, Masuzawa T:** Molecular detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in *Ixodes ricinus* ticks from Istanbul metropolitan area and rural Trakya (Thrace) region of north-western Turkey. *Ticks Tick Borne Dis,* 2, 94-98, 2011. DOI: 10.1016/j.ttbdis.2011.03.004

**28. Aktas M:** A survey of ixodid tick species and molecular identification of tickborne pathogens. *Vet Parasitol*, 200, 276-283, 2014. DOI: 10.1016/j.vetpar.2013.12.008

**29. Aktas M, Altay K, Dumanli N:** Molecular detection and identification of *Anaplasma* and *Ehrlichia* species in cattle from Turkey. *Ticks and Tick-borne Dis*, 2, 62-66, 2011. DOI: 10.1016/j.ttbdis.2010.11.002

**30. Aktas M, Ozubek S:** Bovine anaplasmosis in Turkey: First laboratory confirmed clinical cases caused by *Anaplasma phagocytophilum*. *Vet Microbiol*, 178, 246-251, 2015. DOI: 10.1016/j.vetmic.2015.05.021

**31. Düzlü Ö, İnci A, Yıldırım A, Önder Z, Çiloğlu A:** Köpeklerde kene kaynaklı bazı protozoon ve rickettsial enfeksiyonların real time PCR ile araştırılması ve saptanan izolatların moleküler karakterizasyonları. *Ankara Univ Vet Fak Derg,* 61, 275-282, 2014. DOI: 10.1501/Vetfak_0000002642

**32. Aktas M, Altay K, Dumanli N, Kalkan A:** Molecular detection and identification of *Erlichia* and *Anaplasma* species in Ixodid ticks. *Parasitol Res*, 104, 1243-1248, 2009. DOI: 10.1007/s00436-009-1377-1

**33. Dreher UM, de la Fuente J, Hofmann-Lehmann R, Meli ML, Pusterla N, Kocan KM, Woldehiwet Z, Braun U, Regula G, Staerk KD, Lutz H:** Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. *Clin Diagn Lab Immunol*, 12, 1177-1183, 2005. DOI: 10.1128/CDLI.12.10.1177-1183.2005

**34. Ogden NH, Casey AN, Woldehiwet Z, French NP:** Transmission of *Anaplasma phagocytophilum* to *Ixodes ricinus* ticks from sheep in the acute and post-acute phases of infection. *Infect Immun*, 71, 2071-2078, 2003. DOI: 10.1128/IAI.71.4.2071-2078.2003

**35. de la Fuente VJ, Torina A, Caracappa S, Tumino G, Furla R, Almaza C, Kocan KM:** Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily. *Vet Parasitol*, 133, 357-362, 2005. DOI: 10.1016/j.vetpar.2005.05.063

**36. Torina A, Agnone A, Blanda V, Alongi A, D'Agostino R, Caracappa S, Marino AM, Di Marco V, de la Fuente J:** Development and validation of two PCR tests for the detection of and differentiation between *Anaplasma ovis* and *Anaplasma marginale*. *Ticks Tick Borne Dis*, 3, 283-287, 2012. DOI: 10.1016/j.ttbdis.2012.10.033

**37. de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, von Loewenich FD, Grzeszczuk A, Torina A, Caracappa S, Mangold AJ, Naranjo V, Stuen S, Kocan KM:** Sequence analysis of the msp4 gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol*, 43, 1309-1317, 2005. DOI: 10.1128/JCM.43.3.1309-1317.2005

**38. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S:** MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol*, 28, 2731-2739, 2011. DOI: 10.1093/molbev/msr121

**39. Woldehiwet Z:** The natural history of *Anaplasma phagocytophilum*. *Vet Parasitol*, 167, 108-122, 2010. DOI: 10.1016/j.vetpar.2009.09.013

**40. Chastagner A, Dugat T, Vourc'h G, Verheyden H, Legrand L, Bachy V, Chabanne L, Joncour G, Maillard R, Boulouis H-J, Haddad N, Bailly X, Leblond A:** Multilocus sequence analysis of *Anaplasma phagocytophilum* reveals three distinct lineages with different host ranges in clinically ill French cattle. *Vet Res*, 45, 114, 2014. DOI: 10.1186/s13567-014-0114-7

**41. Strašek Smrdel K, von Loewenich FD, Petrovec M, Avšič Županc T:** Diversity of ankA and msp4 genes of *Anaplasma phagocytophilum* in Slovenia. *Ticks Tick Borne Dis,* 6, 164-166, 2015. DOI: 10.1016/j.ttbdis.2014.11.008

**42. Aydın L, Bakirci S:** Geographical distribution of ticks in Turkey. *Parasitol Res*, 101 (Suppl. 2): 163-166, 2007. DOI: 10.1007/s00436-007-0694-5

**43. Vatansever Z, Gargili A, Aysul NS, Sengoz Z, Estrada-Peña A:** Ticks biting humans in the urban area of Istanbul. *Parasitol Res*, 102, 551-553, 2008. DOI: 10.1007/s00436-007-0809-z

**44. Gargili A, Kar S, Yilmazer N, Cerit C, Sonmez G, Sahin F, Gunseli Alp H, Vatansever Z:** Evaluation of ticks biting humans in Thrace province, Turkey. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-A): S141-S146, 2010. DOI: 10.9775/kvfd.2009.2555