Identification of Ticks on Tortoises (*Testudo graeca*) and Investigation of Some Pathogens in these Ticks in Kahramanmaraş, Turkey

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Abstract: This study was made to identify the tick types causing infestation in tortoises living in orchards in the city centre of Kahramanmaraş and the microorganisms they carried. Eighty-four tortoises were included in the study. Species determination of ticks collected from animals in which infestation was seen and their microbiological analyses were conducted. Species determination of 272 ticks on 60 of 84 (71.4%) tortoises taken for the study was made. The tick supernatants were used to detect CCHFV antigen by means of commercial ELISA test kits and aerobic, anaerobic cultures were performed. All ticks (180 of which were male and 92 of which were female) were detected as *Hyalomma aegyptium*. 98 aerobic bacteria were isolated in bacteriological treatments of ticks. These bacteria were as follows: 38 *Bacillus sp*., 23 *Diphtheroid bacilli*, 21 CNS, 7 *Pasteurella* sp., 6 *Micrococcu*s sp. and 3 *Enterobacter aerogenes*. CCHFV antigen was not identified in ELISA testing. It is clearly seen in our study that hard ticks live on vertebrate host animals, like the tortoise, not only in rural and mountainous areas but also in crowded city centres and may carry various microorganisms. Additionally, these animals should be held under the control.
Key words: Tick infestations; *Ixodidae*; Tick-borne diseases

Kaplumbağalardaki (*Testudo graeca*) Kenelerin İdentifikasyonu ve Kahramanmaraş'taki Bu Kenelerdeki Bazı Patojenlerin Araştırılması

Özet: Bu çalışma Kahramanmaraş şehir merkezindeki meyve bahçelerinde yaşayan kaplumbağalarda enfestasyona neden olan kene tiplerini ve taşıdıkları mikroorganizmaları tanımlamak için yapıldı. Çalışmaya seksen dört kaplumbağa alındı. Enfestasyonun görüldüğü hayvanlardan toplanan kenelerin türleri belirlendi ve mikrobiyolojik incelemeleri yapıldı. Çalışma için alınan 84 kaplumbağanın 60'ındaki 272 kenenin tür belirlemesi yapıldı. Kene örneklerinde CCHV antijeni belirlemek için ticari ELISA testi kullanıldı. Ayrıca bu örneklerin aerobik ve anaerobik olarak kültürleri yapıldı. Tüm kenelerin (180 erkek, 92 dişi) *Hyalomma aegyptium* türü olduğu saptandı. Kenelerin bakteriyolojik incelemelerinde 98 aerobik bakteri izole edildi. Bu bakteriler şöyleydi: 38 *Bacillus* sp., 23 difteroid basil, 21 CNS, 7 *Pasteurella* sp., 6 *Micrococcu*s sp. ve 3 *Enterobacter aerogenes*. ELISA testinde CCHFV antijeni bulunmadı. Bizim çalışmamızda açıkça görülmektedir ki sert keneler sadece kırsal ve dağlık alanlarda değil aynı zamanda kalabalık şehir merkezlerinde kaplumbağalar gibi omurgalı konakçı hayvanlarda yaşamaktalar ve çeşitli mikroorganizmalar taşıyabilirler. Ayrıca, bu hayvanlar kontrol altında tutulmalıdır.
Anahtar kelimeler: Kene infestasyonları; *Ixodidae*; Kene kaynaklı hastalıklar

INTRODUCTION

The species *Testudo graeca*, defined in *Systema Naturae*, is a tortoise inhabiting Northern Africa, the Middle East, and Europe. Tortoises are herbivorous and vertebrate reptile animals. Unlike other reptiles, tortoises, being popular pets of children, are kept in houses in addition to their natural environment (Turkozan et al., 2005; Tavassoli et al., 2007). *Hyalomma* ticks (hard tick, field tick), especially those in the Ixodidae family, may live in the leg and tile regions of these organisms living in orchards, gardens, forests, and rural areas and are normally harmless to humans. Ticks, which can change their skins by transition from larva to nymph to maturity on tortoises, form a potential risk in environments where these animals live and can lead to various infections by attacking humans (Sonenshine, 2005; Ulrike et al., 2005).

Worldwide, tick sourced infections are primarily Crimean-Congo hemorrhagic fever virus (CCHFV), but also include *Anaplasmosis*, *Babesia* spp., *Rickettsiosis*, *Ehrlichiosis*, *Francisella tularensis* (tularemia), *Coxiella burnetii* (Q-fever), tick encephalitis (Flavivirus), tick paralysis, relapse fever infecting with the tick, and Lyme disease. Lyme disease observed in humans due to the tick bite is caused by the *Borrelia burgdorferi sensu lato* complex (*B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii*), which is a spirocket structured bacteria common in temperate regions.

In addition to the specific infections mentioned above, ticks can act as mechanical or biological vectors of many Gram negative (*Brucella melitensis*, *Salmonella enteritidis*, *Pasteurella pneumotropica*,

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Aeromonas hydrophila, *Pseudomonas aeruginosa*) and Gram positive (*Bacillus cereus, Erysipelothrix rhusiopathiae, Listeria monocytogenes*) bacteria, thereby constituting a hazard for human health. In addition, ticks may cause toxications, allergic reactions, and abscesses, depending on allergens in saliva (Gurbuz et al., 2010; Arıkan et al., 2009; Stojek & Dutkiewicz, 2004).

In this study, the ticks on *Testudo graeca* living in natural areas in Kahramanmaraş were classified, and the presence of possible pathogen microorganisms in these ticks was investigated.

MATERIAL and METHODS

Design and specimen collection

This study applies a descriptive method. In different orchards and gardens close to the city centre in Kahramanmaraş, 84 adult tortoises determined as *Testudo graeca* were inspected for ticks. 272 adult ticks were collected from the back leg and tail regions of 60 tortoises in which the tick infection was seen, and were sent to Kahramanmaraş Sütçü Imam University Medicine Faculty Research Laboratory for the determination of species and for pathogen analysis.

Microbiological and parasitological analysis

In the study, 272 adult ticks were examined for determination of the gender and species under the stereo microscope and were identified in the species level according to the classification criteria (such as capitulum, palp, festoon, cervical canal, anal shield, accessorial shield, and scutum colour) (Dumler & Rosen-Feld, 2000; Walker et al., 2003). Each tick was washed in sterile salty water (0.85% NaCl) and after their outside sections were disinfected with 70% ethyl alcohol, they were washed in salty water again. Each tick was placed into tubes with sterile distilled water and were then homogenized with the homogenizator (Daihan HS-30E, DAIHAN Scientific, Korea) at 3000 rpm for 3 minutes; they were then centrifuged at 4000 rpm for 30 minutes and supernatants were extracted (Arıkan et al., 2009; Stojek & Dutkiewicz, 2004). The tick supernatants were used to detect CCHFV antigen by means of aerobic, anaerobic culture, and commercial ELISA test kits. To make the culture, each tick suspension was incubated in aerobic and anaerobic media at 37 °C for 24-48 hours by sowing into two agars: one with 5% sheep blood (Merck, Darmstadt, Germany) and the other being eosin methylene blue (EMB, Merck) agar. Reproductions in the end of the incubation were evaluated with routine microbiological methods (Dumler & Rosen-Feld,2000); Koneman et al., 2006). ELISA testing (VectoCrimea, CHF-antigen ELISA test kit, Vector-Best, Novosibirsk, Russia) was used to determine the CCHFV antigen; instructions of the producer firm were followed.

Ethical approval

The study was approved by the Ethics Committee at the Medical School of the University of Kahramanmaraş Sütçü İmam. All subjects provided their oral informed consent before participating in the study.

RESULTS and DISCUSSION

Scutums of ticks were dark brown. Their cervical canals were short and deep and the front end sections of anal shield had a sharp structure and no lateral canals. Their festoons were available and apparent and their accessorial shield were detected as united with genital canals. It was determined that all 272 hard ticks (180 males, 92 females) were adult and pertained to the species *Hyalomma aegyptium* connected to the genus *Hyalomma* from the metastriata subfamily (Figures 1-4).

In bacteriological inspections, 98 aerobic bacteria were isolated. These bacteria were 38 *Bacillus* sp., 23 *diphtheroid bacilli*, 21 *coagulase negative staphylococcus* (CNS), 7 *Pasteurella* sp., 6 *Micrococcus* sp. and 3 *Enterobacter aerogenes* (Table 1). Anaerobic bacteria were not found in the culture examination. In ELISA testing, CCHFV antigen was not detected.

Ticks from the family Ixodidae lead to numerous diseases, such as CCHFV, Lyme borreliosis, human granulocytic anaplasmosis, tularemia, typhus, monocytotropic ehrlichiosis, Q fever, and tick encephalitis (Gurbuz et al., 2010; Arıkan et al., 2009; Goodman, 2005; Hayes, 2005; Torres &Schossberg, 2000). In 2948 tick cases seen from 2002 up to 2010 in Turkey, 147 citizens have lost their lives due to the CCHFV disease and the importance of tick sourced zoonotic pathogens is increasing (Albayrak et al., 2010). Nevertheless, in a study made on the school of health students, knowledge level concerning CCHFV was found to be low (Ozer et al., 2010).

In Turkey, it having a temperate climate with its wide tame and wild animal assets, hard tick species, which can be a vector of CCHFV, and many other pathogens live (Aydin & Bakirci, 2007). In particular, that hard ticks connected to the genus *Hyalomma* breed carry and infect fatal CCHFV, which lead to fear and panic in many cities in the summer months. In our study, when *T.graeca* tortoises commonly inhabiting orchards and gardens in the city centre were randomly selected and examined for ticks, it was determined that *H.aegyptium* was a tick species inhabiting these animals. In similar research conducted in Iran, hard ticks were found on 14 of 32 *T.graeca;* all were identified as *H.aegyptium* (n: 117) (Tavassoli et al., 2007).

Figure 1. A large number of ticks (*Hyalomma aegyptium*) collected from the pre-anal area and the axilla of the hind limbs of the tortoise.

Figure 2. *Hyalomma aegyptium* (dorsal-male).

Figure 3. *Hyalomma aegyptium* (dorsal-female).

Figure 4. *Hyalomma aegyptium* (ventral-male).

Table 1. Distribution of pathogenic microorganisms isolated from *Hyalomma aegyptium* on *Testudo graeca*

Microorganisms	**Adult male tick**	**Adult female tick**	**Total**
Bacillus sp.	22 (57.9 %)	16 (42.1 %)	38 (100.0)
Diphtheroid bacilli	5 (21.7 %)	18 (78.3 %)	23 (100.0)
CNS	12 (57.2 %)	9 (42.8 %)	21 (100.0)
Pasteurella sp.	2 (28.6 %)	5(71.4 %)	7 (100.0)
Micrococcus sp.	1 (16.7 %)	5(83.3 %)	6(100.0)
Enterobacter aerogenes	3 (1 %)	0 (0.0)	3 (100.0)
CCHFV	0 (0.0)	0 (0.0)	0 (100.0)
Total	45 (45.9%)	53 (54.1%)	98 (100.0)

Tortoises commonly inhabiting Europe, North Africa, the Middle East, and Southwest Asia are prevalent hosts of *H.aegyptium* in particular, and they threaten human health because these ticks carry Lyme disease, *Theileriazis* and *Pasteurella tularensis*, and many other pathogens (Siroky et al., 2006; Tavassoli et al., 2007; Guner et al., 2003). 1327 ticks on a total of 211 tortoises, (*T. graeca, T. hermanni* and *T.marginata*) from among wild animal fauna in Balkan countries, such as Greece, Bulgaria, Croatia and Romania were classified; the most commonly found hard tick was detected as *H.aegyptium*. Moreover, in the study, *Haemaphysalis sulcata, H. inermis,* and *Rhipicephalus sanguineus* were identified (Siroky et al., 2006).

Worldwide, many studies aiming towards the classification of ticks and pathogens they carry have been made. In a comprehensive research in which the tick fauna populations in Turkey were studied, widespread hard tick species and subspecies in Mediterranean region were found to include *Ixodes ricinus, R.annulatus, R.bursa, R. sanguineus, R.turanicus, Dermacentor marginatus, D.niveus, H. aegyptium, H.a. anatolicum, H. a. excavatum, H. detritum, H. dromedarii, H. parva, H.punctata* and *H.sulcata* (Aydin & Bakirci, 2007).

In a research into tick infestation in tame animals, in Kayseri city, the most often seen *Hyalomma* sp. ticks on animals were found to be *H.a.anatolicium, H.a.excavatum* and *H.detritum*, and in Burdur region *H.marginatum* and *H. a.excavatum* (Yay et al., 2004). More than 800 tick species have been identified worldwide, and 32 tick species have been identified in Turkey; ticks connected to *Hyalomma* breed are known to be the most widespread tick species in Turkey. These ticks are found on tame animals, such as cows, sheep, and goats, and in small vertebrate wild animals. These ticks they mostly become active between April and September (Tavassoli et al., 2007; Arıkan et al., 2009).

In Oman, CCHFV antigen was detected in 7-8 % of animals bearing hard ticks using the ELISA test. Moreover, it was determined that CCHFV was seen more often (30.3 %) in individuals who have physical contact with animals in which the tick infestation was resent or who are employed in cattle breeding (Williams et al., 2000). In the Northern Anatolia region of our country, CCHFV was scanned in ticks retrieved from tame animals and tortoises through the antigen ELISA test with results as follows: 33.87 % CCHFV antigen in Samsun, 4.34 % in Ordu, 8.86 % in Giresun, 6.09 % in Sinop, 7.40 % in Amasya, 5.08 % in Tokat and 8.06 % in Sivas (Albayrak et al., 2010). These results can be accepted as meaningful when evaluated together with the CCHFV disease cases developing in people living in that region of our country. In our study CCHFV antigen was not met in the research made with the antigen ELISA.

This situation is in harmony with the fact that CCHFV disease is yet to be seen in tick bite cases in Kahramanmaraş city. However to say with confidence that there are no ticks carrying CCHFV in our city, detailed researches including into large cattle and small cattle and into other hard tick species must be made.

It was reported that death occurred in one person with CCHFV disease due to a tick bite in Kayseri, a neighboring city, in May 2010. For this reason, it would be imprudent to say that ticks carrying CCHFV won't invade our city. Moreover, ticks can carry not only CCHFV, but also hundreds of other pathogens mechanically or biologically. In Poland, in research into ticks, important human pathogens, such as *Pasteurella pneumotropica, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* (Gram-negative bacteria) were isolated (Stojek&Dutkiewicz, 2004). Gram-negative *Pasteurella* sp. and *E. aerogenes* and Gram-positive CNS species, which are isolated from ticks examined in our study, bear a risk of various human infections.

To conclude, hard ticks continue to be an important health problem in terms of CCHFV, Lyme and other pathogens between April and September in our country. It is clearly seen in our study and other researches that hard ticks live on vertebrate host animals, like the tortoise, not only in rural and mountainous areas but also in crowded city centres and may carry various microorganisms. To completely eliminate ticks from the ecosystem is manifestly not possible.

However the tick species living in every region of our country must be identified in detail in terms of pathogens and reservoirs they carry, and the dangers they represent must be contended with through biological, chemical, environmental, and personal protection methods.

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REFERENCES

Albayrak, H., Ozan, E., Kurt, M. 2010. An Antigenic İnvestigation of Crimean-Congo Hemorrhagic Fever Virus in Hard Ticks from Provinces in Northern Turkey. Tropical Animal Health Production, 42: 1323-1325.

Arıkan, D., Tıras, Ü., Saraçoğlu, D., Tasar, M.A., Dallar, Y. 2009. Evaluation of the Cases Appealed as Crimean-Congo Hemorrhagic Fever. Ege Journal of Medicine, 48: 29–31.

Aydin, L., Bakirci, S. 2007. Geographical Distribution of Ticks in Turkey. Parasitology Research, 101:163–166.

Dumler, J.S., Rosen-Feld, A. 2000. *in*:Microbiology and Laboratory Diagnosis of Tick Borne Diseases. Cunhu BA. (Ed). Tick Borne Infectious Diseases, Diagnosis And Management. New York, USA, 15–50 pp.

Gürbüz, M.K., Erdoğan, M., Doğan, N., Birdane, L., Cingi, C., Cingi, E. 2010. Olgu Sunumu: Kene Isırması ile Oluşan İzole Fasiyal Paralizi. Türkiye Parazitoloji Dergisi 34: 61–64.

Goodman, J.L. 2005. *in*: Human granulocytic anaplazmozis. Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (eds). Tick-borne Diseases of Humans. Washington DC, USA, 218pp.

Guner, E.S., Hashimoto, N., Kadosaka, T., Imai, Y., Masuzawa, T. 2003. A novel, fast-growing *Borrelia* sp. isolated from the hard tick *Hyalomma aegyptium* in Turkey. Microbiology 149:2539–2544.

Hayes, E.B. 2005. *in*: Tularemia. Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (eds). Tick-Borne Diseases of Humans. Washington DC, USA, 207pp.

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C., Woods, G.L. 2006. Guidelines for the Collection, Transport, Processing, Analysis, and Reporting of Cultures From Specific Specimen Sources. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia, Lippincott Co., 2-66 pp.

Ozer, A., Miraloglu, M., Ekerbicer, H.C., Cevik, F., Aloglu, N. 2010. Knowledge Levels About Crimean Congo Hemorrhagic Fever Among Midwifery and Nursing Students in Kahramanmaraş, Turkey. Southeast Asian Journal of Tropical Medicine Public Health, 41: 77–84.

Sonenshine, D.E. 2005. *in*: The Biology of Tick Vectors of Human Disease. Goodman J.L., Dennis D.T., Sonenshine D.E. (eds). Tick-Borne Diseases of Humans. Washington DC, USA, 12–36 pp.

Siroky, P., Kla´ra, J., Petrzˇelkova, K.J., Kamler, M., Andrei, D., Mihalca, A.D., Modry, D. 2006. *Hyalomma aegyptium* as Dominant Tick in Tortoises of the Genus Testudo in Balkan Countries, with Notes on Its Host Preferences. Experimental and Applied Acarology, 40: 279–290.

Stojek, N.M., Dutkiewicz, J. 2004. Studies on the Occurrence of Gram-Negative Bacteria *In* Ticks: *Ixodes ricinus* As a Potential Vector of *Pasteurella*. Annals of Agricultural and Environmental Medicine 11: 319–322.

Torres, J.M., Schossberg, D. 2000. *in*: Tick Paralysis. Cunhu, B.A. (ed). Tick Borne Infectious Diseases, Diagnosis and Management. New York, USA, 103–110pp.

Türkozan, O., Olgun, K. 2005. A Preliminary Survey of *Testudo graeca* Linnaeus 1758 Specimens from Central Anatolia, Turkey. Turkish Journal of Zoology 29: 255–262.

Tavassoli, E., Asiabi, N.R., Tavassoli, M. 2007. *Hyalomma aegyptium* on Spur-thighed Tortoise (*Testudo graeca*) in Urmia Region West Azerbaijan, Iran. Iranian Journal of Parasitology 2: 40–47.

Ulrike, G.M., Steven, D.J., Timothy, J.K. 2005. *in*: A Different Kind of Host for Human Pathogenes. Goodman, J.L., Dennis, D.T., Sonenshine, D.E. (eds). Tick-Borne Diseases of Humans. Washington, USA, 37pp.

Walker, A.R., Bouattour, A., Camicas, J.L., Estradapeňa, A., Horak, I.G., Latif, A.A., Pegram R.G., Preston P.M. 2003.Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Edinburgh, Scotland: Bioscience Reports.

Williams, R.J., Busaidy, S., Mehta, F.R., Maupin, G.O., Wagoner, K.D., Awaidy, S., Suleiman, A.J.M., Khan, A.S., Peters, C.J., Ksiazek, T.G. 2000. Crimean-Congo Haemorrhagic Fever: A Seroepidemiological and Tick Survey in the Sultanate of Oman. Tropical Medicine and International Health, 5: 99–106.

Yay, M., Yazar, S., Aydın, L., Şahin, İ. 2004. Investigation of Tick Species on Sheep and Cattle around of Kayseri. Erciyes University Journal of Health Sciences, 13: 25–29.