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Original Article
Özgün Araştırma

Molecular investigation of the relationship between vector tick and host in Lumpy Skin Disease

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Abstract: *Lumpy Skin Disease Virus* (LSDV), belonging to the *Capripoxvirus* genus of the *Poxviridae* family, causes significant infections in cattle, especially in African and Middle East countries. LSD is an arboviral disease that spreads with bloodsucking flies as the primary source of infection. However, in experimental studies, nucleic acid belonging to the LSDV has been detected in ticks, and it has been reported that ticks may also play a role in transmission. Within the scope of this study, it is aimed to investigate the vector tick-host relationship of the disease by collecting blood and tick samples from cattle, sheep, and goats in Samsun, Sivas, and Tokat provinces, where LSD infections are intensely detected in the Black Sea Region in Turkey. For this purpose, ticks and blood samples were collected from 88 cattle, 511 sheep, and 108 goats with tick infestation between March 2016 and October 2017. A total of 2508 ticks were collected from these animals, whose blood samples with EDTA were taken, and the ticks were classified according to species. Blood samples and ticks were tested to reveal the presence of LSDV nucleic acids by real-time PCR, and LSDV nucleic acids could not be detected in both blood samples or ticks.

Keywords: LSDV, Tick, Vector, real-time PCR

Sığırların Nodüler Ekzantemi hastalığında vektör kene ile konak arasındaki ilişkinin moleküler araştırılması

Özet: *Poxviridae* familyasının *Capripoxvirus* cinsine ait olan Sığırların Nudüler Ekzantemi Hastalığı Virusu (SNEHV), özellikle Afrika ve Ortadoğu ülkelerinde sığırlarda önemli enfeksiyonlara neden olmaktadır. SNEH, birincil enfeksiyon kaynağı olan kan emici sineklerle yayılan arboviral bir hastalıktır. Ancak deneysel çalışmalarda kenelerde SNHEV'ye ait nükleik asit saptanmış ve bulaşmada kenelerin de rol oynayabileceği bildirilmiştir. Bu çalışma kapsamında, Türkiye'de Karadeniz Bölgesi'nde yeralan ve SNEH enfeksiyonlarının yoğun olarak tespit edildiği Samsun,Sivas ve Tokat illerinden sığır, koyun ve keçilerden kan ve kene örnekleri toplanarak hastalığın vektör-kene-konak ilişkisinin incelenmesi amaçlanmıştır. Bu amaçla Mart 2016-Ekim 2017 tarihleri arasında kene enfestasyonu olan 88 sığır, 511 koyun ve 108 keçiden kene ve EDTA'lı kan örnekleri alındı. Kan örnekleri alınan bu hayvanlardan toplam 2508 kene toplandı ve keneler türlerine göre sınıflandırıldı. Real time PCR ile SNHEV nükleik asitlerinin varlığını ortaya çıkarmak için kan örnekleri ve keneler test edildi ve hem kan örneklerinde hem de kenelerde SNHEV nükleik asitleri tespit edilemedi.

Anahtar kelimeler: SNEHV, Kene, Vektör, real time PCR

Introduction

Lumpy Skin Disease (LSD) is one of the cattle's important arboviral diseases caused by LSDV, in the genus *Capripoxvirus* from the *Chordopoxvirinae* subfamily of the *Poxviridae* family (Tuppurainen et al., 2013a; Lubinga et al., 2014). The disease affects water buffalo and cattle of all ages (Weiss, 1968; Ahmed et al., 2021). The mortality rate is generally between 1-3%. However, it can reach up to 40% (Coetzer, 2004). And it is characterized by smallpox lesions in the digestive and respiratory tracts and skin. Besides, fever, enlarged superficial lymph nodes, keratitis, salivation, and nasal discharge were also observed (Coetzer, 2004; Tuppurainen et al., 2011; Menasherow et al., 2014; Lubinga et al., 2015). Besides, skin lesions can occur as a result of ulcerative lesions occurring on the skin (Green, 1959).

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LSDV is on the list of diseases which are obliged to be reported by World Organization for Animal Health (WOAH). The LSD, also named Cattle's Nodular exanthema, was first seen in 1929 in Zambia. The LSDV spread to various African countries in the following years (OIE, 2021; Tuppurainen et al., 2021). Currently, LSDV is endemic in the Middle East and Africa, causing an economic loss in cattle. Recent outbreaks, especially in the Middle East and Asia, pose a risk of spreading the disease to Europe (Tuppurainen and Oura, 2012; Tuppurainen et al., 2021). The disease was first detected in Turkey in 2013. It is thought that The LSDV has entered Turkey via cattle smuggling and refugees and their animals from northern Syria and Iraq, which is endemic in the regions for LSDV (Sevik and Dogan, 2017; Albayrak et al., 2018). The risk of spreading LSDV from Syria and Iraq to Turkey is due to the lack of animal disease policies in the Middle East region, insufficient laboratory tests, and ineffective control and contact with international organizations such as OIE (Tuppurainen and Oura, 2012).

The occurrence of the disease is associated with hot and humid weather conditions and the abundance of flies (Tuppurainen et al., 2011). Furthermore, high temperatures, heavy rainy seasons, and the presence of water ponds cause an increase in the population of blood-feeding arthropods that transmit vector-borne diseases such as LSD (Tuppurainen and Oura, 2012). It has long been thought that more than one fly species transmit LSDV (Weiss, 1968; Tuppurainen and Oura, 2012). The virus was isolated from *Stomoxys calcitrans* and *Biomyia fasciata* in 1960s (Du toit and Weiss, 1960; Weiss, 1968). Mechanical transmission has been demonstrated in *Stomoxys* flies and *Aedes aegypti* mosquitoes (Kitchen and Mellor, 1986; Chihota et al., 2001).

Various viruses belonging to the *Flaviviridae* family (Tick-Borne Encephalitis, Louping ill, etc.), *Bunyavivirade* family (Crimean Congo Haemorrhagic fever, etc.), and *Reoviridae* family (Colorado Tick Fever, etc.) have been detected from various tick species (Tuppurainen et al., 2011). We have limited knowledge about where or how LSDV can survive in cattle during the inter-epidemic period or its target reservoir. Therefore, studies about tick distribution may provide possible information about the sudden reappearance of LSDV even years after its appearance. Some of the ticks, feed on a variety of mammals and birds and can transmit agents such as LSDV (Tuppurainen and Oura, 2012).

This study aimed to investigate the role of the tick in the transmission of LSDV by investigating the LSDV nucleic acid in ruminants in different seasons.

Material and Methods

Ethics Statement

We designed all study protocols and procedures following the national legislative rules and ethical standards, under validation order by Samsun Veterinary Control Institute Scientific Ethics Committee, Ministry of Agriculture and Forestry, the Republic of Turkey (No: 26/01/2015/5/35, Date: 26 January 2015).

Sampling and Sampling Area

Samples were collected from Samsun (41°"N 36°"E), Sivas (39°"N 37°"E), and Tokat (40°"N 36°"E) provinces, where LSD cases have been reported previously (Figure 1). Especially between April and October, when ticks are active, samples were taken to the field every month for two years. Before taking samples from the animals, their general physical examination was performed and their body temperature was measured. Body temperatures were normal and no clinical symptoms in all animals. A total of 707 EDTA blood samples, 88 of which from cattle, 511 of which from sheep, and 108 of which from goats, were collected. These blood samples were delivered to the laboratory under cold conditions. In addition, a total of 2508 ticks were collected from animals of which blood was taken (Table 1).

Preparing homogenizes and DNA extractions

Ticks were classified according to various characteristics (genus, species, sex, saturation). Afterward, ticks were prepared for DNA extraction according to Tuppurainen et al., 2015. A total of 745 tick pools were created. According to their size, one to ten ticks were placed in each 2 ml centrifuge tube. For this purpose, the ticks were cut into small pieces, and tick samples were placed in 2 ml centrifuge tubes with 3 mm steel beads. 500-750 µl of PBS was added to the centrifuge tubes, and the samples were homogenized for 5 minutes at maximum speed (50 Hz) in the Qiagen Tissue lyser. After homogenization, the samples were centrifuged at 4400 rpm for 15 minutes at +4 °C. The supernatants were stored at -20 °C for later use.

Nucleic acid extraction was performed from the blood and tick homogenized supernatants using with a High Pure Viral Nucleic Acid Kit (Roche) following the manufacturer's instructions. The obtained nucleic acids were stored at -20 °C to be used in the real-time PCR.

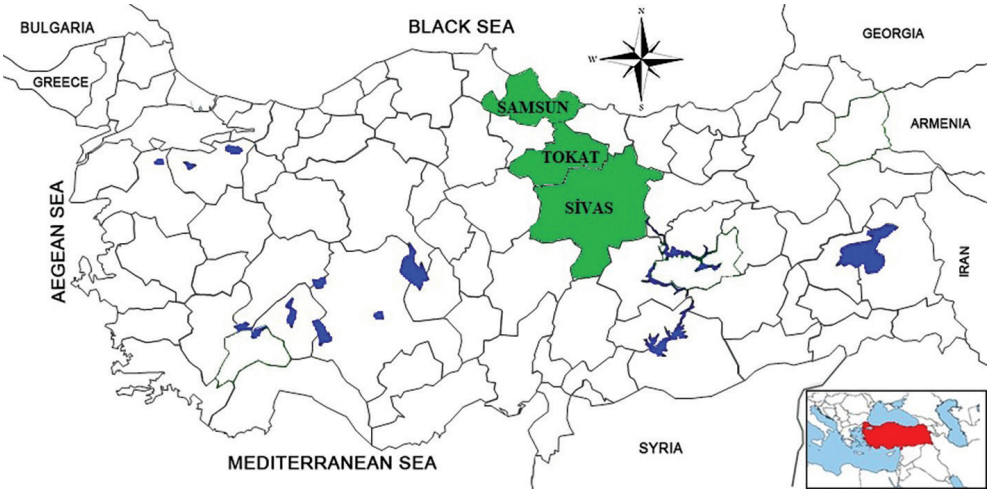

Figure 1. Provinces (Samsun, Tokat, and Sivas) in which samples were collected.

Table 1. Tick samples are distributed according to species, region, and gender.

Tick species	Samsun		Sivas		Tokat		Total
	male	female	male	female	male	female	
Dermacentor marginatus	2	-	165	185	35	47	434
Haemaphysalis sulcata	-	-	1	-	272	451	724
Haemaphysalis punctata	-	-	6	7	10	60	83
Rhipicephalus spp	-	17	6	85	-	2	110
Rhipicephalus bursa	5	-	22	8	11	12	58
Rhipicephalus turanicus	79	57	271	306	-	6	719
Haemaphysalis spp.	-	-	5	24	-	98	127
Hyalomma spp.	-	-	-	4	1	13	18
Hyalomma marginatum	-	-	19	6	78	100	203
Hyalomma dendriticum	-	-	-	-	16	-	16
İxodes ricinus	2	14	-	-	-	-	16
Total	**88**	**88**	**495**	**625**	**423**	**789**	**2508**

Real-time PCR

The real-time PCR was performed to detect the P32 gene of LSDV using Taq DNA Polymerase (Thermo, Cat No: EP0401). For this purpose, 2.5 µl 10X Taq buffer, 10 mM dNTP, 0.8 nM of both primers (CaPV-074F1 5′-AAAACGGTATATGGAATAGAGTTGGAA-3′, CaPV-074R1 5′-AAA TGAAACCAATGGATGGGA-TA-3′), 0.4 nM of probe (CaPV-074P1 5′-FAM-TG-GCTCATAGATTTCCT-TAMRA-3′), 2 mM $MgCl_2$, 1,25 unit Taq polymerase and 5 µl of template DNA (not measured) were mixed. The assays were carried out in Light Cycler 2.0 (Roche, Menheilm, Germany) using the following amplification program: 95 °C for 2 min; and 40 cycles of 95 °C for 15 s and 60 °C for 1 min (Bowden et al., 2008).

Results

Identification of Ticks

Ticks collected from animals were classified according to species, region, and gender. A total of 2508 ticks were collected. *Haemaphysalis sulcata* is the most found tick, especially in Tokat province. *Rhipicephalus turanicus* is the second most found tick species, especially in Sivas province. *Hyalomma marginatum* and *Dermacentor marginatus* were also found widely (Table 1).

Real-time PCR results

Samples obtained from 707 EDTA blood and 745 tick pools in total were negative in terms of LSDV nucleic acid.

Discussion

Although LSDV DNA could not be obtained from EDTA blood and tick samples collected from Sivas, Tokat, and Samsun, notable information about the predominant tick species population in these regions has been obtained (Table 1). *Haemaphysalis sulcata, Rhipicephalus turanicus*, *Dermacentor marginatus, Hyalomma marginatum, Haemaphysalis punctata, and Rhipicephalus bursa are the most collected tick species in the study area. Adult ticks found in this study, are generally active in spring and autumn. Haemaphysalis sulcata, Rhicephalus turanicus*, and *Dermacentor marginatus* are the most found tick species in this study. They are three-host ticks and have a wide distribution area especially reported in Mediterranean climates (Dantas-Torres et al., 2017; Keskin et al., 2013; Stanko et al., 2021). *They were found as a vector of many infectious diseases (*Hornok, 2017; Pfäffle et al., 2017; Santos-Silva et al., 2017; Vatansever, 2017a; Vatansever, 2017b). Although LSDV has not been detected in sheep and goats, ticks on sheep and goats were included in the experiment as they were kept with Cattle and could be random hosts.

In a previous study conducted with the 3 most common tick species (*Rhipicephalus appendiculatus*, *Amblyomma hebraeum*, *Rhipicephalus decoloratus*) in Africa, LSDV was detected in different life forms of these ticks fed on cattle experimentally infected with LSDV. These data strongly suggest that LSDV can be spread among host animals by ixodid ticks (Tuppurainen et al., 2011). LSDV was also detected in Bulgaria from *Hyalomma marginatum* and *Rhipicephalus bursa* (Alexandrov, 2016). And from *Dermacentor marginatus* and *Hyalomma asiaticum* in Kazakhstan in 2016 (Ornbayev et al., 2021). In another study, *Rhipicephalus appendiculatus* male ticks were fed from cattle that were experimentally infected with LSDV, and then these ticks were transferred to non-LSDV-infected cattle. As a result of the study, cattle also showed symptoms of LSD and were observed to recover in a short time. It has also been found that *Rhipicephalus appendiculatus* males transmit LSDV by feeding on skin without visible lesions. Thus, it was stated that viremic animals without lesions can be a source of infection In addition, it was reported for the first time that *Rhipicephalus decoloratus* ticks could play a role in the transovarial transmission of LSDV (Tuppurainen et al., 2011; Lubinga et al., 2013a; Tuppurainen et al., 2015). The finding of transovarial transmission of LSDV in female ticks (*A. hebraeum, R. appendiculatus, and R. Decoloratus*) indicates the potential to be reservoir hosts for LSDV. (Tuppurainen et al., 2013b; Lubinga et al., 2013b). It has been stated that ticks can play an active role in both mechanical and transtadial transmission and play an important role in the epidemiology of LSD disease (Lubinga et al., 2013a; Lubinga et al., 2015). In another study, viral antigen was detected in salivary glands, hemocytes, singanglia, ovaries, testicles, fat bodies, and midgut of *A. hebraeum* and *R. appendiculatus* ticks. Ticks have been evaluated as a biological potential for transmission of LSDV, as the virus has been shown to penetrate the midgut wall and infect various tick organs (Lubinga et al., 2014).

Besides, LSDV has also been detected in ticks collected from animals naturally infected with LSDV. The virus was also detected in tick samples collected from the field during LSD outbreaks in Egypt and South Africa and it was observed that LSDV remained infectious until 35 days in cell lines but did not grow. It was also concluded that intracellular or extracellular survival of the virus in tick tissues might be more important than active replication of the virus in tick cells (Tuppurainen et al., 2015). LSDV was detected by PCR on adults, eggs, nymphs, and larvae of *R. annulatus* ticks collected from animals naturally infected with LSDV (Rouby et al., 2017).

According to the distribution of the tick species in this study, it is seen that the tick species (*Rhipicephalus spp, H. marginatum,* and *R. turanicus*) may transmit LSDV mechanically are quite common in Sivas and Tokat regions. However, we could not detect LSDV in a total of 2508 ticks that we examined in this study should not mean that the disease will not be spread by ticks. This can be possible that the animals from which samples collected are not viremic, even if the animals were infected. In addition to this, as the transmission of LSD by insects or ticks is likely to be only mechanical, the viral loads in tick mouthparts are likely to be low, and probably pooling of samples has diluted it even more. Consequently, this hypothesis is getting stronger and should also be taken into account and research by further studies.

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Conflict of Interest: The author declares that there are no competing interests

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