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RESEARCH ARTICLE

Serological and Epidemiological Investigation of Bluetongue, Maedi-Visna and Caprine Arthritis-Encephalitis Viruses in Small Ruminant in Kirikkale District in Turkey [1]

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Summary

Viral infections cause important problems and significant economic loses in sheep and goats that can be protected by an investigation of infections. This study aimed to determine the sheep and goat viral infections including bluetongue virus (BTV), maedi-visna virus (MVV) and caprine arthritis-encephalitis virus (CAEV) in Kirikkale city located in Central Anatolia region of Turkey. Blood samples collected from 279 sheep and 146 goats were tested by ELISA, RT-nested PCR and nested PCR. It was detected that antibody response to BTV (49.8%), CAEV (7.5%) and MVV (19.4%) were seropositivity values in all serum samples. BTV (2.87%) and MVV (9.25%) antigens were detected in seropositive whole blood samples via RT-nested PCR and nested PCR but not CAEV. It was found that double positive animals (14.6%) for MVV and BTV in sheep but not goat. As a conclusion sheep and goat were infected with naturally BTV, CAEV and MVV in Kirikkale region. This study showed that this the first report for BTV, MVV and CAEV infections from Kirikkale region. Therefore infections are needed to be further investigations to determine detailed survey studies.

Keywords: *Sheep, Goat, Bluetongue virus, Caprine arthritis-encephalitis virus, Maedi-visna virus*

Kırıkkale Yöresinde Bulunan Küçük Ruminantlarda Mavidil, Maedi-Visna ve Caprine Arthritis-Encephalitis Enfeksiyonlarının Serolojik ve Epidemiyolojik Araştırılması

Özet

Viral enfeksiyonlar, koyun ve keçilerde yapılan araştırmalar ile önlenebilen önemli sorunlara ve ekonomik kayıplara neden olur. Bu çalışmada mavidil virüsü (BTV), maedi-visna virüs (MVV), caprine arthritis-encephalitis virüs (CAEV)'lerini içeren viral enfeksiyonların, İç Anadolu bölgesinde yer alan Kırıkkale ilinde bulunan koyun ve keçilerde belirlenmesi amaçlanmıştır. Kan örnekleri 279 koyun ve 146 keçiden toplandı ve ELISA, RT-nested PZR ve nested PZR ile test edildi. Serum örneklerinde antikor cevabı BTV (%49.8), CAEV (%7.5) ve MVV (%19.4) seropozitiflik oranı ile tespit edildi. Seropozitif tam kan örneklerinde RT-nested PZR ve nested PZR ile, BTV ve MVV antijenleri sırasıyla (%2.87) (%9.25) oranlarında belirlendi fakat CAEV belirlenemedi. Koyunlarda hem MV hem de BT'ye karşı seropozitif hayvanların oranı %14.6 oranında saptandı. Sonuç olarak Kırıkkale yöresinde koyun ve keçilerde BTV, CAEV ve MVV enfeksiyonlarının doğal varlığının ortaya konulduğu bu çalışma, bu yörede sözü geçen enfeksiyonlarla ilgili yapılan ilk çalışmadır. Bu nedenle enfeksiyonların belirlenmesi için daha detaylı sürvey araştırmalarına ihtiyaç vardır.

Anahtar sözcükler: *Koyun, Keçi, Mavidil virüsü, Caprine arthritis-encephalitis virüs, Maedi-visna virüs*

INTRODUCTION

Bluetongue (BT) is an arthropod-transmitted disease and bluetongue virus (BTV), the etiological agent of bluetongue disease of wild and domestic ruminants which results in significant economics loses. Etiological agent is a non-enveloped double-stranded RNA virus belonging to genus orbivirus of the family Reoviridae [1]. Twenty-four

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serotypes of BTV have been recognized world wide. The disease can cause up to 100% morbidity and with 0-50% case fatality rates in sheep [2]. The vast majority of BT infections are clinically unapparent. The virus is known to be distributed around the world in countries located in the tropics and subtropics, where *Culicoides* species are present. Only around 50 of the 1.500 known *Culicoides* species have been shown to be capable of developing a fully disseminated transmissible BTV infection [3]. BTV was first described in the Cape Colony of southern Africa after merino sheep were introduced into the region in the late 18th century, and was subsequently recognized in other parts of Africa, Europe, the Middle East and Indian sub-continent, the Americas, and Asia [4]. Bluetongue disease has been almost found all region of Turkey since the first outbreak seen in Turkey was in Syria border 1944-1947 [5-7]. Survey studies are the important to epidemiology of BTV because it was hypothesized that Turkey could a possible the BT gateway through European countries [3,8]. The distribution of *Culicoides* biting midges is known to be influenced by weather and climate conditions such as temperature, humidity, and rainfall [9]. To date there is no evidence that any outbreak of BTV or any study BTV infection and scientific data about kind of *Culicoides spp* in the Kirikkale city where located near the Kizilirmak river that is the longest river in Turkey. Diagnosis of BTV disease is used many techniques such as virus isolation detection of viral RNA with PCR or RNA hybridization, antigen detection IFAT, ELISA, antibody detection ELISA [10,11]. Although there is a vaccine for preventing BTV infections, an animal vaccinated and protected from infection with one serotype of BTV is not protected from subsequent infections with other viral serotypes [12].

Small ruminant lentiviruses (SRLV) cause two different diseases that are called maedi-visna virus (MVV) in sheep and in goat's caprine arthritis-encephalitis virus (CAEV) are considered genetically as a single pathogen causing a multisystemic inflammatory disease [13,14]. MVV and CAEV are a single-stranded RNA virus of the genus lentivirus in the family Retroviridae [15]. SRLVs include cause persistent infections in target organs for instance the mammary gland, the carpal joints, the central nervous system and the lungs [16]. While MVV recognized in the Iceland in 1933, CAEV first recognized in the United Sates in 1974 [17]. Infection is distributed almost worldwide and is responsible for economic losses related to the drop of milk production, lameness and interstitial pneumonia [18,19]. In Turkey, the presence of a MVV infection was first revealed among sheep based on pathological observation conducted in 1975. Following first observation numerous studies have demonstrated that infection is widespread in Turkey [20-22]. In Turkey, even though CAEV was firstly reported in 1994 there have been documented a few case and sero-epidemiological studies about CAEV [23-27]. To date, there is no evidence that any outbreak of MVV or any CAEV infection and scientific data in the Kirikkale, Central Anatolia Turkey.

MATERIAL and METHODS

Study Area and Sample Collection

The present study was conducted on two hundred and seventy nine sheep and a hundred forty six goats during the period from April to December 2010 at the Kirikkale, Turkey. Samples were collected from all sheep and goat within herds under investigation at the same time of clinical examination. However animals used in this study did not show any apparent clinical signs of BTV, MVV and/or CAEV. Sera and whole blood samples were collected from sheep and goat in Kirikkale province. While whole blood samples were collected in EDTA coated tubes (Vacutest, K3 EDTA; Arzergrande, Italy), blood samples were collected into tubes (Vacutest, Arzergrande, Italy). Blood samples allowed to clot at room temperature and centrifuged at 1.200 rpm for 10 min on the same day and sera were collected and kept at -20°C until ELISA work.

Enzyme Linked Immunosorbent Assay (ELISA)

A commercial ELISA kit (Pourquier ELISA Maedi-Visna/CAEV serum Screening, France) was used for the detection of MVV antibody response according to the manufacturer's instructions. A commercial ELISA kit [Pourquier Bluetongue Competitive ELISA version: P00450/06 (Institude Pourquier, France)] was used for the detection of BTV antibody response according to the manufacturer's instructions. Samples were analyzed and calculated with an automated ELISA reader at 450 nm (SIRIO S® Elisa Reader, Indonesia).

PCR Technique

To detect MVV or CAEV antigen, firstly DNA was extracted from blood using a spin column system (DNAsy Blood and Tissue Kit, Qiagen, Germany) according to the manufacturer's instructions. To detect CAEV antigen, the first round PCR was run in reaction mixture contained 5 µl cDNA, 25 mM Tris-HCl pH 8.9, 50 mM KC1, 3 mM $MgCl_2$, 2 mM of dNTP mix, 10 pmol of each external primer 5'-CAAGCAGCAGGAGGGAGAAGCTG-3' and 5'-TCCTACCC CCATAATTTGATCCAC-3' and 3 U Taq DNA polymerase (MBI Fermentas) the expected PCR product size 296 bp. Ten microliters nested PCR reactions were performed in a reaction mixture with the same reagent composition using the internal primers 5'-GTTCCAGCA ACTGCAAACA GTAGCAATG-3' and 5'-ACCTTTCTGCTTCTTCATTTAATTT CCC-3' the expected PCR product size 196 bp [15]. To detect MVV antigen, the first round PCR was run in reaction mixture contained 5 µl cDNA, 25 mM Tris-HCl pH 8.9, 50 mM KCl, 2 mM of dNTP mix, 10 pmol of each external primer 5'-CAACARGGIGGIATMATAGAYTCIGG-3' and 5'-AR TGIGTRTARTCIACYTGCCA-3' and 3 U Taq DNA polymerase (MBI Fermentas) the expected PCR product size 412 bp. Ten microliters nested PCR reactions were performed in a reaction mixture with the same reagent

composition using the internal primers 5′-GGG ATMATA GAYTCGGGRTATCARGG-3′ and 5′-TGGGTRTARTCGACYTGC CARTG-3′ the expected PCR product size 404 bp [28]. MVV positive control was provided kindly by Dr. Dilek Muz that is worked in the Department of Virology Faculty of Veterinary Medicine University of Mustafa Kemal, Turkey. Reaction conditions for nested PCR were as follows; 95ºC for 2 min, followed by 32 cycles of 95ºC for 1 min, 60ºC for 1 min, 72ºC for 1 min and finally a cycle of 72ºC for 5 min. To detect BTV antigen, firstly RNA was extracted from blood using a spin column system (QIAamp Viral RNA mini kit, Qiagen, Germany) according to the manufacturer's instructions. Random primer (1.25 mM random primer; Promega, Madison, WI, USA) was used in the first step of cDNA synthesis. For this purpose, 10 µl viral RNA was combined with 1 µl (0.5 µg) random primer and preheated at 70°C for 10 min to denature secondary structures. The mixture was cooled rapidly, and 2 µl (100 mM) dNTPs, 5 µl 5X RT buffer, 1 µl M-MLV RT (200 IU/µl Promega, USA), 1 µl RNasin (MBI Fermentas) and 5 µl H_2O were added reaching to the total volume of 25 µl. The RT mix was incubated at 37°C for 60 min and stopped by heating at 95°C for 10 min. The yield of cDNA was checked by PCR signal generated from the internal standard housekeeping gene, *Ovies aries* glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (GenBank accession number: DQ386891). The GAPDH forward primer was 5′- AGCTCGTCATCAATGGAAAGGC-3′, and the GAPDH reverse primer was 5′-ATGGCGTGGACAGTGGTCATAA-3′. The cDNA stock was stored at -20°C until study. The first round PCR was run in reaction mixture contained 5 µl cDNA, 25 mM Tris-HCl pH 8.9, 50 mM KC1, 2 mM of dNTP mix, 10 pmol of each external primer 5′-GTTC TCTAGTTGGCAACCACC-3′ and 5′-AAGCCAGACTGTTTCCC GAT-3′ and 3 U Taq DNA polymerase (MBI Fermentas) the expected PCR product size 274 bp. 10 µl nested PCR reactions were performed in a reaction mixture with the same reagent composition using the internal primers 5′-GCAGCATTTTGAGAGAGCGA-3′ and 5′-CCCGA TCATACATTGCTTCCT-3′ the expected PCR product size 101 bp [29]. Reaction conditions nested PCR reactions were as follows; 95ºC for 2 min, followed by 32 cycles of 95ºC for 40 s, 55ºC for 1 min, 72ºC for 40 s and finally a cycle 50ºC 3 min and of 72ºC for 5 min. BTV vaccine strain was using as positive control provided kindly by Prof. Dr. Aykut Ozkul that is worked in the Department of Virology Faculty of Veterinary Medicine University of Ankara, Turkey. All nested-PCR reactions were carried out in BO-PCR-5 thermal cycler (Hamburg, Germany). The InGenius LHR (Syngene, Cambridge, UK) was used to see PCR amplicons by using ethidium bromide staining after 1.5% agarose gel electrophoresis.

RESULTS

It was collected samples from 425 sheep and goat in the Kirikkale city, Central Anatolia of Turkey. It was found that rate of antibody response to BTV were in sheep 139 out of 279 (49.8%) seropositivity value. It was found that rate of antibody response to CAEV were 11 out of 146 (7.5%) seropositivity values in the goat. It was determined that rate of antibody response to MVV were 54 out of 279 (19.4%) seropositivity values in the sheep *(Table 1)*. Double seropositive animals 41 out of 279 (14.7%) for MVV and BTV were determined in sheep but not in goat. To determine BTV antigen, 139 whole blood samples (antibody positive for BTV) submitted for BTV diagnosis were tested by RT-nested PCR. Firstly, we checked whether RT-PCR assay was working or not. Therefore *Ovies aries* GAPDH gene was targeted as an internal control. All samples were shown GAPDH signal *(Fig. 1A)*. After confirming RT reaction by using GAPDH primers, first round of nested-PCR was carried out by using external primers which could be amplificated at the NS1 of the BTV genome and are considered to amplify all BTV genome. PCR amplicon of NS1 of the BTV genome was not demonstrable in first round nested PCR including positive control for PCR *(Fig. 1B)*. When second round of PCR was carried out internal primers for BTV, 4 out of 139 (2.9%) whole blood samples were detected as a positive (*Table 2* and *Fig. 1C*). Detection of CAEV was carried out eleven samples that are seropositive for CAEV nested PCR. However PCR signal were not detected for CAEV in goat by using CAEV specific primers *(Table 2)*. Detection of MVV was carried out fifty four samples that are MVV positive antibody response nested PCR. PCR amplicon for MVV was not demonstrable

Table 1. *Prevalence of BTV, MVV and CAEV in sheep and goat from Kirikkale city in Turkey by using ELISA*

Tablo 1. *Kırıkkale'de koyun ve keçilerde ELISA ile BTV, MVV ve CAEV prevalansı*

Number of Animals Used in This Study	MVV-CAEV	BTV
Number of Goat	146	-*
Number of Sheep	279	279
Number of Seropositives Animals	65	139
Number of Seropositives Goat	11	-
Number of Seropositives Sheep	54	139
Number of Total Animals	425	279

** Goat did not tested for BTV (Keçiler BTV için test edilmedi)*

Table 2. *Number of RT- nested PCR or nested PCR positive samples from whole blood that were coincide with seropositive samples for BTV, CAEV, MVV*

Tablo 2. *BTV, CAEV, MVV seropozitif tam kan örneklerinden, RT-PCR-nested PZR veya nested PZR pozitif örnek sayıları*

PCR Results	CAEV	MVV	BTV
Positive Goat	-	-	-*
Positive Sheep	-	5	4
Number of Animals Used in for PCR	11	54	139

** Goat did not tested for BTV (Keçiler BTV için test edilmedi)*

in first round nested PCR including positive control for PCR. When second round of PCR was carried out internal primers for MVV, 5 of 54 (9.3%) *(Table 2)* samples were shown expected PCR product size *(Fig. 1D)*.

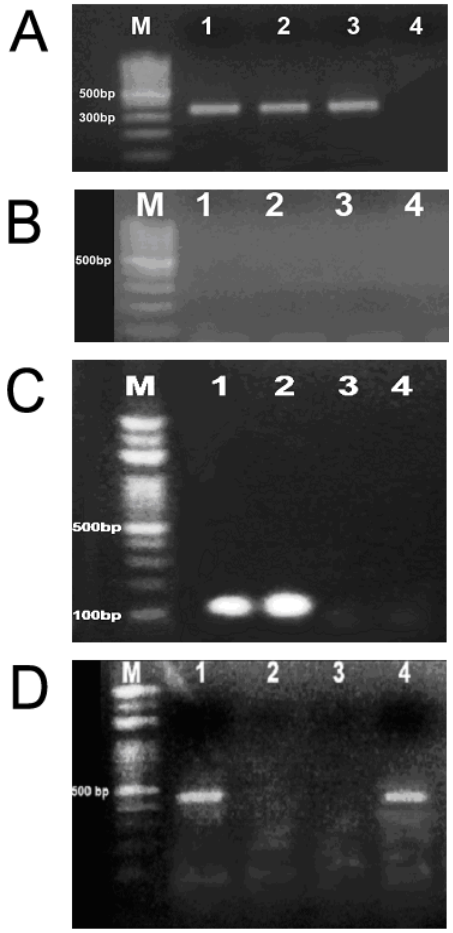

Fig 1. RT-nested PCR with RNA extracted from clinical samples for BTV and nested-PCR with DNA extracted from clinical samples for MVV (whole blood). **A.** RT-PCR control, RT-PCR was carried out *Ovies aries* GAPDH primers. Whole blood samples lane 1 (positive control for BTV), lane 2 (positive clinical sample), lane 3 (negative clinical sample) and lane 4 (dH_2O), Lanes M: molecular length marker. **B.** First round RT-nested PCR for BTV was carried out BTV external primers. Whole blood samples lane 1 (positive control for BTV), lane 2 (positive clinical sample), lane 3 (negative clinical sample) and lane 4 (dH_2O), Lanes M: molecular length marker. **C.** Second round RT-nested PCR for BTV was carried out BTV internal primers. Whole blood samples lane 1 (positive control for BTV), lane 2 (positive clinical sample), lane 3 (negative clinical sample) and lane 4 (dH_2O), Lane M: molecular length marker. **D.** Second round nested PCR for MVV was carried out MVV internal primers. Sample lane 1 (positive sample), lane 2 (negative clinical sample), lane 3 (dH_2O) and lane 4 (positive control for MVV), Lane M: molecular length marker

Şekil 1. Klinik örneklerden elde edilen RNA'lar ile BTV için RT-nested PZR (tam kan) ve klinik örneklerden elde DNA ile MVV için nested-PZR. **A.** RT-PZR kontrol, RT-PCR *Ovies aries* GAPDH primer ile kuruldu . Tam kan örnekleri hat 1 (BTV için pozitif kontrol), hat 2 (pozitif klinik örnekler), hat 3 (negatif klinik örnekler) ve hat 4 (dH_2O), hat M: moleküler ağırlık markeri. **B.** BTV için ilk round nested RT-PZR, BTV dış primerleri ile kuruldu. Tam kan örnekleri hat 1 (BTV için pozitif kontrol), hat 2 (pozitif klinik örnekler), hat 3 (negatif klinik örnekler) ve hat 4 (dH_2O), hat M: moleküler ağırlık markeri. **C.** BTV için ikinci round nested RT-PZR, BTV iç primerleri ile kuruldu. Tam kan örnekleri hat 1 (BTV için pozitif kontrol), hat 2 (pozitif klinik örnekler), hat 3 (negatif klinik örnekler) ve hat 4 (dH_2O), hat M: moleküler ağırlık markeri. **D.** MVV için ikinci round nested PZR MVV iç primerleri ile kuruldu. Hat 1 (pozitif klinik örnek), hat 2 (negatif klinik örnek), hat 3 (dH_2O) ve hat 4 (MVV için pozitif kontrol), hat M: moleküler ağırlık markeri

DISCUSSION

Many disease problems can be solved by an investigation of animal populations rather than the individual. The measurement of the amount of infectious and non-infectious diseases in a population assists in determining their importance and efficacy of control campaign [30]. This study was carried out to determine the BTV, MVV and CAEV infections in Kirikkale city of Turkey.

Currently, most vaccines used are modified live vaccines, but a restricted number of inactivated vaccines are also available and new types of vaccines are being developed. The first epizootic of BT outside Africa was confirmed in sheep on Cyprus in 1943 and a vaccine was successfully used to control outbreaks of BT on Cyprus in 1946 and 1947. At the same time, BT was reported in Israel, Turkey and probably Syria [31]. Prior to 1998, BTV only occurred sporadically in Europe and following 1998 is known as the invasion of the Western and Eastern Mediterranean basin by *Culicoides imicola*, a tropical midge. In 2008, one further step was reached in the invasion of the European continent [32]. It was reported that 14.5% of sampled animals have BT virus specific antibodies in Eastern and South-eastern regions of Turkey [25]. It was announced that BTV seroprevalence was 4.68% in sheep in Konya region of Turkey [33]. Although BTV virus has different 24 serotypes, virus has already been determined on the worldwide with only type 4, 9, 16 recognized within Turkey [2]. Another study showed that BTV seroprevalence was found 91.76% and the specific frequencies were 72.16%, 42.05% and 36.93% for serotypes 4, 9 and 16 in 352 cattle in north-eastern Anatolian cities [34]. It was showed that the seroprevalance of bluetongue was determined as 3% (6/200) in sheep, 11% (22/200) in cattle [35]. The present study was carried out in Kirikkale city that is situated in central the Anatolia region of Turkey in Asia. It lays 39º 52′ 53″ north of the equator and 33º 26′ 46″ meridians east of Greenwich. Kirikkale has a coastline with the majority lying along the Kizilirmak River. The northern part of the region is hilly and in many places at altitude ranges between 700 and 1450 meter. Extreme recorded temperatures range from -10°C to 35°C at Kirikkale. Additionally there is no vaccination for BTV in this city in sheep or goat. In present study, 139 out of 279 (49.8%) sheep have shown BTV specific antibodies *(Table 1)*. Unfortunately goat samples did not incorporate in the study in order to limited fund. In this study was found that PCR positive for BTV samples was 4 out of 139 (2.9%) whole blood *(Fig 1C* and *Table 2)*. It suggested that four sheep is viremic in the sampling time. It was suggested that vaccination of sheep or goat will help to take under control of BTV and *Culicoides spp* will be combated according this results. This is first report BTV infection for Kirikkale. BTV was found by South African, French, American, and Icelandic researchers. Extensive research into the pathology, etiology, and epidemiology of this slowly progressive and ultimately fatal disease was

confirmed in several countries [31,36]. BTV infection is need to further investigations to determine detailed sero-epidemiological studies in goat and cattle and the circulated serotypes to found vaccine which should be contain serotype(s).

CAEV and MVV are considered to be genetically distinct but antigenically related pathogens of goats and sheep. SRLV infections seen on farms develop after long incubation and a slow progression of disease to death but in nature they may also have short latency and cause acute leukoencephalitis and/or acute arthritis and pneumonia in young kids or lambs with exceptionally high mortality. SRLV is persistent infection a feature that share with immunodeficiency-causing lentiviruses such as MVV, CAEV [37]. Animals can not be effectively vaccinated against SRLVs and MVV is prevalent in sheep populations worldwide. For this reason seropositive sheep or goat represent a marker of wild type infection. To date, MVV seroprevalence data obtained in Turkey have been found such as 23.9%, from 3.8% to 41.2%, and 1.2%. Recent study showed 15.3% prevalence observed in the Istanbul city [20-22]. Additionally studies with regard to MVV in Turkey that seropositivity was determined 26.7%, 23.9% and 10% respectively [21,38-40]. It was announced that MVV sero-prevalence during the study period ranged from 24% to 39% in Latxa sheep and from 77% to 80% in Assaf sheep [41]. In the present study it was found that rate of antibody response to MVV were 54 out of 274 (19.4%) seropositivity values in the sheep *(Table 1)*. PCR finding for MVV showed 5 out of 54 (2.9%) animals were detected as a positive (*Fig 1D* and *Table 2*). CAEV infects mostly goats and has a global distribution in the world. Serological data was shown in Australia, USA and, UK prevalence rates of 82%, 73%, 4.3%, respectively [24]. Perspective of in the neighbor of Turkey although there is no data with associated in the Iran, Iraq, Syria, Azerbaijan, Armenia, Bulgaria and Rumania, CAEV was just reported in Greece [42]. To date it was suggested that there is no study in CAEV free countries of neighbor of Turkey. The serologic examination was carried out by AGID technique in 16 out of 808 (1.9%) for CAEV in Turkey [26]. Even though one reported that all of the sampled goats were negative for CAEV antibodies eastern and south-eastern Anatolia, another researchers was reported 7 out of 675 goats (1.03%) sampled were positive for CAEV antibodies with cELISA, but no sero-positivity was detected with AGID in the same region [24,25]. Researchers were carried out ELISA for detecting antibody against to CAEV both adult goat (n=75) and their kids (n=70). While adult goat did not show any clinical sign for CAEV and were serological positive for CAEV, their kids showing arthritis and encephalitis were confirmed serological positive for CAEV using ELISA in Nevsehir city that was very close to Kirikkale [23]. In the present study it was found that rate of antibody response to CAEV were 11 out of 146 (7.5%) in the goat *(Table 1)*. However serological finding did not supported by PCR. For diagnosing the infection, specific antibody detection methods as AGID, ELISA are most frequently used. Molecular biological techniques can be hampered by the low viral load in the blood and the pronounced heterogeneity of the viral genomes [43]. In the present study it was suggested that virus could not be detected for low viral load and maybe real time PCR could be solve this problem. As a conclusion sheep and goat were infected with naturally BTV, CAEV and MVV in the Kirikkale region. Additionally some sheep were co infected with BTV and MVV. But there is no vaccination or prevent to these infection. This study showed that this is first report BTV, MVV and CAEV infection in the Kirikkale and these infections are still available in the Central Anatolia. Therefore infections are needed to be further investigations to determine detailed survey studies.

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RESEARCH ARTICLE

Orta Karadeniz Bölgesinde Ruminant ve Tek Tırnaklılarda Kan Emici Sineklerle Nakledilen Bazı Arboviral Enfeksiyonların Seroprevalansı

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Özet

Bu çalışmada, Karadeniz Bölgesinde yer alan 5 ilde (Samsun, Sinop, Ordu, Amasya ve Tokat) bulunan 200 sığır, 200 koyun ve 223 tek tırnaklıdan (114 at, 67 eşek, 42 katır) alınan kan örnekleri kullanıldı. Kan serumu örneklerinde mavidil antikorlarını tespit etmek için competitive ELISA (cELISA), akabane ve üç gün hastalığı antikorlarını belirlemek için blocking ELISA ve equine infeksiyöz anemi antikorlarının tespiti için ise agar jel immunodiffuzyon testi (AGID) kullanıldı. Elde edilen verilere göre mavidil enfeksiyonunun seroprevalansı koyunlarda %3, sığırlarda %11; akabane enfeksiyonunun seroprevalansı koyunlarda %0.5, sığırlarda %22; üç gün hastalığı seroprevalansı ise sığırlarda %13.5 olarak belirlendi. Tek tırnaklılarda equine infeksiyöz anemi virusuna karşı antikor tespit edilemedi.

Anahtar sözcükler: *Akabane, equine infeksiyöz anemi, Karadeniz bölgesi, Mavidil, Seroprevalans, Üç gün hastalığı*

Seroprevalence of Some Arboviral Infections Transported Blood Sucking Insects in Ruminants and Equids in Middle Blacksea Region in Turkey

Summary

In this study, serum samples collected from 200 cattle, 200 sheep and 223 equids (114 horse, 67 donkey, 42 mule) in five province include (Samsun, Sinop, Ordu, Amasya and Tokat) in Blacksea Region. The seroprevalances were detected for bluetongue by competitive ELISA (cELISA), for akabane and bovine ephemeral fever by blocking ELISA, and for equine infectious anemia by agar gel immunodiffusion test (AGID). According to obtained data, the seroprevalance of bluetongue was recorded as 3% (6/200) in sheep, 11% (22/200) in cattle, the seroprevalance of akabane was recorded as 0.5% (1/200) in sheep, 22% (44/200) in cattle, the seroprevalence of bovine ephemeral fever infection was found as 13.5% (27/200) in cattle. No antibody against EIAV was detected in equids.

Keywords: *Akabane, Equine infectious anemia, Blacksea region, Buetongue, Seroprevalence, Bovine ephemeral fever*

GİRİŞ

Vektörler vasıtasıyla evcil hayvanları enfekte eden viruslar, çeşitli formlarda enfeksiyon tablosu oluşturarak önemli ekonomik kayıplara neden olmaktadır. Ülkemizde mavidil (BT, bluetongue), akabane (AKA), üç gün hastalığı (BEF, bovine ephemeral fever) ve equine infeksiyöz anemi (EIA) vektörlerle nakledilen önemli viral enfeksiyonlardandır.

Mavidil virusu *Reoviridae* familyasında, *Orbivirus* genusu içinde yer alır. Etkenin bilinen 24 serotipi vardır [1,2]. Koyun, keçi, sığır, manda, geyik, antilop, ceylan, deve gibi birçok evcil ve yabani ruminant etkenin konakçı spektrumundadır. Sığırlar, enfeksiyonun en önemli rezervuarı olup, genellikle klinik belirti göstermezler. Koyun, keçi ve geyik gibi bazı yabani ruminantlarda enfeksiyon, ateş, depresyon, salivasyon artışı, mukoprulent burun akıntısı, yüz ödemi, ağız mukozasında hiperemi ve ülser, koronitis, kas güçsüzlüğü gibi klinik belirtileri takiben ölümle sonuçlanır [3,4]. Enfeksiyonun epidemiyolojisinde rezervuar, vektör ve iklim en önemli faktörlerdir [5].

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Mavidil virusuna vektörlük yapan sineklerin *(Culicoides spp.)* şimdiye kadar 1210 türü belirlenmiş olup, bunlardan yalnız 15 tanesi mavidil virusunun bulaşmasında rol oynamaktadır. Mavidil virusunun transovarial nakli söz konusu değildir [6]. Bugün için mavidil enfeksiyonu genellikle tropik ve subtropik bölgelerde görülse de, 50. kuzey paralel ile 35. güney paralel arasındaki tüm coğrafyada enfeksiyonun varlığı bilinmektedir [7,8].

Akabane virusu (AKAV) *Bunyaviridae* familyasında, *Orthobunyavirus* genusu içinde yer alır [9]. Enfeksiyon koyun, keçi ve sığırlarda arthrogriposis-hydranencephaly (AH), abort, fötal mumifikasyon, erken doğum ve ölü yavru doğumu gibi konjenital anomalilere neden olur. Enfeksiyon sığırlarda subklinik seyreder [10-12]. Afrika, Asya ve Avustralya'da enfeksiyonun varlığı bilinmektedir [13-17].

Üç gün hastalığı virusu (BEFV), *Rhabdoviridae* familyası içinde *Ephemerovirus* genusu içinde yer alır [18]. Sığır ve mandalarda ani beden ısısı artışı, depresyon, topallık gibi belirtilerle başlayıp, kısa bir süre içinde iyileşme olduğundan dolayı üç gün hastalığı olarak adlandırılmaktadır [19]. Bugün için enfeksiyonun varlığı, Avustralya, Japonya, Afrika, Asya ve Ortadoğu'da bilinmektedir [13,14,16,20,21].

Akabane virusu ile üç gün hastalığı virusunun bulaşmasında *Aedes, Culex, Anopheles* ve *Culicoides* genusu içindeki bazı sinek türleri rol almaktadır [11,19,22].

Equine infeksiyöz anemi virus (EIAV), *Retroviridae* familyasında, *Lentivirus* genusu içinde yer alır [23]. Enfeksiyon sadece tek tırnaklılarda görülmekte olup, trombositopeni, anemi, aşırı kilo kaybı, bedenin alt kısımlarında ödem, değişken bir ateş ile karakterize bir klinik tablo oluşturur [24]. Enfeksiyona yakalanıp iyileşen tek tırnaklılar virusu ömür boyu taşırlar [25]. Enfeksiyon dünyada yaygın olarak görülmektedir [24].

Equine infeksiyöz anemi virusunun bulaşmasından, *Tabanidae* familyasında, *Stomoxys, Chrysops, Tabanus, Hybomitra* genusunda yer alan bazı sinekler ile iatrojenik faktörler sorumludur [22,24].

Bu çalışmada, Karadeniz Bölgesinde ruminant ve tek tırnaklılarda sokucu sineklerle nakledilen bazı viral hastalıkların seroprevalansının ortaya konulması amaçlanmıştır.

MATERYAL ve METOT

Serum örnekleri

Çalışmada, 2007 yılında Karadeniz Bölgesinde (Samsun, Sinop, Ordu, Amasya, Tokat) halk elinde yetiştirilen 200 adet sığır, 200 adet koyun ve 223 adet tek tırnaklıdan (114 at, 67 eşek, 42 katır) kan serumları örneklendi *(Tablo 1, Şekil 1)*. Sığır ve koyun kan serumları her ilçedeki aynı yerleşim birimlerinden eşit sayıda olmak üzere 10'ar adet temin edildi. Serum örnekleri 56°C'de 30 dakika süreyle inaktive edildikten sonra kullanılıncaya kadar -20°C'de saklandı.

Tablo 1. *Serum örneği alınan iller ve ilçeleri*
Table 1. *Provinces and their towns in which serum samples were obtained*

Samsun	Sinop	Ordu	Amasya	Tokat
Ayvacık	Merkez	Korgan	Göynücek	Turhal
Asarcık	Boyabat	Mesudiye	Merkez	Merkez
Tekkeköy	Durağan	Perşembe	Suluova	Almus
Terme	Gerze	Gülyalı	Merzifon	Reşadiye

Şekil 1. Serolojik tarama için örneklemenin yapıldığı yerler
Fig 1. Locations of flocks and herds sampled for serosurvey

Enzyme Linked Immunosorbent Assay (ELISA)

Örneklenen sığır ve koyun kan serumları mavidil antikorları yönünden cELISA, AKAV ve BEFV antikorları yönünden, blocking ELISA ile incelendi. Bu amaçla ticari olarak temin edilen cELISA (VMRD Inc.,Pulman, WA, USA) ve blocking ELISA (EMAI, Camden, NSW, Australia) kitlerinden yararlanıldı. Testler üretici firmaların prosedürüne uygun olarak yapıldı. Test pleytleri, 450 nm filtre absorbans değerleri okunarak, sonuçlar hesaplandı.

Agar Jel Immunodiffüzyon Testi (AGID)

Örneklenen tek tırnaklı kan serumları EIAV antikorları yönünden ticari olarak temin edilen AGID test kiti (IDEXX, Westbrook, USA) ile kontrol edildi. Test üretici firmanın prosedürüne göre yapıldı.

BULGULAR

Kan örneklerinde mavidil virusuna karşı seropozitiflik oranı sığırlarda %11, koyunlarda %3 olarak tespit edildi. Materyal sağlanan iller için seropozitif oranları sığırlarda

%2.5-25 olarak belirlenirken, sadece iki il için koyunlarda seropozitiflik (%5 ve %10 oranlarında) saptandı. AKAV enfeksiyonu için seropoziflik oranları sığırlarda %22, koyunlarda %0.5 olarak bulundu. Materyal sağlanan illerden Ordu, Samsun ve Sinop'ta sığırlarda AKAV'a karşı seropozitiflik oranları sırasıyla %62.5, %40 ve %7.5 olarak belirlendi. Örneklenen koyunlarda ise AKAV'a karşı sadece Tokat (%2.5) ilinde seropozitiflik tespit edildi. BEFV enfeksiyonu yönünden seropozitiflik oranı örneklenen populasyon için %13.5 olarak bulundu. İllere göre BEFV'a karşı seropozitiflik oranları Sinop, Amasya ve Samsun'da sırasıyla %37.5, %27.5, %2.5 olarak belirlendi *(Tablo 2)*. Tek tırnaklılardan sağlanan kan örneklerinin tümü EIAV'e karşı antikorlar yönünden negatif olarak belirlendi.

Tablo 2. *BTV, AKAV ve BEFV seropozitif hayvanların sayı ve oranları*
Table 2. *The number and ratio of BTV, AKAV and BEFV seropositive animals*

İl	Adet *	Sığır Serumu Ab (%)			Koyun Serumu Ab (%)	
		BTV	AKAV	BEFV	BTV	AKAV
Samsun	40	8 (20)	16 (40)	1 (2.5)	-	-
Sinop	40	1 (2.5)	3 (7.5)	15 (37.5)	2 (5)	-
Ordu	40	10 (25)	25 (62.5)	-	-	-
Amasya	40	1 (2.5)	-	11 (27.5)	4 (10)	-
Tokat	40	2 (5)	-	-	-	1 (2.5)
Toplam	**200**	**22 (11)**	**44 (22)**	**27 (13.5)**	**6 (3)**	**1 (0.5)**

** Her iki tür için test edilen serum sayılarıdır*

TARTIŞMA ve SONUÇ

Bu çalışmada, Karadeniz Bölgesindeki 5 ilde (Samsun, Sinop, Ordu, Amasya ve Tokat) sığır, koyun ve tek tırnaklı (at, eşek, katır) hayvanlarda kan emici sineklerle bulaşan bazı viral hastalıkların seroepidemiyolojisi araştırıldı. Elde edilen veriler, bölgede farklı türlere ilişkin kan emici sineklerle bulaşan viral hastalıklar yönünden yapılan ilk bildirimdir.

Türkiye'de ilk defa 1944 yılında tespit edilen mavidil enfeksiyonuyla ilgili, Doğu ve Güneydoğu Anadolu [13,20,26-28], Akdeniz ve İç Anadolu [29-32], Marmara ve Ege Bölgesi'nde [16,33,34] koyun ve sığırlarda pek çok çalışma yapılmıştır. Enfeksiyonun seroprevalansı koyunlarda %1-36.04, sığırlarda %2.3-88 arasında tespit edilmiştir. Bu çalışmada ise Karadeniz bölgesinde incelenen 5 ilde bulunan 20 ilçede yetiştirilen sığırlarda %11, koyunlarda ise %3 oranında seropozitiflik saptandı. Bu çalışmanın sonuçları, ülkemizde yapılmış diğer çalışmalarla kıyaslandığında, mavidil virus enfeksiyonun Karadeniz bölgesi'nde daha düşük seroprevalansa sahip olduğu sonucuna varıldı. Bölgede en yüksek mavidil seropozitifliğinin tespit edildiği Ordu (%25) ve Samsun (%20) illerinde, özellikle baraj gölü bulunan sulak alanlar açısından zengin ilçeler (Samsun'da Tekkeköy ve Ayvacık, Ordu'da Gülyalı ve Perşembe ilçeleri) için pozitif hayvan sayısının %50'ye kadar ulaştığı belirlendi. Bu yüksek seropozitiflik oranının, enfeksiyonu taşıyan vektörün bu alanlardaki yaygınlığının bir göstergesi olabileceği kabul edildi. Bölgede karasal iklimin hüküm sürdüğü illerde (Amasya, Tokat) seropozitiflik oranlarının düşük olmasının da (%2.5-10), enfeksiyonu taşıyan vektörler ve iklim koşulları ilişkisi çerçevesinde değerlendirilmesi gerektiği düşünüldü.

Sığırların enfeksiyon için rezervuar olması, bölgedeki küçük ruminantlar için potansiyel bir tehlike oluşturmaktadır. Türkiye'de mavidil enfeksiyonu seroprevalansının koyun ve sığırlarda beraber araştırıldığı çalışmaların [13,27,30,31,33] bazılarında koyunlarda, bazılarında ise sığırlarda daha yüksek seropozitiflik tespit edilmiştir. Bu çalışmada ise, seroprevalans koyunlara oranla sığırlarda daha yüksek bulunmuştur. Çalışmalarda seroprevalansın sığır ve koyunlarda farklı oranlarda tespit edilmesi, örneklenen hayvan sayısına, ırk duyarlılığına, yaşa, örnekleme dönemi gibi faktörlere bağlı olduğu düşünülebilir.

Türkiye'de sığır ve koyunlarda akabane virus enfeksiyonuyla ilgili yapılan çalışmalarda [13,16,20], enfeksiyonun koyunlarda %8.1, sığırlarda %0.14-27.98 oranları arasında olduğu bildirilmiştir. Bu çalışmada ise akabane enfeksiyonunun seroprevalansı koyunlarda %0.5, sığırlarda %22 olarak saptandı. Yapılan çalışmalarla karşılaştırıldığında, özellikle Ordu (%62.5) ve Samsun (%40) illerinde sığırlarda yüksek oranda seropozitiflik tespit edildiği belirlendi. Sığırlarda seropozitifliğin sahilde yer alan illerde görülmesi ve özellikle bu illerin sahilde yer alan ilçelerinde %90'a varan bir seropozitiflik tespit edilmesi, vektörlerin nemli, sulak ve rakımı düşük yerlerde daha yoğun ve akabane virus yönünden oldukça yüksek oranda taşıyıcı olduğunu ortaya koymaktadır. Bu verilerden hareketle, son yıllarda sıklıkla Ordu ve Samsun illerinde klinik olarak ihbarı yapılan amorosis (kör buzağı) olgularının etiyolojisinde rol oynayabilecek etkenler içinde akabane virusunun da değerlendirilmesi gerektiği düşünülmektedir.

Ülkemizde yapılan çalışmalarda [13,16] üç gün hastalığı enfeksiyonunun seroprevalansı sığırlarda %8.0-9.2 arasında olduğu saptanmıştır. Bu çalışmada, enfeksiyonun seroprevalansı %13.5 olarak bulundu. Yapılan çalışmalarla kıyaslandığında, özellikle Sinop (%37.5) ve Amasya (%27.5) illerinde enfeksiyonun yüksek oranda bulunduğu görülmektedir. Enfeksiyonun hem nemli ve ılıman hem de karasal iklimin hüküm sürdüğü illerde görülmesi, buna karşın benzer konumdaki diğer illerde (Tokat, Ordu) hiç belirlenmemesi, vektörün her iki iklim kuşağında bulunmasına karşın populasyon dinamiğinde mikroklimanın etkili olduğu düşünülmektedir.

Türkiye'de EIAV enfeksiyonunun seroprevalansına ait daha önceki çalışmalarda [35-38] olduğu gibi bu çalışmada da seropozitiflik ortaya konamadı.

Sonuç olarak bölgede kan emici sineklerle bulaşan enfeksiyonların, serolojik veriler yanında virolojik olarak da araştırılması gerekmektedir. Sığırlarda mavidil ve akabane seroprevalansının Ordu ve Samsun illerinde diğer illere göre daha yüksek olması ve aynı hayvanlarda hem mavidil hem akabane antikoru tespit edilmesi bulaşmada rol oynayan vektörün her iki virusu da taşıyor olabileceği ihtimalini akla getirmektedir. Bölgede sinek populasyonlarında etken izolasyon çalışmalarının yapılmasının her iki enfeksiyonla mücadele için de faydalı olabileceği gibi bölge için enfeksiyonu taşıyan vektör türünün tespiti de gerçekleştirilmiş olacaktır.

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