



Molecular investigation of most important viruses causing abortion in small ruminants: the importance of akabane virus

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ABSTRACT

Objective. This study was aimed to investigate the presence of pestivirus, schmallerberg virus (SBV), akabane virus (AKAV), and determined the seasonal distribution in small ruminant abortion cases **Materials and methods.** A total of 164 small ruminant aborted fetuses (39 goats and 125 sheep) were investigated in the West Turkey between the years of 2015 and 2019. While the SBV and pestivirus were examined with real-time RT-PCR, the AKAV was examined with nested-PCR. **Results.** In this study, while four sheep (2.3%) were found to be pestivirus positive, nine sheep and one goat (5.9%) were found to be AKAV positive. SBV was detected in none of the animals. According to the results of the studies carried out, AKAV, a vector-borne virus, was detected more than other viruses in sheep and goat abortions in West Turkey. A positive sample of akabane virus detected in sheep fetuses was sequenced based on the partial S segment. This sample showed 99-100% similarity to the Adana-15, Aksu-1, and Aksu-2 isolates previously detected in Southern Turkey. Phylogenetic analysis showed that the sequenced isolate in this study was clustered within genogroup Ib. Another important piece of data revealed in this study was the determination of the seasonal distribution of sheep and goat abortions. Abortion cases which started to increase in November reached a peak in February and declined till August. **Conclusions.** This is the first study in the Aegean Region (including seven provinces) in which three important viral agents were simultaneously investigated in small ruminant aborted fetuses.

Keywords: Abortion; akabane virus; arboviral diseases; pestivirus; schmallerberg virus (*Source: CAB Thesaurus*).

RESUMEN

Objetivo. Este estudio pretende investigar la presencia de pestivirus, virus de Schmallenberg (SBV), virus akabane (AKAV) y determinó la distribución estacional en abortos de pequeños rumiantes. **Materiales y métodos.** Se investigó un total de 164 fetos abortados de pequeños rumiantes (39

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cabras y 125 ovejas) en el oeste de Turquía entre 2015 y 2019. El SBV y el pestivirus se examinaron con RT-PCR en tiempo real y el AKAV se examinó con PCR anidada. **Resultados.** En este estudio, cuatro ovejas (2.3%) resultaron positivas para pestivirus y nueve ovejas y una cabra (5.9%) resultaron positivas para AKAV. No se detectó SBV en ningún animal. Como resultado, el AKAV, un virus transmitido por vectores, se detectó más que otros virus en abortos de ovejas y cabras en el oeste de Turquía. Se secuenció una muestra positiva de virus akabane detectada en fetos de ovejas basándose en el segmento S parcial. Esta muestra encontró una similitud del 99-100% con los aislados de Adana-15, Aksu-1 y Aksu-2 detectados anteriormente en el sur de Turquía. El análisis filogenético mostró que el aislado secuenciado en este estudio estaba agrupado dentro del genogrupo Ib. Además, se determinó la distribución estacional de los abortos de ovejas y cabras. Los casos de aborto que empezaron a aumentar en noviembre alcanzaron un pico en febrero y disminuyeron hasta agosto. **Conclusiones.** Este es el primer estudio en la región del Egeo (incluidas siete provincias) en el que se investigaron simultáneamente tres importantes agentes virales en pequeños fetos abortados de rumiantes.

Palabras clave: Aborto; enfermedad transmitida por vectores; pestivirus; virus akabane; virus de Schmallenberg (*Fuente: CAB Thesaurus*).

INTRODUCTION

Abortions are one of the most important problems which influence husbandry by causing significant economic loss (1). The most important causes of abortion are infectious agents such as parasites, bacteria and viruses. Viruses are one of the most prominent causes of abortion in small ruminants (2,3,4,5).

AKAV and SBV, which are a vector-borne diseases, belong to the genus *Orthobunyavirus*, from *Peribunyaviridae* family. They are closely associated with tinaroo, aino, shamonda and peaton viruses. AKAV and SBV are an enveloped, three segmented (S, M, L) and negative-polarity RNA viruses (6,7). AKAV causes reproductive disorders (abortion, premature births and stillbirths), congenital anomalies such as arthrogyrosis-hydranencephaly which are also called AH syndrome (7,8). SBV leads to fever, decrease in milk yield in cattle and stillbirth and abnormalities in sheep and goats (9). AKAV and SBV are transmitted by *Culicoides* biting mite (10,11). Pestivirus is a single stranded, enveloped, negative-polarity RNA virus and it can cause infection in wild animals such as chamois, deer and antelope in addition to domestic animals such as cattle, sheep, pig and camel. Pestivirus infections are seen in a great number of countries in the world and they cause significant economic loss due to abortions they cause (12).

The Aegean Region is one of the seven geographical regions in Turkey and it is in the West of Turkey. There is a large number of husbandry trade and many wetlands and agriculture areas in this region. Human travel and mobility have also roles in the spread of diseases in afore-mentioned area. In addition,

the incidence of arboviral diseases (such as AKAV) is high due to warm characteristics of the Aegean Region. For this reason, it has become important to investigate diseases that cause abortion in animals.

The aim of the present study is to investigate the presence of pestivirus, SBV, AKAV, and determined the seasonal distribution in small ruminant abortion cases which occurred in the Aegean Region between the years 2015-2019.

MATERIALS AND METHODS

Sampling. The study was conducted on abortions of sheep and goats collected for routine diagnosis from the Aegean region in western Turkey between 2015 and 2019. In total, 164 aborted fetuses from 39 goats and 125 sheep were examined with real time RT-PCR for pestivirus and SBV, nested RT-PCR for AKAV.

Fetal tissues including brain, lung, spleen, lymph nodule and liver were aseptically obtained during necropsy. An equal amount of sample of each organ of the aborted fetuses were pooled and homogenized with 5 ml 1X PBS (Phosphate-Buffered Saline) (Sigma-Aldrich) and centrifuged at 3500 rpm and 4 °C for 30 min. The samples were stored at -80°C until testing.

RNA Extraction. Total nucleic acid extraction was performed by using MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany) according to the manufacturer's instruction. RNA extracts were maintained at -80 until testing.

Real-Time and Nested PCR Assay. Real Time RT-PCR for amplifications of pestivirus and SBV viral RNA were performed with real-time Ready Virus Master (Roche, Germany). Primers and probes used for the amplification of 5'-UTR region of pestivirus and partial S segments of SBV have been previously published by Hoffmann et al (13) and Bilk et al (14), respectively. Nested PCR for amplifications of AKAV viral RNA were performed with one-step RT-PCR kit (Roche, Germany) using primers published by Akashi et al (15).

Sequencing and Phylogenetic Analysis. Sequence analysis was done as forward and reverse. Nucleotide sequence was evaluated using the DNADynamo software. The consensus nucleotide sequence that was obtained were confirmed using the basic local alignment search tool (BLAST) at the National Central for Biotechnology Information (NCBI). The nucleotide sequence in this study and other sequences taken from NCBI were analyzed and aligned using the program MEGA version X. The phylogenetic tree was constructed with the neighbour-joining method using by Kimura 2-parameter model, a bootstrap value of 1000 replicates for all methods and only values above 50% are reported.

RESULTS

A total of 164 aborted fetuses were obtained from the cases of sheep and goat abortions that occurred in the West Turkey from January 2015 to

June 2019. In the study, the annual distribution of abortions was examined; the number of the abortion cases was the highest in 2019 [47] and followed by 2016 [46], 2018 [36], 2017 [25], 2015 [10]. According to the monthly distribution of sheep-goat abortion materials, abortion cases begin to increase in November, peak in February, and decrease until August (Figure 1).

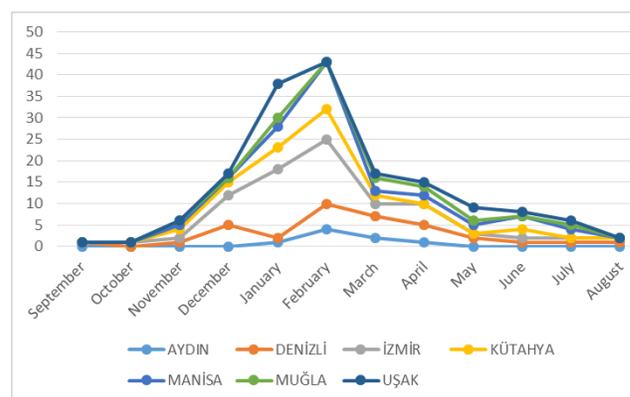


Figure 1. The monthly distributions of sheep-goat abortion fetuses. The graph shows similar results for each province.

The pestiviruses and SBV were tested using real-time PCR, and AKAV was tested using nested PCR. Pestivirus and AKAV were found in four (2.3%) and 10 (nine sheep, one goat) (5.9%) of the cases, respectively (Table 1). In most of the samples examined in the study, more AKAV was detected than pestivirus. SBV was not detected in the 164 aborted fetuses in this study.

Table 1. Number of abortion according to animal species, geographical location and provinces.

Provinces	Geographical position	Sheep			Goat			TOTAL		
		No. fetuses	Positivity		No. fetuses	Positivity		No. fetuses	Positivity	
			Aka	Pesti		Aka	Pesti		Aka	Pesti
AYDIN	37°49'51.1"N 27°51'47.0"E	7	-	-	1	-	-	8	-	-
DENİZLİ	37°46'57.3"N 29°05'48.3"E	22	2	1	6	-	-	28	2	1
İZMİR	38°25'27.9"N 27°08'34.5"E	33	1	-	19	1	-	52	2	-
KUTAHYA	39°24'18.7"N 29°36'46.6"E	19	-	2	2	-	-	21	-	2
MANİSA	38°36'53.4"N 27°25'45.7"E	25	1	1	4	-	-	29	1	1
MUĞLA	37°14'49.5"N 28°21'25.5"E	6	3	-	4	-	-	10	3	-
USAK	38°40'27.4"N 29°24'18.6"E	13	2	-	3	-	-	16	2	-
TOTAL		125	9	4	39	1	-	164	10	4

It was determined that most of the sheep-goat abortion cases in the Aegean Region were in Izmir Province and followed by the provinces of Manisa, Denizli, Kutahya, Usak, Mugla, Aydin.

The sheep-goat abortions that were found to be AKAV positive were detected in January [2], February [2], April [4], May [1], and July [1]. Thus, most of the AKAV positive cases occurred in April. Although AKAV is a vector-borne disease, it was also found to occur in the winter months. No abnormality (hydranencephaly and arthrogryposis) was found in the abortions of nine sheep and one goat that were found to be AKAV positive.

Turkish isolates obtained from previously studies (AKSU-1, AKSU-2 and Adana-15) showed the highest nucleotide acid homology (100%) to KMAF-SA isolate (Genbank accession number: MW679535) in this study. The phylogenetic tree generated based on S segment sequencing showed that KMAF-SA is in genogroup Ib, clustering with Adana-15, AKSU-1 and AKSU-2 (Figure 2).

DISCUSSION

In this study, pestivirus, SBV, and AKAV, which are three crucial agents that cause abortions in small ruminants, were examined using PCR. This is the first study in the Aegean Region (including seven provinces) to simultaneously investigate three important viral agents in aborted fetuses. Pestivirus and AKAV were found in 2.3% and 5.9% of the cases, respectively. SBV was not found in any of the sheep and goat abortion cases. AKAV was found in more of the abortion cases than pestivirus. As in the study conducted by Sevik (16) in Southern Turkey, AKAV was found in higher incidence in sheep abortions in comparison to pestivirus and SBV was not detected in any sample.

AKAV infection is widely seen in Australia, the Middle East countries, Southeast Asia and some African countries (10). Also, the presence of AKAV in small ruminants in Turkey has been reported in previous studies (16,17,18). In addition to factors related to the ecosystem, such as global warming, seasonal changes, and the presence of wet areas, animal transportation, changes in demographic structure, and increases in human travel play an important role in the occurrence of vector-borne diseases, such as AKAV (19,20,21). The Aegean Region, which is in the western part of Turkey, is a subtropical region that is characterized by hot and humid summers and rainy and warm winters. The vast number of water sources and agricultural areas in the region create significant habitats for vectors. Moreover, due to the fact that the Aegean Region is an industrial, agricultural, and tourism area, intense human and animal mobility make it a potential location for vector-borne diseases.

Climatic factors and the geographical location of the Aegean Region are suitable for the year-round circulation of *Culicoides*, so they play a prominent role in the spread of vector-borne diseases. AKAV re-emerges in ruminants in Turkey with irregular intervals of 4-6 years (17,21). In this study, the molecular detection of AKAV occurred between 2016 and 2017. AKAV was not detected in 2015, 2018, and 2019. The results obtained in the study may explain the fact that the disease is seen as endemic over a period of 4-6 years and that adult animals in the regions where it occurs are immune to the disease. Thus, AKAV was not detected in 2018 and 2019. AKAV was detected in the Western Region of Turkey in March in a study performed by Oguzoglu et al (17). In the present study, AKAV was detected in January,

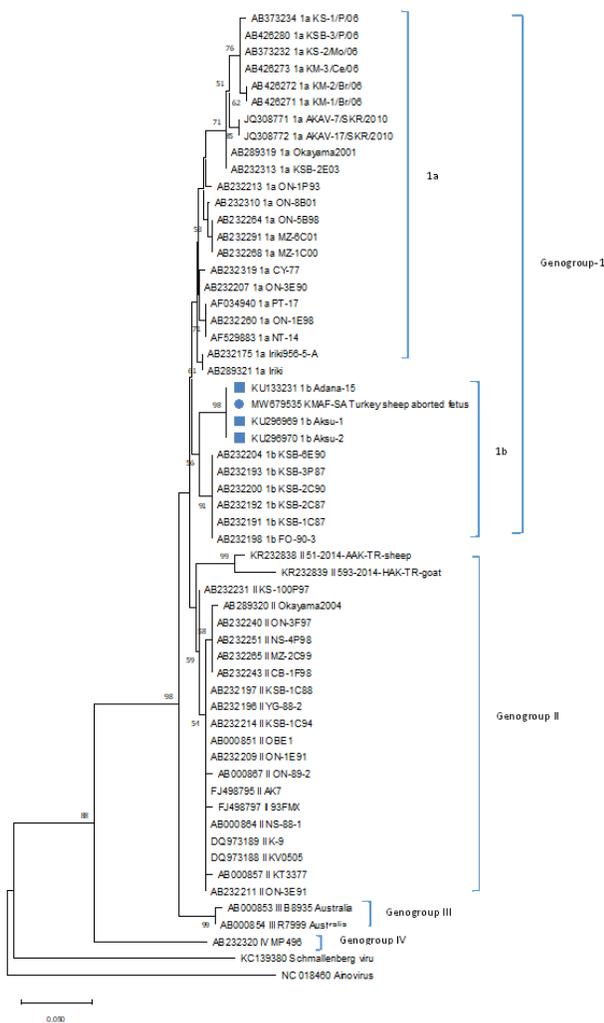


Figure 2. Phylogenetic tree of AKAV isolates from Turkey and other countries.

February, April, May, and July. The fact that the agent was detected mostly in winter months was an indicator that biting mites circulate during each season in the Aegean Region of Turkey.

In this study, no abnormality (hydranencephaly and arthrogryposis) was seen in the sheep and goat abortion cases in which we found AKAV. In small ruminants, the gestation period in which the animals are most sensitive to the agent ranges between days of 28 and 56. In the last gestation period, the abnormality incidence is very low (10). In this study, there was no definite information about in which period of gestation the abortions occurred.

In previous studies in Turkey, AKAV isolates obtained in sheep were determined as genogroup Ib and II (16). The phylogenetic tree generated based on S segment sequencing showed that KMAF-SA is in genogroup Ib.

In the previous studies carried out in the field, SBV infection have been reported in the Germany, Netherlands, Denmark, Norway, Belgium, France, Luxembourg, England, Italy, Spain, Switzerland, Sweden, Austria, Finland, Poland, Ireland, Czech Republic, Estonia, Hungary, Slovenia, Croatia, Lithuania, Greece, Russia, Serbia, and Romania (3). SBV was not found in any of the sheep and goat abortion samples. This is in agreement with the previous study conducted in the Mediterranean region of Turkey (16). However, a previous study conducted in the Marmara region (northern Turkey) reported that SBV was found in two sheep abortions (22). In another study conducted in the south of Turkey, SBV was found in 13.11% of sheep blood samples and in 13.33% of goat blood samples; blood samples were taken from animals one to six days after abortion (18). In this context, it suggests that blood samples taken from the mother rather than abortion samples for SBV may be more useful in detecting the nucleic acid of the SBV.

Pestivirus was found in 52.3% of sheep abortions in the Marmara region, which borders Europe (23), and in 98 of 396 (24.74%) sheep abortions in a study including the Marmara region and a few eastern cities (24). In a study conducted in the Black Sea region, which is also in the north, pestivirus was found in 91.9% of sheep abortions and 100% of goat abortions (25). In contrast, pestivirus was detected in only 10.4% of sheep abortions and also it was not found in any goat abortions in a study conducted in the south (26). Gur (27) reported that in the western province

of Afyonkarahisar, pestivirus was detected in 2 of 568 sheep abortions. The pestivirus results in the present study differed from those reported in several previous studies (23,25,28), but aligned with others where there were low incidences reported (26,27,29,30). Some ideas can be put forward to possibly explain the different results. One reason is that the ELISA test method is more sensitive for pestivirus (31) and that this method was used by Tuncer-Goktuna et al (23) in their study. The second reason could be the different sampling methods used in the studies. Third, although the 5' UTR gene region is a conserved region in all pestiviruses, it may not have detected new pestivirus genotypes, such as HoBi-like or single base changes, which may have affected primer annealing (31,32).

Serological studies conducted in the Marmara and Black Sea regions of Turkey showed that pestivirus infections in goats were not common (33,34). Pestivirus was also not found in goats in other studies conducted in Turkey using antigen ELISA (23,26). In the present study, pestivirus RNA was not found in goats.

In conclusion, viral agents that cause sheep and goat abortions in the Aegean region, which is in west Turkey and has a subtropical climate, have been examined molecularly. This is the first study in the Aegean Region (including seven provinces) in which three important viral agents were simultaneously investigated in small ruminant aborted fetuses. According to this study, the akabane virus caused sheep and goat abortions in this region and was followed by pestivirus. However, SBV was not found in the study. Therefore, screening for vector-borne viruses should be performed in the long term, particularly for the akabane virus, and large-scale insecticide-based vector control programs should be implemented when increases in virus are detected. The findings that the vector-borne akabane virus was the most common viral agent in sheep and goats in the Aegean Region and that SBV was detected in other regions of the country, while it was not found in Aegean Region where climatic conditions are warmer and the possibility of the incidence of vector-borne diseases is higher should be the subject of future studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

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