

The Investigation of Pestivirus and Rift Valley Fever Virus Infections in Aborted Ruminant Foetuses in the Blacksea Region in Turkey

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Summary

In this study, aborted foetuses (cattle, sheep and goat) in either coastal or inland Blacksea region of Turkey were surveyed for the presence of nucleic acids from pestiviruses (bovine viral diarrhoea virus-BVDV, border disease virus-BDV) and rift valley fever virus (RVFV) by reverse transcriptase polymerase chain reaction assay (RT-PCR). Pestivirus nucleic acids were found in 39 of 53 aborted calves (73.6%), in 34 of 37 aborted lambs (91.9%) and in 2 of 2 aborted kids (100%) by RT-PCR. In addition RVFV has been investigated in the same foetuses, however RVFV nucleic acid has not been confirmed in any samples.

Keywords: Aborted foetus, RT-PCR, Pestivirus, RVFV, Turkey

Karadeniz Bölgesinde Ruminant Atık Fötüslerinde Pestivirus ve Rift Vadisi Humması Virus Enfeksiyonlarının Araştırılması

Özet

Bu çalışmada, Karadeniz bölgesinin sahil ve iç kesimlerinden elde edilen atık sığır koyun ve keçi fötüsleri pestiviruslar (bovine viral diare virus-BVDV, border hastalığı-BDV) ve rift vadisi humması virus (RVFV) nükleik asitleri yönünden reverz transkriptaz polimeraz zincir reaksiyonu (RT-PZR) ile test edildi. RT-PZR ile 53 atık buzağının 39'unda (%73.6), 37 atık kuzunun 34'ünde (%91.9) ve 2 atık oğlağın tamamında (%100) pestivirus nükleik asidi tespit edildi. Ayrıca aynı atık fötüslerin tamamında RVFV araştırıldı, fakat hiçbirisinde RVFV nükleik asidi tespit edilemedi.

Anahtar sözcükler: Atık fötüs, RT- PZR, Pestivirus, RVFV, Türkiye

INTRODUCTION

Pestiviruses are enveloped, single-stranded RNA viruses that can infect ruminants and camelids. Pestiviruses belong to the *Pestivirus* genus of the *Flaviviridae* family and include border disease virus (BDV), bovine viral diarrhoea virus-1 (BVDV-1), BVDV-2 and classical swine fever virus (CSFV) and a tentative giraffe species ^{1,2}. Infection with pestiviruses can result in severe economic and reproductive losses in ruminants. Many studies have shown that pestiviruses are not highly host-specific. It has been reported that BVDV can infect not only cattle but also sheep, swine, goat, deer and giraffe; BDV infects sheep, swine and goats ³. Rift Valley Fever virus (RVFV) belongs to the family *Bunyaviridae*, genus *Phlebovirus*, and was first isolated in

1930 during an investigation of a large epizootic in Kenya. RVFV is a zoonosis and primarily transmitted by mosquitoes and causes a potentially severe disease among both humans and animals. RVFV affects ruminants and is characterized by high rates of abortion and death in young animals. Economic consequences of this disease can be devastating. RVFV has been detected across Africa, from Senegal to Madagascar and from Egypt to South Africa. In 2000, RVFV has been reached the Arabian Peninsula ⁴. Due to the increasing range of the virus, the high numbers of competent vector species present in currently RVF-free regions, such as Europe, the intensification of international trade in live animals, and the unknown impact of climate



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change, several national and international agencies have issued warnings about the heightened risk of introduction of RVFV into RVF-free countries ⁵.

Abortions in domestic ruminants are big problem in both the Blacksea region and Turkey. Especially, abortion rate caused by viral agents (pestiviruses) is dramatically increasing in the recent years ⁶. Additionally, due to global warming, vector-borne viral infections extend toward the north of world. The Blacksea region is the most rainfall region of Turkey. We wonder whether RVFV transmitted by mosquitoes is transmitted to Turkey. The aims of this study were to survey organ pools obtained from a variety of aborted ruminant fetuses in the Blacksea region of northern Turkey for the presence of nucleic acids from pestiviruses and RVFV.

MATERIAL and METHODS

Sample Collection and Processing

A total of 92 aborted fetuses obtained from different small private farm and fetuses were collected different time and different locations. The clinical symptoms of any infections were not observed and no vaccine (against pestiviruses) was used in the herds and flocks including fetuses sampled for molecular study. Aborted fetuses were collected between January and December of 2009 in the northern of Turkey (Table 1). It has been tested for pestivirus and RVF virus infections. A total of 92 organ pools, including the brain, lung, spleen, lymph nodule and liver (460 organ samples) from aborted lambs, kids and calves, were examined. Samples were placed in 2 ml of PBS diluent with MagNA Lyser Green Beads (Roche, Mannheim, Germany) and were homogenized at 3.000×g for 3 min by MagNa Lyser (Roche, Mannheim, Germany). Homogenates were centrifuged in Eppendorf tubes at 12.000×g for 3 min to remove the suspended solids, without removing the beads. The supernatants were stored at -80°C until testing.

RNA Extraction, Reverse Transcriptase PCR Assay

Viral RNA was extracted from 350 µl of organ supernatant by using the MagNA Pure LC RNA Isolation Kit III (Roche, Mannheim, Germany) and stored at -80°C. PCR were performed with Titan One-tube RT-PCR system (Roche, Mannheim, Germany) as per manufacturer's instructions. The genomic region encoding the highly conserved 5'-UTR of the pestivirus genome was amplified using panpesti generic primers (324/326) ⁷. Primers (RVFV1 and RVFV2) were selected from published sequence of small (S) RNA genome segment, which codes for nucleoproteins and non structural proteins of RVFV ⁸. Briefly, RT-PCR was performed in a 50 µl volume containing 5 µl of viral RNA, 1 µl of each primers, 10 µl of 5x RT buffer, 1 µl of 10 mM dNTPs, 1 µl of enzyme mix, 2.5 µl of 100 mM DTT, 0.25 µl of Rnase inhibitor (10 U/ µl), and 28.25 µl of HPLC water. PCR products were analyzed in 1% agarose gels after electrophoresis at 100 V for 30 min. The amplicons were observed under ultraviolet light (Fig. 1 and 2). NADL strain obtained from the Ankara University Faculty of Veterinary Medicine Virology Department was used as positive control for pestivirus. Positive control RNA of RVFV (56/74, South Africa) was kindly provided by Dr. Alejandro Brun (Centro de Investigación en Sanidad Animal (INIA), Madrid, Spain).

RESULTS

A total of 92 organ pools was tested by RT-PCR for pestiviruses and RVFV. No RVFV genomic RNA was detected in any aborted fetuses. However, pestivirus nucleic acids were found in 39 of 53 aborted calves (73.6%), in 34 of 37 aborted lambs (91.9%) and in 2 of 2 aborted kids (100%). Out of 92 aborted ruminants fetuses tested, 75 (81.5%) were found as pestivirus positive. Positivity rates varied between 50%-100% in calves and 66%-100% in lambs in the province basis (Table 1).

Table 1. Number of sampled aborted fetuses according to geographical position of locations and provinces

Tablo 1. Coğrafi konum ve illere göre örneklenen atık fütüs sayıları

Provinces	Geographical Position	Lamb		Calf		Kid		Total	
		No. Foetuses	Positivity (%)	No. Foetuses	Positivity (%)	No. Foetuses	Positivity (%)	No. Foetuses	Positivity (%)
Sinop	42°01' N, 35°09' E	4	4 (100)	3	2 (66.6)	-	-	7	6 (85.7)
Sivas	39°45' N, 37°02' E	4	4(100)	2	1 (50)	-	-	6	5 (83.3)
Amasya	40°39' N, 35°51' E	5	4 (80)	8	5 (62.5)	1	1 (100)	14	10 (71.4)
Samsun	41°17' N, 36°20' E	9	8 (88.8)	31	24 (77.4)	-	-	40	32 (80)
Trabzon	41°00' N, 39°43' E	5	5 (100)	-	-	-	-	5	5 (100)
Tokat	40°19' N, 36°43' E	4	4 (100)	4	3 (75)	-	-	8	7 (87.5)
Rize	41°02' N, 40°31' E	3	3 (100)	3	3 (100)	-	-	6	6 (100)
Giresun	40°55' N, 38°24' E	3	2 (66.6)	2	1 (50)	1	1 (100)	6	4 (66.6)
Total		37	34 (91.9)	53	39 (73.6)	2	2 (100)	92	75 (81.5)

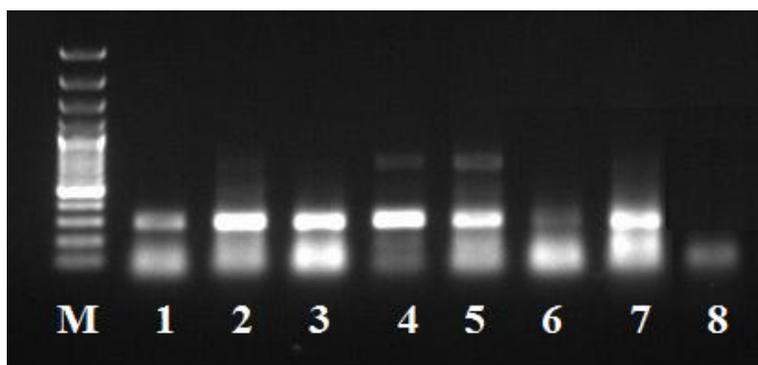
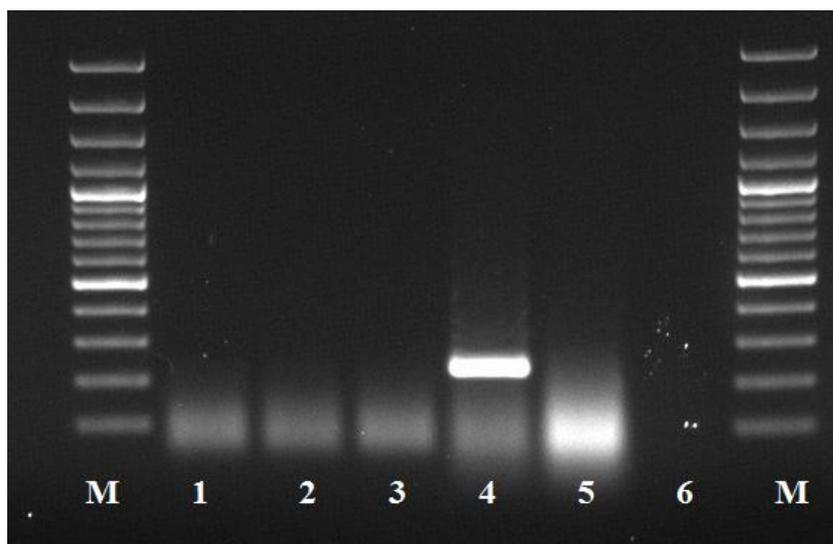


Fig1. Agarose gel electrophoresis of pestivirus PCR positive specimens and controls. **Lines M:** 100 bp DNA Ladder, **Line 1-6:** positive, **Line 7:** positive control (288bp), **Line 8:** negative control

Şekil 1. Pestivirus PZR agaroz jel elektroforezde pozitif örnekler ve kontroller. **M:** 100 bp DNA merdiven, **1-6:** pozitif örnekler, **7:** pozitif kontrol, **8:** negatif kontrol

Fig 2. Agarose gel electrophoresis of RVFV PCR negative specimens and controls. **Lines M:** 100 bp DNA Ladder, **Line 1-3,6:** negative, **Line 4:** positive control (240 bp), **Line 5:** negative control

Şekil 2. RVFV PZR agaroz jel elektroforezde negatif örnekler ve kontroller. **M:** 100 bp DNA merdiven, **1-3,6:** negatif örnekler, **4:** pozitif kontrol, **5:** negatif kontrol



DISCUSSION

Pestivirus infections in large and small ruminants are widespread worldwide with a wide range of seroprevalences depending on the management of animal husbandry. Pestivirus infections are congenital viral diseases of ruminants and also, economically important infectious agents of even-toed ungulates (order *Artiodactyla*), causing considerable losses notably in cattle and swine ². In this study, pestivirus RNA was found in 39 of 53 aborted calves (73.6%), in 34 of 37 aborted lambs (91.9%) and in 2 of 2 aborted kids (100%) by RT-PCR. Many virological studies have been performed in Turkey for pestiviruses. The presence of pestiviruses were reported between 0.9% and 12.8% in ruminants in different part of Turkey ^{6,9-16}. The genetic typing of pestiviruses isolated from cattle and sheep in Turkey has shown that a new subgroups of BVDV-1, BVDV-2 and BDV is widespread ¹⁰⁻¹⁴. Moreover, the Turkish BVDV-2 field isolates showed the highest similarity with North America BVDV isolates ¹¹. These similarities are interesting because the herds sampled included animals imported from the North America. It is known that the introduction of infected animals may play a significant role in the distribution of new infectious agents on a herd or even country level ¹¹. In addition, an ovine isolate was identified as first member of BVDV-2 ¹⁴. The geographic distribution

of BVDV-1 subgenotypes were detected in Turkey. BVDV-1/1 was detected as predominant subgenotype ^{12,14}. BVDV-1/1 also was determined as predominant subgenotype in Samsun province ¹⁴. In our study, the most aborted fetuses cases were detected in Samsun provinces, and also many pregnant cattle were imported in the same provinces. In addition, there was a frozen sperm production centre, which functioned as a sperm bank and provided service to other neighbour provinces of Samsun. It can be speculated that BVDV strains could have been transferred to another by the frozen sperm of those animals.

Seroprevalence in the flock of sheep varies from region to region, but the average percentage is between 18.9% and 74.5% ^{6,17}. The rate of bovine abortion associated with pestivirus has been estimated by many researchers. In cattle rearing areas of America the rate is 2%, for Argentina as high as 25%, and for United Kingdom as many as 27% of bovine abortions have been associated with pestiviruses, often in conjunction with opportunistic pathogens ¹⁸. The presence of pestiviruses (81.5%) detected in this study is higher than the previously reported studies ^{6,9-15}. Pestiviruses in aborted fetuses are likely to be under-detected for two reasons. First, the actual abortion may be undetected or appropriate samples may not be submitted for laboratory investigation. Secondly, even when samples

are submitted, the cause of the abortion is often not identified¹⁹.

The another reason of the higher percentage of pestiviruses in that region can be use of live vaccines purpose of control programme some viral infections for instance peste des petits ruminants, sheep and goat pox and etc. Pestiviruses are frequent contaminants of modified live virus vaccines produced on primary ovine and bovine cells²⁰ and may play a role in pestivirus maintenance and/or dissemination in the region.

The RT-PCR has been a successful molecular tool for research purposes and diagnosis for pestivirus infections. This has allowed the detection of pestivirus RNA in a variety of clinical samples such as blood, tissues, serum and swabs²¹. It has been used fresh organ samples of aborted fetuses in this study. Different material such as blood and organ samples are used in the diagnosis of pestivirus infections⁶. The detection rates of pestivirus antigens from blood and organ samples from clinically healthy and infected animals were detected as 0.7% and 10.4%, respectively by Hasircioğlu et al. The organ samples are more valuable than blood samples as the diagnostic material from ruminants in transplacental infections⁶. When considering the wide-spread practice of communal grazing in the restricted area of small ruminants with cattle, the type of pestivirus infection circulating in small ruminants and the possibility of interspecies transmission is of particular interest. Epidemiologically, the most important feature of pestiviral infections is the ability to generate persistently infected (PI) offspring when the first infection of the mother occurs within a certain time frame of pregnancy. By shedding large amounts of virus throughout their lives, PI animals represent the main source of infection for susceptible hosts². There is no special effort to detect PI animals in flocks and herds and also no vaccination, eradication and control programme for pestiviruses is present in Turkey.

Reservoir-vector-climate trio was very important at the epidemiology for the RVFV infection, like other vectorborne diseases. Climate conditions of the Blacksea region were more suitable for *Culicoides spp.* The reservoir for RVFV is mosquitoes, particularly *Aedes* species. In the African outbreaks, *Aedes lineatopinnus* was the primary reservoir. Given are average annual values of heat, humidity, and rainfall of the Blacksea region [13.0 (4.2-22.1)°C, 71%, 842.6 mm³]; but, annual rainfall of same region in 2009 is 1042.3 mm³. In this respect, when the results of general annual rainfall average and annual rainfall average of 2009 year were compared, annual rainfall average of 2009 year was found to be higher than general annual rainfall average. Additionally, annual heat changes are more dramatic in the Blacksea region²². Following heavy rainfalls, the pooling water gives the eggs a proper environment to hatch. These newly hatched infected mosquitoes then seek an feeding source (human or animal). Higher vector

activity cause to increase in vector-dependent diseases⁴. There are great number of factors which influence the epidemiology of RVFV infection (season, time of breeding, presence of sheep, uncontrolled animal movement). It has been reported that infection predominantly occurs in fall, a rainy and damp season. The Blacksea region is a damp area which receives a great quantity of rain.

To date is not any report about RVFV in Turkey and Europe, but due to global warming, this infection can be transmitted by mosquitoes north of Mediterranean sea⁵. Increased rainfall has been accompanied by increased abortions, so we need to consider RVFV. This is the first investigation on RVFV in Turkey. At the same time, no viral nucleic acid was detected in aborted fetuses in the middle Blacksea region of Turkey in this study. Although mosquito species known to transmit RVFV have been observed in Turkey²³, there has been no report of acutely infected humans and animals in Turkey. This study has shown that the RVFV is not prevalent in the northern of Turkey in where the climatic conditions are more rainy and humidity. To better understand RVFV transmission in Turkey, additional studies focusing on major vectors (e.g. mosquitoes) are needed.

In conclusion, this study demonstrates percentage of pestiviruses in aborted ruminant fetuses, more than what has been previously reported^{6,9-11}. In the future, these results can be deeper reflect due to the studies on molecular typing of pestiviruses. While we did not detect RVFV in aborted fetuses surveyed in this study, continued efforts may reveal more information about the role of different mosquito species in RVFV maintenance and/or dissemination in the region.

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