



RESEARCH ARTICLE

Survey of four important viruses causing abortion in cattle in Aegean Region

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Ege Bölgesi'nde sığırlarda abortusa neden olan dört önemli virus üzerine bir araştırma

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Öz

Amaç: Çalışmanın amacı, Türkiye'nin Ege Bölgesi'nde 2015-2019 yılları arasında sığır abortus vakalarında Bovine herpesvirus-1 (BHV-1), Schmallerberg virus (SBV), Akabane virus (AKAV) ve pestivirus'ün rolünü araştırmaktır.

Gereç ve Yöntem: Bu çalışmada, 2015-2019 yılları arasında Türkiye'nin Ege Bölgesi'nden toplanan 204 aborte fetüs örneği incelenmiştir. Bu amaçla Bovine herpesvirus-1 (BHV-1), Schmallerberg virus (SBV) ve pestivirus taraması için real-time PCR tekniği kullanılırken, Akabane virus (AKAV) için ise nested-PCR tekniği kullanılmıştır.

Bulgular: İncelenen örneklerin 25'i (%12.3) pestivirus pozitif, 1'i (%0.5) Akabane pozitif ve 1'i de (%0.5) BHV-1 pozitif bulundu. SBV tespit edilmedi. Bu sonuçlara göre, Ege Bölgesi'nde en fazla aborta neden olan viral etkenin pestivirüs olduğu belirlendi. Laboratuvara en fazla abort materyalinin 2018 yılında gönderildiği, bunu 2019, 2017, 2016 ve 2015 yıllarının takip ettiği belirlendi.

Öneri: Bu çalışmanın sonuçlarına dayanarak, Ege bölgesinde tespit ettiğimiz etkenler gelecek değerlendirmelerde dikkate alınmalı, kontrol ve izleme çalışmaları oluşturulmalıdır.

Anahtar kelimeler: Abort, Akabane virus, Bovine herpesvirus-1, pestivirus, Schmallerberg virus

Abstract

Aim: The aim of the study was to investigate the role of Bovine herpesvirus-1 (BHV-1), Schmallerberg virus (SBV), Akabane virus (AKAV) and pestivirus in cattle abortion cases in the Aegean Region of Turkey between 2015 and 2019.

Materials and Methods: In this study, 204 bovine abortions that occurred in the Aegean region of Turkey between the years 2015 and 2019 were investigated. While BHV-1, SBV, and pestivirus were examined with real-time PCR, AKAV was examined with nested-PCR.

Results: In our study, 25 (12.3%) of the samples analyzed were positive for pestiviruses, 1 (0.5%) was positive for Akabane, and 1 (0.5%) was positive for BHV-1. SBV was not detected. According to these results, the viral agent causing most abortions in the Aegean region was found to be pestivirus. The most abortion-related material was sent to the lab in 2018, followed by 2019, 2017, 2016, and 2015.

Conclusion: Based on the results of this study, future evaluations should consider the cattle abortion agents detected in the Aegean region, and control and monitoring studies should be established.

Keywords: Abortion, Akabane virus, bovine herpesvirus-1, pestiviruses, Schmallerberg virus



Introduction

Abortion is caused by infectious agents such as viruses, bacteria, fungi and parasites, as well as non-infectious causes such as hormones, nutritional disorders and genetic factors (Peter 2000). Abortions are considered normal at a rate of up to 1-2% in herds. However, higher rates of abortion cases are considered a serious economic loss (Bagley 1999). Viruses are one of the most important causes of abortion in ruminants. Akabane virus (AKAV), Schmallenberg virus (SBV), Bovine herpesvirus-1 (BHV-1) and pestivirus have been shown to be the main viral pathogens causing abortion in cattle, sheep and goats (Inaba et al 1975, Kurogi et al 1975, Anderson 2007, Yilmaz et al 2014, Aslan et al 2015).

Pestivirus is an enveloped, small (about 40–60nm), positive-polarity, single-stranded RNA virus classified within the Flaviviridae family (Becher et al 2003). Pestiviruses have been renamed and classified into 11 species, according to the International Committee of Taxonomy Virus (ICTV). Accordingly, they are named as BVDV-1 (Pestivirus A), BVDV-2 (Pestivirus B), border disease (Pestivirus D), classical swine fever (Pestivirus C), and HoBi-like (Pestivirus H). BVDV-1 subtypes range from 1a to 1q, while BVDV-2 subtypes range from 2a to 2d. BVDV-3 (pestivirus H), a member of the pestivirus genus HoBi-like pestivirus, has been detected in cattle and buffalo (Mishra et al 2014). BVDV has been grouped in two biotypes, cytopathic (cp) and non-cytopathic (ncp) according to the morphological changes it causes in cell culture (Fulton et al 2000). Pestiviruses cause serious reproductive disorders in pregnant animals, including abortions, physical and congenital anomalies and the births of persistently infected animals (Nettleton et al 1998, Evans et al 2017).

The BHV-1 genome is approximately 135 kb in size, enveloped, double-stranded DNA virus (Harrison 2001). BHV-1 is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, which belongs to the Herpesviridae family. BHV-1 isolates have been grouped into three different subtypes, BHV-1.1, BVH-1.2a and BHV-1.2b, which are distinguished by genetic differences and varieties in clinical symptoms (Metzler et al 1985). BHV-1.1 and BVH-1.2a cause respiratory and genital system disorders and abortions. While BVH-1.2b is not seen in abortion cases, it has fewer pathogenic characteristics when compared with the other subtypes (Edwards et al 1990, Miller et al 1991, Spilki et al 2004). The most important source of transmission of this type of BHV-1 is through artificial insemination with infected semen and infected bulls (Kupferschmied et al 1986, Wrathall et al 2006). The virus becomes latent in sacral or trigeminal ganglia following primary infection and can be transmitted/shed with or without clinical symptoms with the effect of stress factors (Narita et al 1976, Ackermann et al 1984).

AKAV and SBV are members of the Simbu serogroup in the

Orthobunyavirus genus, which belongs to the Peribunyaviridae family, and they are carried by *Culicoides* biting midges (Rasmussen et al 2014). AKAV and SBV are enveloped, 3-segmented (S: Small, M: Medium, L: Large) and negative-polarity RNA viruses (Kirkland 1988, Maclachlan and Dubovi 2016). AKAV is closely associated with Aino, Tinaroo, Peaton and Schmallenberg viruses and causes reproductive disorders (e.g., abortion, preterm births and stillbirths) in cattle, sheep and goats, as well as congenital anomalies such as arthrogriposis-hydranencephaly, i.e., AH syndrome (Kurogi et al 1985, Maclachlan and Dubovi 2016). SBV was found for the first time in Germany in November 2011, and the disease has been detected throughout Europe since then (Lievaart-Peterson et al 2015). The disease causes fever, decreased milk yield in cattle, stillbirth and other anomalies in sheep and goats (Tarlinton 2012).

The aim of the present study is to research the role of pestivirus, BHV-1, SBV and AKAV in cattle abortion cases that occurred in the Aegean region between the years 2015 and 2019.

Material and Methods

Samples

In accordance with the regulation on the working procedures and principles of animal experimentation ethics committees (8. article k subparagraph); ethics committee decision is not required for studies with dead animals or tissue, slaughterhouse materials and abort fetuses.

Aborted fetuses from cattle that underwent routine diagnosis in a virology laboratory at İzmir/Bornova Veterinary Control Institute (İzmir, Turkey) between 2015 and 2019 were investigated in this study. A total of 204 aborted fetuses were tested in Aegean Region of the west Turkey (Table 1).

The pool consisting of the brain, lung, spleen, lymph node and liver from each aborted fetuses were homogenized with 5 ml Eagle Minimum Essential Medium (EMEM) (SIGMA-ALDRICH, United Kingdom) and centrifuged at 3500 rpm and 4°C for 15 min. The supernatants were stored at -80°C until testing.

RNA Extraction and PCR Assays

Total nucleic acid was extracted from 200 µl of supernatant using MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Germany) according to the manufacturers instructions. Real-Time RT-PCRs for detection of Pestivirus and SBV were performed by using Real-Time Ready Virus Master (Roche, Germany). Primers and probes used for the amplification of 5'-UTR region (132 bp) of pestivirus and partial S segments (88 bp) of SBV have been previously published by Hoffmann et al (2006) and Bilk et al (2012), respectively. Real-Time PCR



Table 1. Number of samples collected from abortion cases in the Aegean region in 2015-2019 by provinces

Provinces	2015	2016	2017	2018	2019	Total
Aydın	0	2	3	22	20	47
Denizli	0	0	0	13	10	23
İzmir	0	0	1	6	6	13
Kütahya	1	6	0	9	6	22
Manisa	3	5	3	14	12	37
Muğla	0	5	3	13	4	25
Uşak	0	12	0	19	6	37
TOTAL	4	30	10	96	64	204

for amplifications of BHV-1 viral DNA were performed with The LightCycler® 480 Probes Master (Roche, Germany). Primers and probe used for the amplification of a 97 bp sequence of the glycoprotein B (gB) gene has been previously published by Abril et al (2004). Nested PCR for amplifications of AKAV viral RNA of 230 bp were performed by using One-Step RT-PCR Kit (Roche, Germany) using primers published by Akashi et al (1999). PCR products of AKAV were run on 1.5% agarose gel stained with ethidium bromide in TAE buffer and photographed using equipment from Vilber Lourmat (France).

Results

The annual distribution of the materials was examined, it was determined that the most abortion samples were sent to the laboratory in 2018 (96), followed by 2019 (64), 2017 (30), and 2016 (10), respectively. Interestingly, only four samples were sent in 2015 (Table 1).

Abortion samples sent to the laboratory were mostly from the Aydın province, followed by the cities of Manisa, Uşak, Muğla, Denizli, Kütahya, and Izmir, which are located in the Aegean region.

Pestivirus, BHV-1 and AKAV were found to be 25 (12.3%), 1 (0.5%), and 1 (0.5%) respectively (Table 2). Pestivirus was detected in more samples than AKAV and BHV-1 examined in the study (p-value < 0.00001). SBV was not detected from the 204 aborted fetuses in this study (Table 2).

In our study, no malformations were detected in abortions with pestivirus and no signs of hydranencephaly and artrogryposis were detected in AKAV positive.

Discussion

Abortions cause significant economic loss in dairy cattle breeding and this can be prevented by researching the diseases

Table 2. Number of sampled aborted fetuses and positivity according to geographical position of locations and provinces

Provinces	Geographical location	No. fetuses	Positive			
			AKA	Pestivirus	BHV-1	SBV
Aydın	37°49'51.1"N 27°51'47.0"E	47	-	4	-	-
Denizli	37°46'57.3"N 29°05'48.3"E	23	-	4	1	-
İzmir	38°25'27.9"N 27°08'34.5"E	13	-	2	-	-
Kütahya	39°24'18.7"N 29°36'46.6"E	22	-	-	-	-
Manisa	38°36'53.4"N 27°25'45.7"E	37	-	6	-	-
Muğla	37°14'49.5"N 28°21'25.5"E	25	1	4	-	-
Uşak	38°40'27.4"N 29°24'18.6"E	37	-	5	-	-
TOTAL		204	1	25	1	-





which cause abortions and taking the necessary precautions. In this study, pestivirus, SBV, BHV-1, and AKAV, which cause a significant number of the abortion cases for cattle in the Aegean region, were examined molecularly. Viruses are one of the most important causes of abortion in ruminants. AKAV, SBV, BHV-1, and pestiviruses have been shown to be the primary viral pathogens which cause abortion in cattle, sheep, and goats (Inaba et al 1975, Kurogi et al 1975, Anderson 2007, Yildirim et al 2011, Yilmaz et al 2014, Aslan et al 2015). In our study, pestivirus, BHV-1, and AKAV rates were determined to be 12.3%, 0.5%, and 0.5%, respectively. SBV was not determined in this investigation. When compared to the other viruses studied, pestiviruses were discovered in a larger number of abortions. Pestiviruses were found at higher rates in abortions than BHV-1, contrary to prior studies (Kirkbride 1992, Yang et al 2012, Morrell et al 2019). When the present study and previous studies are taken into consideration, it is known that pestiviruses are among the most important causes of abortion cases in Turkey (Albayrak and Ozan 2012, Tuncer-Goktuna et al 2016, Bulut et al 2018).

Pestiviruses were detected in 31 of 60 cattle abortions in a retrospective research conducted in the Marmara region of northern Turkey, which borders Europe, similar to the current study (Tuncer-Goktuna et al 2016). In another study conducted in the Black Sea region of northern Turkey, pestivirus was detected in 28.5% of cattle abortions (Albayrak and Ozan 2012). In a study performed by Sevik (2018) in the province of Afyonkarahisar in western Turkey, pestivirus was determined with a rate of 22.2%. In the present study, pestiviruses were found at low rates when compared with some studies (Albayrak et al 2012, Albayrak and Ozan 2012, Bulut et al 2018), but at high rates when compared with other studies (Gur 2009, Hasircioglu et al 2009, Yang et al 2012, Morrell et al 2019). Despite this, pestiviruses should be considered as one of the most important problems in cattle abortions. The possible reasons for different detection rates in different studies could be the number of sampled farms, the number of sampled animals, and the differences in test methods.

In transplacental infections, which are caused by pestiviruses, foetal deaths can occur in any period of pregnancy. Factors such as the biotype and dose of the virus and the foetal development period in which the infection develops determine the course of the infection in the foetus (Nettleton et al 1998). The fact that there were no malformations in the foetus materials which is pestivirus positive in the study can be explained in this way. In pestiviruses cases, malformations can be seen in the fifth and ninth months of pregnancy (Schweizer and Peterhans 2014), but in our study, there was no definite information about the period of pregnancy in which the abortions occurred.

BHV-1 can be a latent in trigeminal ganglions and sacral ganglion following field isolate infection or attenuated vaccination. Reactivation to a latent infection can occur during stressful situations, such as transport and delivery or the application of corticosteroids (Nandi et al 2009). In our study, which was based on gB gene region, DNA was detected only in 1 of the 204 samples. Although varying prevalence of seropositivity was obtained in studies conducted with BHV-1 in Turkey (Aslan et al 2015, Yavru et al 2015), the virus was either not found (Yavru et al 2015) or found in very low rates (Aslan et al 2015). The results of our study were similar to the results of other studies previously performed in different regions of the country and the prevalence of the agent was detected to be low (Aslan et al 2015, Tuncer-Goktuna et al 2016). The BHV-1 virus was detected at a low rate in this study, which is consistent with prior studies that found a low virus rate. On the other hand, the high seropositivity in the studies mentioned above might be attributed to the latency of the virus, and vaccinated animals.

Vaccines developed against viral and bacterial diseases have long been used in preventive medicine. Intensive breeding and integrated husbandry are the most popular activities in the Aegean region, which is the subject of our study. The number of family-owned farms is quite low. Thus, vaccination is very common against pestiviruses and BHV-1 in husbandry. It is highly possible that the viral agents that were the subjects of this study were found to be less prevalent in Aegean region compared with other regions due to periodical vaccinations that are common in intensive husbandry.

As in the case of the SBV, the vector of AKAV is *Culicoides* (Kirkland 2015). AKAV has also been reported in Africa, the Middle East, Southeast Asia, Australia, and Turkey (St George 1989). In Turkey, AKAV has been molecularly detected in small ruminants (Oguzoglu et al 2015, Sevik 2017), however; AKAV nucleic acid was not found in any of the cattle in east (Yildirim et al 2015). Turkey is in a subtropical region, and climatic factors play a significant role in the spread of arboviral infections. The climate is suitable for year-long circulation of *Culicoides*. In this study, the molecular detection of AKAV in aborted calves was reported for July 2017. AKAV was not detected in the years 2015, 2016, 2018, and 2019. AKAV reappears in ruminants in Turkey at irregular intervals of 4–6 years (Oguzoglu et al 2015, Oguzoglu 2018). The AKAV positivity in an aborted calf in our study was found in the province of Mugla, the southernmost part of the Aegean region. Hydranencephaly and arthrogryposis were not detected in AKAV-positive abortions. Cattle are the most sensitive to the agent between a gestation period of 3–6 months. The incidence of abnormality caused by this infection is very low in the last gestation period (Kirkland 2015).

SBV has an endemic course in European countries (Wernike et al 2017). Following the first detection of SBV in Germany in





2011, it spread throughout all of Europe, following which a decrease in outbreaks was noticed after 2014. However, SBV caused epidemics by re-emerging in Belgium in 2016, and in France and other countries in 2017 (Muskens et al 2012, Sohier et al 2016, Delooz et al 2017, Wernike et al 2017). In this study, primers designed by Bilk et al (2012), which targeted the S segment used in European countries, were used. Studies conducted in different countries using the same protocol reported identifying the agent in abortions, stillbirths, and malformed fetuses (Bilk et al 2012, Hoffmann et al 2012, Muskens et al 2012). Our study used the same protocol, and SBV was not detected in any of the abortion samples. However, RNA was determined in 2 sheep abortions by a study conducted in the Marmara region of Turkey (Yılmaz et al 2014), and the presence of the agent was shown serologically (Azkur et al 2013).

Conclusion

The viral agents causing cattle abortions in the Aegean region, which is located in the western part of Turkey, were examined. According to this study, pestiviruses cause the highest number of cattle abortions in this region, followed by BHV-1 and AKAV. However, SBV was not detected as the cause of any of the cattle abortions. When the results of the study are evaluated overall, a general increase can be seen in abortion cases in Aegean region in terms of years. Based on the results of this study, future studies should take into account the Aegean region's livestock abortion agents control and monitoring studies should be established. In addition, agents such as BTV, BHV-4 and BHV-5, which cause abortions in cattle, should also be studied thoroughly in the future. Lastly, since the region has a sub-tropical climate, the presence of AKAV and SBV should be researched in detail in biting midges.

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Conflict of Interest

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Ethical Approval

This study was carried out with the permission of the Izmir/ Bornova Veterinary Control Institute Animal Experiments Local Ethics Committee report 26.05.2021.

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