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Original Article
Özgün Araştırma

Investigation of OvHV-2 virus in small ruminants in Western Turkey

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Abstract: Malignant catarrhal fever (MCF) is a fatal disease of ruminants and has a worldwide distribution. The MCF virus (MCFV) group has currently known to contain 10 members. Ovine gammaherpesvirus 2 (OvHV-2) is one of the members of MCFV group and has economically importance in cattle. Sheep is the reservoir host of OvHV-2 while domestic goat is naturally susceptible species, although infection is generally asymptomatic in goat. In this study, the presence of OvHV-2 was investigated in sheep and goats by hemi-nested PCR in Aegean Region of west Turkey. Total of 138 sheep and goat samples which consist of 73 whole blood, 39 internal organs and 26 aborted fetuses were investigated. Thirty-seven blood samples, four fetal samples, and seven internal organ samples were found to be OvHV-2 positive. While 47 of the total sheep samples were found to be positive, only one goat sample was found positive. Similarly, previous studies have shown that prevalence of OvHV-2 in goats lower than sheep.

Key words: Goat, malignant catarrhal fever (MCF), ovine gammaherpesvirus 2 (OvHV-2), sheep

Türkiye'nin batısındaki küçükbaş hayvanlarda OvHV-2 virusunun araştırılması

Özet: Malignant Katharal Fever (MCF) ölümcül bir geviş getiren hastalığıdır ve dünya çapında bir dağılıma sahiptir. MCF virusu (MCFV) grubunun şu anda 10 üye içerdiği bilinmektedir. Ovine gamaherpesvirus-2 (OvHV-2), MCFV grubunun üyelerinden biridir ve sığırlarda ekonomik olarak öneme sahiptir. Koyunlar, OvHV-2'nin rezervuar konağıdır ve keçiler ise doğal olarak duyarlı türdür, ancak enfeksiyon genellikle keçilerde asemptomatiktir. Bu çalışmada, OvHV-2'nin Türkiye'nin batısında yer alan Ege Bölgesindeki koyun ve keçilerdeki varlığı hemi-nested PCR yöntemi ile araştırılmıştır. Yetmiş üç tam kan, otuz dokuz iç organ ve yirmi alt abort fetüsten oluşan toplam yüz otuz sekiz koyun ve keçi örneği incelendi. Otuz yedi kan örneği, dört abort fötus ve yedi iç organ örneği OvHV-2 virusu yönünden pozitif bulunmuştur. Toplam koyun örneklerinin kırk yedisi pozitif olarak bulunurken sadece bir keçi örneği pozitif bulunmuştur. Önceki çalışmalar ile benzer olarak keçilerde OvHV-2 prevalansının koyunlardan daha düşük olduğunu görülmüştür.

Anahtar kelimeler: Keçi, koyun, malignant kataral fever (MCF), Ovine gammaherpesvirus 2 (OvHV-2)

Introduction

Malignant catarrhal fever is a letal disease in cattle, water buffalo, American bison, and other wild ruminants (Russell et al. 2009; Stahel et al. 2013). It is characterized by lymphoid cell accumulations in non-lymphoid organs, lymphocytic hyperplasia in lymphoid organs and vasculitis (Anonymous, 2018). The typical clinical findings of the disease are severe nasal discharge and fever (O'Toole et al. 2002). In addition, inflammation, erosion and mucopurulent exudation (in upper respiratory tract, oral mucosa and eyes), swollen nodules, lameness and neurological findings can be observed (Li et al. 2005).

The MCF virus (MCFV) group is currently known to contain 10 members. Six of these (AlHV-1, AlHV-2, OvHV-2, CpHV-2, MCFV-WTD and Ibex-MCF) cause naturally disease in susceptible species while one of these (HipHV-1) cause disease experimentally in rabbits and others viruses (Gemsbok-MCFV, Muskox-MCFV, Aoudad-MCFV) are yet to be associated with disease (Li et al. 2014). All members of the MCFV group belong to the genus *Macavirus*, sub-family *Gammaherpesvirinae*, family *Herpesviridae* (ICTV 2020). Herpesviruses are enveloped, 120–250 nm with icosahedral symmetry. The genome consists of double-stranded, linear DNA 180 kb long (Knowles 2011). The OvHV-2 genome consists of 130,930 bp, with a G+C-rich. The genome have 73 ORFs and 62 of them demonstrate homology with other gammaherpesviruses (Hart et al. 2007).

OvHV-2 and AlHV-1 are reported to be the major causing agents of the disease that cause more significant economic loss in ruminants (Stahel et al. 2013). Although AlHV-1 does not cause disease in its natural host, the African antelopes, it causes dis-

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ease in cattle in Africa and various ruminant species in zoos all over the world (Horner and Tham 2003). OvHV-2 is widespread in the sheep populations around the world and causes sheep-associated malignant catarrhal fever (SA-MCF) in some ruminants (Cunha et al. 2012; O'Toole and Li 2014). It is reported that the infection is generally subclinical in sheep, which are the reservoir host for OvHV-2. However, MCF-like clinical symptoms such as fever and nasal discharge were reported to occur in sheep exposed to a high dose of OvHV-2 via aerosol route (Li et al. 2005; Sood et al. 2013; O'Toole and Li 2014). Domestic goat is one of the naturally susceptible species for OvHV-2. Infection in goats is generally asymptomatic, but OvHV-2 induced MCF like lesions have been reported (Jacobbsen et al. 2007; O'Toole and Li 2014).

Although malignant catarrhal fever caused by OvHV-2 is reported to be sporadic in cattle and other species, it may cause outbreaks when cattle are husbandried with other species susceptible to MCF or somehow in contact (Li et al. 2000; Schultheiss et al. 2000; Brenner et al. 2002; Otter et al. 2002; Twomey et al. 2002; Li et al. 2008; Neimanis et al. 2009; Vinod Kumar et al. 2014). MCF cases caused by SA-MCF have been reported in several regions in Europe, Canada, North and South Africa, Asia, and Middle East (Brenner et al. 2002; Benazzi et al. 2004; Neimanis et al. 2009; Gelaye et al. 2013; Vinod Kumar et al. 2014).

In this study, the presence of DNA of OvHV-2 was investigated by hemi-nested PCR in sheep and goat raised in the Aegean region of Turkey.

Materials and Methods

Samples and Preparation

The samples used in the study were randomly selected from the routine diagnostic materials sent to the Virology department of İzmir / Bornova Veterinary Control Institute. A total of 138 sheep and goat samples consist of 73 whole blood, 39 internal organs and 26 aborted fetus were collected from 19 different goat and sheep herds from 7 provinces of the Aegean region located in west of Turkey. Distribution of the samples as provinces are shown in Table 1. Whole blood samples were used directly. The internal organ and fetus samples were homogenized in PBS (1/10 v/v) then homegenate was centrifuged at 3000 rpm and 15 min and supernatants were used for DNA extraction. In fetus samples, if internal organs available, same protocol as adult animals were used whereas whole fetus was used in cases of early abortion in which internal organs were not developed.

DNA extraction and Hemi-nested PCR

Commercial kit (DNeasy Blood and Tissue Kit, Qiagen, Germany) was used for DNA extraction and extraction was carried out according to the manufacturer's instructions.

DNA amplification was carried out by hemi-nested PCR protocol targeting the DNA polymerase gene described previously (Flach et al. 2002). For the first and second amplification POL1: 5'-GGC (CT) CA (CT) AA (CT) CT ATG CTA CTC CAC-3 'and POL2: 5'-ATT (AG) TC CAC AAA CTG TTT TGT-3', POL2 and OHVPol: 5'-AAA AAC TCA GGG CCA TTC TG-3' primer sets were used, respectively. The expected amplicon length for the first and second PCR products were 386 bp and 172 bp, respectively. PCR reaction was carried out by using a commercial kit (Fast cycling PCR kit, Qiagen, Germany). The reaction mixture contained 10 µl Fast PCR Mastermix, 5 µl DNA template, 3 µl H_2O and 1 µl (10 µM) of each primer. For the first run thermocycle profile was; initial denaturation at 95°C 15 min, following 25 cycles of DNA denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, primer extension at 72 °C for 1 min and final extension step at 72°C for 10 min. The conditions of the second run were the same as the first run but were performed in 30 cycles instead of 25 cycles. Final PCR products were analyzed by in 1.5 % agarose (containing ethidium bromide) gel electrophoresis and visualised under UV light source. Positive and negative controls were used in each PCR analysis.

Results

In this study, a total of 138 samples were examined and 48 samples were found positive. According to sample type, 37 of 73 (51%) blood samples, 4 of 26 (15%) fetal samples, and 7 of 39 (18%) internal organ samples were found to be positive. Consistent with the literature, it was observed that the rate of OvHV-2 virus in sheep is higher than in goats. Figure 1 shows the PCR visualization.

Distribution of the samples and PCR results as provinces are sum up in Table 1.

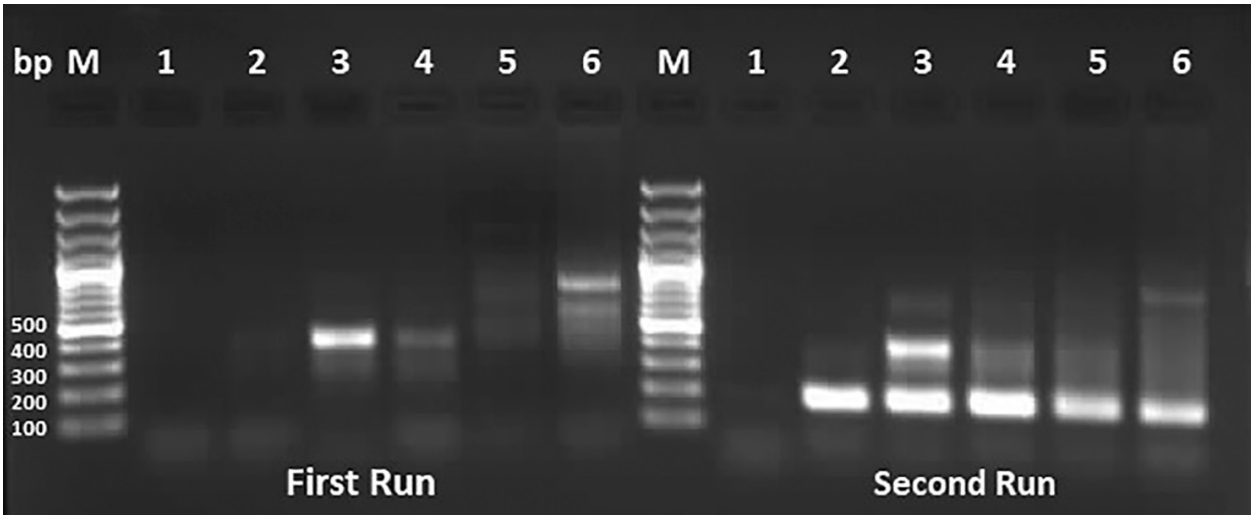

Figure 1. PCR result image. bp: base pair, M lane:100-bp ladder; 1: negative control, 2: positive control, 3: organ samples; 4,5: whole blood samples; 6: fetus.

Table 1. Distribution of samples by provinces

Provinces	Whole blood				Internal organ				Aborted fetus				Total	Positive Rate %
	Sheep		Goat		Sheep		Goat		Sheep		Goat			
	P	N	P	N	P	N	P	N	P	N	P	N		
İzmir	1	6	0	1	2	10	0	8	2	7	1	2	40	15
Aydın	7	5	-	-	0	3	-	-	0	2	-	-	17	41
Denizli	0	1	-	-	1	0	-	-	0	1	0	1	4	0
Kütahya	1	1	-	-	2	1	0	2	1	1	0	1	10	40
Manisa	7	3	0	5	1	0	-	-	0	2	0	1	19	42
Muğla	21	6	0	8	1	3	0	2	-	-	0	2	43	51
Uşak	-	-	-	-	0	3	-	-	0	2	-	-	5	0
Total	37	22	0	14	7	20	0	12	3	15	1	7	138	34

P: positive N:negative, -: not sampled

Discussion and Conclusion

SA-MCF is the most common form of the disease and leads to outbreaks with high mortality rates in cattle kept together with sheep all over the world (O'Toole et al. 2002; Schultheiss et al. 2000; Otter et al. 2002; Twomey et al. 2002; Li et al. 2008).

The presence of MCF in Turkey have been severely reported as follows; Yazıcı et al. (2006) reported that antibodies were not present in the blood serum of the 150 randomly selected apparently healthy sheep in the Samsun province while antibodies were present in the blood serum samples of 18 of the 29 apparently healthy cattle (62%). Yeşilbağ (2007) reported that seroprevalence was 97.5%, 96%, 15%, respectively in sheep, goat and cattle in Marmara Region. Yıldırım et al. (2012) detected DNA of OvHV-2 in 2 samples in 7 nasal and 9 conjunctival swab samples collected from sheep.

In this study, the presence of OvHV-2 was investigated in the whole blood, internal organ and aborted fetus samples from seven provinces situated in the Aegean Region. In goat, all of 14 whole blood and 12 internal organ samples were found negative while OvHV-2 virus DNA was detected in just one of 8 fetus samples. In sheep, OvHV-2 virus DNA was detected in 37 of the whole blood samples (62.7%), 7 of the internal organ samples (25.9%), 3 of the fetus samples(16.6%), and 45.1% of all sheep samples. Similarly, previous studies have showed that prevalence of OvHV-2 in goats lower than

sheep (Wiyono 1999; Banumathi et al. 2008; Khudhair et al. 2020).

It is reported that OvHV-2 transmission is generally horizontal way, while vertical transmission is rarely. Disease-free herds could be created by separating the lambs at the proper time (at 2.5 months) but adult sheep are as susceptible to the disease as lambs (Li et al. 1998, 2000). Although nasal secretion is reported to be the major route of transmission, a study found that the agent is present in 27 different organs and tissues including the genitalia, which suggests that the agent may be transmitted through other routes as well (Li et al. 2001; Hüssy et al. 2002; Li et al. 2004,). Headley et al. (2015) reported transplacental transmission in cattle. Dashty (2015) identified the OvHV-2 virus in cattle uterus and foetal placenta and reported that transuterine transmission may be occurred. Although the OvHV-2 DNA could not be identified in the uterus of the sheep, detection of the viral DNA in the placental epithelium, stromal fibroblasts, and vascular endothelial cells indicates that could be possible intrauterine fetal infection. Li et al. (1998) found no DNA or antibodies in 4 fetus samples collected from OvHV-2 positive sheep in last 5 days of the pregnancy, and no antibodies in the 118 serum samples collected from the lambs of OvHV-2 positive sheep before presuckling lambs. However, in two (twins) out of 77 lambs, the OvHV-2 virus was detected in the peripheral blood leukocyte (PBL) samples using the PCR technique. In this study, four aborted samples found to be positive.

Prevalence of MCF-antibodies in goats in the Marmara region, which is bordering to the Aegean region, was reported 96% (Yeşilbağ 2007). In our study all whole blood and internal organ samples obtained from goats were found to be negative for DNA of OvHV-2. When we compare these two studies, it is thought that the differences in results may be due to the methods used.

In conclusion, the fact that the OvHV-2 virus was detection in sheep is 45% in the Aegean region shows that more extensive investigation of the virus presence in sheep and cattle population in the region, is needed In addition, detection of the virus in 4 (s-3, g-1) aborted fetuses shows that detailed studies are needed regarding the transplacental transmission of the OvHV-2 virus in sheep and goat.

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