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Research Article

Virological Investigation of Border Disease Infection in Sheep with Abortion Problem

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ABSTRACT

Pestiviruses are important pathogens to economic losses in cattle, sheep, goat and pigs. Border disease virus (BDV) caused by pestiviruses is characterized by low birth weight and congenital disorders in lambs, while infection is subclinical in adult sheep. Transmission of border disease between animals is through direct contact. However, since persistently infected animals are natural sources of the disease, large outbreaks may occur if a persistent infected animal enters the susceptible herd. In this study, the aim was to investigate the presence of BDV by a PCR assay using primers specific for the DNA fragment of the 5 'NC region by taking blood samples from 75 sheep with abortion problems found on Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic. As a result of the test, all animals were found as negative. Testing of a variety of sample types collected from a larger number of animals is recommended in order to determine the epidemiological existence of border disease.

Keywords: Abortion, Pestivirus, Border disease, PCR

Abort Problemli Koyunlarda Border Disease Enfeksiyonunun Virolojik Olarak İncelenmesi

ÖZET

Pestiviruslar sığır, koyun, keçi ve domuzlarda ekonomik kayıplara yol açan önemli patojenlerdir. Pestivirusların neden olduğu Border Disease Virusu (BDV), kuzularda düşük doğum ağırlığı ve konjenital bozukluklarla karakterize edilirken, yetişkin koyunlarda enfeksiyon subklinik seyretmektedir. Hayvanlar arasında Border hastalığının bulaşması doğrudan temas yoluyla olur. Bununla birlikte, persiste enfekte hayvanlar hastalığın doğal kaynakları olduğundan, persiste enfekte bir hayvan duyarlı sürüye girerse büyük salgınlar meydana gelebilir. Bu çalışmada Selçuk Üniversitesi Veteriner Fakültesi İç Hastalıkları Kliniğine gelen abort problemli 75 koyundan kan örnekleri alınarak, 5 'NC bölgesinin DNA fragmanına özgü primerlerin kullanıldığı PCR testi ile BDV varlığının araştırılması amaçlanmıştır. Test sonucunda tüm hayvanlar negatif bulunmuştur. Sonuç olarak, Border hastalığının epidemiyolojik varlığını ortaya koymak için daha fazla hayvandan daha çeşitli örneklemeler yapılması tavsiye edilmektedir.

Anahtar Kelimeler: Abort, Pestivirus, Border hastalığı, PCR

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Introduction

Border Disease (BD) disease was first reported in 1959 in sheep and goats in the border region between Wales and England and then spread to the whole world (Hughes et al., 1959). Border disease virus (BDV) is from the genus *Pestivirus* of the family *Flaviviridae* (Fauquet et al., 2005). Although BDV affects a wide variety of animals, it mainly infects sheep and goats (OIE, 2017). It is in the same genus as the classical swine fever virus and Bovine Viral Diarrhea Virus (BVDV) 1 and 2 (Oguzoglu et al., 2009). Various studies are showing that pestiviruses have no host specificity. In this context, it can be said that BDV can infect sheep, pigs, goats, cattle as well as deer and giraffes (Paton 1995; Oguzoglu et al., 2010). BDV is divided into 2 biotypes according to whether it has a cytopathogenic effect (CP) or not (Tabash et al., 2009). In recent studies based on sequence analysis and phylogenetic tree, it has been divided into at least 8 genotypes (Caruso et al., 2017; Cerutti et al., 2019). BDV can be transmitted both horizontally and vertically. Vertical transmission is particularly significant in terms of the epidemiological role of the disease. Infection of the foetus in the early period of pregnancy leads to the birth of "persistently infected lambs". Persistently infected animals infect susceptible animals in the herd with the virus shedding with their secretions and extracts (Dahhir et al., 2019). It is also called "hairy shaker disease" or "fuzzy lamb syndrome" due to abnormal hair formation observed in new-born animals (Oğuzoğlu, 2012). Low birth weight, ataxia, occasional enteric dysfunction, mucosal disease-like lesions have also been reported in BDV-infected sheep and lambs (Berriatua et al., 2006). There are no significant clinical findings observed in adult sheep and goats other than abortion (Thabti et al., 2005). Serological studies conducted have revealed that the seroprevalence of BDV varies between 5% and 50% from country to country or from region to region (Cabezón et al., 2010), (Kaiser et al., 2016), (Saeed 2020). The prevalence of BDV has been reported in many countries such as Turkey, Austria, Japan, and Italy (Ataseven et al., 2006; Krametter-Froetscher et al., 2008; Giangaspero et al. 2011; Giammarioli et al. 2015). In Turkey, it was detected in many regions such as Afyonkarahisar (Gür, 2009), Black Sea region (Albayrak et al., 2012), Kars (Yilmaz et al., 2014), Konya (Avcı, 2010), Eastern and Southeastern regions (Ataseven et al., 2006). Diagnostic methods of BDV include techniques such as virus isolation, immunohistochemistry, ELISA, etc. (Dagleish et al., 2010; Strong et al., 2010; Al-Rubayie and Hasso, 2014). It has been reported that detection of BDV from clinical samples is difficult, but RT-PCR is a very successful molecular technique. It has been stated that RT-PCR enables the detection of pestivirus DNA from various samples such as blood, tissue, serum, and swap (Edmondson et al., 2007).

In this study, the aim was to investigate the presence of BDV by PCR test in blood samples from sheep with abortion problems who were taken to Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic.

Material and Methods

Leukocyte samples were collected from 75 sheep with abortion problems between the ages of 1 and 5-years who were taken to Selcuk University Faculty of Veterinary Medicine Internal Medicine Clinic between November 2019 and December 2020. Blood samples were taken from the jugular vein and transferred to sterile tubes containing EDTA.

Viral RNA extraction was performed according to the procedure specified by the manufacturer (QIAamp Viral RNA Mini Kit/ Cat No./ID: 52906) in the kit. The primer used was the RNA fragment (288bp) of the 5' NC region in the NADL strain of BVDV type 1 reported by Vilček et al. (1994). Primer 324 was 5'ATG CCC WTA GTA GGA CTA GCA 3' (position at NADL 108-128, W= A or T) and primer 326 was 5' TCA ACT CCA TGT GCC ATG TAC 3'(NADL 395-375). QIAGEN OneStep RT-PCR Kit (Cat No./ID: 210212) was used for PCR. Positive control, negative control and 50µL final reaction mixture for each sample (10µl 5x, 1 µl dNTP (10mM), 2 µl forward primer (10pmol), 2 µl reverse primer (10pmol), 1 µl enzyme mix, 29 µl water, 5 µl RNA) were prepared. The thermal cycle program is 1-minute denaturation at 94 ˚C, 1-minute annealing at 56 ˚C, 1-minute extension at 72 ˚C. After 35 cycles performed in this manner, the PCR products were run on a 2% agarose gel. PCR bands were examined under UV light in the presence of ethidium bromide.

Ethical approval: All procedures and animal care complied with the guidelines of the Selcuk University Veterinary Faculty Ethics Committee (Ethical approval number 2019/06 on 2019155).

Results

In this study, leukocyte samples taken from sheep with abortion problems were found to be negative in terms of pestivirus antigen.

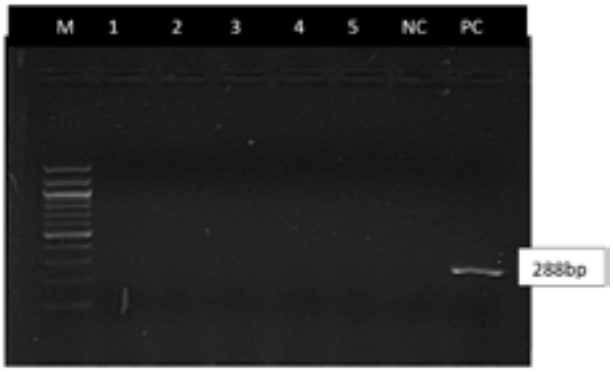

Figure 1. M: Marker, 1: Sample No 1, 2: Sample No 2, 3: Sample No 3, 4: Sample No 4, 5: Sample No 5, NC: Negative Control, PC: Positive Control

Discussion

Pestiviruses are among the most important causes of reproductive disorders and immunosuppression and are responsible for significant economic losses in farms. Bovine Viral Diarrhea Virus (BVDV) and BDV are closely related genetically. Both can infect cattle, sheep, goats, pigs, and non-domestic ruminants (Nettleton and Entrican, 1995). Currently there is no vaccination, control, or eradication program for Border Disease in Turkey.

Abortion observed in sheep may be caused by other infectious agents other than BDV, such as *Neospora caninum* (Dubey and Lindsay 1990), Listeria (Chand and Sadana, 1999), *Brucella* sp. (Ocholi et al., 2005).

Hasırcıoğlu et al. (2009) serologically and virologically investigated the presence of pestivirus in sheep and goats that had abortions. Therefore, they examined serum and leukocyte samples from 735 sheep, 35 goats, and tissue samples from 48 abortions/stillborn lambs by Enzyme-Linked ImmunoSorbent Assay (ELISA). As a result, 475 (64.6%) of 735 sheep with abortion and 2 (5.7%) of 35 goats with abortion were found to be positive for pestivirus antibodies. Besides, they stated

Table 1. The ages of the animals sampled

Number	Age of Animal	Number	Age of Animal
1	3 year-old	39	1.5 year-old
2	5 year-old	40	1.5 year-old
3	5 year-old	41	2 year-old
4	1 year-old	42	2 year-old
5	1 year-old	43	1.5 year-old
6	3 year-old	44	2 year-old
7	2 year-old	45	3.5 year-old
8	1 year-old	46	4 year-old
9	4 year-old	47	4.5 year-old
10	5 year-old	48	3 year-old
11	1.5 year-old	49	2 year-old
12	2.5 year-old	50	2.5 year-old
13	3 year-old	51	4 year-old
14	3 year-old	52	1.5 year-old
15	1.5 year-old	53	3 year-old
16	5 year-old	54	1 year-old
17	4.5 year-old	55	2.5 year-old
18	4 year-old	56	2 year-old
19	3 year-old	57	4 year-old
20	5 year-old	58	3 year-old
21	5 year-old	59	2 year-old
22	1.5 year-old	60	2.5 year-old
23	1.5 year-old	61	3 year-old
24	1 year-old	62	3 year-old
25	2.5 year-old	63	1.5 year-old
26	3 year-old	64	5 year-old
27	4 year-old	65	1 year-old
28	2 year-old	66	4 year-old
29	4.5 year-old	67	1.5 year-old
30	3 year-old	68	1 year-old
31	2 year-old	69	2 year-old
32	4 year-old	70	1 year-old
33	1.5 year-old	71	3 year-old
34	2 year-old	72	1 year-old
35	3 year-old	73	5 year-old
36	2 year-old	74	4 year-old
37	1 year-old	75	2.5 year-old
38	3 year-old		

that while the presence of pestivirus antigen was detected in 5 (0.7%) of the leukocyte samples collected from sheep with abortion and in the waste foetus tissue samples of these animals, they could not detect the presence of antigen in any of the goats with abortion.

Berber and Sözdutmaz (2013) investigated the presence of pestivirus by ELISA in abort samples obtained from sheep in Elazığ, Malatya, and Tunceli provinces. They determined 36 (22.5%) of 160 samples as positive.

Ural and Erol (2017) serologically and virologically investigated pestivirus infection in sheep and goats in Aydın and İzmir provinces. They found that 41.40% (53/128) of goat samples and 47.59% (158/332) of sheep samples were seropositive, but whole blood samples were negative in terms of antigen. They pointed out that although no viraemic animals were detected in sheep and goats, the detection of seropositivity indicated that pestiviruses were circulating in the region.

To investigate the presence of persistent pestivirus in sheep and lambs infected with Contagious ecthyma (CE) infection in a sheep farm in Sakarya Gülyaz (2016) examined leukocyte samples from 18 sheep and 26 lambs by nested RT-PCR method and found negative for the presence of pestivirus nucleic acid.

Avcı (2010) found 11 out of 1000 sheep leukocyte samples (1.1%) as positive for the presence of BDV antigen, while 989 (98.9%) were negative. All 500 lamb leukocyte samples were determined as negative for the presence of BDV antigen.

In the study by Valdazo-González et al. (2006), they sampled 423 sheep, including 5 herds, and detected only 11 (2.6%) as virus-positive. In the second sampling they performed to confirm the presence of persistent infection, 6 of them (1.4%) were found positive. Braun et al. (2014) tested 1170 sheep for BDV by RT-PCR in their study to investigate whether BDV was transmitted to cattle from sheep grazing on the same pasture, and they found that only 8 (0.68%) were positive. Mokhtari ve Manshoori (2018) found that 9 (9%) of the foetal fluids from 100 sheep that had abortions were positive. Braun et al. (2013) found 2,384 sheep negative for BDV by quantitative RT-PCR, while 310 (13.5%) of 2291 sheep were found to be positive for BDV antibodies by ELISA. The antigen results of this study are similar to ours. However, we could not comment on the disease history because we did not examine it serologically. Kaleibar et al. (2014) found 10 (11.36%) out of 88 sheep positive by nested RT-PCR. The reason for this might be the increase in virus titer as they inoculated leukocyte samples into cell culture in their study. Ali et al. (2015) examined a total of 382 pneumonic lung tissues, including 305 sheep and 77 goats, for pestivirus antigen by ELISA, they found 32 (10.5%) sheep and 9 (11.7%) goats positive.

In Burgu's (2003) study, viremia due to pestivirus was found in 1 out of 108 fetuses. It was found that as a result of pestivirus control of other organs of this fetus, the presence of pestivirus was detected in the lung, liver, brain, intestine, and kidney of the same fetus as a result of control of other organs of that foetus. Pestivirus was detected from the amniotic fluid and placentoma samples of the mother of the same fetus, the leukocyte sample was found to be negative.

Burgu et al. (2001) examined a total of 1460 sheep in 9 different farms for persistent infections, they found that only 1 sheep was "transiently viraemic" and none was persistently infected. Therefore, they interpreted that although there were no acute or persistent infected animals in the herd at the time of sampling, there may have been acutely infected animals in the herd before.

Lambs are protected from disease until about 2 months of age by the passive immunity provided by high-quality colostrum they will receive immediately after birth. However, in order to have IgG in the colostrum the animals should be vaccinated before birth in relation to this factor. To prevent BDV, persistently infected animals should be identified and removed from the herd while healthy animals should be vaccinated. Double sampling should be done to identify animals with PI. This should include re-sampling animals that are positive for BDV antigen 1 month after the initial analysis. Those whose tests are positive again should be considered persistently infected and sent to the slaughterhouse. Vaccination aims to clinically protect seronegative animals and to prevent or at least limit the birth of PI lambs, along with protecting

foetuses in the early stages of pregnancy. Depending on the individual serological status of the animals, vaccination should be administered to the entire herd or only to seronegative animals. Sudden temperature changes, transport, weaning, transfer, sudden change in care and feeding conditions are the main stress factors for the animals. Since the expected level of immunity from the vaccine may not be achieved in animals exposed to stress, it is recommended that vaccinations be given during the period when stress in the herd is lowest. Currently there is no commercially available vaccine for BDV. The vaccines against BVDV in Turkey are administered to sheep at half or a quarter of the dose used for vaccination of cattle.

It should be ensured that illegal animal transport, which plays a major role in the spread of BDV, is also brought under control.

In summary, the study evaluated the leukocyte samples from 75 animals and it is recommended that the negative results obtained should be evaluated in parallel with the study conducted by Burgu (2003) along with leukocyte samples in amniotic fluid and placenta samples. It is recommended that a large number of samples from a wide population be tested to investigate the presence of BDV. Eradication programs should be developed to identify and remove persistently infected animals from the flock. It should be noted that sheep may serve as reservoirs for pestiviruses observed in other species. In addition, the activities of the animals (through introduction of illegal animals, transfers between them or imports from neighbouring countries) should be carefully monitored. This will prevent the spread of the disease to larger populations. In addition, animal breeders and veterinarians should be informed about this infection and thus cases should be screened for BDV. The informative study results will contribute to the economic benefits of the country as well as scientific data. In studies conducted on molecular genetic typing of BDV in our region, this will be an important step for studies on vaccination which is the most effective method to prevent the disease.

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