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RESEARCH ARTICLE

# Investigation of Pestivirus Infections in Aborted Sheep and Goats in Burdur Region

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## Summary

In this study, the presence of pestivirus reported as an important cause of abort cases in sheep and goats was investigated serologically and virologically in aborted sheep and goats. For this purpose, serum and leukocyte samples from 735 aborted sheep and 35 aborted goats, tissue samples (lung, spleen, brain) from 48 aborted or stillborn lambs were collected and these samples were tested by ELISA for the presence of pestivirus antigen and antibody. 475 (64.6%) of 735 aborted sheep and 2 (5.7%) of 35 aborted goats were seropositive for the presence of antibody to pestivirus. Also, the presence of pestivirus antigen was detected in only 5 (0.7%) of leukocyte samples collected from aborted sheep and in only 5 (10.4%) of tissue samples of aborted lambs belonging to them, whereas not detected in any of aborted goats. In conclusion, the results of this study that was done the detection of specific antigen and the detection of high seroprevalence in aborted sheep and aborted lambs belonging to them have brought up that infections of pestivirus are very widespread in Burdur province and that pestivirus is an important cause of abort cases in sheep in this province.

**Keywords:** *Sheep, Goat, Pestivirus, ELISA, Abortion*

# Burdur Bölgesinde Abort Yapmış Koyun ve Keçilerde Pestivirus Enfeksiyonunun Araştırılması

## Özet

Bu çalışmada, koyun ve keçilerde abort olgularının önemli bir nedeni olarak bildirilen pestivirus varlığı abort yapmış koyun ve keçilerde serolojik ve virolojik yönden araştırıldı. Bu amaçla abort yapmış 735 adet koyun, 35 adet keçiden serum ve lökosit örnekleri ve 48 adet abort / ölü doğmuş kuzudan doku örnekleri toplandı. Bu örnekler pestivirus antijen ve antikor varlığı yönünden ELISA ile incelendi. 735 adet abort yapmış koyunun 475 (%64.6) adedi ve 35 adet abort yapmış keçinin 2 (%5.7)'si pestivirus antikorları yönünden pozitif tespit edildi. Ayrıca, abort yapan koyunlardan toplanan lökosit örneklerinin 5 (%0.7)'inde ve bu hayvanlara ait atık fetus doku örneklerinde pestivirus antijen varlığı belirlenirken, abort yapmış keçilerin hiçbirinde antijen varlığı belirlenmedi. Sonuç olarak, Burdur bölgesinde abort yapan koyunlarda ve yavrularına ait doku örneklerinde spesifik antijen varlığı ve yüksek seroprevalans tespitinin, pestivirus enfeksiyonunun bu bölgede yaygın ve koyunlarda gözlenen abort olgularında önemli bir nedeni olduğunu ortaya koymaktadır.

**Anahtar sözcükler:** *Koyun, Keçi, Pestivirus, ELISA, Abort*

## INTRODUCTION

Sheep and goats are among the species of animals used by man for the production of meat, milk, leather and wool. Whether the purpose of rearing sheep and goats is production of milk, wool or meat, these processes are dependent on the ability of the animals to reproduce successfully. Any decrease in the total number of reproductive animals in a flock will cause important economic losses that could close the operation. Infertilization results from two main factors: non-infectious and infectious factors. Non-infectious factors include genetic and nutritional or toxic causes. Infectious ones are the most important factors causing abortions in the small ruminants. Pestiviruses are among viral infections causing abortion in sheep and goats [1].

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Pestiviruses are enveloped, single-stranded, positive sense RNA viruses. The genus pestivirus within the family Flaviviridae consists of four accepted species: border disease virus (BDV), bovine viral diarrhoea virus-1 (BVDV-1), BVDV-2 and classical swine fever virus (CSFV) and a tentative Giraffe species [2].

All pestiviruses are important veterinary pathogens causing economic losses in cattle, sheep and pigs. Besides the important economical losses, pestiviruses may compromise the normal immune response to other pathogens and increase the severity of other infections in sheep [3]. The current classification of pestiviruses refers to the host species from which they were recovered [4]. However, numerous investigations have indicated 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 [5]; CSFV infects mainly pigs but experimentally also cattle and goats [6,7]. The disease is characterized by abortions, barren ewes, stillbirths and the birth of small weak lambs in a flock. Clinical disease in goats is rarely reported [8]. In ruminants, vertical transmission plays an important role in the epidemiology of pestiviruses. Infection of foetuses before they have an immune system can result in the birth of persistent infected (PI) animals. Neverthless, in contrast to sheep and cattle, It has been reported that persistent infection in goats is rare [9]. Pestiviruses in pregnant goats usually cause abortions, malformations and necrotic placentitis rather than PI offspring [10]. Pestivirus has been isolated from only a few naturally infected goats [11,12]. Loken [13] suggests that PI sheep, and in particular cattle, are the main suppliers for pestivirus infections in goats. Lambs born to these infected dams are always persistently viraemic. Rams that are persistently infected have poor quality, highly infective semen and reduced fertility [14,15]. Due to their negative impact on reproduction and health, they cause substantial economic losses to the sheep and cattle industries [16].

Serological surveys have demonstrated widespread natural infection with pestiviruses in sheep and goats in many parts of the world [9,10,17-22].

The purpose of this study was to investigate the presence of pestivirus antibodies and antigens in aborted sheep and goats in Burdur province, and to determine the role and importance of pestiviruses in the etiology of abortion cases that lead to economic losses in rearing sheep and goat.

## MATERIAL and METHODS

***Animals and Flocks:*** Between 2005 and 2007, samples were collected from aborted animals in 40 sheep and 3 goat flocks in Burdur and its districts. Of flocks sampled 80% had <100 and 20% had 100-300 animals. Most of sheep were indigenous breeds such as Merino, Dağlıç. Goats were Anatolian Black Goat Breed. Farmers reported that clinical disease sings such as abortions, stillbirths, birth of small weak lambs with or without tremors were observed in flocks sampled in previous years. Sheep and goats were housed together but in some flocks sheep and cattle were housed together. In addition, these animal species was using the same pastures. No pestiviral vaccines had been used in animals in flock's samples.

***Blood Samples and tissue samples from aborted foetuses:*** For serological and virological studies, serum and leucocyte samples from 735 aborted sheep and 35 aborted goats in Burdur and its districts, tissue samples (brain, lung, spleen) from 48 aborted or stillborn lambs were collected. Blood samples from aborted sheep and goats were always collected from the jugular vein. Blood samples for sera were collected into tubes containing no anticoagulant. Then, the samples were centrifuged and the serum was separated. Leucocytes were prepared from blood samples collected into tubes with EDTA by a standard method. Sera and leucocytes were stored at -20°C until used. In addition, tissue samples collected into sterile stainer from aborted foetuses were kept at -20°C until used.

***Antigen detection by ELISA in leucocyte and abortion samples:*** To determine the presence of antigen in these samples a commercial BVDV antigen ELISA kit (Institut Pourquier, France) was used. Tissue samples (brain, lung, spleen) from aborted foetuses and leucocyte samples collected aborted sheep and goats were prepared as reported in the test procedure. ELISA was performed according to the procedure described by the manufacturer. The ELISA test results were expressed as positive and negative.

***Antibody detection by ELISA in serum samples:*** Pestivirus antibodies in serum samples collected

from aborted sheep and goats were detected by using commercial BVD/MD/BD P80-ELISA kit (Institut Pourquier, France) according to the procedure described by the manufacturer. The ELISA test results were expressed as positive and negative.

## RESULTS

475 (64.6%) of 735 serum samples collected from aborted sheep and 2 (5.7%) of 35 serum samples collected from aborted goats were found as seropositive by ELISA for pestivirus antibodies *(Table 1)*. In addition, the presence of pestivirus antigen was detected in only 5 (0.7%) of leukocyte samples collected from aborted sheep and in only 5 (10.4%) of tissue samples of aborted lambs belonging to them *(Table 2)*. None of 35 leucocyte samples obtained aborted goats were detected as positive for pestivirus antigens *(Table 1)*.

***Table 1.*** *Results of antibody-ELISA in serum samples and antigen-ELISA in leucocyte samples*

***Tablo 1.*** *Serum örneklerinde ELISA-antikor ve lökosit örneklerinde ELISA-antijen sonuçları*

| Animals | Number of animal | ELISA antibody | | Positive (%) | ELISA antigen | | Positive (%) |
|---|---|---|---|---|---|---|---|
| | | - | + | | - | + | |
| **Sheep** | 735 | 260 | 475 | 64.6 | 730 | 5 | 0.7 |
| **Goat** | 35 | 33 | 2 | 5.7 | 35 | 0 | 0 |
| **TOTAL** | 770 | 293 | 477 | 70.3 | 765 | 5 | 0.7 |

***Table 2.*** *Results of Antigen-ELISA in tissue samples obtained from aborted foetuses*

***Table 2.*** *Doku örneklerinde ELISA-antijen sonuçları*

| Animals | Number of animal | ELISA | | Positive (%) |
|---|---|---|---|---|
| | | Negative | Positive | |
| **Tissue samples (Sheep)** | 48 | 43 | 5 | 10.4 |
| **TOTAL** | 48 | 43 | 5 | 10.4 |

## DISCUSSION

Pestiviruses are a major cause of reproductive failure and immunosupression, and cause substantial economic losses in livestock industries through their impact on reproduction and health. However, BVDV and BDV are not strictly host specific and can cross infect cattle, sheep, goats, pigs and non-domesticated species [16]. No vaccination, eradication and control programme for BVDV is present in Turkey.

The presence of pestivirus infections which the prevalence rates were founded between 0.06-3.0 percent has been determined in previous virological studies carried out in Turkey [23-25]. Burgu et al.[23] found the presence of antigen in 14 (3%) of 478 blood samples collected from aborted sheep and 8 (10%) of 74 tissue samples collected from aborted lambs. Oğuzoğlu et al.[26] detected the presence of pestivirus antigen by ELISA in 6 (2%) (three sheep and 3 goats) of 297 blood samples from 146 sheep and 151 goats from 8 small ruminant flocks with abortion history in the Turkish provinces of Aydın and Burdur. The investigators have first carried out the genetic characterization of BDV in small ruminants in Turkey. Çokçalışkan [25] found as positive the presence of pestivirus antigen in one (0.93%) of 108 foetuses collected from slaughtered sheep.

In this study, the presence of pestivirus antigen was detected in 5 (0.7%) of 735 leucocyte samples collected from aborted sheep and 5 (10.4%) of 48 tissue samples collected from aborted lambs while not detected in any of blood samples collected from aborted goats. The prevalence rates of pestivirus founded in tissue samples in the present study were founded in agreement with rate reported by Burgu et al.[23], but high than one reported by Çokçalışkan [25]. In blood samples collected sheep, this rate was founded lower than ones reported by Burgu et al.[23] and Oğuzoğlu et al.[26]. Although the presence of pestivirus antigen by ELISA was found in three of blood samples collected from goats by Oğuzoğlu et al.[26], we could not detected pestivirus antigen by ELISA in blood samples collected from goats. Pestivirus antigen by ELISA in aborted goats could not be also detected in the study done in Eastern and Southeastern Anatolia in Turkey by Ataseven et al.[27] and being in agreement with the results of this study.

In Turkey, too many serologic studies in cattle have been carried out about pestivirus infections that are seen as widespread in all over the world, but too many studies in small ruminants have not about pestivirus infections. In this study, the positivity rate was found as 64.6% (475/735) in aborted sheep and 5.7% (2/35) in aborted goats. In a seroepidemiological study conducted in sheep

and goats in various areas of Quebec by Lamontagne and Roy [18], the seroprevalence rates of BVD were found as 10.9% (73/699) in sheep and 16% (16/100) in goats. Krametter-Froetscher et al. found a seropositivity rate of 29.4% (1448/4931) in sheep in Austria [22]. In another study, the same investigators found a seropositivity rate of 11.5% (63/549) in goats in Austria [21]. They reported that the seroprevalence rates of pestivirus in sheep and goats were significantly higher on farms with cattle. The seroprevalence rate of pestivirus observed in sheep in the present study was high than ones reported by Lamontagne and Roy [18], Krametter-Froetscher et al.[22]. In this study, this rate in goats was found a little low than ones reported by Lamontagne and Roy [18], Krametter-Froetscher et al.[21]. But, the seroprevalence rate of pestivirus in goat flocks with abortion history in the southeast region of Turkey has been found high (30.2%) [27]. The results of investigation done in the southeast region of Turkey have show that pestivirus infections in goats in this region are more widespread.

The seroprevalence rates of BVD were found 42.8% (232/541) in aborted sheep by Burgu et al.[23], 36.3% (160/440) in healthy sheep by SNT by Şimşek et al.[28], 41.33% (31/75) in pregnant sheep by SNT by Çokçalışkan [25] and 18.94% (463/2444) in healthy sheep by SNT by Okur-Gümüşova et al.[29]. We found higher rates than ones reported above. But, in this study, high seropositivity detected for pestivirus could be because of cross reaction known between BVDV and BDV. In above studies, the BVDV seroprevalence, but not BDV seroprevalence, in sheep has been investigated by using the cytopathogenic BVDV-NADL strain. In addition, many factors such as the structure of herd sampled, individual and regional differences as well as sensitivity of test used may affect the results. Okur-Gümüşova et al.[29] reported that among the prevalence rates of pestivirus in coastal and inland areas of Turkey were significantly differences due to climate characteristics and climate factors could play a major role in virus spread.

In Burdur province, the presence of pestivirus in cattle was reported in a previous serologic study carried out by Kale et al.[30]. Both BVDV and BDV can infect sheep and cattle. Vilcek et al.[31]. demonstrated that sheep may naturally be infected not only with BDV, but also with BVDV types I and II. Berriatua et al.[32] reported that BDV prevalence in sheep could be a major risk factor for pestiviral infection of other species, particularly cattle that share communal pastures with sheep. The spread between the host species create problem in the struggle of disease [15]. In this region, this situation should be taken into account in control studies and farmers should be informed with respect to BDV infections. Besides, in this study, sheep in some of flocks sampled were observed to be kept and to be pastured together with cattle.

The genetic typing of pestiviruses isolated from cattle in Turkey has shown that a new subgroup of BVDV-1 is predominant and widespread [33]. In addition, the genetic characterization of pestiviruses isolated from sheep and goats in Turkey has revealed that the isolates belong to a new BDV subgroup being different from the known BDV subgroups [26]. Moreover, an ovine isolate was identified as first member of BVDV-2 reported in Turkey by Yeşilbağ et al.[33]. In addition, the same investigators indicated the presence of BVDV-2 in cattle serologically.

Pestivirus control is based on identifying and eliminating PI animals and preventing infection of susceptible pregnant ewes. As a control strategy, it may be recommended that persistently infected animals can be kept together with the other animals in the flock prior to gestation to engender flock immunity and to avoid exposing sheep or goats to infection during pregnancy. Close herding for at least 3 weeks, preferably indoors, is necessary for pestivirus to spread effectively, but exposure should stop two months before the start of the breeding season [1].

In conclusion, the results of investigation indicate that natural infection with pestivirus is more widespread in sheep in Burdur vicinity and reveal that pestivirus infections have to be considered as one of the major pathogens in the cases of abortion seen in this region. Also, the widespread distribution of pestiviruses in the region requires the implementation of pestivirus control programmes based on identifying and eliminating PI animals and highlights the potential risk of sheep as a pestivirus reservoir for other species when the risk of spread between species is considered. Further investigations are needed to truly understand the role of goats in the epidemiology of disease in Burdur province and the effect of pestiviruses in the cases of abortion seen in goats in this region.

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