

## Peste Des Petits Ruminants (PPR) Virus Infections in Goats in the Eastern Anatolia of Turkey

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### Summary

In this study, a totally 98 materials were used consist of different samples such as blood, oro-nasal swap, lung, spleen and lymph node from goats (n=38) in the 28 flocks suspected the PPRV infection as clinically and macroscopic pathologic remarks. The goats that used in this study housed in the 11 different provinces of Eastern Anatolia. RT-PCR was used for the diagnosis of PPRV infection. PPRV nucleic acid was detected in 50 of 98 materials by RT-PCR. According to the results of RT-PCR, the PPRV nucleic acid was detected in 39.2% (11/28), 44.7% (17/38) and 45.4% (5/11) of the flocks, sampled animals and provinces in eastern anatolia, respectively. Diagnostic value of necropsy materials such as lymph node, spleen, lung and of clinical samples such as oro-nasal swap and blood were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR. Data showed that PPRV infections of the goats were widespread in the Eastern Anatolia region. Additionally, it is determined that RT-PCR is sensitive and reliable method in the diagnosis of PPRV infections.

**Keywords:** Goat, PPR, RT-PCR

## Türkiye'nin Doğu Anadolu Bölgesindeki Keçilerde Peste Des Petits Ruminants (PPR) Virus Enfeksiyonları

### Özet

Bu çalışmada, klinik ve makroskopik patolojik olarak PPRV enfeksiyonundan şüphelenilen 28 farklı sürüdeki keçilerden (n=38) alınan kan, oral-nazal swap, akciğer, dalak ve lenf nodülü gibi farklı örneklerden toplam 98 materyal kullanıldı. Bu çalışmada kullanılan keçiler Doğu Anadolu Bölgesi'nin 11 farklı ilinde yetiştiriliyordu. PPRV enfeksiyonunun teşhisinde RT-PCR kullanıldı. RT-PCR tekniğiyle 98 materyalin 50'sinde PPRV nükleik asidi belirlendi. RT-PCR sonuçlarına göre, PPRV nükleik asidi sürülerin %39.2'si (11/28), örneklenen hayvanların %44.7'si (17/38) ve Doğu Anadolu Bölgesinde örnekleme yapılan illerin %45.4 (5/11)'inde tespit edildi. RT-PCR ile PPRV enfeksiyonunun teşhisinde oral-nazal swap ve kan gibi klinik örnekler ve lenf nodülü, dalak ve akciğer gibi nekropsi materyali teşhis değeri yüksek materyal olarak belirlendi. Bu bilgiler keçilerin PPRV enfeksiyonunun Doğu Anadolu'da yaygın olduğunu ve PPRV enfeksiyonunun teşhisinde RT-PCR'in duyarlı ve güvenilir bir metod olduğunu gösterdi.

**Anahtar sözcükler:** Keçi, PPR, RT-PCR

### INTRODUCTION

Peste des petits ruminants (PPR) is associated with PPRV, a morbillivirus (family Paramyxoviridae) closely related to the rinderpest virus as well as the viruses of canine distemper in dogs, phocine distemper in seals, and measles in human <sup>1</sup>. PPR, also known as goat plague, kata and pseudo-enteritis complex <sup>2-5</sup>, is similar clinically to rinderpest. The diseases occurs mostly in goats and sheep. Infection rates in sheep

and goats rise with age, and rapidly fatal in young animals <sup>6,7</sup>. PPRV infection results is an acute, highly contagious disease, and characterized by fever, anorexia, necrotic stomatitis, ulceration of mucous membranes, and inflammation of the gastrointestinal tract leading to severe diarrhoea, purulent ocular and nasal discharges, and respiratory distress <sup>6,8,9</sup>. Morbidity and mortality rates vary but may reach 90-100%.



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These rates are usually lower in endemic areas, where mortality may be 20% or less, and serosurveillance is sometimes the only indicator of infection<sup>6,9</sup>. Four lineages of PPRV have been identified; lineage 1 and 2 viruses in west Africa, lineage 3 in east Africa, Arabia and southern India, and lineage 4 in the Middle East and Asia subcontinent, reaching east as far as Nepal and Bangladesh. PPR was first described<sup>10</sup> in West Africa and for 30 years was thought to be confined to this area. The disease has since been recognized as endemic in West and Central Africa<sup>11</sup> and in the north-east of the continent, Sudan<sup>9,12,13</sup>, Kenya and Uganda<sup>14</sup> and Ethiopia<sup>15,16</sup>. In 1987 it appeared in the Middle East and has since then been confirmed in Jordan<sup>17</sup>, Pakistan<sup>18</sup>, southern India<sup>19,20</sup>, Turkey<sup>21-23</sup> and Israel<sup>15</sup>.

The existence of PPR infection in Turkey was declared officially in 1999<sup>8</sup> but, previous reports of the presence of the virus in the country<sup>24,25</sup>, and since then many outbreaks have been reported<sup>21-23,26,27</sup>. Ozkul et al.<sup>21</sup> isolated PPR viruses from 2 separate field cases in Turkey. These viruses belonged to lineage 4, to which goats seem to be more susceptible than sheep. The disease results in high mortality, especially among young goats, although the frequency of the disease is higher in older goats<sup>22,28</sup>.

In 2004, an outbreak of PPR was detected in Thrace, i.e., the European part of Turkey<sup>29</sup>. As in rinderpest, close contact with an infected animal or contaminated fomites is required for the disease to spread. Large amounts of the virus are present in all body excretions and secretions, especially in diarrhetic feces. Infection is mainly by inhalation but could also occur through the conjunctiva and oral mucosa<sup>1</sup>.

Laboratory confirmation of suspected cases is necessitated by the clinical similarity of rinderpest. Enzyme-linked-immunosorbent assay (ELISA) is now routinely used<sup>30,31</sup>. Virus isolation and differential neutralization in cell culture are slow, tedious and of low efficiency. Immunocapture<sup>32</sup> and reverse-transcription polymerase chain reaction (RT-PCR) followed by nucleotide sequencing<sup>33-35</sup> are the current diagnostic methods for all morbillivirus infections.

In this study, we investigated the prevalence of PPRV infection in goats by regions in east of Turkey and to assess the diagnostic values of the virus isolation and RT-PCR techniques in the diagnosis of PPR infections.

## MATERIAL and METHODS

From 01.06.2010 to 25.03.2011, a totally 98 materials (24 lymph node, 6 nasal and oral swap, 26 lung, 22 spleen and 20 defibrinated blood) from goats (n=38) in the 28 flocks suspected in the PPRV infection as clinically, housed in the 11 different provinces east of Turkey (Adıyaman, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkari, Malatya, Muş, Şırnak, Siirt, Tunceli). Collected samples from 28 flocks different suspected to have PPR were submitted to the Elazığ Veterinary

Control and Research Institute for necropsy and virological examination.

**Detection of PPR virus:** RT-PCR was performed for the detection of PPR virus. The reaction was carried out with a PPRV-specific primer set (PPRVF1b: AGTACAAAAGAT TGCTGATCACAGT and PPRVF2d: GGGTCT CGAAGG CTAGGC CCGAATA) originally designed by Forsyth and Barrett<sup>34</sup> to amplify a 448-bp cDNA product from the F gene. A lyophilized live PPR vaccine, produced by the Etlik Veterinary Control and Research Institute Ankara, Turkey, was used as the positive control. RNA was extracted from the positive control or tissue homogenate from the field samples using RNeasy Mini Kit<sup>36</sup>, (Qiagen, Germany) according to manufacturer's protocol. The RT-PCR was performed with Qiagen One- Step RT-PCR kit (Qiagen, Germany). The 20 µL reaction mixture contained 7 µL Molecular Grade Water, 0.8 µL 10 pmol of forward and reverse primers, 4.0 µL buffer, 0.8 µL dNTP mix, 0.8 µL enzyme mix, 4.0 µL 5x Q-Solution, 2.6 µL template RNA. The thermocycling profile was as follows: reverse transcription at 50°C for 30 min, initial denaturation and activation of polymerase at 94°C for 15 min, followed by 35 cycles of denaturation, annealing and extension at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, respectively, and final elongation at 72°C for 7 min (Thermal Cycler, Techne Plus). The RT-PCR products were analysed by electrophoresis at 80V for 2 h on 1.5% agarose gel stained with ethidium bromide. PCR products with a molecular size of 448 bp were considered indicative for PPRV<sup>34</sup>.

Goats with clinical signs of PPRV infection were developed fever, anorexia, dehydration, dullness, mucopurulent ocular nasal discharge, lacrimation, conjunctivitis, dyspnea and diarrhea. Most clinical cases of mouth, erosion, ulceration and necrosis on the lip, gingiva, buccal cavity, tongue and palate.

Blood samples were collected from jugular vein in EDTA containing and nonheparinised vacutainer tubes for analysis RT-PCR and C-ELISA. Blood samples were centrifuged for 10 min at 1500 rpm and destined with pipette the buffy coat than buffy coat stored in -80°C until used. Oro-nasal swaps centrifuged at 3.000 xg for 5 min remove the suspended solids. The supernatants were stored at -80°C until used. Necropsy was performed on short times after death, and tissue samples for virologic analyses; fresh samples of the lung, spleen, and lymph nodes. were placed in 2 mL of PBS diluent (1/10 w/v) with MagNA Lyser Green Beads (Roche, Mannheim, Germany) and were homogenized at 3.000xg for 3 min by MagNA Lyser (Roche, Mannheim, Germany). Homogenates were centrifuged in Eppendorf tubes at 12.000xg for 3 min to remove the suspended solids, without removing the beads. The supernatants were stored at -80°C until assayed with reverse transcriptase-polymerase chain reaction (RT-PCR).

Competitive enzyme linked immunosorbent assay (C-ELISA) was used for serological detection of PPRV specific antibodies. Serum was collected from 15 clinically ill kids

analysed for the detection of antibodies against to PPR by a commercial C-ELISA kit (ID vet-veterinary diagnostic kits, France). The ELISA was performed according to the manufacturer's instruction as described elsewhere <sup>37</sup>.

## RESULTS

Informations belong to focal no and name, the number of sick animals in accepted to laboratory, the number of sampled material and sampling time of the goats in flocks of PPRV positive determined was showed [Table 1](#).

Eleven different provinces brought goat flocks (n=38) with PPRV infection suspicious in the east of Turkey was shown in [Fig. 1](#).

PPRV antibodies were detected by C-ELISA in all serum samples of clinically sick kids. PPRV was isolated and identified in RT-PCR from the tissue, such as blood samples and oro-nasal swabs collected from all the sick goats. Moreover we can observe the specific 448 bp band obtained from the DNA amplification of F protein-coding gene using the primers PPRVF1b: AGTACAAAAGATTGCTGATCACAG T and PPRVF2d: GGGTCTCGAAGG CTAGGCCCGAATA ([Fig. 2](#)).

## DISCUSSION

Common clinical signs of infection with PPRV such as high fever, anorexia, necrotic stomatitis, dehydration, ulceration of mucous membranes and inflammation of the gastrointestinal tract leading to severe diarrhea, purulent ocular

**Table 1.** Informations belong to focal no and name, the number of sick animals in accepted to laboratory, the number of sampled material and sampling time of the goats in flocks of PPRV positive determined

**Tablo 1.** PPRV pozitif belirlenen sürülerdeki keçilerin örnekleme zamanları, örneklenen materyal sayısı, laboratuara kabul edilen hasta hayvanların sayısı, odak numarası ve odak isimlerine ait bilgiler

Focal No	Focal Name	The Number of Sick Animals in Accepted to Laboratory	The Number of Sampled Material	Sampling Time
1	Adıyaman	2	8	01.06.2010
2	Bingöl	1	5	05.09.2011
3	Bitlis	1	3	05.09.2011
4	Bitlis	1	2	22.06.2011
5	Bitlis/Mutki	1	3	23.03.2011
6	Diyarbakır/Çermik	2	10	07.07.2011
7	Elazığ	3	3	30.03.2011
8	Elazığ/Baskil	1	4	20.07.2011
9	Elazığ	1	4	19.09.2011
10	Elazığ/Palu	1	1	10.10.2010
11	Elazığ	1	3	07.02.2011
12	Elazığ/Keban	1	1	26.04.2011
13	Elazığ/Palu	1	1	23.03.2011
14	Elazığ	1	2	02.12.2010
15	Hakkari	3	3	07.07.2011
16	Malatya/Doğanşehir	1	2	05.09.2011
17	Malatya/Yeşilyurt	3	9	21.06.2011
18	Malatya	1	1	20.07.2011
19	Malatya/Arapkir	1	3	30.05.2011
20	Malatya/Arapkir	1	3	25.03.2011
21	Malatya	1	3	13.04.2011
22	Malatya/Yeşilyurt	1	3	25.03.2011
23	Muş	3	3	03.09.2011
24	Şırnak	1	3	30.03.2011
25	Siirt	1	3	30.03.2011
26	Tunceli/Mazgirt	1	3	22.06.2011
27	Tunceli/Hozat	1	5	07.02.2011
28	Tunceli	1	4	25.03.2011
<b>TOTAL</b>		<b>38</b>	<b>98</b>	<b>2010-2011</b>

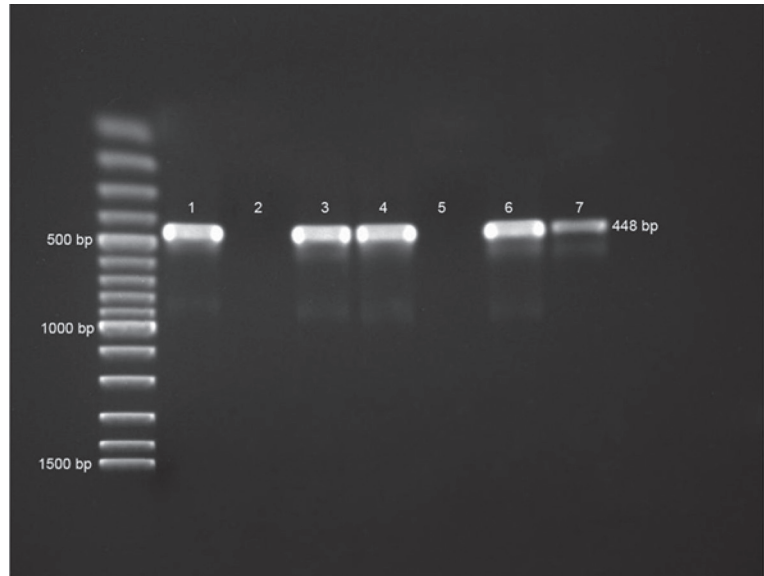


**Fig1.** Eleven different provinces brought goat flocks (n=38) with PPRV infection suspicious in the east of Turkey (The provinces were shown with bold)

**Şekil 1.** Türkiye'nin Doğusu'ndaki 11 farklı ilden PPRV enfeksiyonu şüphesiyle getirilen keçi sürüleri (n=38) (İller koyu ile gösterildi)

**Fig 2.** Agarose Gel Electrophoresis of PPRV. PCR Positive and Negative Specimens and Controls. Line 1-Positive Control, Line 2-Negative Control, Line 3- Positive Lymph Node Sample, Line 4- Positive Lymph Node Sample, Line 5-Negative Sample, Line 6-Positive Blood Sample, Line 7-Positive Oral-Nasal Swap Sample

**Şekil 2.** PPRV' nin Agarose Gel Electrophoresisi. PCR pozitif ve negatif örnekler ve kontroller. Çizgi 1- Pozitif Kontrol, Çizgi-2 Negatif Kontrol, Çizgi 3- Pozitif Lenf Nodülü Örneği, Çizgi 4- Pozitif Lenf Nodülü Örneği, Çizgi 5- Negatif Örnek, Çizgi 6- Pozitif Kan Örneği, Çizgi 7- Pozitif Oral-Nasal Swap Örneği



and nasal discharges, lacrimation and respiratory distress were observed in the present study which were also reported elsewhere <sup>38-42</sup>.

PPR has been reported in a number of countries in the region including Pakistan, Nepal, Bangladesh, Turkey, Afghanistan, Iran and India <sup>20,24,43,44</sup>. Although the clinical and postmortem findings may be sufficient for the diagnosis of PPR in the endemic areas, yet laboratory confirmation is essential for definitive diagnosis. We were reveal clinical and virologic findings all of animals suspected to have PPR were submitted to the Elazığ Veterinary Control and Research Institute for examination.

Forsyth and Barret <sup>34</sup> reported that viral nucleic acid was

detected in the samples of blood, conjunctival swap, lymph nodule and spleen, however no viral nucleic acid was detected in the samples of lung, oral swap and nasal swap by RT-PCR. They reported that sample of lymph nodule should be preferred to the other samples as the sample of necropsy. Similarly, Albayrak and Alkan <sup>45</sup> reported that the PPRV nucleic acid from nasal and conjunctival swap samples from the infected animals were detected, but no viral nucleic acid was detected in the samples of blood samples and oral swap by RT-PCR. They reported that the samples of nasal and conjunctival swaps are more valuable as the diagnostic material from animals with clinical symptoms. We determined that the diagnostic value of necropsy materials such as lymph node, spleen, lung, oro-nasal swap

and blood were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR. In the our study, different materials such as blood, swap samples (nasal, oral) and tissue samples are used in the diagnosis of PPRV infection. Positivity rates as 54.2% (13/24), 66.6% (4/6), 45.0% (9/20), 46.2% (12/26), 46.2% (12/26) were detected in the samples of lymph nodule, nasal and oral swap, blood, lung, spleen respectively. The results of our study were similar reveal with results of other investigations.

The goats seem to be more susceptible than sheep and the disease results in high mortality, especially among young goats, although the frequency of the disease is higher in older goats<sup>6,7,22,28</sup>. Similarly, the goats of the age was between 7 day and 6 month age in the study. Sarker et al.<sup>46</sup> investigated the prevalence of PPR according to age categories and they have identified as prevalence of PPR in goats with age categories adult (>1 year), young (between 4 to 12 months) and sucklers (between 1 to 3 months) was found to be 10.2%, 31.1% and 13.1%, respectively. In the present study we also have identified as the prevalence of PPR in goats with age categories adult (>1 year), young (between 4 to 12 months) and sucklers (between 1 to 3 months) was found to be 2.6%, 60.5% and 36.8%, respectively. Singh et al.<sup>47</sup> also assessed that the disease is most prevalent in the goats less than one year. According to these report, we thought that the increased susceptibility of young goats were might be due to malnutrition, poor immunity and poor examine the affected goats management systems.

In the Turkey, Eastern of Anatolia is one of the regions made intensive of livestock. Region has 34.3% about of small ruminants in the Turkey<sup>48</sup>. As Eastern Anatolia shares a border with five Middle East countries, it is possible that the virus entered Turkey via uncontrolled animal movement from neighboring countries where PPRV is endemic.

Previously, the clinic and laboratory studies in sheep and goats for PPR were applied different province of Turkey such as Afyonkarahisar<sup>49</sup>, Burdur<sup>50</sup>, Mardin<sup>51</sup>, Batman, Denizli, Cihanbeyli (Konya), Amasya, Sakarya Eskişehir, Malatya, Sivas, Isparta, Van, Aydın, Muğla<sup>21,22</sup>, Edirne<sup>29</sup>, Kırıkkale<sup>52</sup> Samsun, Sinop, Amasya, Tokat, Rize, Trabzon, Giresun, Ordu<sup>45</sup>, and gazelle in Ceylanpınar (Şanlıurfa)<sup>53</sup>. Although, the PPR investigation was made in Eastern Anatolia province such as Malatya, Elazığ ve Van<sup>21</sup>, this study was more comprehensive related to Eastern Anatolia province such as Adıyaman, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkari, Malatya, Muş, Şırnak, Siirt and Tunceli.

Albayrak and Alkan<sup>45</sup>, PPR nucleic acid detected in 26 of 124 materials by RT-PCR. According to the results of RT-PCR, the PPRV infections were diagnosed in 44.1% (34/51) and 31.5% (18/57) of the flocks and sampled animals, respectively. Similarly, in the present study, PPRV nucleic acids were detected in 50 of 98 materials by RT-PCR. According to the results of RT-PCR, the PPRV infections were diagnosed in 39.2% (11/28), 44.7% (17/38) and 45.4% (5/11) of the

flocks, sampled animals and provinces in Eastern Anatolia, respectively. Data showed that PPRV infections of the goats were widespread in the east anatolia region. Additionally, it is determined that RT-PCR is sensitive and reliable method in the diagnosis of PPRV infections.

PPRV infection in Turkey needs continuous screening by reliable diagnostic systems as pointed out elsewhere. Meanwhile, adequate prophylactic approaches using local virus strains should be considered as part of the control and eradication policies. This will help in immobilizing and suppressing infections before the spread from neighboring countries.

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