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SHORT COMMUNICATION

Molecular Detection of Peste des Petits Ruminants Virus from Different Organs/Tissues of Naturally Infected Animals [1]

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Summary

PPR virus (PPRV) detection rate in different organs/tissues of naturally infected sheep and goats was investigated. In order to achieve this, 24 animals, each from different flocks, with PPR suspect were examined for the presence of PPRV nucleic acid in different organ/tissues by reverse transcriptase PCR (RT-PCR) and real time RT-PCR. Virus neutralizing test (VNT) was used for the determination of PPRV specific antibody response. Real time RT-PCR and RT-PCR were found positive in all of lung, spleen, liver and lymph node samples tested in each of 16 VNT-positive animals and were negative in all samples from VNT-negative animals (n=8).

Keywords: *Peste des petits ruminants virus, Tissues, RT-PCR, Real time RT-PCR*

Doğal Enfekte Hayvanların Farklı Organ/Dokularında Koyun ve Keçi Vebası Virusunun Moleküler Tespiti

Özet

Doğal enfekte koyun ve keçilerin farklı organ/dokularından PPR virusunun (PPRV) tespit oranının araştırılmasının amaçlandığı bu çalışmada, her biri farklı sürüye ait PPR şüpheli 24 hayvan, farklı organ/dokularda PPRV varlığı yönünden konvansiyonel reverz transkriptaz polimeraz zincir reaksiyonu (RT-PCR) ve real time RT-PCR yöntemleri ile incelenmiştir. PPRV spesifik antikor yanıtını belirlemek için virus nötralizasyon testi (VNT) kullanılmıştır. Real time RT-PCR ve RT-PCR yöntemleri ile test edilen 16 VNT pozitif hayvanın bütün akciğer, dalak, karaciğer ve lenf nodülü örnekleri pozitif olarak tespit edilirken, VNT negatif hayvanlardan (n=8) elde edilen bütün akciğer, dalak, karaciğer ve lenf nodülü örnekleri negatif olarak belirlenmiştir.

Anahtar sözcükler: *Koyun ve keçi vebası virusu, Dokular, RT-PCR, Real time RT-PCR*

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants that is characterized by fever, an erosive stomatitis, broncho-interstitial pneumonia, diarrhoea and enteritis [1]. Clinical disease is seen in sheep and goats, and seroprevalence rate in sheep and goats rises with age. However, cattle, buffaloes and camels can become infected but there is little or no evidence of symptoms associated with their infection [2].

The causative agent, peste des petits ruminants virus (PPRV), is a member of the *Morbillivirus* genus in the *Paramyxoviridae* family. It is antigenically closely related to rinderpest virus (RPV), measles virus (MeV), canine distemper virus (CDV), and morbilliviruses of marine mammals [3]. Genetically, PPRV isolates can be grouped into four distinct lineages on the basis of partial sequence analysis of the fusion protein (F) and nucleoprotein (N) genes [4].

PPR occurs in West and Central Africa, Central and Southern Asia, the Middle East and Arabia [5]. It was first histochemically described in Turkey in 1993 [6]. Sequence analysis on the basis of the F gene of the isolates from

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outbreaks of PPR in Turkey has revealed that the virus was closely related to the members of lineage 4 [7].

Competitive ELISA and virus neutralization are routinely used for serological diagnosis of PPR, but gold-standard test remains the virus neutralizing test [8]. Virus isolation and differential neutralization in cell culture are slow, takes 2-3 weeks to complete, and of low efficiency. With advances in molecular biology, new diagnostic tests like PCR are available for detecting PPRV genomic material. Reverse transcription-PCR (RT-PCR) provides rapid, sensitive and reliable diagnosis of the disease [9]. However, these are being replaced by more sensitive and robust real time RT-PCR assays [10]. Organ (lymph node, spleen, lung, and liver) and swab specimens (ocular and nasal) can be used in PCR for the detection of PPRV genomic material [9]. In routine diagnostic laboratories all organ/tissues and swab specimens are not always available for testing. The aim of this study was to investigate PPR virus detection rate in different organs/tissues of naturally infected sheep and goats by reverse transcriptase PCR (RT-PCR) and real time RT-PCR. We used these two tests to compare the obtained results for each samples.

MATERIAL and METHODS

Samples and Positive Control

During January 2011 and May 2012, 24 animals, each from different flocks in 4 provinces (Konya, Antalya, Aksaray and Niğde), suspected to have PPR were submitted to the Veterinary Control Institute, Konya, Turkey. Blood serum samples were collected before necropsy. Except lymph nodes other organs/tissues were collected from all of cases. All tissue samples were kept at -85ºC prior to sample preparation and the RT-PCR assays. A total of 86 tissue samples of lung (n=24), liver (n=24), spleen (n=24) and mesenteric lymph nodes (n=14) collected from 24 animals (15 sheep and 9 goats), aged between 1 and 24 months, were tested. Lyophilized freeze-dried live PPR vaccine (Nigeria75/1 vaccine strain) obtained from the Division of Virology, Etlik Central Veterinary Control and Research Institute, Ankara, Turkey, was used as the positive control.

Virus Neutralizing Test

A virus-neutralization test for PPRV was performed in microtiter plates according the OIE recommendations [8]. Sera with VNT titres of >1:10 were considered positive.

RNA Extraction and Conventional RT-PCR

Viral RNA was extracted from tissue samples (25-30 mg) using a robotic extraction method (MagNA Pure LC 2.0 System, Roche Applied Science, Indianapolis, IN, USA) with the Magna Pure LC total nucleic acid isolation kit. One-step RT-PCR was used for the detection of PPRV RNA. Primers (PPRVF1b: 5´AGTACAAAAGATTGCTGATCACAGT and PPRVF2d: 5´GGGTCTCGAAGGCTAGGCCCGAATA) based on F protein coding gene of virus which amplify a 448 bp product [7] were used.

One-step Real Time RT-PCR

Real time RT-PCR amplification and detection was performed using LightCycler 2.0 real time PCR machine (Roche Applied Science, Indianapolis, IN, USA) with the one step RT-PCR kit (Qiagen, Germany). The primers in the N region PPRVF: AGAGTTCAATATGTTRTTAGCCTCCAT and PPRVR: TTCCCCARTCACTCTYCTTTGT and probe: Fam-CACCGGAYACKGCAGCTGACTCAGAA Tamra were used [10]. The samples that had a Ct value <35 were considered positive.

RESULTS

Of 24 sera tested, 16 (66.6%) were antibody-positive for PPRV by VNT. CPE of PPRV/N75/1 was observed at 4 days post-inoculation in wells. RT-PCR was found positive in all of lung, spleen, liver and lymph node samples tested in each of positive animals (n=16) and was negative in all samples from negative animals (n=8). The positivity rates were 100% (16/16), 100% (16/16), 100% (16/16) and 100% (11/11) for lungs, livers, spleens and lymph nodes, respectively *(Table 1)*.

The amplification of PPRV specific 448 bp fragment from nucleic acid of test samples and positive control (vaccine strain Nigeria75/1) were described as positive reaction *(Fig. 1)*.

Of the 86 tissue samples, 59 samples collected from positive animals (n=16) were positive for PPRV by real time RT-PCR and Ct values ranged from 18 to 33. All the 27 tissue samples collected from negative animals (n=8) were found negative for PPRV. Ct value of positive control (vaccine strain Nigeria75/1) was found 25. *Table 2* presents the mean Ct and SD values of tissue samples examined.

DISCUSSION

PPR was officially reported to OIE in Turkey for the first time in September 1999 [11], but there had been previous reports on its occurrence [6]. Serological studies carried out in Turkey show that prevalence of PPRV infection varies between 8.39% and 47.17% [7,12].

PPR can be confused clinically with contagious caprine pleuropneumonia (CCPP) or pasteurellosis, and hence the clinical observations for both diseases should always be confirmed by a laboratory test. Serological assays such as ELISA designed to detect the presence or absence of antiviral antibodies are used for confirmation of freedom from disease [8]. Neutralization and isolation of virus in cell culture is technically difficult and time-consuming and

Table 1. *RT-PCR results in various tissues of sheep and goats with PPR*
Tablo 1. *PPR ile enfekte koyun ve keçilerin farklı dokularının RT-PCR sonuçları*

Case	Species	Oral Cavity Lesions	RT-PCR Findings			
			Lung	Spleen	Liver	Lymph Node
1	Sheep (lamb)	p[a]	p	p	p	*[b]
2	Sheep (lamb)	p	p	p	p	*
3	Sheep (lamb)	p	p	p	p	*
4	Sheep (lamb)	p	p	p	p	p
5	Sheep (lamb)	p	p	p	p	p
6	Sheep (lamb)	p	p	p	p	p
7	Sheep	p	p	p	p	*
8	Sheep	p	p	p	p	p
9	Sheep	p	p	p	p	p
10	Sheep	p	p	p	p	p
11	Sheep	p	p	p	p	p
12	Sheep	p	p	p	p	p
13	Goat	p	p	p	p	p
14	Goat	p	p	p	p	p
15	Goat	p	p	p	p	p
16	Goat	p	p	p	p	*

[a] *Positive;* [b] *Not examined*

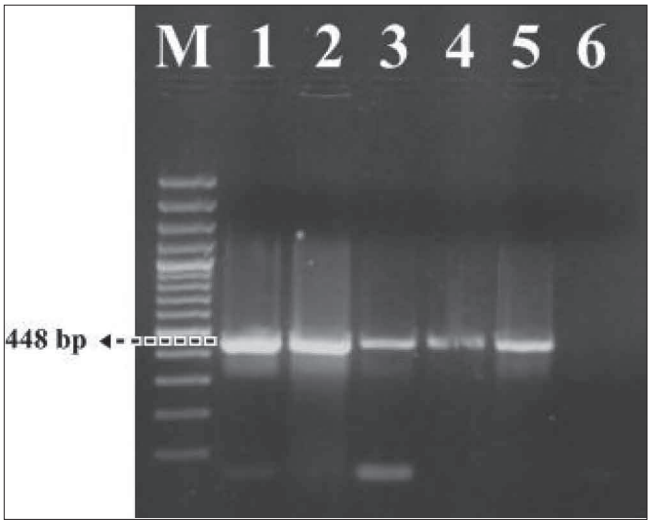

Fig 1. Agarose gel electrophoresis of RT-PCR product based on F gene of PPRV, **M:** Molecular marker of 100 bp, **Lane 1:** Positive control (Nigeria75 /1), **Lane 2-5:** Sample of lymph node, lung, liver, spleen, respectively, from the same animal, **Lane 6:** Negative control. Lymph nodule bands were comparably stronger than other tissue samples

Şekil 1. PPR virusunun F genine dayalı RT-PCR ürünlerinde agaroz jel elektroforezi, **M:** 100 bp moleküler marker, **1:** Pozitif kontrol (Nigeria75 /1), **2-5:** Sırasıyla aynı hayvana ait lenf nodülü, akciğer, karaciğer ve dalak örnekleri, **6:** Negatif kontrol. Lenf nodülü bandları diğer doku örneklerininkinden nispeten güçlü idi

Table 2. *Cycle threshold (Ct) values of tissue samples*
Tablo 2. *Farklı dokuların eşik döngüsü (Ct) değerleri*

Tissue Samples	n[a]	Mean Ct	± s.e.m[b]	SD[c]
Lung	16	25.73	1.45	5.03
Liver	16	26.04	1.19	3.77
Spleen	16	24.87	1.37	3.88
Lymph node	11	22.49	0.92	2.44

[a] *Number of samples;* [b] *Standard error of mean;* [c] *Standard deviation*

thus is not suitable as a routine diagnostic assay [4]. As an alternative to isolation, several RT-PCR and real time RT-PCR assays based on the fusion, the nucleoprotein, or the matrix protein genes were developed for the rapid and specific detection of PPRV [4,13]. In this study, one conventional RT-PCR targeting the F gene and one real time RT-PCR targeting the N gene were used to determine whether the organ/tissues had viral RNA.

For diagnosis of PPR, samples of conjunctival discharges, nasal secretions, buccal and rectal mucosae and anticoagulant-treated blood from live animals or lymph nodes, especially the mesenteric and bronchial nodes, lungs, spleen and intestinal mucosae from necropsied animals are recommended [8]. Success of detecting the virus in blood depends on the time of sampling (viraemic phase) and there are more chances of missing the presence of PPRV in the blood. Mahajan *et al.*[14] reported that the ocular and nasal swab samples are most valuable diagnostic material in case of live animals whereas tissue samples should be preferred in case of dead animals for diagnosis of PPRV infection. In a previous study, Albayrak and Alkan [15] have observed that maximum positive rate with tissue samples (50%, 22.58% and 17% in lymph nodes, spleen and lungs, respectively) followed by nasal (25%) and conjunctival (10%) swab samples whereas no blood and oral swab samples were positive for PPRV nucleic acid by RT-PCR.

Most of the time, tissue samples from necropsied animals have been submitted to the Division of Molecular Microbiology, Veterinary Control Institute, Konya, Turkey for molecular detection of PPRV. All required tissues/ organs are not available in every cases. Therefore, in this study, we aim to investigate detection rate of PPRV in different tissue samples (lungs, livers, spleens and lymph nodes) by RT-PCR and real time RT-PCR. RT-PCR and real time RT-PCR were found positive in all of lung, liver, spleen and lymph nodes samples tested in each of positive animals (n=16) and were negative in all samples from negative animals. Furthermore, the results of F gene-based conventional RT-PCR were consistent with the results of N gene-based real time RT-PCR. Therefore, these two methods are suitable for PPR diagnosis.

In the study, different organ/tissues samples from necropsied animals were used for diagnosis of PPRV

infection in small ruminants and the diagnostic value of lungs, livers, spleens and lymph nodes were assessed with RT-PCR and real time RT-PCR assays and it was concluded that in molecular diagnosis of PPR infection lung, liver, spleen and mesenteric lymph node samples can equally be used for PPR virus detection.

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