Kafkas Univ Vet Fak Derg
15 (3): 423-428, 2009
DOI:10.9775/kvfd.2009.024-A

RESEARCH ARTICLE

Immunohistochemical Detection of Peste des Petits Ruminants (PPR) Viral Antigen from the Cases of Naturally Occurring Pneumonia in Sheep [1]

Yavuz Selim SAGLAM * Ahmet TEMUR **

[1] This research was supported by Research Fund of Atatürk University (BAP, 2004/230)
* Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum - TURKEY
** Veterinary Control and Research Institute, Pathology Lab, Erzurum - TURKEY

Makale Kodu (Article Code): 2009/024-A

Summary

In this study, the presence and distribution of Peste des Petits Ruminants (PPR) viral antigen in sheep pneumonia were investigated in Erzurum province. For this purpose, a total of 70 pneumonic sheep lungs were examined both histopathologically and immunohistochemically. Tissue sections were routinely stained with hematoxylin and eosin and, immunohistochemical staining procedure. The presence of PPR viral antigen was detected in 8 (11.42%) out of 70 pneumonic lungs Microscopic findings as necrotic bronchitis, bronchiolitis, syncytial cells of pulmonary tissues together with viral intracytoplasmic or intranuclear inclusion bodies were observed in some samples of lungs. PPR viral antigen was detected frequently in the cytoplasm and rarely in the nucleus, including the luminal surfaces and in the cytoplasm of alveoli epithelial cells, bronchial glands, bronchial and bronchiolar epithelial cells, syncytial cells and alveolar macrophages. Viral antigen was seen mostly in lungs with interstitial pneumonia. The results of the present study showed that the presence of PPR in pneumonic lungs of sheep obtained was 11.42% in the region of Erzurum and it has a role in aetiological factors in naturally occurring pneumonia in sheep.

Keywords: *Peste des petits ruminants, Immunohistochemistry, Sheep*

Doğal Enfekte Koyun Pnömonilerinde Küçük Ruminant Vebası (PPR) Hastalığı Viral Antijeninin İmmunohistokimyasal Olarak Belirlenmesi

Özet

Bu çalışmada, Erzurum çevresinde koyun pnömonilerinde küçük ruminant vebası (PPR) hastalığı viral antijeninin varlığı ve dağılımı araştırıldı. Bu amaçla toplam 70 adet pnömonili koyun akciğeri histopatolojik ve immunohistokimyasal olarak incelendi. Doku kesitleri rutin olarak hematoksilen - eosin ve immunohistokimyasal yöntemler ile boyandı. İncelenen 70 pnömonili akciğerin 8 (%11.42)'inde PPR viral antijeni saptandı. Histopatolojik olarak, akciğer örneklerinin çoğunda, nekrotik bronşitis, bronşiolitis, multinüklear sinsitial hücreler ile intrasitoplazmik ya da intranükleer inklüzyon cisimcikleri gözlendi. PPR viral antijenin alveol epitellerinde, bronşial bezlerde, bronş ve bronşiol epitel hücrelerinde, sinsitiyal hücrelerde ve makrofajlarda sıklıkla intrasitoplazmik, nadiren intranükleer olarak saptandı. Bu viral antijenin çoğunlukla interstisyel pnömonili akciğerlerde lokalize olduğu görüldü. Çalışma sonuçları, Erzurum çevresinden sağlanan pnömonili koyun akciğerlerinde PPR viral antijeni varlığının %11.42 olduğunu ve hastalığın koyun pnömonilerinin etiyolojisinde rol aldığını gösterdi.

Anahtar sözcükler: *Küçük ruminant vebası, İmmunohistokimya, Koyun*

INTRODUCTION

Peste des petits ruminants (PPR) is an infectious disease of sheep and goats which is characterised by high fever, mucopurulent nasal and ocular discharges, erosive and ulcerative stomatitis, gastroenteritis and pneumonia [1-4]. Morbidity and mortality rates vary but can be as high as 100 and 90%, respectively. These levels are usually lower in endemic areas and mortality rate can be as low as 20% [1,5].

The causative agent peste des petits ruminants virus (PPRV) is classified in the morbillivirus genus in the family Paramyxoviridae, a genus that also includes Rinderpest virus (RPV), Canine distemper virus, human Measles virus [3,6]. PPRV and RPV infections of small ruminants are clinically similar so the differential diagnosis has to be made between these two diseases when they coexist in an area [4,7]. PPR was first reported

İletişim (Correspondence)

+90 442 6314193/1002

yssaglam@atauni.edu.tr

from West Africa in the early 1940s [8] and it was later found in parts of sub-Saharan Africa for several decades and in the Middle East and southern Asia [9]. The outbreaks of PPR are also recently reported in Turkey [10-12].

The principal pathological findings of PPR are seen in the digestive and respiratory systems. Lung lesions in PPR, which are not seen in rinderpest [1,2,13,14], are important in differential diagnosis [14]. As with rinderpest, there are usually severe erosive, necrotic stomatitis and enteritis [15].

Diagnosis of PPR is mainly based on by neutralization and isolation of virus in cell culture [16], competitive enzyme linked immunosorbent assay (C-ELISA) and nucleic acid hybridization [4,7,16]. More recently, an alternative and very sensitive technique such as reverse transcription-polymerase chain reaction (RT-PCR) was described for spesific detection of PPRV [7,17,18]. Also immunohistochemical (IHC) studies have been performed on experimentally and naturally occurring PPR infections of sheep and goats [1,11,12,14,19-21].

Therefore, the aim of this study was to detect the presence and distribution of PPR viral antigen in sheep pneumonia by IHC technique in Erzurum province of Eastern Turkey.

MATERIAL and METHODS

Samples of pneumonic sheep lungs were obtained from departments of pathology (Atatürk University Faculty of Veterinary Medicine and Erzurum Veterinary Control and Research Institute) and the lungs suspected with pneumonia were referred for diagnosis in Erzurum province of Eastern Turkey. All the samples were collected in 2005 and 2006 from different flocks and regions.

Macroscopically, pneumonia was detected in a total of 70 sheep lungs which were examined histologically and immunohistochemically for the presence of PPR viral antigen. Tissue samples of pneumonic lungs were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm, and stained by routine methods with haematoxylin and eosin (HE). After, sections were examined under a light microscope.

To detect the presence and distribution of PPR viral antigen in tissue samples of pneumonic lungs, Avidin-Biotin Complex Peroxidase (ABC-P) technique [1,14,19] was applied according to the staning procedure of commercial immunoperoxidase kits (CadenzaTags peroxidase kit with AEC, Shandon Inc. Pittsburgh, PA, USA). For these purposes, sections that were separated from same tissues prepared for the histopathological examination were passed xylene, alcohols series and digested with 0.1% trypsin solution. To quench endogenous peroxidase activity and reduce background staining, the sections were treated with 3% hydrogen peroxide in 70% methanol for 5 min at room temperature and then washed in buffer for 2 min. All slides were treated with Protein Blocking Agent to reduce non-specific binding of antibodies. Every section was applied with polyclonal rabbit-anti-rinderpest virus (RPV) hyperimmun serum (the anti-RPV serum was kindly supplied from Etlik-Ankara Central Veterinary Control and Research Institute) diluted 1:100 as a primary antibody, and incubated 30 min in room temperature in a humidified chamber. The sections were incubated with biotinylated commercial secondary antibody (OmniTags Biotinylated Secondary "Anti Rb, Gt, Mo" 55757- 11/2007) and streptavidin-peroxidase reagent. As a chromogen 3-Amino-9-ethylcarbazole (AEC) was applied to the sections. All sections were counterstained with Mayer's haematoxylin, washed in tap water, covered with lamella using gelatine and observed for signs of ppr antigen under a light microscope. Instead of the primary antibodies, phosphate buffered saline solution (PBS) was used to every section control slide. Immunoperoxidase staining procedure was performed with manual immunostaining equipment (Sequenza Immunostaining -Shandon).

RESULTS

In the present study, the presence and distribution of PPR viral antigen in pneumonic lungs were detected with IHC technique. Presence of PPR viral antigen was found 8 (11.42%) out of 70 samples.

Histopathological findings as necrotic bronchitis, bronchiolitis and interstitial pneumonia were observed in lungs. Also there were alveolar macrophages and syncytial cells in the alvoli *(Fig1)*. These changes in lungs were characterized with interstitial pneumonia (n=5), catarrhal bronchopneumonia (n=2) and fibrinous pneumonia (n=1 out of 8 positive samples). The pathognomonic findings as intracytoplasmic inclusion bodies were seen in alveolar *(Fig 2)* and bronchi-bronchiolar epithelial cells *(Fig 3)* as well as intra-nuclear inclusion bodies in the pneumocytes in some samples. Additionally, exudates with characterized desquamated epithelial cells and mononuclear cell infiltration were observed in the bronchi and bronchiolar lumina *(Fig 3)*.

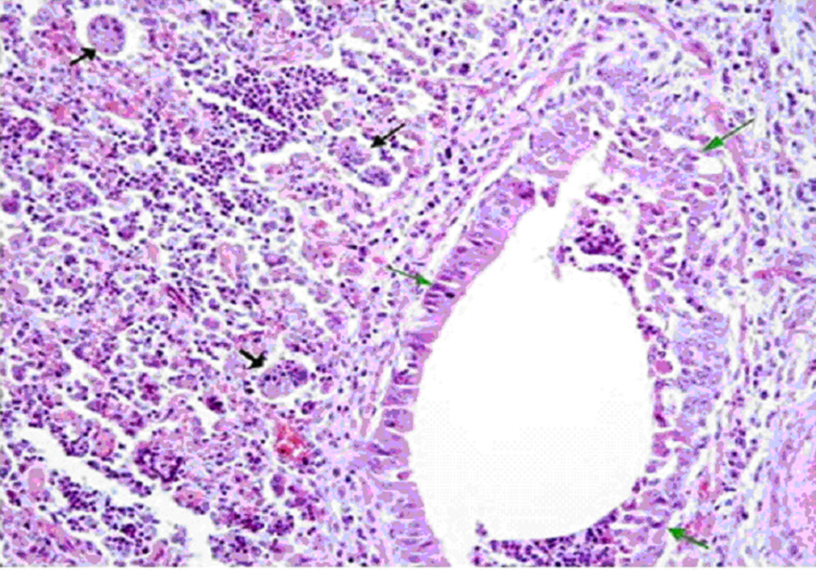
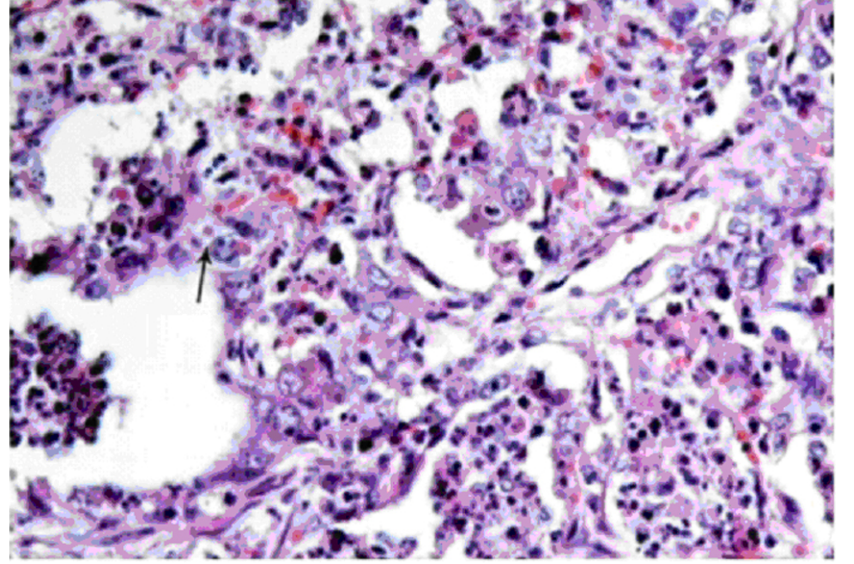


Fig 1. Necrotic bronchiolitis (green arrows) and syncytial cell formation (black arrows) in alveolar lumina. H.E. x 290

Şekil 1. Nekrotik bronşiolitis (yeşil ok) ve alveollerde sinsityal hücre yapıları (siyah ok). H.E. x 290

Fig 2. Intracytoplasmic inclusion bodies in alveoli epithelial cells. H.E. x 360

Şekil 2. Alveol epitel hücrelerinde intrasitoplazmik inklüzyon cisimcikleri. H.E.x360



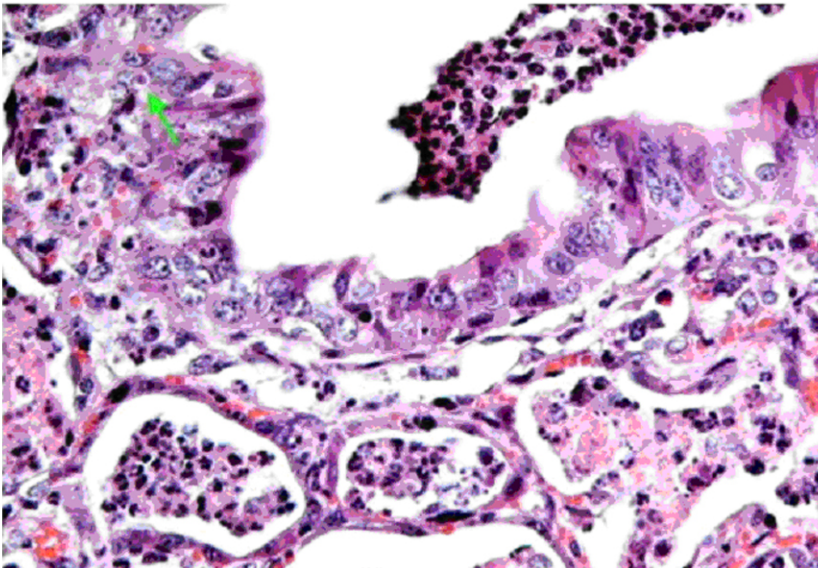


Fig 3. Intracytoplasmic inclusion bodies in the bronchi epithelial cells and showing exudates in the bronchi lumina . H.E. x 400

Şekil 3. Bronş epitel hücrelerinde intrasitoplazmik inklüzyon cisimcikleri ve bronş lumeninde eksudat görünümü. H.E. x 400

Peste des petits ruminants viral antigen was detected particularly at the luminal surfaces and in the cytoplasma of alveoli epithelial cells *(Fig 4)* and rarely in the nuclei of alveolar, bronchi and bronchiolar epithelial cells, alveolar macrophages and syncytial cells. Also the antigen was detected in the cytoplasma of epithelial cells of bronchial glands *(Fig 5)*. Viral antigen was observed strongly at the luminal surfaces of alveoli epithelial, bronchi and bronchiolar epithelial cells with bronchial glands. Viral antigens were seen mostly in lungs with interstitial pneumonia.

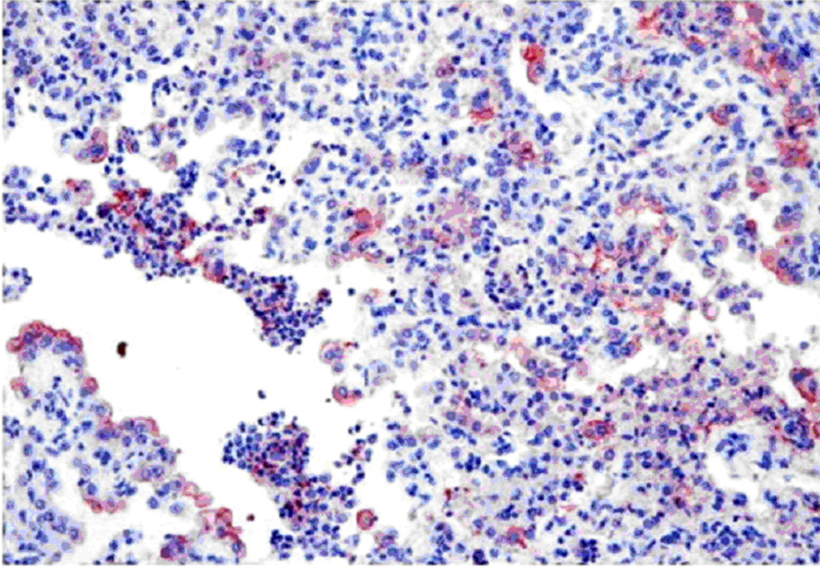


Fig 4. Positive immunoreactions at the luminal surfaces and in the cytoplasma of alveoli epithelial cells. ABC-P x 310

Şekil 4. Alveol epitel hücrelerinin sitoplazmasında ve lumene bakan kısmında pozitif immunoreaksiyonlar. ABC-P x 310

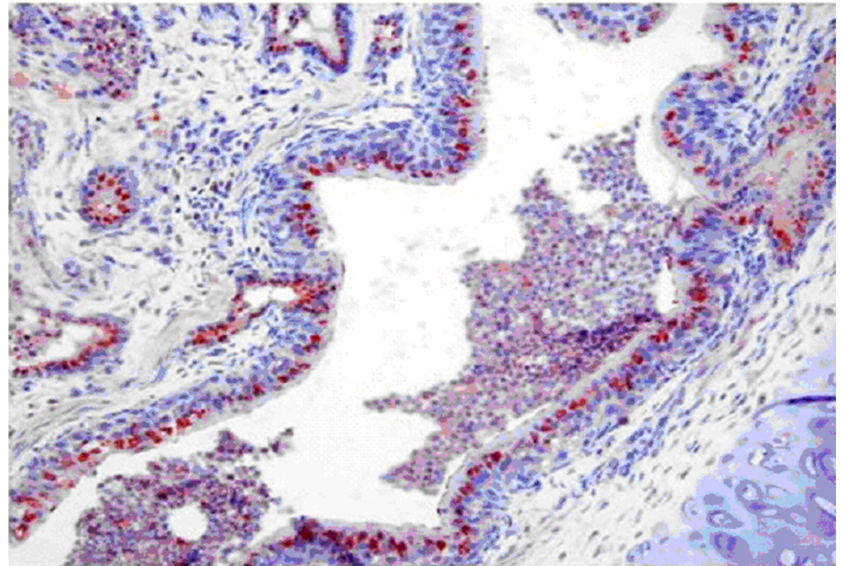


Fig 5. Positive immunoreactions in the cytoplasma of epithelial cells of bronchial glands and bronchi. ABC-P x270

Şekil 5. Bronşial bezlerin ve bronş epitel hücrelerinin sitoplazmalarında pozitif immunoreaksiyonlar. ABC-P x 270

DISCUSSION

Peste des petits ruminants virus is not easy to isolate in cell culture from pathological samples and the isolation process takes 2-3 weeks to complete if successful [7]. But it was reported that RT-PCR-ELISA is a sensitive, specific and semi-quantitative technique to detect PPRV from large numbers of field samples collected from endemic areas [18]. On the other hand, the results of some studies suggested that immuno-histochemical examinations could be an alternative method in the absence of more sophisticated methods of laboratory diagnosis of PPRV infection [11,22]. It was also emphasized that, in such cases, the immuno-histochemical detection of PPR viral antigen is of great value and immunolabelling of the antigen was essential for differential diagnosis [1,11,12,21].

Peste des petits ruminants was first reported by Alcigir et al.[1] in Turkey and affected animals were

originated from Eastern Turkey. Then, different new PPR outbreaks were reported in our country [11,12,23-25]. In this study, the presence of PPR viral antigen was found in 8 (11.42%) out of 70 samples. More recently, some local reports in Turkey showed that the PPRV infection in pneumonic lungs of goats were found to be 40% [11], while the prevalence of clinical cases ranged from 8.3 to 30% [23]. In other study [10], it was described that the prevalence for PPRV infection varied from 0.87% to 82.6% and was higher in sheep (29.2%) than in goats (20%). The animal health authorities also reported that seventy eight PPR outbreaks occurred in 2005 in Turkey [25]. In the present study although the presence of PPR in sheep is not too high but the present values are very important and are considered high for the aetiology of pneumonia in sheep. In recent years, many outbreaks have been reported both in Turkey and the neighboring countries [26,27], presumably indicating a high incidence of PPRV infection. All the previous investigations and the present findings clearly show a marked rise in the global incidence of the infection.

In the PPR infections, the pulmonary lesions were characterized by bronchitis, bronchiolitis or broncho-interstitial pneumonia, and the presence of syncytial cells formations and viral inclusion bodies [1,2,11,14]. In this study, similar microscopic findings such as necrotic bronchitis, bronchiolitis, interstitial pneumonia and syncytial cells of pulmonary tissues together with viral intrasitoplasmic or intranuclear inclusion bodies in some samples were observed. In some cases it was not observed any presence of viral inclusion bodies that are important for diagnosis of PPR. Similary, it was reported that [12] eosinophilic intracytoplasmic inclusion bodies and syncytial cells were detected in the some of bronchopneumonia cases examined. Yener et al.[11] suggested that the absence of viral inclusion bodies could be due to the animals in acute phase of disease or recovering from the PPR. In a previous experimental study [17], it was reported that the syncytial cells were observed during the terminal stage of infection. Indeed, the results of the present study showed that the viral inclusion bodies could not be observed in each positive case. But, detection of PPR viral antigen was essential for differential diagnosis.

In the present study, the polyclonal rabbit-anti-rinderpest virus (RPV) serum as primary antibody was used successfully for the IHC detection of PPRV antigens on tissue samples because of their strong cross-reactivity, as shown in previous PPR studies [1,11,12,14]. In the present study, PPR viral antigen was detected particularly in the cytoplasma of alveolar, bronchial and bronchiolar epithelial cells, bronchial glands. The antigen was also detected in alveolar macrophages and the syncytial cells of lung tissues. The present immunohistochemical findings have also been reported in previous experimental studies [14,22] and naturally cases [1,12,14]. The latter researchers observed that the amount of IHC staining was correlated directly with the severity of the inflammatory process. Yener et al.[11] described that the immunoreaction in the type II pneumocytes, syncytial cells and alveolar macrophages were weak. It was observed that the immuno-reactions were strong at the luminal surfaces of alveoli epithelial, bronchial and bronchiolar epithelial cells with bronchial glands.

In conclusion, the presence of PPR viral antigen in pneumonic lungs of sheep in the region of Erzurum was obtained as 11.42 %, lower than those reported in the previous studies [10,11,23]. Additionally, the viral antigen was seen mostly in lungs with interstitial pneumonia and there was a close relationship between the presence of PPR viral antigen and the pneumonia in sheep.

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