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BHV-1 Antigen Detection in Paraffinized Lung Sections of Pneumonic Sheep Lung Using Immunohistochemistry

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Abstract:

BACKGROUND: Respiratory tract infections caused by some viruses with cattle origin have been demonstrated in sheep and goats.

OBJECTIVES: The main goal of this study was to determine Bovine Herpes virus type 1 BHV1antigen in formalin-fixed paraffin-embedded lung tissue of pneumonic sheep, using immunohistochemistry (IHC) staining method.

METHODS: For this purpose, the lungs of 4079 sheep, which were raised in various farms in the Garmsar district and surrounding areas and were brought to the local abattoir for slaughtering between April and September 2016, were examined.

RESULTS: Macroscopic pneumonia findings were detected in different lobes particularly in the apical and cardiac lobes of the lungs of 259 sheep (6.35%). The rates of mild, moderate and severe consolidations observed in the pneumonic lungs were 59.8%, 26.3 % and 11.6 %, respectively. Pneumonias were microscopically classified in sheep as interstitial pneumonia (49.8%), suppurative bronchopneumonia (15.7%), bronchointerstitial pneumonia (11.1 %), and parasitic pneumonia (14.3%). A total of 220 pneumonic lungs, excluding parasitic pneumonia, examination with immunohistochemistry (IH) in terms of BHV1 antigen, were considered. BHV1 antigen was determined to be 8.63 % by the immunohistochemistry (IHC) method.

CONCLUSIONS: In conclusion, the presence of viral antigen in lung tissues of sheep may indicate that natural pneumonia may be induced by BHV1 or possibly other species-specific herpesviruses. Moreover, it is suggested that sheep might have a role in the transmission of this virus to cattle.

Keywords:

BHV1, Immunohistochemistry, Lung, Pneumonia, Sheep

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Introduction

Infections of the respiratory tract caused by some viruses with cattle origin have been demonstrated in small ruminants (Caswell and Williams, 2007; Sharp and Nettleton, 2007). Although the causative agents are rarely demonstrated, many viruses associated with respiratory system diseases in cattle have been implicated in natural and experimental infections in sheep and goats (Thiry et al., 2006). Bovine herpesvirus 1 (BHV1), which is one of the most important emerging diseases of domestic and wild cattle (Biswas et al., 2013), is a DNA virus in the genus Varicella virus in the family Herpesviridae (Ezzi et al., 2013) and causes huge economic loses (Biswas et al., 2013). BHV1 includes three subtypes, 1 and 2a which are associated with respiratory disease (IBR), 2b is identified with reproductive disease (Infectious Pustular Vulvovaginitis, IPV) and 3 which is referred to as encephalitis (Ezzi et al., 2013). Moreover, cross transmission of ruminant herpesviruses has been reported among ruminant species (Giuliani and Sharma, 1995; Hage et al., 1997; Lehmkuhl et al., 1985; Shankar and Yadav, 1987; Yesilbag and Dagalp-Bilge, 2003).

BHV1 is readily transmitted and has worldwide distribution. BHVI has been eradicated in Denmark, Finland, Norway, Sweden, Austria, Germany and some parts of France (Ezzi et al., 2013). The detection of antibodies against BHV1 in sheep indicates that this species may play a role in the epidemiology of BHV1, but it has been suggested that they have no major role in the transmission of BHV1 infections from sheep to cattle (Çeribasi et al., 2016).

Although latency and reactivation of BHV1 in goats have previously been demonstrated (Six et al., 2001) no data are currently available in sheep.

Moreover, experimental infections in lambs with BHV1 and adenoviruses usually produce lesions which are confined to the respiratory tract (Belak et al., 1976; Cutlip and Lehmkuhl, 1986; Cutlip and Lehmkuhl, 1983; Giuliani and Sharma, 1995).

BHV1 was isolated from a single lamb suffering from a respiratory disease (Trueblood et al., 1978).

It was reported that natural BHV1 infection has caused severe respiratory disease and keratitis in two goats, where the virus was recovered from the eyes and nose (Mohanty et al., 1972). Ciliary destruction and markedly decreased mucosiliar cleaning in the respiratory tract have been reported in both BHV1 and BAV3 infections (Cutlip et al., 1996; Jericho, 1983).

Although BAV3 and BHV1 pathogenicity have been well defined in cattle (Caswell and Williams, 2007; Jericho, 1983; Narita et al., 2000; Narita et al., 2003), few natural pneumonia cases have been reported in sheep and goat as a result of these viruses (Mahmoud and Ahmed, 2 009).

Due to similar pulmonary lesions in BAV3 and BHV1 , routine histopathologic examination has been reported to be insufficient for the diagnosis, infections (Caswell and Williams, 2007).

The confirmative diagnosis of these two infections including BAV3 and BHV1 is made by the following techniques: virus isolation in cell culture, PCR, electron microscopy, serum neutralization analysis, fluorescence antibody and immunoperoxidase techniques

(Debey et al., 2001; Mahmoud and Ahmed, 2009; Narita et al., 2003; Narita et

al., 2000; Okurgumusova et al., 2007).

The purpose of this study was to determine the persistence and prevalence of BHV1 antigen in formalin-fixed and paraffin embedded lung tissues of pneumonic sheep in the Garmsar district and surrounding areas, Semnan Province, in Iran, using immunohistochemistry (IH) staining technique.

Materials and Methods

Sample collection: The lungs of 4079 sheep, which were raised in the Garmsar district and surrounding areas and were brought to the abattoir between April and September 2016, were examined post-slaughtering. Macroscopic pneumonic lesions were found and detected in different lobes particularly in the apical and cardiac lobes of the lungs belonging to 259 sheep. The tissue samples taken from affected lungs were fixed in10 % buffered formalin.

Gross and histopathological examination.

The severity of pneumonia in all pulmonary lobes was scored based on the extent of consolidation. Based on the lesions on pulmonary lobes and the volumes of the lobes involved, less than 10 %, between 10 % and 20%, and more than 20 %, lesions determined were evaluated as "mild", "moderate" and "severe", respectively.

Tissue samples taken from grossly consolidated lungs were fixed in10 % buffered formalin for 48 h and were embedded in paraffin wax before sectioning. The tissues were then stained with haematoxylin and eosin (H&E), and finally examined under light microscopy.

Immunohistochemistry (IH) staining method was applied to the total number of 220 lungs, which were microscopically characterized as having suppurative bronchopneumonia, bronchointerstitial pneumonia, and interstitial pneumonia, but not lung with parasitic pneumonia.

Immunohistochemistry: Tissue sections were immunohistochemically processed to assess the expression of herpes virus antiserum (polyclonal rabbit antibody to herpes simplex virus type 1 (BHSV 1); catalog number RP 018; 1.100 dilution, Diagnostic BioSystems, CA, USA), using routine avidin-biotin-peroxidase complex techniques. Selected sections were stained for immunohistochemistry and processed according the manufacturer's instructions. The paraffin-embedded, 5-µm sections were attached to glass slides coated with poly-L-lysine and dried overnight at 37°C to optimize adhesion. Sections were de-paraffinized in multiple xylene baths, and rehydrated in sequentially graduated ethyl alcohol baths. To reduce non-specific background staining due to endogenous peroxidase, slides were incubated in hydrogen peroxide in methanol for10 min. The sections were washed twice in phosphate buffer solution (PBS) before 5-min incubation in blocking and overnight at 4°C incubation with primary antibody. They were rinsed four times in PBS, and then incubated with a biotinylated polyvalent antibody for 10 min at room temperature. After three washes in PBS, streptavidin peroxidase was applied for 10 min at room temperature, and the slides were rinsed four more times in PBS. EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) were used as seconder kit. Tissues were further incubated for 20 min at room temperature in a solution of DAB (3,3'-diaminobenzidine) chromogen. After a final wash in PBS, tissues were counterstained with Mayer's hematoxylin, washed in water, and cover slips were applied with

mounting media. For negative control primary antibody omitted the slides.

Results

Gross pathological findings: ossly examined post-slaughtering, and pneumonic lesions were detected in the different lobes of the lung particularly in the apical and cardiac lobes in 259 cases (6.35%). The rates of mild, moderate and severe consolidations observed in different lobes of pneumonic lungs were 59.8 %, 26.3 % and 11.6 %, respectively (Figs.1, 2 and 3). Generally, the lesions in different lobes were characterized as irregular lobular atelectatic foci and patchy or confluent consolidated purple-red or grey foci.

Histopathological findings: In microscopical examination, pneumonias were classified in sheep as interstitial pneumonia (49.8%), suppurative bronchopneumonia (15.7%), bronchointerstitial pneumonia (11.1%), and parasitic pneumonia (14.3%). Suppurative bronchopneumonia, bronchointerstitial pneumonia, and interstitial pneumonia, excluding parasitic pneumonia, were determined in 220 sheep lungs which were examined for the presence of BHV1 antigen using immunohistochemistry (IH) staining technique.

Immunohistochemistry (IH) findings: Of the 220 pneumonic lungs, BHV1 antigen was determined in 19 cases (8.63 %). It was noticed that positive staining was generally present in the pneumonic areas. Although severe immunohistochemistry (IH) staining associated with BHV1 viral antigen was observed generally in the granular appearance and in the cytoplasm of epithelial cells in the airways, in bronchiole associated lymphoid cells and perivascular cell infiltrations as well (Figs. 1, 2), but it was more

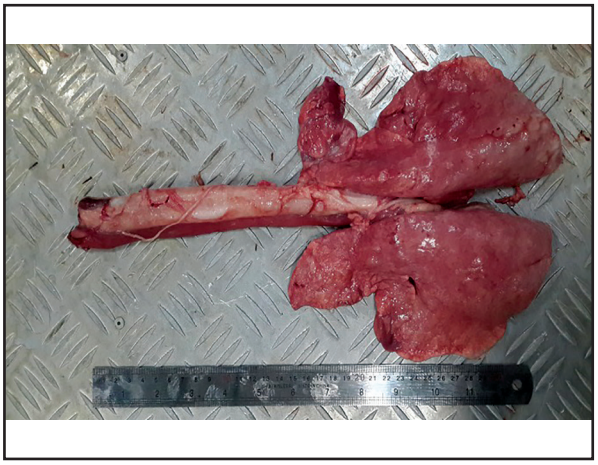

Figure 1. Sheep, lung. Mild pulmonary consolidation.

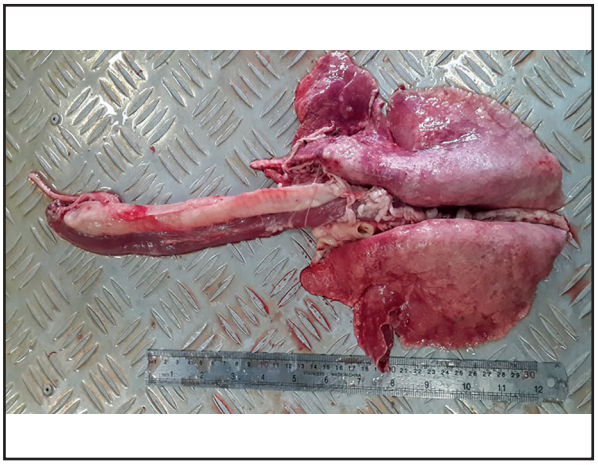

Figure 2. Sheep, lung. Moderate pulmonary consolidation.

Figure 3. Sheep, lung. Sever pulmonary consolidation.

scant in the alveolar epithelium.

No immunopositive staining was observed in tissue from healthy sheep lungs (negative control) (Fig.3).

Discussion

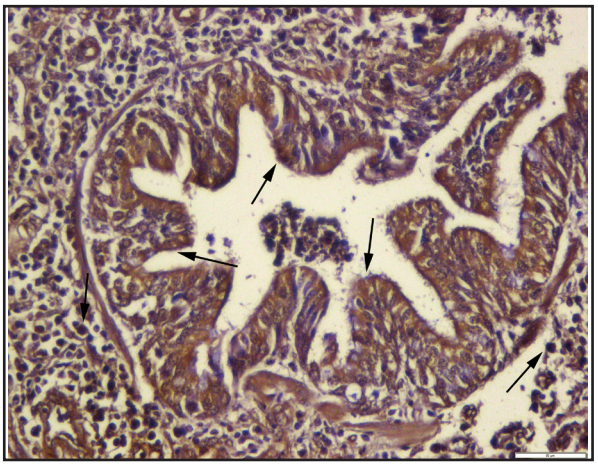

Figure 4. Sheep, lung. BHV1 positivity was detected in bronchiolar epithelium (black arrow) and perivascular cell infiltrations (white arrow). × 1000.

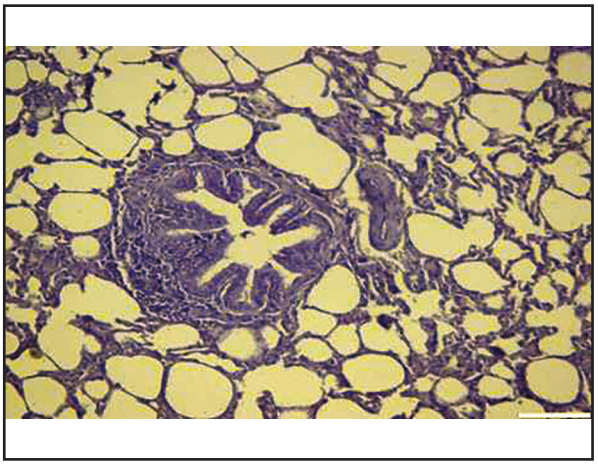

Figure 5. Sheep, lung. No immunopositive staining was observed in tissue from healthy sheep lungs. × 100.

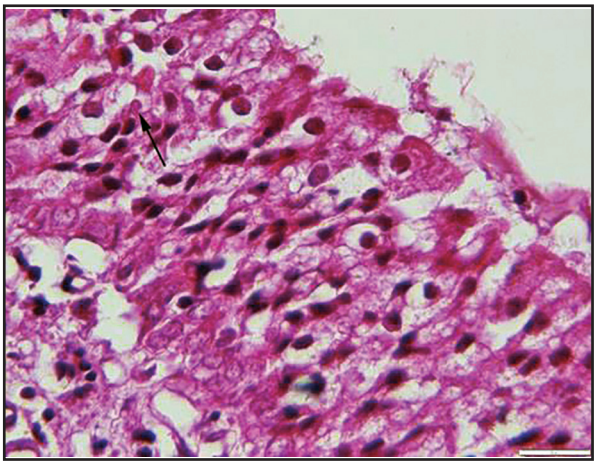

Figure 6. Sheep, lung. Presence of intranuclear inclusion body (arrow). H&E staining. × 1000.

In the present study, BHV1 antigens were determined in 19 (8.63 %) out of 220 sheep, by immunohistochemistry (IH) staining.

In addition, the results of this study are the first in Iran in terms of determination of BHV1 viral antigen by immunohistochemistry (IH) staining in lung tissues of sheep with natural pneumonia.

In the experimental infection of calves with BHV1, viral antigens were observed in bronchi, bronchioles and alveolar epithelium by immunohistochemistry (IH) staining (Narita et al., 2000).

The (IH) findings of the present study, in terms of the distribution and localization of BHV1 viral antigen in lungs, are consistent with the results of previous studies performed in sheep and goat and cattle (Narita et al., 2003; Narita et al., 2000).

In addition, it is epidemiologically important to determine localization of viral agents throughout the epithelium of the respiratory tract in sheep and goats, in terms of the spread of antigens to susceptible animals by nasal secretions and coughing (Caswell and Williams, 2007).

It has been reported that experimental adenovirus and BHV1 infections are microscopically characterized by proliferative bronchiolitis, degeneration, desquamation or hyperplasia of bronchial and alveolar type II epithelium, atelectasis, lymphocyte, macrophage and neutrophil infiltrations, thickening of the interalveolar septum and intranuclear inclusions in endothelial and epithelial cells in ruminants

(Belake et al., 1980; Cutlip et al.,1996; Cutlip and Lehmkuhl, 1 986; Lehmkuhl et al., 1997; Narita et al., 2000; Sharp and Nettleton, 2007).

The histopathological findings of the present study were similar to the results of previous studies, including the presence of intranuclear inclusion bodies (Fig- ..).

In previous studies, serological evidence was obtained for the presence of BHV1 in sheep and goats. Other researchers reported the presence of BHV1 at the rate of5.4 % in lambs in their seroepidemiological study (Lehmkuhl et al., 1985; Mahmoud and Ahmed, 2009).

The percentage of BHV1 isolates was determined as 5.8 % by PCR in the lung tissues of sheep and goats raised in Egypt, and it was suggested that this situation may be due to overcrowding and bad hygienic measures, which play a role in the transmission of respiratory disease in an animal population (Mahmoud and Ahmed, 2009).

The prevalence of BHV1 antibodies was found to be between 11.2 and 13 % in goats and between 0 and 2.9 % in sheep raised in Africa (Jesset and Rampton, 1975; Maurice and Provost, 1970; Taylor et al., 1977).

Different studies reported 6.9% and 13.2% of tested goats were BHV1 positive in Canada and USA respectively, while BHV1 seropositivity was 10.8 % in sheep (Elazhary et al., 1984).

In recent years, seroprevalence of several viruses causing bovine respiratory diseases was studied in different bovine populations in several regions of Iran. The average was 30-100%, up to 100% and 20-80% for BHV-1, PI3V and BVDV, respectively (Hajikolaei, et al., 2007, Sakhaee, et al., 2009, Badieei, et al., 2010, Shirvani, et al., 2012).

Besides, it has been reported that BHV1 prevalence ranges from 0.7 to 5.52% in goats (Ataseven et al., 2010) and from 2.44 to 9.6% in sheep in Turkey (Albayrak et al., 2007; Ataseven et al., 2010; Yesilbag and Dagalp-Bilge, 2006).

In another study conducted on cattle in the Elazig province of Turkey, prevalance of BAV3 and BHV1 was detected as 5.26 % and 2.43 % by IP and 6.88 % and 4.45 % by DFAT, respectively (Ceribasi et al., 2014).

When all the data obtained so far for BHV1 positivity are considered, it is plausible to suggest that urgent prevention measures are required in order to control these infections in Iran.

Conclusion: In conclusion, in the present study, BHV1 antigen was determined as 8.63 % by IH staining technique in pneumonic sheep lungs. The presence of viral antigens in the lung tissues of sheep may indicate that natural pneumonia may be induced by BHV1, or possibly other species-specific herpesviruses. In addition, it is thought that sheep might have a role in transmission of these viruses to cattle.

Aknowledgments

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Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۴، ۳۲۱-۳۱۳

شناسایی آنتی ژن BHV-۱ در مقاطع بافتی پارافینه متغلق به ریه گوسفندان مبتلا به پنومونی با استفاده از تکنیک ایمونوهیستوشیمی

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چکیده

زمینه مطالعه: عفونت‌های مجاری تنفسی با عامل برخی ویروس‌های با منشاء گاوی در گوسفند و بز نشان داده شده است.

هدف: هدف اصلی از این مطالعه بررسی آنتی ژن BHV ۱ در نمونه بافت ریه، تثبیت شده در فرمالین و قالب‌گیری شده در پارافین، متعلق به گوسفندان مبتلا به پنومونی با استفاده از تکنیک رنگ آمیزی ایمونوهیستوشیمی بود.

روش کار: به همین منظور ریه های متعلق به ۴۰۷۹ راس گوسفند، که در مزارع دامپروری شهرستان گرمسار و مناطق اطراف پرورش داده شده و جهت کشتار بین ماه های فروردین تا شهریور سال ۱۳۹۵ به کشتارگاه نیمه صنعتی این شهرستان آورده شده بودند مورد معاینات پس از کشتار قرار گرفتند.

نتایج: یافته‌های ماکروسکپیک پنومونی در لوب‌های مختلف بویژه در در لوب‌های راسی و کاردیاک ریه‌های متعلق به ۲۵۹ راس گوسفند (۶/۳۵٪) شناسایی و ثبت شد. درجات ملایم، متوسط و شدیدی کبدی شدن در ریه‌های پنومونیک به ترتیب در ۵۹/۸٪، ۲۶/۳٪ و ۱۱/۶٪ ریه‌ها مشاهده شد. در معاینات میکروسکپی پنومونی در گوسفندان مورد مطالعه تحت عناوین پنومونی بینابینی (۴۹/۸٪)، برونکوپنومونی چرکی (۱۵/۷٪)، پنومونی برونکواینترستیشیال (۱۱/۱٪)، و پنومونی انگلی (۱۴/۳٪). در مجموع با حذف ریه‌های مبتلا به پنومونی انگلی، ۲۲۰ ریه پنومونیک به منظور شناسایی آنتی ژن BHV ۱ تحت مطالعات میکروسکپی با استفاده از تکنیک ایمونوهیستوشیمی قرار گرفتند. آنتی ژن BHV ۱ در ۸/۶۳٪ ریه‌ها شناسایی شد.

نتیجه‌گیری نهایی: در نهایت حضور آنتی ژن BHV ۱ در بافت ریه گوسفندان می‌تواند بیانگر این نکته باشد که پنومونی طبیعی در گوسفندان ممکن است با منشاء BHV ۱ بوجود بیاید. بعلاوه تصور می‌شود BHV ۱ می‌تواند به عنوان یک عامل مستعد کننده برای بروز پنومونی‌های باکتریایی ثانویه عمل کند.

واژه‌های کلیدی:
BHV۱ ، ایمونوهیستوشیمی، ریه، پنومونی، گوسفند

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