Original Article. DOI: 10.22059/ijvm.2019.262877.1004913 *Iranian Journal of Veterinary Medicine*

Denovirus Antigen Detection in Paraffinized Lung Sections of Pneumonic Goat Lungs Using Immunohistochemistry

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Abstract:

BACKGROUNDS: Diseases affecting the respiratory tract of sheep and goats are one of the most important factors which limit production of these species on a world-wide basis.

OBJECTIVES: The main goal of this study was to determine Adenovirus (AdV) antigen in formalin-fixed paraffin-embedded lung tissue of pneumonic goats, using immunohistochemistry (IHC) staining method.

METHODS: For this purpose, the lungs of 402 goats, 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 26 goats (%6.46). 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 goats as interstitial pneumonia (n=15) (57.69%), suppurative bronchopneumonia (n=4) (15.38%), bronchointerstitial pneumonia (n=3) (11.53%), and parasitic pneumonia (n=4) (15.38%). A total of 22 pneumonic lungs, excluding parasitic pneumonia, examination with immunohistochemistry (IH) in terms of AdV antigen, were considered. AdV antigen was determined to be (13.63 %) (n=3) by the immunohistochemistry (IHC) method.

CONCLUSIONS: In conclusion, the presence of viral antigen in lung tissues of goats may indicate that natural pneumonia may be induced by AdV or possibly other species-specific AdVs. Moreover, it is suggested that AdV might have a role in predisposing this species to secondary bacterial infections.

Keywords:

Adenovirus, Goat, Histopatholog, Immunohistochemistryy, Lung

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Received: 14 January 2019

Accepted: 12 March 2019

How to Cite This Article

Jamshidi, K., Ozmen, O., Rahmani, M., Marvaki, R., Soltan Mohammadi, M. (2019). Denovirus Antigen Detection in Paraffinized Lung Sections of Pneumonic Goat Lungs Using Immunohistochemistry. Iran J Vet Med, 13(2), 143-150. doi: 10.22059/ijvm.2019.262877.1004913

Introduction

Diseases of the respiratory tract of sheep and goats are one of the most important factors which limit production of these species and thus cause major economic loses in small ruminant industry on a world-wide basis (Yesilbag, Güngör,. 2008).

There are 128.7 million animal units (one mature sheep) in Iran. The number of sheep and goats are 52.2 and 25.9 million heads respectively. Per capita consumption of red meat, with meat, milk and eggs are: 12.7, 28.4, 115 and 9.8 kg/person/year respectively.

Livestock production is the principal occupation of the rural inhabitants of Garmsar county (Semnan Province Iran). Sheep and goats are the major livestock species of the province too. Overall productivity of these animals is low due to poor feeding, management factors and prevalence of various viral, bacterial and parasitic diseases. The economic consequences of this situation are profound and include mortalities as well as indirect estimators of morbidity such as increased time to reach market weight, poor feed conversion, higher rates of culling, poor carcass composition, increased condemnation at slaughter and extra cost and time for medication and veterinary services (Valizadeh, 2010).

It has been well demonstrated that some viruses with cattle origin cause respiratory tract infections in sheep and goats (Caswell and Williams, 2007; Sharp and Nettelton, 2007). Although these viruses were rarely determined, it has been revealed that many viruses associated with respiratory system diseases in cattle have been implicated in natural and experimental infections in sheep and goats (Thiry et al., 2007).

Adenovirus infections are most often subclinical, and diseases occur more commonly in the intestinal or respiratory tracts (Debey et al., 2011).

Natural or experimental adenovirus infections in sheep and goat cause lesions mainly in the respiratory tract. In addition, experimental infections in lambs with adenoviruses and BHV1 usually produce lesions, and they are confined to the respiratory tract (Cerebasi et al., 2016).

Although Adenovirus pathogenecity has been well defined in cattle (Caswell and Williams., 2007, Cerebasi et al., 2016), few natural pneumonia cases related to this agent have been reported in small ruminants (Mohammad and Ahmed, 2009; Cerebasi et al., 2016).

Destroyed ciliary activity and markedly decreased mucosiliary cleaning in the respiratory tract have been reported in Adenovirus infections (Cerebasi et al., 2016).

Routine histopathologic examination has been reported to be insufficient for the diagnosis of Adenovirus infections (Caswell and Williams., 2007). The confirmative diagnosis of this infection is made by virus isolation in cell culture, PCR, electron microscopy, serum neutralisation analysis, fluorescence antibody and immunohistochemistry (IH) (Debey, 2011; Mahmoud and Ahmed, 2009; Okurgumusova et al., 2007; Cerebasi et al., 2016).

The purpose of this study was to determine the prevalence of Adenovirus antigen using immunohistochemistry (IH) staining of formalin-fixed, paraffin embedded lung tissues of pneumonic goat slaughtered in the Garmsar municipal slaughterhouse and surrounding areas, Semnan, Northeast Iran.

Materials and Methods

Sample collection: The lungs of 402 goats, which were raised in the Garmsar district and surrounding areas and were brought to the abattoir for slaughtering between April and September 2016, were examined. Macroscopic pneumonia findings were detected only in the apical and cardiac lobes of the lungs belonging to 26 animals. The tissue samples taken from affected lungs were fixed in NBF 10% (10% 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; as 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.

Immunoperoxidase staining: Immunohistochemistry (IHC) staining method was applied to the total number of 22 lungs, which were microscopically characterized as having suppurative bronchopneumonia, bronchointerstitial pneumonia, and interstitial pneumonia, but not lung with parasitic pneumonia (n=4).

Tissue sections were immunohistochemically processed to assess the expression of adenovirus antiserum [adenovirus type 5 antibody (ab6982), 1/100 dilution, Abcam, Cambridge, UK], using routine avidin-biotin-peroxidase complex techniques. Selected sections were stained for immunohistochemistry 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 oC 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 for 10 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) was 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: A total of 402 goat lungs were grossly examined post-slaughtering, and pneumonic lesions were detected in different lobes of the lung particularly in the apical and cardiac lobes in 26 cases (6.47 %). The rates of mild, moderate

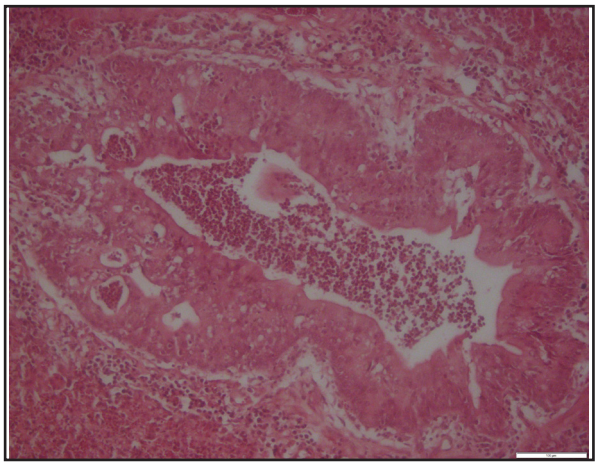

Figure 1. Goat, lung. Bronchiole. Note bronchiolar infiltration of neutrophils.

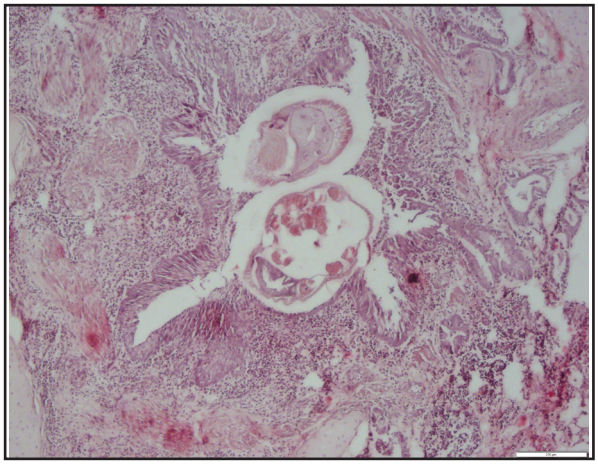

Figure 2. Goat, lung. Bronchus. Note presence of parasite eggs and laves within the bronchus and peribronchial infiltration of inflammatory cells.

and severe consolidation observed in different lobes of pneumonic lungs were 59.8%, 26.3% and 11.6%, respectively. 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 microscopic examination, pneumonias were classified in goats as interstitial pneumonia (n=15) (57.69%), suppurative bronchopneumonia (n=4) (15.38%), bronchointerstitial pneumonia (n=3) (11.53%), and parasitic pneumonia (n=4) (15.38%) (Figs.1, 2). Suppurative bronchopneumonia, bronchointerstitial pneumonia, and interstitial pneumonia, were determined in 22 (84.61%) goat lungs which were examined for the presence of Adenovirus antigen using immunohistochemistry (IHC) staining technique, but not lung with parasitic pneumonia (n=4).

Immunoperoxidase staining (IP) findings: Of the 22 pneumonic goat lungs, AdV antigen was detected in 3 cases (13.63%). It was noticed that positive staining was generally present in the pneumonic areas. Specific IHC staining associated with viral antigen was observed generally in the granular appearance and in the cytoplasm of epithelial cells in the airways. Although severe AdV immunostaining was found in the bronchiolar epithelium, it was scanter in the alveolar epithelium of pneumonic lungs in goat. In addition, AdV antigen was detected in bronchiole associated lymphoid cells. AdV positivity was observed in bronchiolar and alveolar epithelium (Fig.3). No immunopositive staining was observed in tissue from healthy goat lungs, named as negative control (Fig. 4).

Discussion

Previous studies have reported that fluorescent antibody technique (FAT) is an advantageous technique in terms of achieving rapid results because preparation and examination of the samples are performed within a short period of time (Forghani, 2010). However, the efficiency of (FAT) is limited in terms of determining appropriate morphological details in tissues (Cerebasi et al 2016).

While processing formalin-fixed tissues takes more time, the histopathology results of retrospective studies show that formalin-fixed sections are superior to frozen sections in terms of accurate identification of

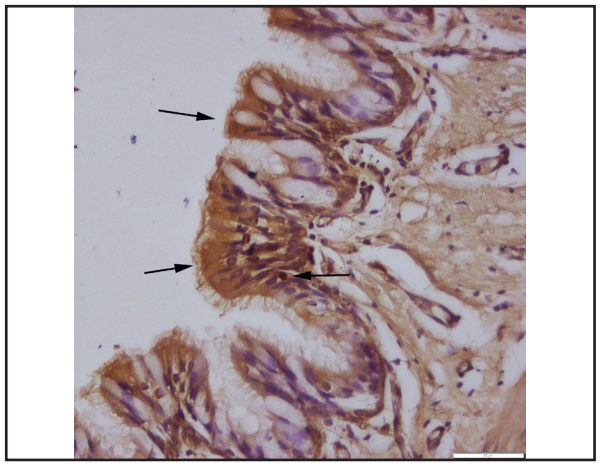

Figure 3. Goat, lung. AdV positivity was detected in bronchiolar epithelium (arrows) and perivascular cell infiltrations (arrows). × 1000.

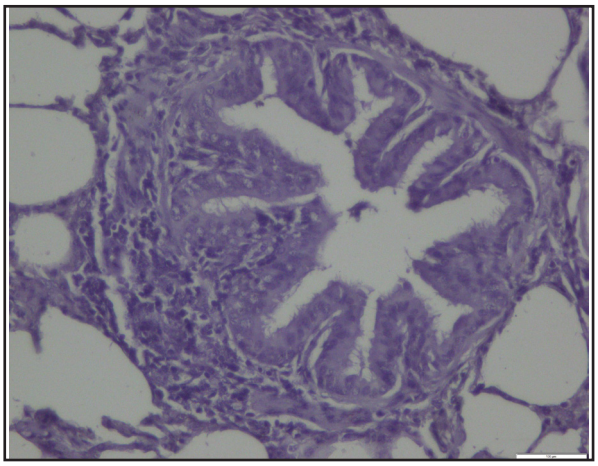

Figure 4. Goat, lung. No immunopositive staining was observed in tissue from healthy goat lungs. × 100.

cell and tissue types.

However, immunogenic epitopes and many antisera used for IHC diagnosis are unreactive in fixed specimens, due to the damaging effect of fixation (Cerebasi et al 2016).

In the present study, AdV antigen was identified in 3 goats (13.63%) by IHC. In addition, the results of this study are the first in Semnan province in terms of determination of AdV viral antigens by IHC in lung tissues of goats with natural pneumonia.

The IHC findings of the present study are consistent with the results of previous studies performed in cattle, in terms of the distribution and localization of AdV viral antigens in lungs (Cerebasi et al 2016).

In addition, it is epidemiologically important to determine localization of viral agent throughout the epithelium of the respiratory tract in 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 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 endotelial and epithelial cells in ruminants (Sharp and Nettelton, 2007).

The histopathological findings of the present study were similar to the results of previous studies, with the exception of inclusion bodies.

Earlier studies have suggested that the determination of viral pneumonia-specific lesions, such as inclusion bodies in experimental adenovirus infections, may depend on many factors, including animal species and age, virulence of agent, amount of virus, infection period and the presence of secondary bacterial infections (Cerebasi et al., 2016).

Thus, definitive diagnosis of AdV associated pneumonia has been reported to be made with PCR, culture, electron microscopy, FAT, IFAT and IHC techniques (Biswas et al., 2013). Moreover, it has been emphasized that failure to determine the virus or antigens by electron microscopy and immunofluorescence staining might be experienced due to low concentrations of virus in the lesions (Cerebasi et al., 2016).

Surveys using bovine adenovirus to de-

tect adenovirus antibodies in goats in Iran (Shirvani et al., 2012), India (Majumder et al.,2015), and the United Sates (Terry et al.,2015) indicate low adenovirus infection rates. Although AdV seropositivity rate was found to be 5.2% in goats in the Marmara region of Turkey (Okurgumusova and Akca, 2007), it was determined as 11.0% by ELISA in Gazelle subgutturosa in the Ceylanpinar region of Turkey (Gur et al., 2008).

In another study conducted on cattle in the Elazig province of Turkey, prevalance of AdV was detected as 5.26% by IHC and 6.88% by DFAT, respectively. When all the data obtained so far for AdV seropositivity are considered, it is plausible (Cerebasi et al., 2014) to suggest that urgent prevention measures are required in order to control this infection in Semnan province.

In conclusion, in the present study, AdV antigen was determined as 13.63% by IHC in pneumonic goat lungs.

The presence of viral antigens in the lung tissues of goats may indicate that natural pneumonia may be induced by AdV, or possibly other species-specific adenoviruses in the study area. In addition, it is thought that goats might have a role in transmission of these viruses to cattle.

Acknowledgments

Authors would like to express their special thanks of gratitude to laboratory staff who helped and gave us the golden opportunity to complete this project in the laboratories of the Department of Pathology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Turkey.

Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۸، دوره ۱۳، شماره ۲،۱۵۰-۱۴۳

شناسایی آنتی ژن آدنوویروس در مقاطع بافتی پارافینه متعلق به ریه بزهای مبتلا به پنومونی با استفاده از تکنیک ایمنوهیستوشیمی

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(دریافت مقاله: ۲۴ دی ماه ۱۳۹۷، پذیرش نهایی: ۲۱ اسفند ماه ۱۳۹۸)

چکیده

زمینه مطالعه: بیماری‌های موثر بر مجاری تنفسی گوسفند و بز یکی از مهم‌ترین فاکتورهای محدود کننده تولید در این گونه‌ها در سراسر جهان می‌باشند.

هـدف: هدف اصلی از مطالعه پیش رو تعیین و شناسـایی آنتی ژن آدنوویـروس (AdV) در بافت ریه متعلق به بزهای مبتلا به پنومونی و تثبیت شده در فرمالین و قالب گیری شده در پارافین با استفاده از تکنیک ایمونوهیستوشیمی می‌باشد.

روش کار: به همین منظور ریه‌های متعلق به ۴۰۲ راس بز، که در مزارع دامپروری شهرستان گرمسار و مناطق اطراف پرورش داده شده و جهت کشتار بین ماه‌های فروردین تا شهریور سال ۱۳۹۵ به کشتارگاه نیمه صنعتی این شهرستان آورده شده بودند مورد معاینات پس از کشتار قرار گرفتند.

نتـایـج: یافته‌های ماکروسکپیک پنومونی در لوب‌های مختلف بویژه در لوب‌های رأسی و کاردیاک ریه‌های متعلق به ۲۶ رأس بز (۶/۴۶ درصد) شناسـایی و ثبت شـد. درجات ملایم، متوسط و شدید کبدی شدن در ریه‌های پنومونیک به ترتیب در ۵۹/۸ درصد، ۲۶/۳ درصد، ۱۱/۶درصد ریه ها مشاهده شد. در معاینات میکروسکپی پنومونی در بزهای مورد مطالعه تحت عناوین پنومونی بینابینی (۱۵) (۵۷/۶۹درصد)، برونکوپنومونی چرکی (۴) (۱۵/۳۸درصد)، پنومونی برونکواینترستیشیال (۳) (۱۱/۵۳درصد)، و پنومونی انگلی (۴) (۱۵/۳۸درصد) شناسایی شدند. در مجموع با حذف ریه‌های مبتلا به پنومونی انگلی، ۲۲ ریه پنومونیک به منظور شناسایی آنتی ژن AdV تحت مطالعات میکروسکپی با استفاده از تکنیک ایمونوهیستوشیمی قرار گرفتند. آنتی ژن AdV در ۳ مورد ریه (۱۳/۶۳درصد) شناسایی شد.

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

واژه‌های کلیدی:
آدنوویروس، بز، هیستوپاتولوژی، ایمونوهیستوشیمی، ریه

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