Assiut University web-site: www.aun.edu.eg

PATHOLOGY AND IMMUNOHISTOCHEMICAL EVALUATION OF CATTLE LUNG SLAUGHTERED AT METROPOLITAN ABATTOIRS IN ASSIUT

AL-HASSAN MOHAMMED MOSTAFA[1], ALAA ELDIN KAMAL YOUSSEF[2], ABDELRAHEIM ATTAAI [3,4]

[1] Pathology and Clinical Pathology, Animal Health Research Institute, Agriculture Research Center, Assiut Regional Lab, Egypt
[2] Food Hygiene Department, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Egypt
[3] Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Assiut University, Assiut, Assiut 71529, Egypt.
[4] Department of Anatomy and Histology, School of Veterinary Medicine, Badr University in Assiut, Nasser City, Assiut, Egypt

Received: 1 April 2024 **Accepted:** 1 August 2024

ABSTRACT

Respiratory diseases continue to be a major cause of clinical manifestation, mortality, production loss, and reduced carcass quality. Given the overlapping clinical manifestations of various causes of bovine respiratory disease (BRD), it is often necessary to conduct pathologic and laboratory investigations to confirm a specific diagnosis in complicated cases. A specific diagnosis is useful to direct antimicrobial or anthelmintic therapy, vaccination programs, and biosecurity practices. Fifty-four lung specimens were collected from adult bulls from Dronka Abattoir, Assiut, Egypt to study the pathological and immunohistochemical alterations of the lung tissue and focus on their relation to the immune cells. Four different forms of lung affections were common; chronic catarrhal pneumonia (fibrosis and hyperplasia with moderate cellular inflammatory cells), chronic interstitial pneumonia (diffuse thickening of the interstitium due to fibrosis, increased cellularity and smooth musculature), pulmonary emphysema (rupture of alveolar walls with communication of alveolar spaces and the appearance of aerated lung tissue) and fibrinous pneumonia (fibrinous exudate with erythrocytic aggregation in the alveoli and bronchiole). In this study, we used a new approach for diagnosing lung affections; CD3-T lymphocytes. CD3-positive T-lymphocytes were in close contact with different pneumonic forms, rather than pulmonary emphysema. The values registered were 13.7, 10.43, 8.40, and 5.19 in chronic catarrhal pneumonia, chronic interstitial pneumonia, fibrinous pneumonia and pulmonary emphysema, respectively. Furthermore, CD3-immunoreactivity is a good marker to recognize different stages of pneumonia. In conclusion, CD3-T lymphocytes could be a beneficial tool for the confirmation of different types of pulmonary pneumonia.

Keywords: Abattoir, pneumonia, Pathology, immunohistochemistry and CD3.

Corresponding author: Al-hassan Mohammed Mostafa
e-mail address: hassanmustafav@gmail.com
Present address: Pathology and Clinical Pathology, Animal Health Research Institute, Agriculture Research Center, Assiut Regional Lab, Egypt

INTRODUCTION

The main causative factors of lower respiratory affection in cows are ingestible toxins, viruses, bacteria, and parasitic larvae (Luke *et al.,* 2023). The economic income of beef products is seriously broken as a result (Prado *et al.,* 2005). Immuno-histochemistry (IHC) is a recent immune-ological manner for checking precisely the immune-pathogenic particles of affected tissues (Olawale *et al.,* 2020). Recent pathological research employs the IHC technique as an alternative method for disease recognition, as it is a more helpful and effective proof aid than other conventional non-specific and molecular approaches which need more stable electrical efforts (Olawale *et al.,* 2020).
Respiratory disease continues to be a major cause of clinical disease, mortality, production loss, and reduced carcass quality. Because the various causes of bovine respiratory disease (BRD) have overlapping clinical manifestations, pathologic and laboratory investigations are often required in those cases for which a specific diagnosis is required. A specific diagnosis is useful to direct antimicrobial or anthelmintic therapy, vaccination programs, and biosecurity practices and to satisfy the curiosity and concern of producers and veterinarians (Jeff Cacwell *et al.,* 2012).

Bovine respiratory disease (BRD) is regarded as the most common illness during the fattening period of cattle in Europe and North America. Assessment of the prevalence of pneumonic lesions at the slaughterhouse can be a good indicator of the relevance of BRD in beef cattle (Pardon *et al.,* 2013). a- The famous bacterial pathogens of respiratory diseases concerning cattle species, along with the most characteristic macroscopical abnormalities, are Pasteurellaceae: *Manhemia haemo-lytica,* and *Pasteurella multocida* (Jeff L Cacwell *et al.,* 2012). The lungs are characterized grossly by cranioventral reddening and firm to hard consolidation; irregularly shaped non-friable foci of coagulation necrosis, interlobular edema (marbling), or fibrinous pleuritis. b- Infection with BVDV or the viruses listed as predisposing causes of bacterial pneumonia. The gross lesions mainly are the cranioventral redness of the lung and firm-to-hard consistency as a result of bacterial bronchopneumonia, whereas the dorso-caudal lung is normal or slightly firm-rubbery. c- Tuberculosis (*M bovis*) leads to single or multiple soft white raised granulomas, often with caseous necrosis and/or mineralization in the center. d- Gross lesions of secondary or opportunistic bacterial pathogens are cranioventral bronchopneumonia, abscesses, and bronchiectasis. The nasal cavity and trachea suffer from multifocal to confluent erosions, covered with fibrin or necrotic debris, distinguished from expectorated lung exudate. e- Heart disease causing pulmonary edema leading to diffuse red-purple heavy lungs, interlobular edema, oozing fluid from the cut surface, and abundant foam or fluid in the trachea. f- *Dictyocaulus viviparus*: acute prepatent disease resulting from larval migration, or chronic patent or post-patent infection. The gross lesions in the acute form are diffusely red lungs, edematous, and firm (interstitial pneumonia). The gross lesions in the chronic form are the presence of adult worms in caudal bronchi, lobular atelectasis, or consolidation of lung tissue, especially in *dorsocaudal* areas of the lung. g- *Ascaris suum* larval migration causes the generalized distribution of lobular atelectasis or consolidation. h- Ingested toxins: L-tryptophan/3-methylindole (lush forage), 4-ipomeanol (moldy sweet potatoes), perilla ketone (purple mint), where the lungs become diffusely edematous and firm (interstitial pneumonia). i- Anaphylaxis causing pulmonary edema and bronchoconstriction leads to diffusely red-purple heavy lungs, interlobular edema, oozing fluid from the cut surface, and abundant foam or fluid in the trachea. j- Hypersensitivity pneumonia gross lesions are diffuse firm and heavy lungs.

El-Seedy *et al*. (2020) reported that for lung affections, the overall prevalence of both *P. multocida* and *M. haemolytica* infections was 26.6% (18.2% for *P. multocida* and 8.4% for *M. haemolytica*). The highest prevalence was reported in EL-Fayoum Governorate, while the lowest prevalence was in Beni-Suef. *P. multocida* was isolated either solely from 4.9% or mixed with other bacteria from 13.3% of the infected calves.

The aim of this work was to focus on the IHC technique as a comparative method, in parallel with H&E ordinary staining, for more understanding of the pathological lesions**.**

MATERIALS AND METHODS

1 -Sample collection:

The materials employed in this study consisted of randomly obtained 54 lung tissues of cattle from Dronka abattoir (Assiut) from different affected lungs, all the samples from male animals and aged from 1-2 years old.

2 -Histopathology

Tissue samples from the lung were fixed in 10% neutral buffered formalin. They were dehydrated by ascending grades of alcohol, cleared by xylene and embedded in paraffin. Sectioning of the tissue was performed at 5 microns thickness and stained with hematoxylin and eosin (H&E) (Bancroft *et al.,* 1996).

3 -Immunohistochemistry

Sections of the lung were prepared for immunohistochemical analysis using an Ultra Tek HRP anti-polyvalent (DAB) staining system (ScyTek Laboratories, West Logan, UT, USA, AMF080). The sections were deparaffinized with xylene, rehydrated in graded ethanol, and washed with distilled water. The sections were heated for 10 min in sodium citrate buffer (0.01 M, pH 6.0) to increase epitope exposure. The sections were left to cool at room temperature and washed with PBS. The endogenous peroxidase activity was quenched with 3% H_2O_2 in distilled water for 15 min, followed by washing with PBS (2X5 min). The sections were blocked with the blocking solution of the kit for 5 min at RT. The sections were incubated overnight at 4 C^o with the diluted rabbit polyclonal anti-CD3 (1:200; Abcam, Cambridge, UK, ab828). Sections were rinsed three times, 5 min each, with PBS and were incubated for 15 min with the secondary Ultra Tek HRP Anti-polyvalent antibody (goat anti-mouse, rat, rabbit and guinea pig IgG) purchased from Scy Tek (TX, USA). Following that, the slides were washed three times for 3 min each with washing buffer. The visualization of the reaction was carried out with Diaminobenzidine (DAB) chromogen diluted with DAB substrate (provided within the same Scy Tek Detection kit) for 10-15 min until the desired staining was achieved. The sections were counterstained with Harris hematoxylin and mounted with mounting media, DPX. The number of immunopositive cells was counted in 5 separate microscopic fields in each slide, and the mean number for each slide was obtained, then the mean ± SE was calculated for each group.

4 -Statistical analysis:

Statistical analysis of the data generated from the study was performed with GraphPad Prism 5. *P* values of <0.05 were considered statistically significant.

RESULTS

1 -Gross examination

Macroscopically, four of the fifty-four collected samples were normal and considered the control. The remaining lung tissues exhibited alterations in coloration (pinkish, reddish and brownish coloration), slight firmness during cutting in some lungs which was a result of consolidation, and some yellowish pus material in a few of them. There were some fibrinous, catarrhal and bloody exudates while cutting sectional parts**.**

Red discoloration of lung tissue commonly results from postmortem pooling of blood as veins dilate after death. Visible reddening indicated specific areas of the lung that should be carefully palpated and sampled histologically. Palpation is a key to effective examination of the lung and should be based on a cut section of the lung to examine the possible contents. Superficial gentle palpation is useless, and the fingers must be pushed deeply.

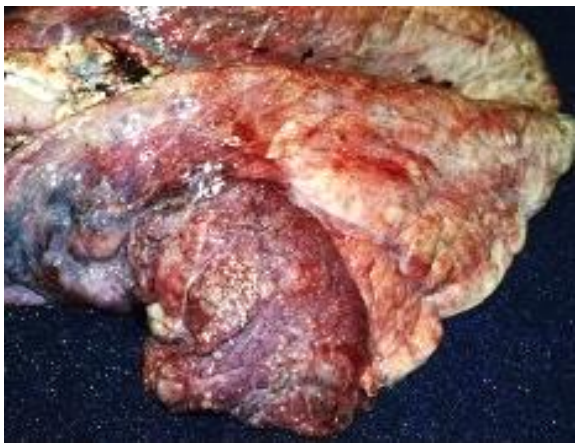

Fig. 1: *Chronic catarrhal pneumonia* is characterized by well-demarcated, purple to gray color and cranioventral reddening and collapse or consolidation, with firm texture and no increase of volume. In some cases, bands of obstructive atelectasis with a variable degree of alveolar emphysema, with round dry friable foci of caseous necrosis.

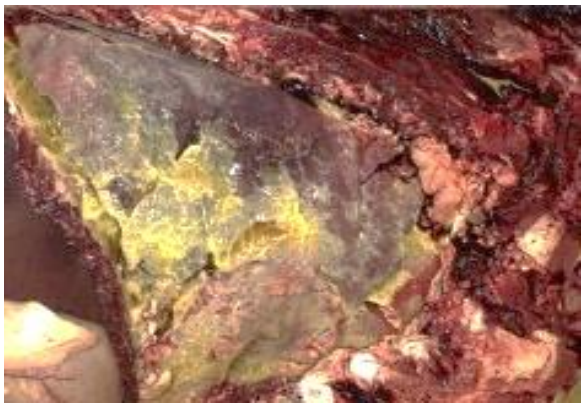

Fig. 2: Fibrinous pneumonia, with consolidation of lung tissue. The dorsocaudal lung of cattle is thick and fibrous. Interlobular and subpleural emphysema, which appears as lines of air-filled bubbles separating the lobules, arises commonly in cattle that are dyspneic for any reason.

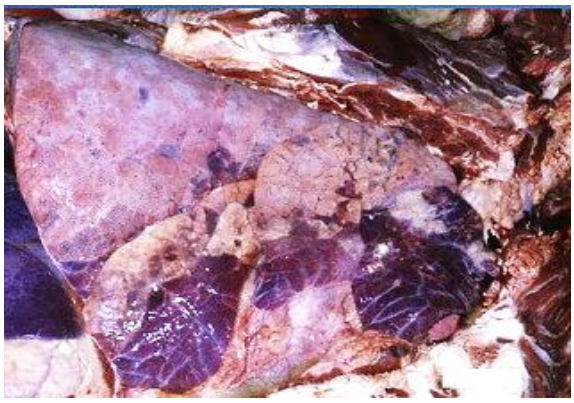

Fig. 3: *Chronic pneumonia*: the cranioventral lung is red-purple and slightly firm-rubbery; the dorsocaudal lung is similar to the other lung with edema and emphysema. On the cut section, affected zones were characterized by the presence of a variable amount of mucopurulent exudate.

2 -Histopathology:

Microscopically, 4 of the examined 54 lungs revealed normal alveoli, bronchi and bronchioles. There was chronic catarrhal pneumonia (22/50), interstitial pneumonia (n=18), pulmonary emphysema (n=4) and fibrinous pneumonia (n=6). Chronic catarrhal pneumonia showed fibrosis and hyperplasia with moderate cellular exudate of macrophages and some neutrophils in the alveoli. Chronic interstitial pneumonia exhibited diffuse thickening of the interstitium due to fibrosis, increased cellularity and smooth musculature. Pulmonary emphysema has a characteristic rupture of alveolar walls with communication of alveolar spaces to the appearance of aerated lung tissue. The characteristic feature of fibrinous pneumonia is fibrinous exudate with erythrocytic aggregation in the alveoli and bronchioles (Fig.4).

Immunohistochemical staining revealed a significant increase of CD3 cells in catarrhal pneumonia, chronic interstitial pneumonia and fibrinous pneumonia than in normal lung. Whereas pulmonary emphysema provoked fewer cells than the other lung infections (Fig.5).

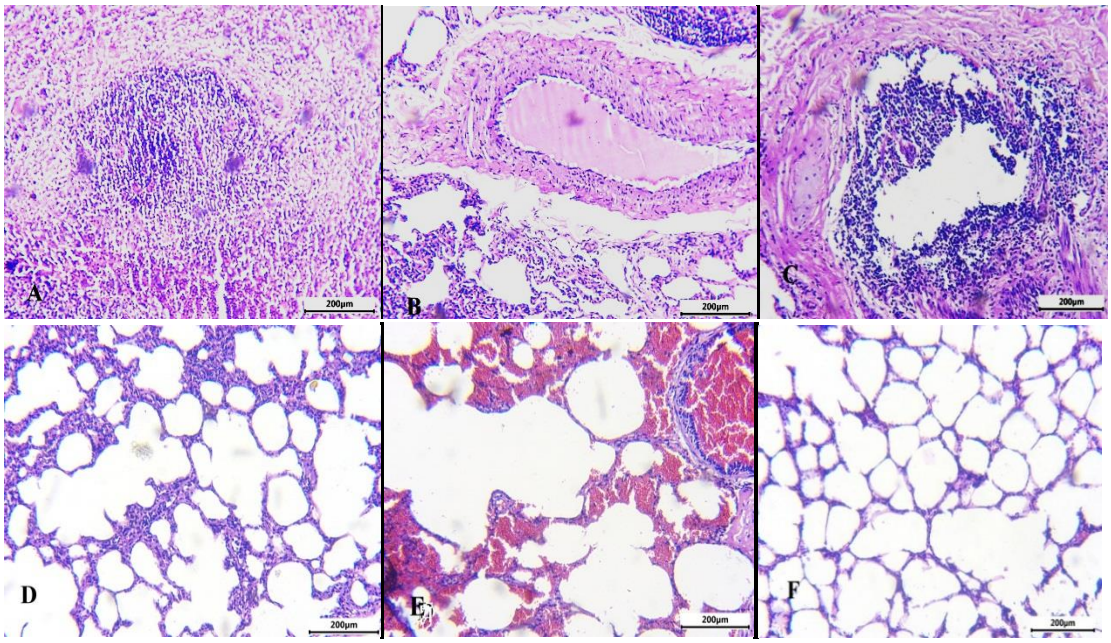

Fig. 4: H&E staining of the lung in different groups. Scale bar = 200 µm. **A), B)** Chronic catarrhal pneumonia showed fibrosis and hyperplasia with moderate cellular exudate in the alveoli. **C)** chronic interstitial pneumonia with marked thickening of the interstitium and diffuse aggregation of mononuclear cells. **D)** pulmonary emphysema with marked aerated alveoli. **E)** fibrinous pneumonia with marked fibrinous exudate within alveoli and bronchioles. **F)** normal alveoli of lung tissue.

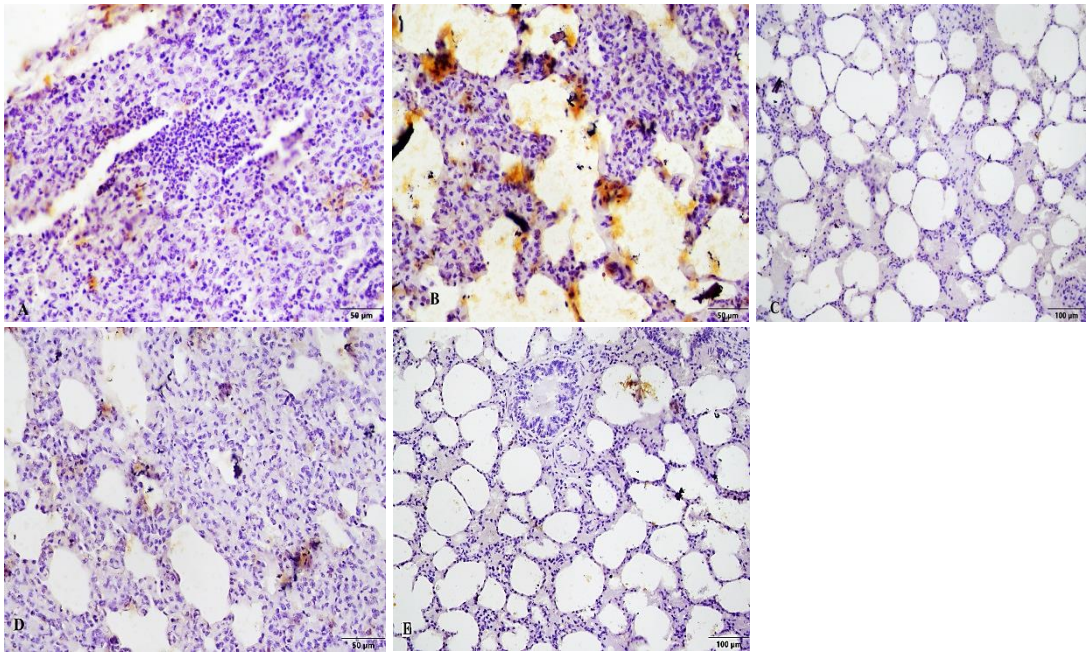

Fig. 5: Immunohistochemical staining of CD3 for different pathological lung affections: A) Chronic catarrhal pneumonia showed a marked increase of CD3 immunoreactivity cells **B)** chronic interstitial pneumonia with marked increase of CD3 immunoreactivity cells **C)** pulmonary emphysema with fewer CD3 immunoreactivity cells **D)** fibrinous pneumonia with high diffusion of CD3 immunoreactivity cells **E)** normal lung tissue with limited CD3 immunoreactive cells.

Table 1: Effect of different pathological affections on the percentage expression of CD3 in the bovine lung:

Group / Immune stain	Chronic catarrhal pneumonia	Chronic interstitial pneumonia	Fibrinous pneumonia	Pulmonary emphysema	Control -ve
CD3	13.70[b]	10.43[b]	8.40[b]	5.19[c]	1.8[a]

[a , b] values do not share a common superscript letter differ significantly at $P < 0.05$.

DISCUSSION

In the present study, 50 cases out of 54 lungs from slaughtered imported beef cattle showed lung abnormalities, 22 cases of chronic catarrhal pneumonia, 18 cases of interstitial pneumonia, while pulmonary emphysema and fibrinous pneumonia were 4 and 6 cases, respectively.

Our data revealed that chronic catarrhal pneumonia is characterized grossly by a well-demarcated, purple to gray color accompanied by some consolidated parts, with firm texture and no enlarged size. On the cut section, pneumonic parts contained a variable amount of mucopurulent exudates inside. Histologically, the main pathognomic features were an inflammatory infiltration formed by neutrophils and, to a lesser extent, macrophages in the alveolar lumina and newly made alveolar epithelization as well. These results were in the same line with (Miguel Fernández *et al.,* 2020).

Interstitial pneumonia, which histopathologically, exhibited widening of interstitial spaces with mucopurulent exudate, dilatation of lymphatic vessels with an inflammatory exudate composed of neutrophils and macrophages and, occasionally multiple foci of coagulative necrosis. These results are in harmony with the previous report **(**Jansen *et al.,* 1976).

Fibrinous pneumonia, in which the lung's gross appearance was surfaced by great thickened parietal and visceral pleura. In addition, it was covered with abundant amounts of yellowish fibrillary material (fibrin). The lungs have well-demarcated solid and swollen areas with evident vascular reactions, such as congestion or fibrin deposition over the pleura of the affected parts. A similar picture was previously described by (Lopez, 2012). Microscopically, an inflammatory exudate is composed of neutrophils and macrophages and, occasionally, small foci of coagulative necrosis. These observations are consistent with previous observations (Miguel Fernández *et al.,* 2020). The alveolar luminae were dilated and filled with fibrinous exudate which appeared as a fibrin network engaged with macrophages, neutrophils and erythrocytes (Lopez, 2012).

The CD markers concerning Bronchus Associated Lymphoid Tissue (BALT) with their histopathological features were previously illustrated in almost all eukaryotes (Pabst, 2022). Interestingly, on the other hand, BALT markers are not described in the respiratory system of adult animals and human beings but are present in young animals and children (Tschernig *et al.,* 1995)**.**

Immunologically, the layer of the mucosa of the lower respiratory tract of large ruminants has two immune mechanisms. One of them depends mainly upon T, B lymphocytes and plasma cells, which are distributed all over the epithelia and the lamina propria. CD3 cells are the most distributed marker in all epithelium of lower respiratory tissues (Heier *et al.,* 2011).

As bovine species reared mainly in open-aired farms, their immune system early developed that provoked high proportions

of CD-expressing T-cells. Furthermore, the bovine pneumonic lung pathogen is the only factor affording lymphatic cell proliferation (Anderson *et al.*, 1986a). Our findings, in harmony with the previous reports, exhibit an accumulative rise of CD3 cells in chronic catarrhal pneumonia, interstitial pneumonia and fibrinous pneumonia, respectively. Whereas, pulmonary emphysema is to a lesser extent than other previously mentioned types.

CONCLUSION

In conclusion, CD3 is a good marker for the diagnosis of bovine pneumonia and other pathological lung affections. It can be used as a confirmatory tool for ordinary pathological methods.

REFERENCES

Anderson, M.L.; Moore, P.F.; Hyde, D.M., and Dungworth, D.L. (1986a): Bronchus associated lymphoid tissue in the lungs of cattle: Relationship to age. Research in Veterinary Science, 41(2): 211–220.

Banchroft, J.D.; Stevens, A. and Turner, D.R. (1996): Theory and practice of histological techniques. Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

El-Seedy, F.R.; Hassan, H.M.; Nabih, A.M.; Salem, S.E.; Khalifa, E.; Menshawy, A.M.S.; Abed, A.H. (2020): Respiratory affections in calves in upper and middle Egypt: Bacteriologic, immunologic and epidemiologic studies. Adv. Anim. Vet. Sci., 8(5): 558-569.

Jeff, L.C.; Joanne, H.; Đurđa, S.; Josepha, D. and Ken, B. (2012): Laboratory and Postmortem Diagnosis of Bovine Respiratory Disease Vet Clin North Am Food Anim Pract. Nov; 28(3): 419–441. Published online 2012 Sep 6. doi: 10.1016/j.cvfa.2012.07.004

Heier, I.; Malmström, K.; Sajantila, A.; Lohi, J.; Mäkelä, M. and Jahnsen,F.L. (2011): Characterisat ion of bronchus-associated lymphoid tissue and antigen-presenting cells in central airway mucosa of children. Thorax, 66(2): 151–156.

Jensen, R.; Pierson, R.E.; Braddy, P.M.; Saari, D.A.; Lauerman, L.H.; England, J.J.; Keyvanfar, H.; Collier, J.R.; Horton, D.P.; McChesney, A.E.; Abarham Benitez and Christie, R.M. (1976): Atypical interstitial pneumonia in yearling feedlot cattle. J. Am. Vet. Med. Assoc.169: 507.

Lopez, A. (2012): Respiratory system, mediastinum, and pleurae. In: Zachary JF, McGavin MD, editors. Pathologic Basis of Veterinary Disease. 5th edition. St. Louis, Miss, USA: Mosby Elsevier; pp.458–538.

Luke, A.J.; Haydock, R.; Kent Fenton; Lauren Sergejewich; Ruud A.W.; Veldhuizen Dani Smerek; Davor Ojkic and Jeff L. Caswell. (2023): Bronchopneumonia with interstitial pneumonia in beef feedlot cattle: Characterization and laboratory investigation. Veterinary Pathology, 60(2): 214– 225.

Miguel, F.; María del Carmen, F.; Francisco, J., G.; Julio, B. and Valentín, P. (2020): Production Significance of Bovine Respiratory Disease Lesions in Slaughtered Beef Cattle. Animals, 10: 1770.

Olawale Olawumi Ola; Ganiyu Adetunji Adeniran; Tony Opoku-Agyemang; Ewurabena Bediako; Oluwagbenga Ishola; Theophilus Aghogho Jarikre, and Benjamin Obukowho Emikpe. (2020): Pathology and immunohistochemical evaluation of lungs of cattle slaughtered at metropolitan abattoirs in Nigeria and Ghana. journal of immunoassay and immunochemistry, Vol 00, Iss 00.

Pardon, B.; Hostens, M., Duchateau, L.; Dewulf, J.; De Bleecker, K.; Deprez, P. (2013): Impact of respiratory disease, diarrhea, otitis and arthritis on mortality and carcass traits in white veal calves. *BMC Vet. Res.* 2013; 9:79. doi: 10.1186/1746-6148-9-79.

Pabst, R. (2022): The bronchus-associated lymphoid tissue (BALT) an unique lymphoid organ in man and animals. Annals of Anatomy, 240: 151833.

Prado, M.E.; Prado, T.M.; Payton, M. and Confer, A.W. (2005): Maternally and Naturally Acquired Antibodies to Mannheimia Haemolytica and Pasteurella Multocida in Beef Calves. Vet. Immunol. Immunopathol, 111(3–4): 301–307.

Tschernig, T.; Kleemann, W.J. and Pabst, R. (1995): Bronchus-associated lymphoid tissue (BALT) in the lungs of children who had died from sudden infant death syndrome and other causes. Thorax, 50(6): 658–660.

التقييم الباثولوجى والمناعة الهستوكيميائية لرئة الماشية المذبوحة فى مجازر مدينة أسيوط

الحسن محمد مصطفى ، علاء الدين كمال يوسف ، عبد الرحيم حسن عطاى

تظل أمراض الجهاز التنفسي سببًا رئيسيًا للمظاهر السريرية والوفيات وفقدان الإنتاج وانخفاض جودة الذبيحة. ونظرًا للمظاهر السريرية المتداخلة لأسباب مختلفة من أمراض الجهاز التنفسي البقري (BRD)، فغالبًا ما يكون من الضروري إجراء تحقيقات مرضية ومخبرية لتأكيد تشخيص محدد في الحالات المعقدة. يكون التشخيص المحدد مفيدًا لتوجيه العلاج المضاد للميكروبات أو المضاد للديدان وبرامج التطعيم وممارسات الأمن الحيوي. تم تجميع أربع وخمسين عينة رئة من الثيران البالغة من مسلخ درنكة، أسيوط، مصر, لدراسة التغيرات المرضية والمناعية الكيميائية لأنسجة الرئة والتركيز على علاقتها بالخلايا المناعية. كانت هناك أربعة أنماط مختلفة من أمراض الرئة شائعة؛ الالتهاب الرئوي القصبي المزمن (التليف وفرط التنسج مع الخلايا الالتهابية الخلوية المعتدلة)، والالتهاب الرئوي الخلالي المزمن (سماكة منتشرة للنسيج الخلالي بسبب التليف وزيادة الخلايا والعضلات الملساء)، وانتفاخ الرئة (تمزق جدران الحويصلات الهوائية مع اتصال الفراغات الحويصلية وظهور أنسجة الرئة المهواة) والالتهاب الرئوي الفبريني (إفرازات فيبرينية مع تجمع كريات الدم الحمراء في الحويصلات الهوائية والشعب الهوائية). في هذه الدراسة، استخدمنا نهجًا جديدًا لتشخيص أمراض الرئة؛ وهو الكشف عن الخلايا الليمفاوية التائية بالاجسام المضادة ل CD3. كانت الخلايا الليمفاوية التائية الإيجابية CD3 على اتصال وثيق بأشكال مختلفة من الالتهاب الرئوي، ماعدا انتفاخ الرئة. كانت القيم المسجلة 13.7 و10.43 و8.40 و5.19 في الالتهاب الرئوي النزلي المزمن والالتهاب الرئوي الخلالي المزمن والالتهاب الرئوي الليفي وانتفاخ الرئة, على التوالي. علاوة على ذلك، فإن التفاعل المناعي لـ CD3 له دلالة جيدة للتعرف على مراحل مختلفة من الالتهاب الرئوي. وفي الختام، يمكن أن تكون الخلايا الليمفاوية التائية CD3 أداة مفيدة لتأكيد أنواع مختلفة من الالتهاب الرئوي.