



A Histopathological and Immunohistochemical Study in the Lung of Clinical Normal guinea pigs

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ABSTRACT

Background and Objective: The emergence of respiratory diseases in mammals is closely linked to good management, as the focus of disease diagnosis is limited to cases where clear clinical symptoms are not present, with the aim of prevention and eradication without treatment. Rats, followed by guinea pigs, are among the small mammals most commonly affected by respiratory problems. This study aimed to identify the macroscopic and microscopic lesions of the guinea pig lung.

Methods:

Naturally, the study included the use of ten animals of both sexes were obtained from Sinjar district, aged between 1-1.5 years, weighing between 1-2 kg, and measuring 20-25 cm in length. After performing the autopsy, the lung was extracted and examined macroscopically.

Results:

The lung consists of two adjacent lungs separated by the mediastinum. The right lung was larger than the left lung and occupied a large space in the thoracic cavity. The relative lung weight results did not show a significant difference between males and females, exception of one male, where the percentage of lung weight was (2.1%). The macroscopic changes included hemorrhage and pulmonary sclerosis in the right, left cranial and in the caudal lobe of lung. Different types of microscopic lesions were identified represented by massive infiltration of inflammatory cell, haemorrhage, proteinases exudate inside the bronchus, enlargement of hyaline cartilage and chondrocytes, fibrosis of the surrounding stroma, loss of the alveolar architectures, presences of edema, necrosis, fatty changes, and variable stage of parasites infestation inside lung tissue. Nonetheless, CD65 were used to target lesions of guinea pigs,

Conclusions:

The results intermediated between high dark, dark and light brown expression. These results indicated that guinea pigs can exhibit microscopic finding and CD65 exhibited the various degree of intensity expression in the absence of respiratory system damage.

Introduction

Guinea pigs are gaining economic importance in many developing countries, and given their importance as productive animals, they are affected by many factors such as inconvenient management of humidity, airflow, temperature, poor diets, house hygiene, making them susceptible to stress factors affecting their immunity status favouring the presenting of different diseases [1,2] where enteritis, pseudo tuberculosis, dermatophytosis and lymphadenitis are caused by several causative agents [3,4]. Pneumonia is a serious illness for Guinea pigs, it has a high morbidity and mortality rate [5] and can be caused by: virus, fungi, bacteria and toxic material. Bacterial causative agents are deemed petty pathogens since they required a previous deterioration of the pleading mechanisms at the lung level colonization [6,7] more frequently epizootics occurs in winter, younger animals and pregnant are more repeatedly affected, other predisposing factors including malnutrition, changes in temperature additionally to deficient or excessive ventilation which is more critical during seasonal change [8,9] respiratory systems infections usually transmission by direct contact, aerosol or through parturition [4]. CD65 is expressed constitutively on monocytes, but in neutrophils it is stored inside the cell, and mobilized to the surface upon priming. Neutrophil expression of CD65 has been shown previously to perform well as a biomarker of infection and even to distinguish bacterial from viral infections. Neutrophil CD64, as well as neutrophil and monocyte [10]. The objective of this work was to determine lung lesions and the expression of CD65 in healthy Guinea pigs.

Material and Methodology

The current study followed the ethics for the animals' research at the Veterinary Medicine College, Mosul University 29-9-2025.

Experimental Animals

The study involved the macroscopic and microscopic examination of ten adult Guinea pig lungs of both sexes, which were divided into two equal groups. Both groups were used for anatomical, histopathological observations and for the detection of pathological findings by immunohistochemistry technique.

Histopath finding

Lung samples were collected from Guinea pigs and placed in 10% formalin solution at room temperature for three days. After that, the samples were dried with rising ethyl alcohol

concentrations (70, 90 and 100%) cleaned with xylene, and immersed in paraffin wax molds. The samples were then cut using a rotary microtome at 4-5µm. The slides were collected on glass plates for routine hematoxylin and eosin staining according to standard protocols after removing the paraffin wax. Simultaneously, a second set of slides was used for immunohistochemical analysis by a pathologist using a digital camera with image processing software and examining by electron microscope [11,12].

Immunohistochemistry finding

Tissue sections underwent xylene dewaxing, rehydrated by ethanol, and washing by phosphate-buffered saline. The endogenous peroxidase activity was then suppressed for 30 minutes by adding a 3% hydrogen peroxide-methanol solution. The tissue sections were then blocked at 25 Celsius for 60 minutes; the slices were then treated with primary antibody overnight at 4 C. Then principal antibody utilized were monoclonal CD65 (Wuhan Biotech with 1:200 dilution) after washing 3 times with poly HRP Goat anti-Mice (Wuhan, China, dilution 1:200) for one hour at 37 C. After 3 times washing, with avidin-biotin complex the section were reveal. With haematoxylin the section was stained for one hour, in distilled water washed, dehydration and covering. The tissue sections were examined using an electron microscope and then photographed by a digital camera (Omax View, USB 2.0 with Megapixel about 9.0, China) [13].

Result

The study involved the macroscopic and microscopic examination of ten adult Guinea pig lungs of both sexes, which were divided into two equal groups. Both groups were used for anatomical, histopathological observations and for the detection of pathological findings by immunohistochemistry technique.

Macroscopic lesions

The current study included a macroscopic examination and morphological diagnosis of each animal after anesthesia Fig (1, 2). Macroscopic changes in the respiratory system, particularly in lung tissue. Consolidation and swelling of lung tissue were observed in fig (3) severe hemorrhage and congestion in all right and left cranial and caudal lung lobe were seen in fig (4,5).



Fig (1) Photomicrograph of Guinea pigs' .Group A



Fig (2) Photomicrograph showed preparing the animal after anesthesia for the purpose of performing the dissection.



Fig (3) Photomicrograph showed swelling and consolidation of guinea pigs lung (black arrow).

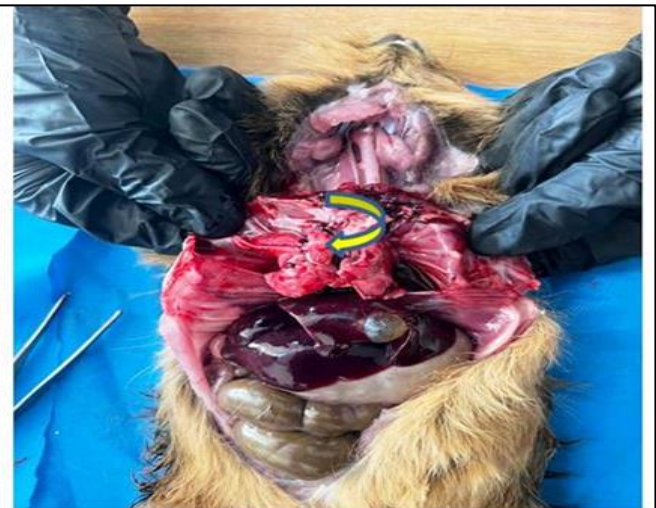


Fig (4) Photomicrograph showed severe hemorrhage of guinea pigs lung (yellow arrow). cranial and caudal lung lobe (blue and yellow arrow)



Fig (5) Photomicrograph of lung severe hemorrhage and congestion in all right and left

Histopathological finding

All histopathological findings observed in this survey study are summarized in table (1) various types of inflammation were detected in the examined lung tissues represented by infiltration of mononuclear and neutrophils cell and fibrosis fig (7,8 and 10) Circulatory disturbances was also noted revealed by recent thrombus , edema and congestion fig (9 and 10) on the other hand growth disturbances revealed hyperplasia was noted in fig (11). Degeneration and necrosis were also noticed which was represented by enlargement of hyaline cartilage and chondrocytes (7). However, proteinases exudate, pulmonary parasitic infection and large emphysema was also noticed in fig (6, 12, and 10).

Table (1): lung lesions categories including characterization, class and types of it

Categories	Lesion characterization	Class	Types
Acute inflammation	infiltration of mononuclear and neutrophils cell	Mild	Progressive
Chronic inflammation	sever obstruction in blood vessels (fibrosis)	Sever	Non- Progressive
Circulatory disturbances	Hemorrhage, edema and congestion	Mild	Progressive
Growth disturbances	Hyperplasia	Moderates	Non- Progressive
Degeneration and necrosis	Alveoli degeneration and necrosis	Sever	Non-Progressive
proteinases exudate	Deposition of proteinase material	Mild - Sever	Progressive& Non- Progressive
Pulmonary parasitic infection	Variable stage of parasites inside lung tissue	Mild- Moderates	Progressive
Emphysema	Lung tissue destructive lesions	Moderates	Progressive

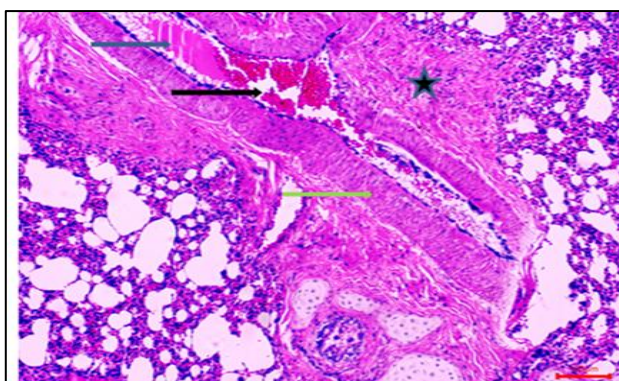


Fig (6) Microscopic pictures of guinea pigs lung showed thickening in the alveolar septa (green arrow), massive infiltration of inflammatory cell (star), haemorrhage (black arrow) and presences of proteinases exudate inside the bronchus, X100,H&E stain.

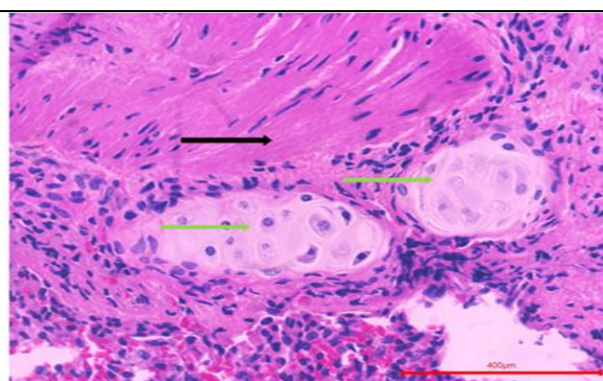


Fig (7) Microscopic pictures of guinea pigs lung showed enlargement of hyaline cartilage and chondrocytes (green arrow), fibrosis of the surrounding stroma (black arrow) X400,H&E stain.

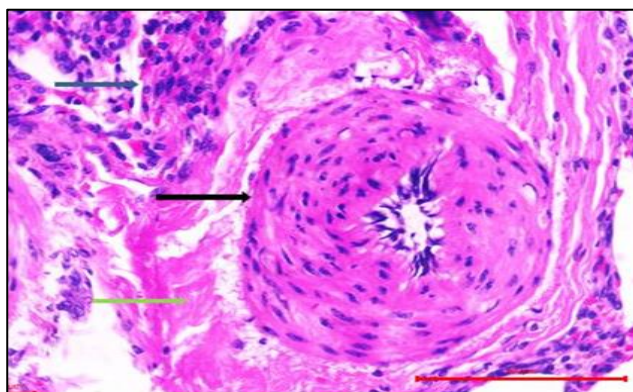


Fig (8) Microscopic pictures of guinea pigs lung showed sever obstruction in blood vessels (fibrosis) black arrow, necrotic area (green arrow), chronic inflammation with massive infiltration, X400, H&E stain.

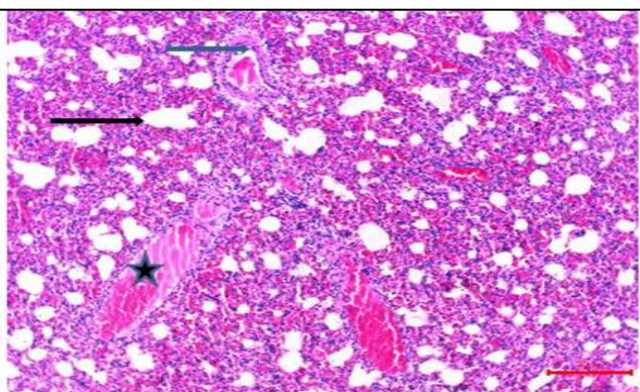


Fig (9) Microscopic pictures of guinea pigs lung showed recent thrombus (star), loss of the alveolar architectures (blue arrow), presences of edema (black arrow) X100, H&E stain.

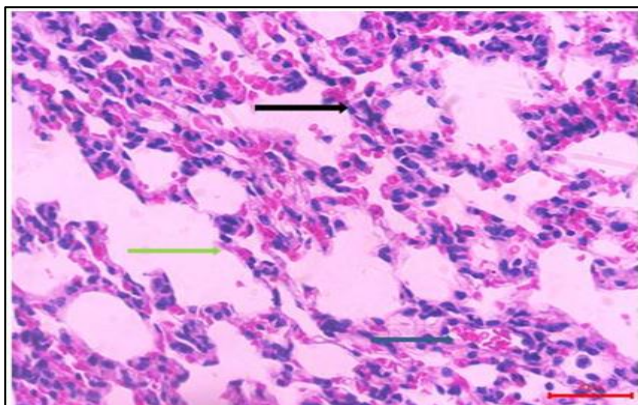


Fig (10) Microscopic pictures of guinea pigs lung showed sever infiltration of mononuclear and neutrophils cell (black arrow), sever congestion (blue arrow) denegation and large emphysema (green arrow) X400,H&E stain.

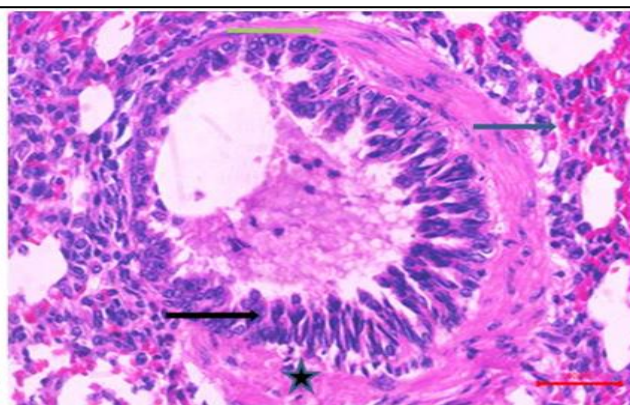


Fig (11) Microscopic pictures of guinea pigs lung showed hyperplasia of bronchus, (black arrow), necrosis (green arrow), fatty changes (star) and hemorrhage (blue arrow) X400, H&E stain.

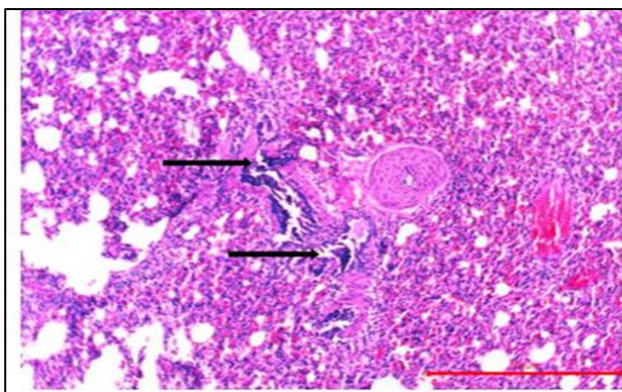


Fig (12) Microscopic pictures of guinea pigs lung showed caseous necrosis (black arrow), X400, H&E stain.

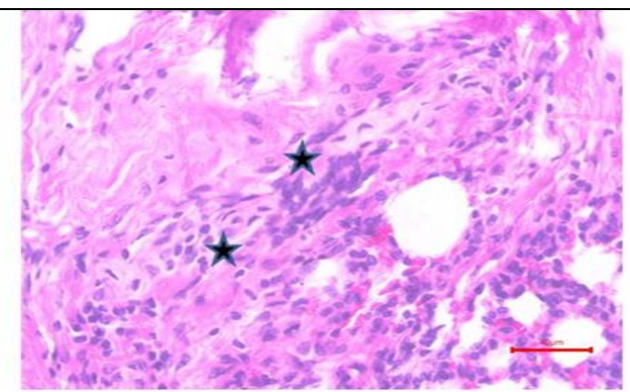


Fig (13) Microscopic pictures of guinea pigs lung showed variable stage of parasites infestation inside lung tissue, X400, H&E stain.

Immunohistochemistry Result

Table (2) showed the gene expression of CD65 in lung regions, which are divided into three expression sites (cytoplasmic, alveoli and bronchi according to the intensity of the color of expression. Figure (14) showed the high dark brown expression for CD65, figure (15) showed the brown expression while figure (16) showed the light brown expression.

Table (2). Grade of brown color distribution in lung parenchyma

CD65 expression	Lung area	Grade of distribution
	cytoplasmic	high dark brown (***)
	alveoli	dark brown (**)
	bronchi	light brown (*)

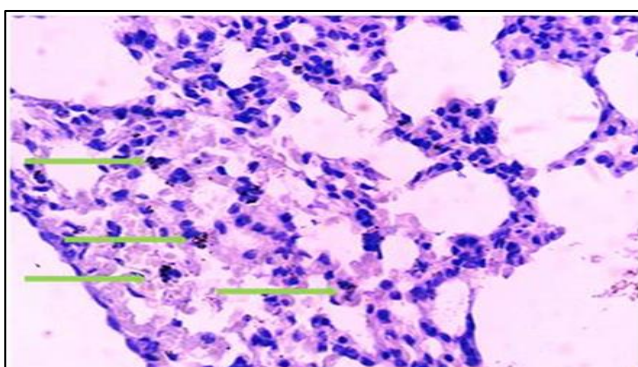


Fig (14) Pulmonary photomicrograph, high dark brown cytoplasmic expression for CD65, X400.

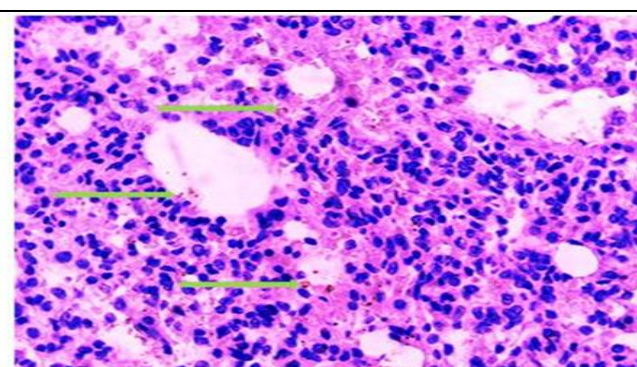


Fig (15) pulmonary photomicrograph, dark brown alveoli expression for CD65, X400.

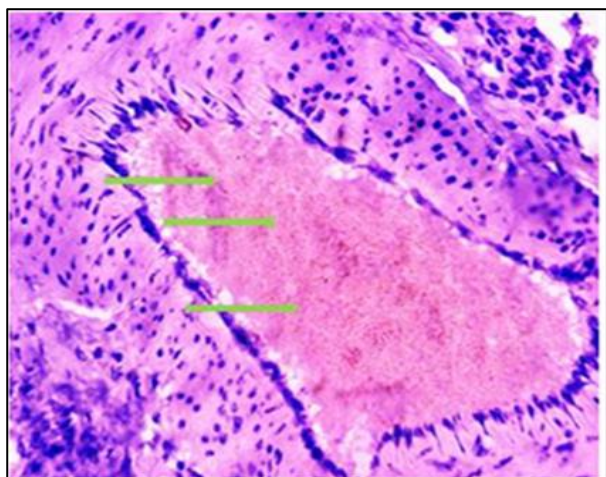


Fig (16) pulmonary photomicrograph, light brown bronchi expression for CD65,X400.

The relative weight of the lung.

The figure (17) shows the lung weight between male and female Guinea pigs .No significant differences in both sex except in the third animal (male) and the relative weight of the lung was (2.1%).

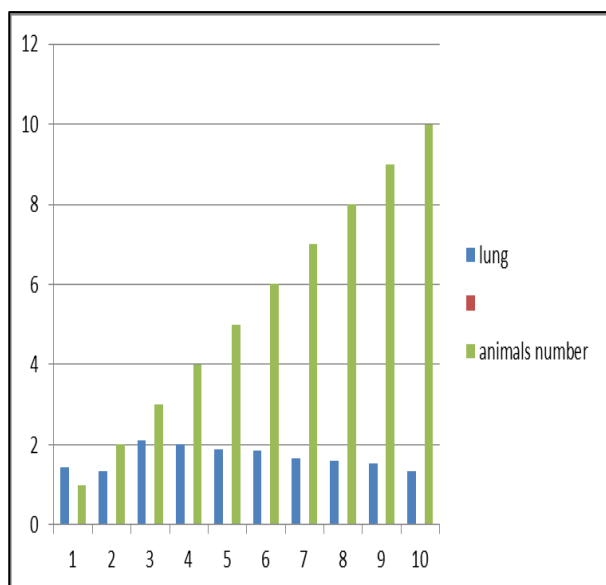


Fig (14) Relative lung weight

Discussion

The rodent animals of all kinds has a greater potential as an animal model of respiratory diseases due to its larger lungs and body when compared with other animals employed in earlier research [14] .As the principal organs of the human and animals respiratory system, the lungs are essential for preserving respiratory health and general well-being. The lungs are especially vulnerable to infections and environmental disturbances because of their anatomical exposure to the external environment [15].The lungs are the main location for co-diseases involving bacterial

and viruses pathogens among respiratory infections, where the innate immune system is quickly triggered to aid in pathogen removal and enhance host survival. The current study was designed to investigate the pathological conditions that affect the lungs of guinea pigs naturally, firstly macroscopically, and secondly microscopically using routine staining and immunohistochemistry techniques. The macroscopic lesions represented by sever haemorrhage and congestion in the right and left cranial lobe could be due to bacterial and viral infection, acute respiratory distress syndrome, impaired blood outflow leading to hypoxia and even death and causes ranging from toxicity, shock and infection [16]. Acute and chronic respiratory tract infections are accompanied by degenerative and proliferative lesions in the larynx, trachea and bronchi. The severity, type, and location of the inflammation depend on the causative agent, leading to ulceration of the mucous membrane epithelium and purulent inflammation. The accumulation of fibrin, mucus, and edema can ultimately lead to the animal's death due to airway obstruction [17]. Lab animals do not spontaneously develop pulmonary emphysema, which is precisely defined as aberrant expansion of the air spaces distal to the terminal bronchiole accompanied by destructive alterations of the alveolar walls. Numerous lung traumas can cause alveolar emphysema in these animals. Pancreatic intra tracheal injections of papain or elastics is used to causes emphysema in rats and mice for experimental purposes [18]. Inhaling cytotoxic particles, can also inducing emphysema through causing sever and focal inflammation according to the most widely accepted hypothesis of the disease's etiology, lung parenchymal inflammation causes an excess of elastase compared to its inactivating protein, which ultimately results in matrix degeneration. Alveolar emphysema has been observed in neonatal rats infected with the Sendai virus by the time they are four months old [19]. Rats and mice lung continue to grow until 10 month of age [19].pulmonary inflammation induced by viral and bacterial infection may present another pathway to develop emphysema in rats .Monocrotaline ,chronic hypoxia and hyperoxia have well been shown to causes chronic pulmonary hypertension in lab animals [20]. At the light level, this disease is characterized by pulmonary vascular abnormalities such as hypertrophy and hyperplasia of smooth muscle and more connective tissue in the adventitia of typically muscular intra-acinar arteries as well as

in the tunica in afflicted arteries ,the internal elastic lamina is evident .Because of the hypertrophy and hyperplasia of pericytes and intermediate cell [21] It is challenging to distinguish between diffuse dilatation of pulmonary capillaries and agonal alterations in the lungs of rats that are killed with toxic material or necropsied after they have died spontaneously for at least 20 minutes, this alterations is of no significance in the absent of other antemortem evidence in the vasculature or lung, always in the sub pleural alveoli ,also may occurs in the euthanized with carbon dioxide of die spontaneously [22].Intra –alveolar congestion and haemorrhage severity is proportional to the toxic material chamber concentration [22].lung epithelium necrosis stimulate mainly intraluminal exudate which will vary from mucopurulent ,fibrinous to serous depending on the timeframe and severity .Few numbers of acute inflammatory cells (neutrophil and macrophage) surround adjacent blood vessels and affected airways .Macrophage are eminent feature of inflammatory infiltration in resolving lesion or in inducing lesion in which epithelial tissue necrosis has occurred. Introduction of irritant materials into parenchyma of lung may inducing necrosis with stated suppurative inflammation (abscess) [23].At the present project we also demonstrated parasitic infection inside lung tissue and these contributed to immunological reaction, environmental pollution and indirect contact with other infected animals [24]. One biomarkers of respiratory system damage were used in the current study,CD65 to compare and evaluate their expression in normal guinea pigs, the expression intensity ranged between dark to light brown and these contributed to the immunity responses , neutrophil roles and other lymphocytes types against bacterial ,viral ,parasitic or even tuberculosis infection inside the respiratory system.Wahlstrom., et al [25] demonstrated that respiratory system lesions affect the efficiency and effectiveness of respiratory cells, and that the loss of these cells causes a loss of lung weight because the cell is the basic unit of the body, thus affecting the overall efficiency and health of the body.

Conclusion

A number of various causative agent of organism are capable of producing spontaneous pulmonary disease in Guinea pig. Different types of microscopic lesions were identified represented by (inflammation, Circulatory disturbances, Growth disturbances, Degeneration and necrosis,

proteinases exudate, pulmonary parasitic infection and Emphysema).The severity of the lung lesions ranged from mild, moderate and severe as well to Progressive and Non- Progressive. IHC expression of CD65 found mild, moderate to high brown color.

Acknowledgments

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Declaration of interests

The authors declare that there are no interesting conflicts.

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Data and availability of material

All data generated and analyzed during this study are included in this published article.

Author contribution

Al-Sabaawy H.B., Sample collection, laboratory techniques, manuscript writing, and revising, Al-salih M.A. and Shahbaa khalil AL-Taee photographing the pictures, Huda Waad, Zobaida Nabeel and Aya Qusay ,drafted manuscript collection.

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دراسة نسيجية مرضية وكيميائية مناعية في رئة خنازير غينيا السليمة سريريا

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الملخص

الخلفية العلمية والاهداف:

ان ظهور أمراض الجهاز التنفسي لدى الثدييات يرتبط ارتباطاً وثيقاً بالإدارة الجيدة، إذ يقتصر تشخيص المرض على الحالات التي لا تظهر فيها أعراض سريرية واضحة، بهدف الوقاية والقضاء على المرض دون علاج. تُعدّ الجرذان، تليها خنازير غينيا، من بين الثدييات الصغيرة الأكثر عرضة لمشاكل الجهاز التنفسي. هدفت الدراسة إلى تحديد الآفات المجهرية والعيانية في رئة خنزير غينيا طبيعياً

المواد وطرائق العمل

شملت الدراسة استخدام عشرة حيوانات من كلا الجنسين، تتراوح أعمارها بين سنة وسنة ونصف، ويتراوح وزنها بين كيلو غرام واحد و اثنان كيلو غرام، ويتراوح طولها بين 20 و 25 سنتيمتراً. بعد إجراء التشريح، تم استئصال الرئة وفحصها عيانياً

النتائج :

. تتكون الرئة من رنتين متجاورتين يفصل بينهما المنصف. كانت الرئة اليمنى أكبر من الرئة اليسرى، وتشغل حيزاً كبيراً في التجويف الصدري. لم تُظهر نتائج الوزن النسبي للرئة فرقاً ذا دلالة إحصائية بين الذكور والإناث، باستثناء ذكر واحد بلغت فيه النسبة المئوية لوزن الرئة 2.1%. شملت التغيرات العيانية نزيفاً وتصلباً رئوياً في الفص الأيمن والأيسر العلوي والسفلي من الرئة. تم تحديد أنواع مختلفة من الآفات المجهرية، تمثلت في ارتشاح كثيف للخلايا الالتهابية، نزيف، إفرازات بروتينية داخل القصبة الهوائية، تضخم الغضروف الزجاجي والخلايا الغضروفية، تليف النسيج الضام المحيط، وفقدان بنية الحويصلات الهوائية، بالإضافة لوجود الوذمة، نخر، تغيرات دهنية، ومراحل متفاوتة من الإصابة الطفيلية داخل أنسجة الرئة. علاوة على ذلك استخدم CD65 لتشخيص آفات رئة خنازير غينيا وترواحت شدة التعبير ما بين اللون الاسود ،البني الداكن والبني الفاتح

الاستنتاج

.تشير النتائج الى ان خنازير غينيا قد اظهرت نتائج مرضية ومناعية نسيجية في رئتها السوية.

الكلمات المفتاحية: D65، خنازير غينيا، تعبير بني فاتح، إصابة طفيلية.