

Histopathological effect of cold environment on pulmonary tissue in Local (Gallus Gallus) and foreign (Turkey broiler) breeds of chickens in Iraq

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Abstract

The present study was carried out on forty local and foreign broiler chickens (twenty for each) (one day old), reared for 35 days under cold environment (January and February-2015). The aim of this study was to assess the histopathological effect of cold stress and the cellular response of pulmonary tissue. All experimental animals were weighed then sacrificed and the lungs were collected and weighed. Specimens were fixed by 10% neutral buffer formalin. Routine histological preparations were carried out. Hematoxylin & Eosin was used for staining. At the end of the experiment, both breeds showed different signs of respiratory response. The work revealed that the pulmonary cellular lesions in foreign breed were more severe and more extensive than in local breed. In local breed, the cellular regenerative response represented by infiltration of immune cells was more efficient, and consequently, the current study revealed that Iraqi local breed was more resistant to environmental stress than European foreign breed which registered a respiratory infection at the end of rearing period. The larger relative size of the lung in European breed may explain the higher injury of its pulmonary tissue. It was concluded that the local breed was more adaptive to cold stress than the foreign breed.

Key words: Pulmonary tissue, Cold environment, histopathology, chicken.

دراسة نسجية مرضية مقارنة لتأثير الجو البارد على النسيج التنفسي بين الدجاج المحلي (Local breed) والدجاج المستورد (European breed) في العراق.

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الخلاصة

أجريت هذه الدراسة على أربعين من الصيصان المحلية والأجنبية بعمر يوم واحد. استغرقت فترة التربية خمسة وثلاثون يوماً تحت ظروف الجو البارد للفترة من (كانون الثاني وشباط) سنة 2015. هدفت التجربة إلى معرفة حجم التأثيرات المرضية النسجية الناتجة عن البرد على النسيج التنفسي بين العرقين. في نهاية التجربة تم وزن جميع الصيصان ثم تمت التضحية بها واستخرجت رئاتها وتم وزنها ثم ثبتت بالفورمالين الداريء المتعادل

10% وأجريت عليها التحضيرات النسجية الروتينية. صبغت الشرائح بصيغة الهيماتوكسلين-أيوسين بينت الدراسة الحالية أن كلا العرقين إستجابا لإجهاد البرد ولكن بطرق مختلفة حيث سجلت إصابات النسيج التنفسي في العرق الأجنبي (والمتمثلة بانسلاخ شديد في الظهارة القصيبية، إحتقان شديد في الشعيرات الأدينية، إفرازات ليفية مصلية واسعة، نزف شديد، التهاب رئوي بيني وإنسلاخ وتقرظظهارة القصيبات الثلاثية) وقد كانت أشد وأكثر إتساعا من التغيرات النسجية في العرق المحلي (والمتمثلة بفرط تنسج النسيج الظهاري واللمفي وارتشاح خلايا وحيدة النواة فضلا عن إحتقان الأوعية الدموية) إضافة لذلك إمتدت الإصابة في العرق الأجنبي إلى أماكن أبعد وأدق في النسيج التنفسي مقارنة بالعرق المحلي الذي تميز بكفاءة تعويضية عالية تمثلت بارتشاح الخلايا المناعية. وتبعاً لهذه التغيرات فإن العرق المحلي يعد أكثر مقاومة للبرد من العرق الأجنبي الذي سجل إصابات مرضية في الجهاز التنفسي في نهاية فترة التربية. إن كبر الحجم النسبي لرئة العرق الأجنبي مقارنة بالعرق المحلي قد يكون السبب وراء إصابة النسيج التنفسي بالمرض. تستنتج الدراسة أن عرق الدجاج الأجنبي واجه صعوبة في التأقلم نسجياً للبرد مقارنة بالعرق المحلي الذي حصل على فرصة أكثر للتأقلم.

Introduction

In spite of the principal function of gas exchange, the respiratory system of birds involved in temperature regulation. In variance with mammals, avian lung has a high gas exchange efficacy (Fedde 1998). Broiler chicken can live comfortably only in a relatively narrow thermal comfort zone of thermo-neutrality. Both low and high temperatures act in a negative way (Blahova et al 2007). The optimal temperature range for efficient production for broiler chickens over 4 weeks of age is 18-21c (Aengwanich and Simaraks 2004). Manning and Wyatt (1990) proved that broiler chickens adapted more easily to lower than to higher temperature. Shinder et al 2002 reported that short-term cold conditioning of chickens at an early age could induce an improvement either in thermo tolerance during cold challenge or in performance of chickens exposed to an optimal environmental temperature. Wandering macrophages are rarely found in the healthy avian respiratory system (Klika et al 1996). The avian respiratory tissue is different from that of other vertebrates, with birds having relatively small lungs with nine air sacs that play an important role in respiration (but are not directly involved in the exchange of gases) (Giesbrecht 1995), so that the large

size of the foreign breed was not proportion to the small size of the lung

Materials and methods

In this study, forty 1-day-old male and female, local and foreign broiler chickens were obtained from a commercial market. The animals were subdivided into four groups; male local, female local, male foreign and female foreign, including 10 chickens in each. The experimental facilities for all groups were the same. All groups were subjected to the same winter temperature till 35th day of age in the animal house, in which the temperature was not adjusted and depended on outdoor climatic conditions. The temperature ranged between 5–15°C. The broilers were reared during cold months (January and beginning of February 2015) to provide cold stress. After the period of rearing, the total body weight were recorded, then all chickens were sacrificed, and the lungs were collected and weighed. The relative weight of the lungs was obtained. Specimens were fixed in 10% neutral buffered formalin. Routine histological techniques were carried out on the specimens of lungs. Hematoxylin and Eosin stain was used. All the Images were uploaded into a computer by means of a digital camera (MEM 1300) through the microscope. T (Ballesteros et al., 2012).

Results

Body weight gain

The current study revealed that at 35 days post-hatching in cold environment, the mean body weight gain of foreign breed was (1230g), was higher than local breed (271.1 g). The relative lung weight of European breed (236.53 g) was also higher than local breed(183.2)(Table 1) . The present results revealed that both, local and foreign breeds were respond differently to cold stress and correspondingly exhibiting different signs of adaptation against that stress. Foreign breed showed more severe histopathological pulmonary changes than local breed and extend deeply in the minor pulmonary tissue leading to a respiratory infection. On the other hand, the study revealed that the immune response of female to environmental stress was higher than that of males.

Histopathological examination

Local breed (Male)

Extensive lymphofollicular hyperplasia in intrapulmonary primary bronchus with appearance of serofibrinous exudates in their lumen (Fig.1). Marked mononuclear cellular (MNCs) infiltration in the wall of parabronchi consists mainly of macrophage and lymphocyte with slight mucosal desquamation (Fig.2). Moderate fibro muscular hypertrophy of bronchiolar wall was recorded associated with lymphoid follicular hyperplasia (Fig.3), while no clear lesions were observed in atria except slight fibrinous deposition associated with mild heterophilic infiltration(fibrino purulent exudate) (Fig.4).

Local breed (Female)

The main characteristic lesions in the pulmonary tissue of this breed was the presence of hyperplastic lymphoid

follicles in the wall of parabronchi together with mucosal hyperplasia accompanied by serous exudates in their lumen (Fig. 5) , as well as evidence of pulmonary congestion, edema and slight thickening of atrial wall due to cellular infiltration (Fig 6). showed marked epithelial hyperplasia in the bronchial mucosa with persistent cilia with diffuse sub mucosal infiltration consists mainly of heterophils and lymphocytes (Fig.7). In addition ,the result showed an intense medial fibro muscular hyperplasia of congested pulmonary arteries with the presence of heterophils and lymphocytes in their lumen (Fig.8) and (Fig.9) showed focal and diffuse lymphocytic infiltration through pulmonary parenchyma.

European breed(Male)

The main histopathological lesions were moderate desquamation of tertiary bronchi with appearance of tissue debris in their lumen . In addition ,evidence of severe hemorrhage ,edema with diffuse serofibrinous exudates mixed with heterophils through pulmonary parenchyma as well as interstitial pneumonia was occurred (Fig 10) . Massive cellular aggregation of heterophils and macrophages mixed with fibrin network occluded the lumen of the primary bronchi with similar infiltration in their epithelial mucosa (Fig.11) while advanced lesion showed moderate to severe sloughing of bronchial mucosa together with moderate destruction in their wall (Fig.12).In addition, the result found severe accumulation of serofibrinous exudate in the wall of atria together with severe congestion of air capillaries . Evidence of severe intrapulmonary connective tissue fibrosis may be observed (Fig 13). Also severe atrophy of pulmonary

atria with disrupted appearance my recorded.

European breed (Female)

Destructive lesions in the wall of atria and tertiary bronchioles were noticed (Fig 14) also extensive with serofibrinous exudates in tertiary

bronchi together with destruction in their wall. (Fig.15) showed moderate to severe fibrinous deposition in tertiary bronchioles associated with cellular infiltration consists mainly of heterophils

Table (1). The mean Body weight (gm) and lung weight (gm) in both local and foreign breeds.

Mean Body weight		Mean Total Lung weight(right & left)		Relative weight	
Local	Foreign	Local	Foreign	Local	Foreign
271.1±1.3	1230±1.6	1.48±0.89	5.19±1.9	183.17	236.53

Discussion

The present study was attempted to correlate between body weight and histopathological examination, however mild and proliferative changes were seen in pulmonary tissue of local breed as compared to those severe lesions that seen in European breed. The lymphoid tissue hyperplasia mainly in the wall of primary bronchi and parabronchi may indicate a good immune response elicited by local breed (Shnawa et al 2014). Also mononuclear cellular infiltration in the parabronchial tissue consist mainly of macrophages and lymphocytes that consider main component of innate immunity, moreover appearance of inflammatory cells in the lumen of blood vessels together with evidence of pulmonary congestion and edema that refers to signs of DTH reaction (Kemp et al 2008).

When a tissue exposed to an environmental stress, Many histological responses were occur, namely; vascular congestion, mononuclear cells infiltration , epithelial and lymphoid hypertrophy and tissue hyperplasia, apoptosis and edema,etc, that was to increase the efficiency and immune defense of the

tissue and to prevent further invasion of different organisms. As a result of these interaction, two pathways were resulted; either tissue survival or death. This was agreed with the hypothesis of Fulda et al 2010. When the respiratory system was subjected to cold environmental stress, the upper respiratory passages were firstly attacked and react with that factor, if the system failed to acclimatized and withstand that stress ,invasion of microorganisms will occur. The present study revealed that most lesions of the respiratory tissue of foreign breed were moderate to severe in intensity but extend deeply in the minor respiratory passages. This is in accordance to (Weiss 1984). The foreign breed failed to overcome and stop the invasion of microorganism that extend deeply to the minor parts of respiratory system, the tertiary bronchioles. When a tissue exposed to any external stress challenge, the first defensive sign is the increase of the blood supply to the effective area providing the area by a defensive leukocytic cells, In addition, increase the number and size of the epithelial, lymphatic and muscular tissues by hyperplasia and hypertrophy. On the

other hand, the regenerative capability of apoptotic cells was increased. Apoptosis eliminates dead or unwanted cells in the lumen of bronchioles. It was concluded that local breed was fairly acclimatized to cold weather comparing to foreign breed. On the other hand, The variation to environmental response between male and female may be attributed to the variation in the activity of humoral and cell-mediated immune response between the different sexes. This is similar to the findings of Leitner et al 1989 who reported that females responded 24-72 hr earlier than males and with higher peak of antibody titers, Moreover, In-vitro proliferation of T-lymphocytes to infection showed an earlier & greater responses in females ,so that we concluded that sex-related

differences in response of broiler chicken may result from a less efficient response in male. Recent studies have revealed that estrogens play an important role in the immune defense and referred to the presence of estrogen receptors on cells-related to immune responses (Cutolo et al 1995). Foreign breed represented by signs of affection like severe sloughing of bronchial mucosa, severe congestion of atrial capillaries, diffuse serofibrinous exudates, severe hemorrhage, interstitial pneumonia and desquamation of tertiary bronchithat ,while Local breed represented by signs of defense like hyperplasia and hypertrophy of pulmonary tissues, blood vessel congestion, leukocytic infiltration and regenerative apoptotic activity.

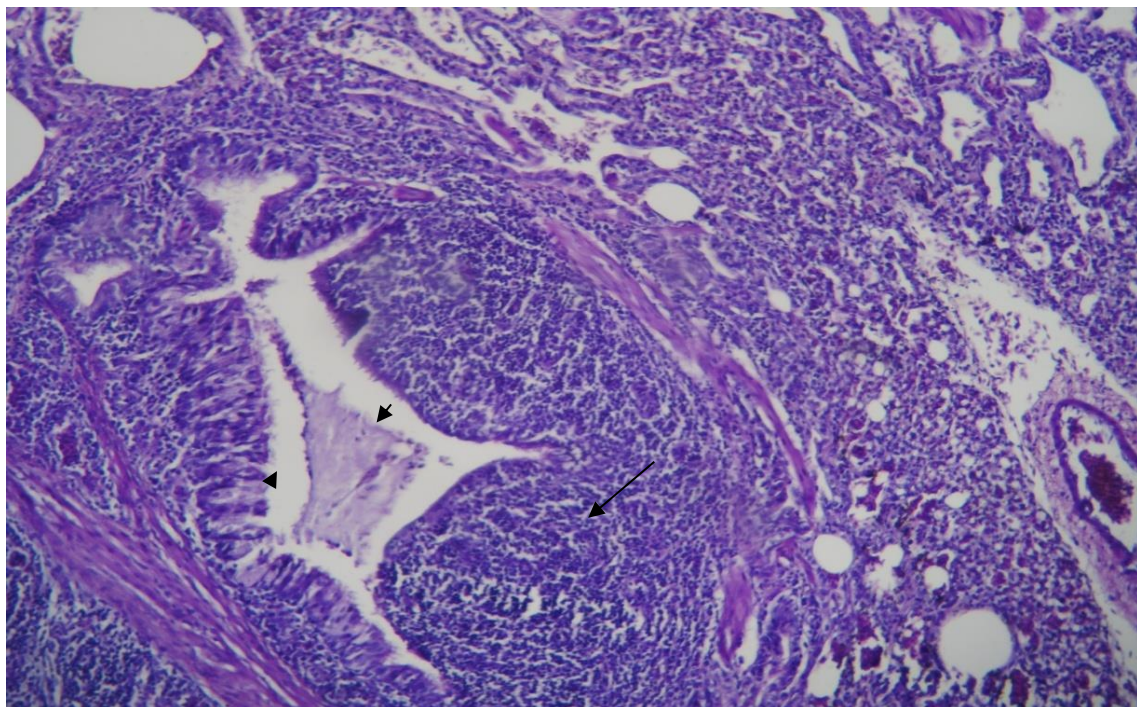


Fig.1 Chicken Lung (Local breed) (Male) exposed to cold environment. Note the presence of hyperplastic lymphoid follicle (long arrow), mucosal hyperplasia(arrow head) accompanied by serous exudates in their lumen(short arrow), pulmonary congestion, edema and slight thickening of atrial wall. X200. H&E stain.

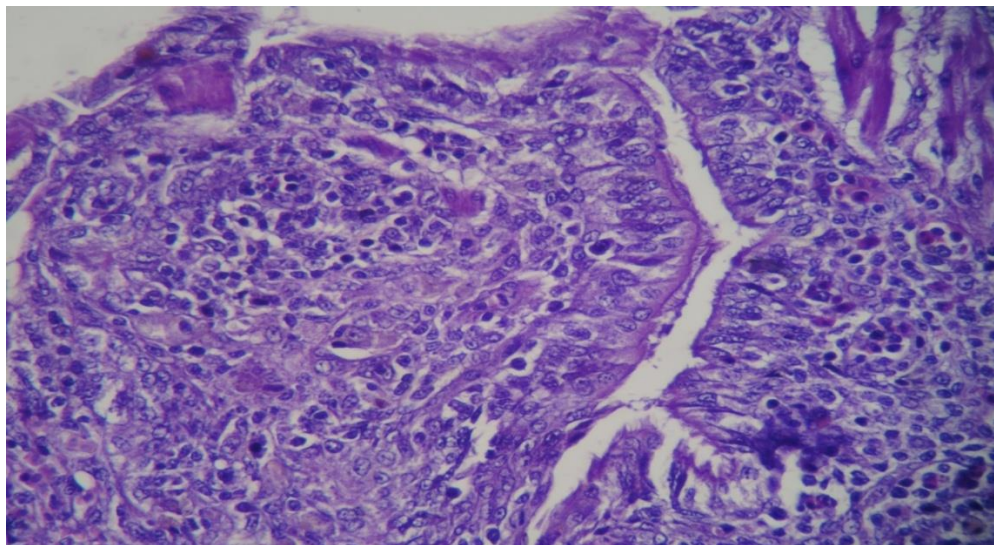


Fig. 2 Chicken Lung (Local breed) (Male) exposed to cold environment. Note leukocytic infiltration especially lymphocyte (short arrow) and macrophage (long arrow) in the wall of parabronchus with slight mucosal desquamation(arrow head). X400. H&E stain

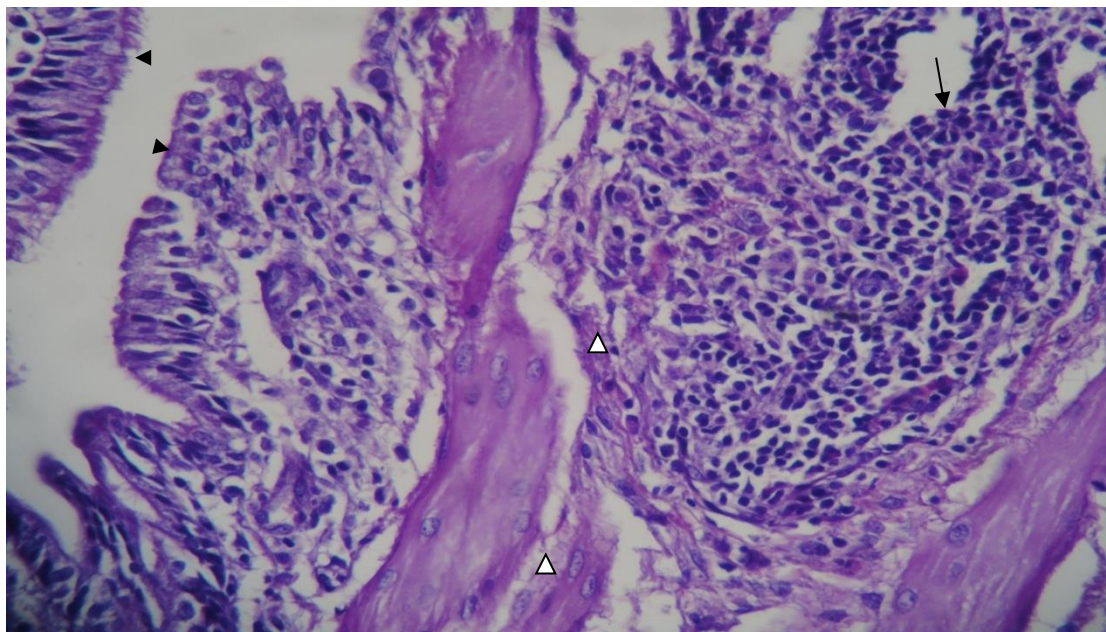


Fig.3 Chicken Lung (Local breed)(Male) exposed to cold environment, shows marked epithelial hyperplasia with persistent cilia (short arrows) , hyperplastic follicles in the wall of parabronchi (long arrow) and moderate fibromuscular hypertrophy (arrow heads). X400. H&E stain.

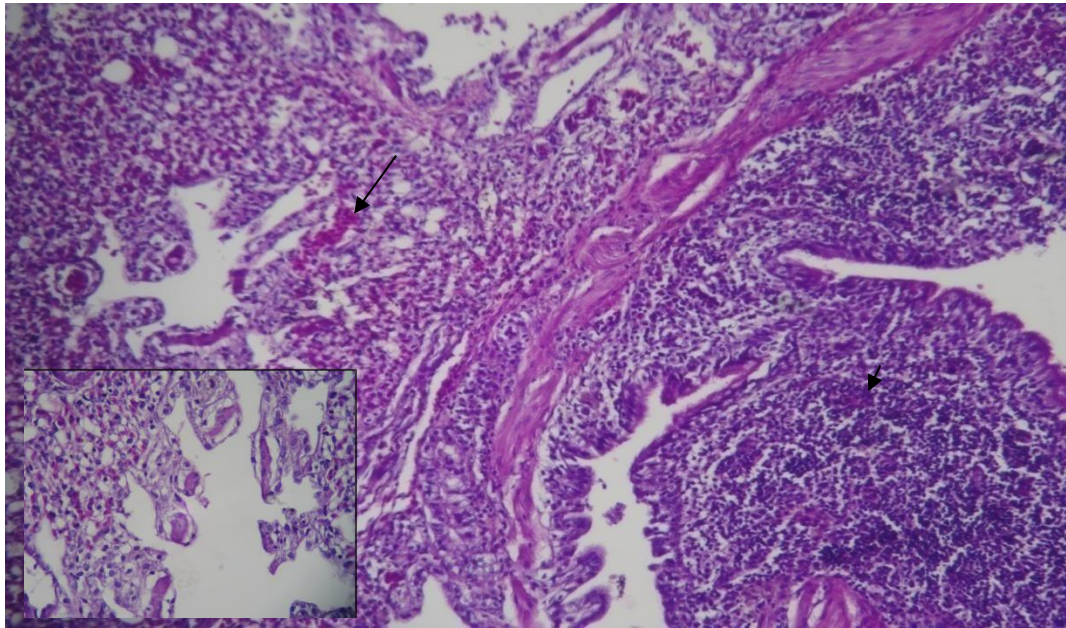


Fig. 4 Chicken Lung (Local breed)(Male) exposed to cold environment. Note the lymphoid follicular hyperplasia (short arrow) in the peri bronchial tissue, slight congestion of air capillaries (long arrows), no clear lesion in pulmonary atria. X200. H&E stain.

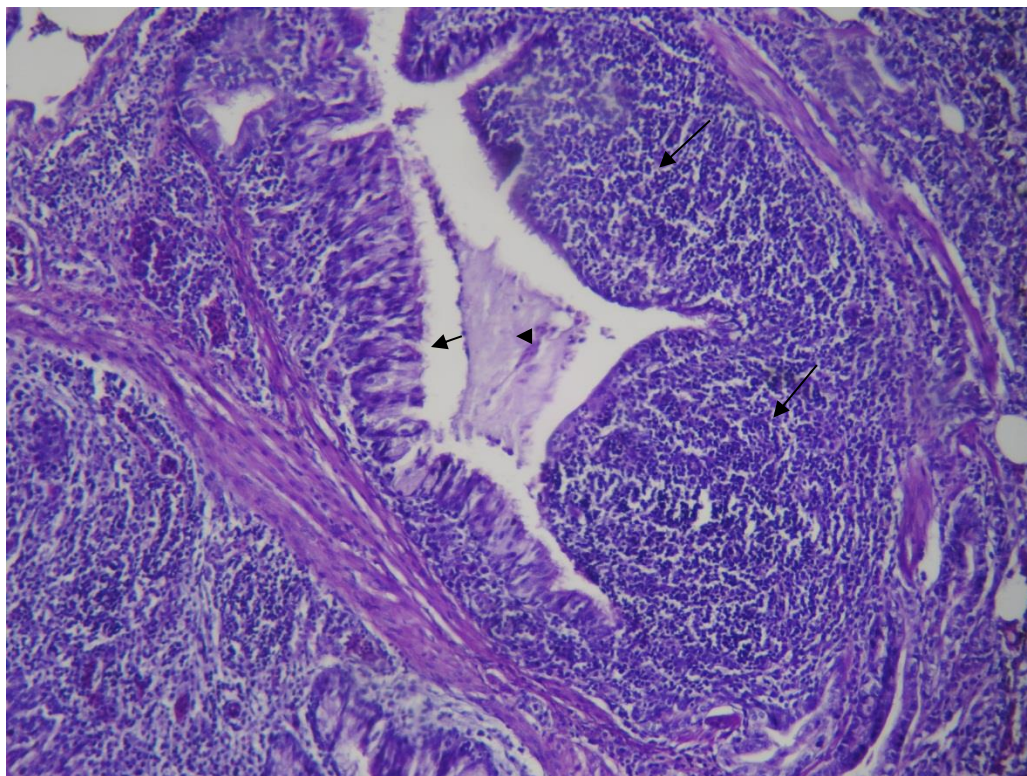


Fig. 5 Chicken Lung (Local breed) (Female) exposed to cold environment .Shows hyperplastic lymphoid follicle in the wall of parabronchus (Long arrows) with mucosal hyperplasia (Short arrow) and luminal serous exudates (arrow head) . X400. H&E stain.

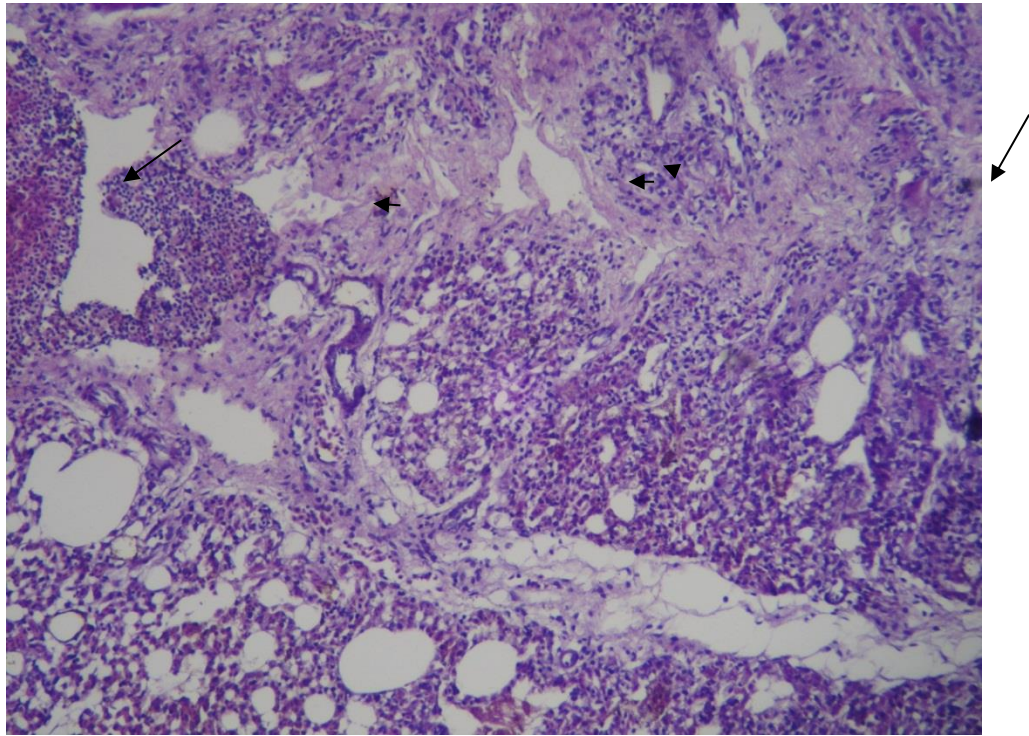


Fig.6 Chicken Lung (Foreign breed)(Female) exposed to cold environment. Shows pulmonary congestion (Long arrows) ,edema(Short arrow) and slight thickening of atrial wall heterophil. X200.H&E stain.

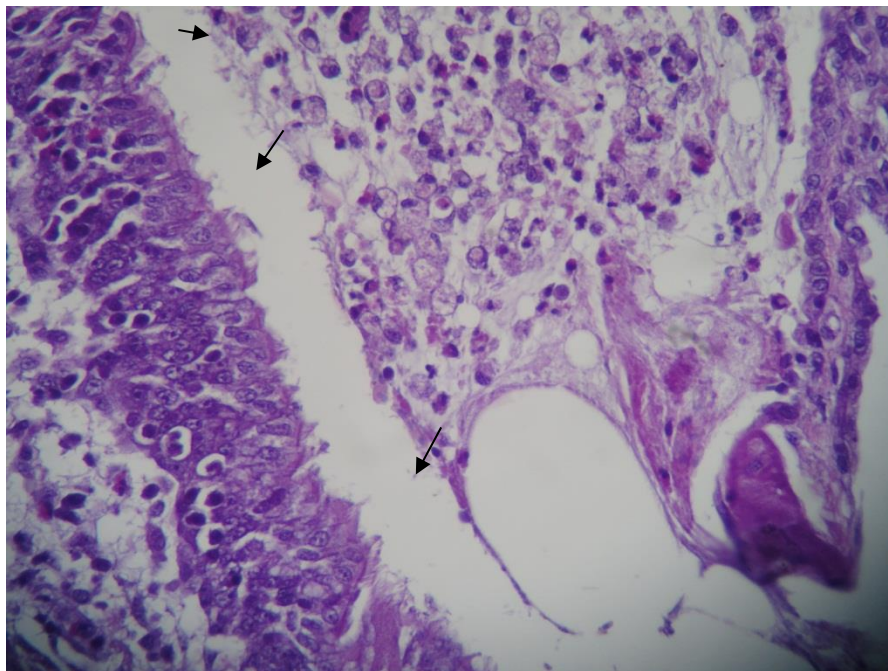


Fig. 7 Chicken Lung (Local breed)(Female) exposed to cold environment. Shows bronchial epithelial hyperplasia with prominent cilia (Long arrows) and luminal fibrino purulent exudates (Short arrow) . X200.H&E stain.

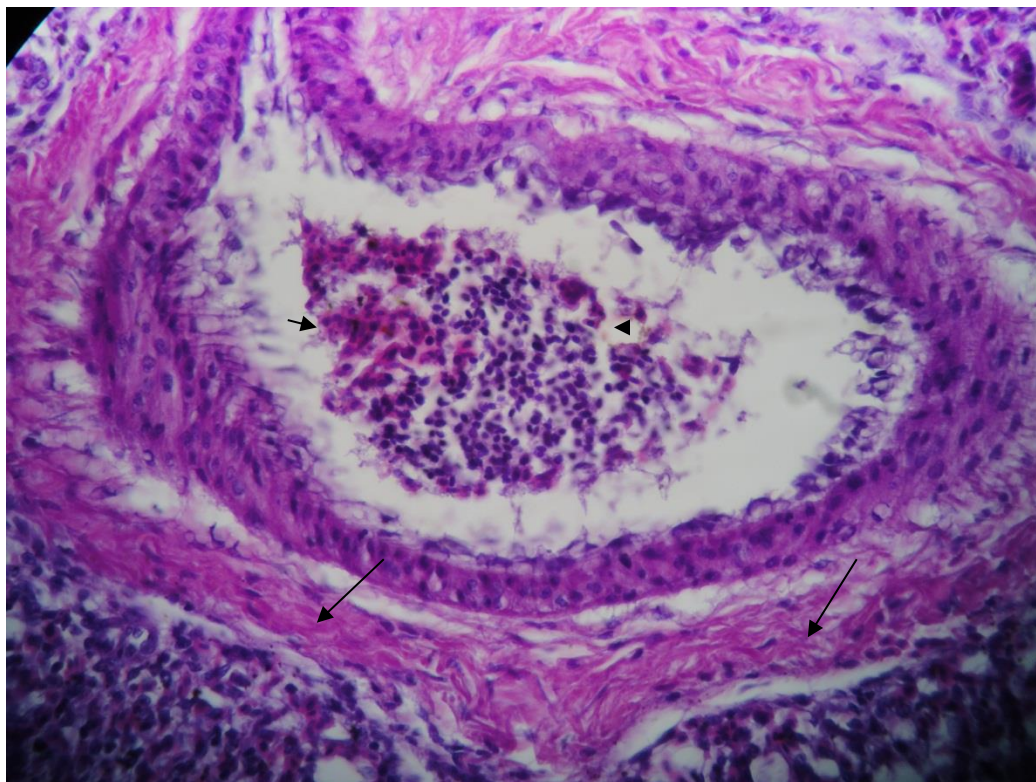


Fig 8. Chicken Lung (local breed)(Female) exposed to cold environment. Shows intense medial fibro muscular hyperplasia (Long arrows) of congested pulmonary arteries with the presence of heterophils (Short arrow) and lymphocytes(Arrow head) in their lumen.X 400 .H&E stain .

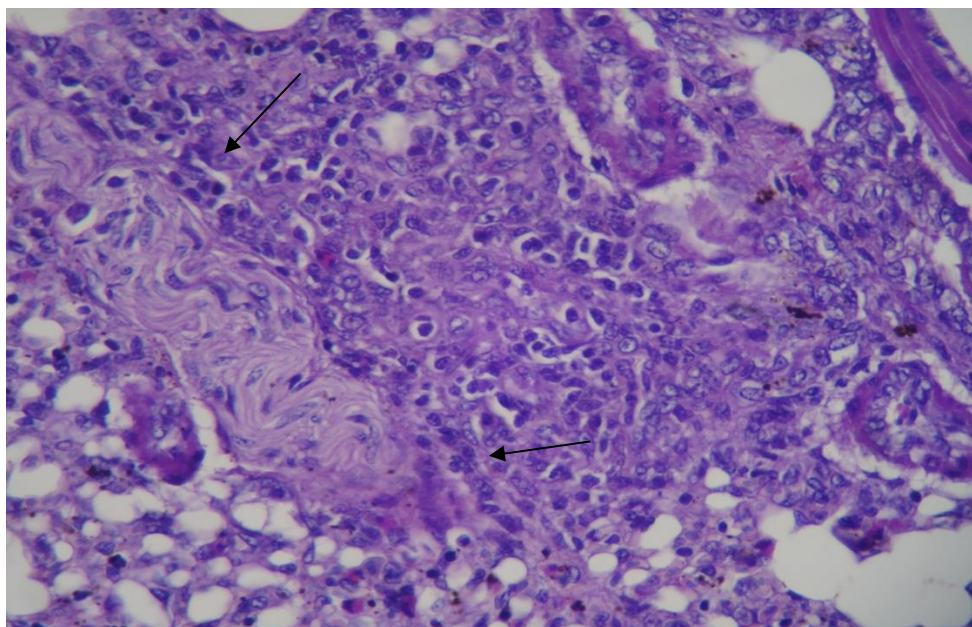


Fig. 9. Chicken Lung (local breed)(Female) exposed to cold environment. shows focal and diffuse lymphocytic infiltration through pulmonary parenchyma(Long arrows). X 200. H&E stain.

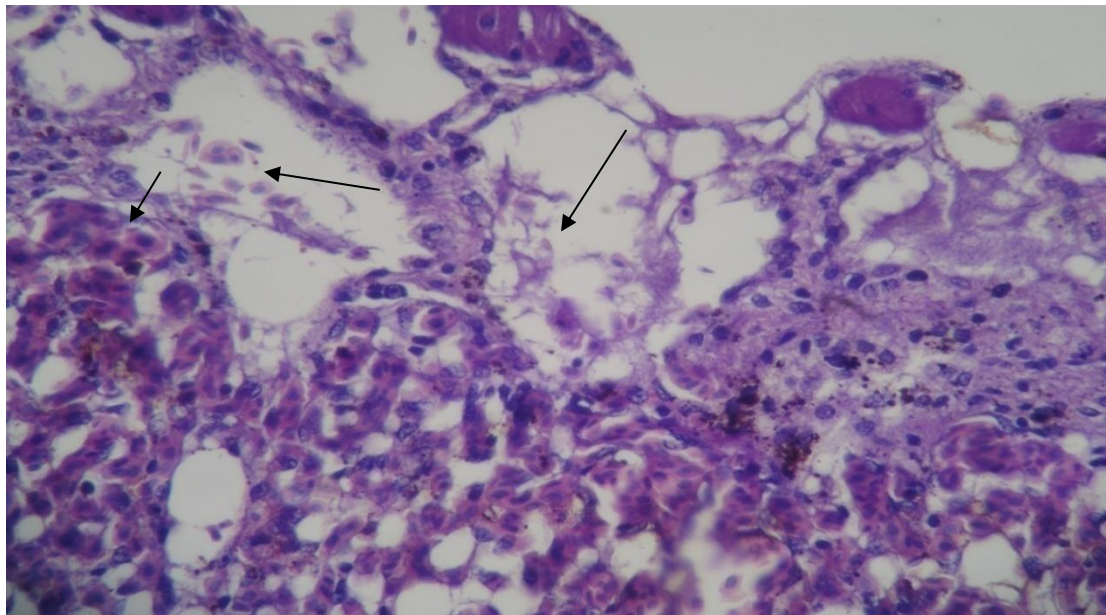


Fig 10 .Chicken Lung (Foreign breed)(Male) exposed to cold environment. Advanced lesion showed severe epithelial destruction(Long arrows). The wall of atria & tertiary bronchus is accompanied by congestion(Short arrow). X200.H&E stain

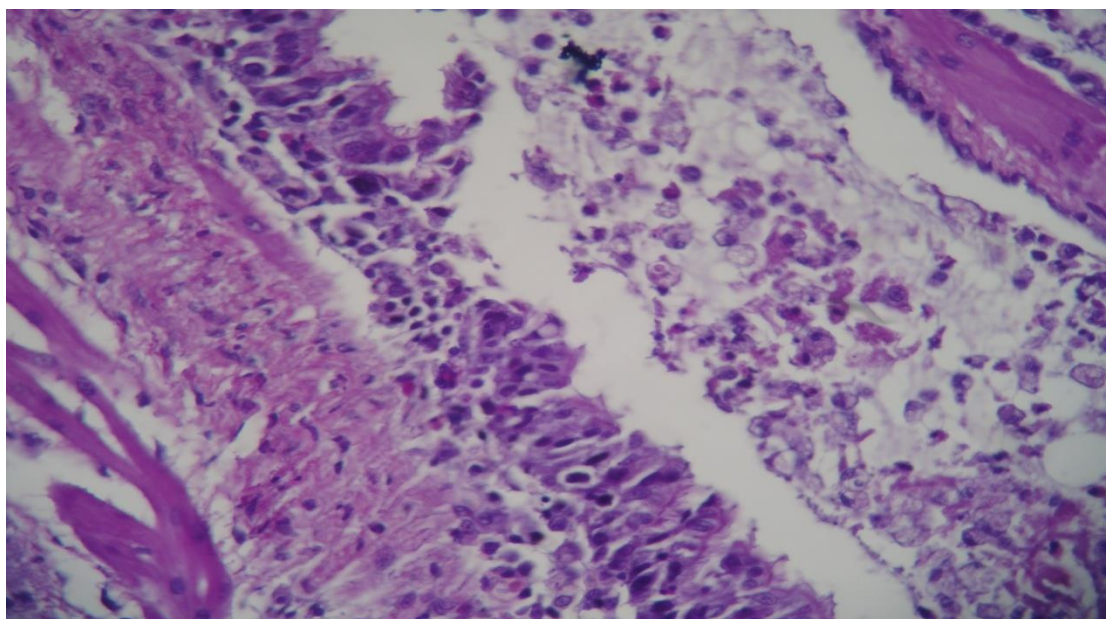


Fig 11. Chicken Lung (Foreign breed)(Male) exposed to cold environment. Severe accumulation of fibrinopurulent exudates (long arrow) together with moderate sloughing of bronchial mucosa. X400.H&E stain.

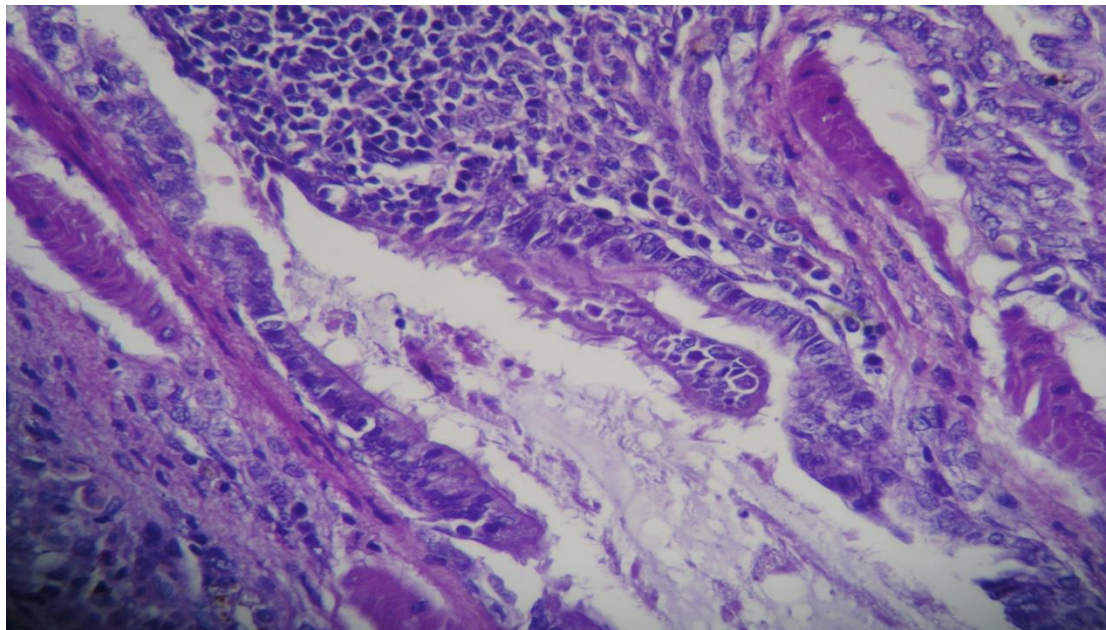


Fig 12. Chicken Lung (Foreign breed)(Male) exposed to cold environment. Note the advanced lesion with moderate to severe sloughing of bronchial mucosa and moderate destruction of their wall(arrows).X200.H&E stain.

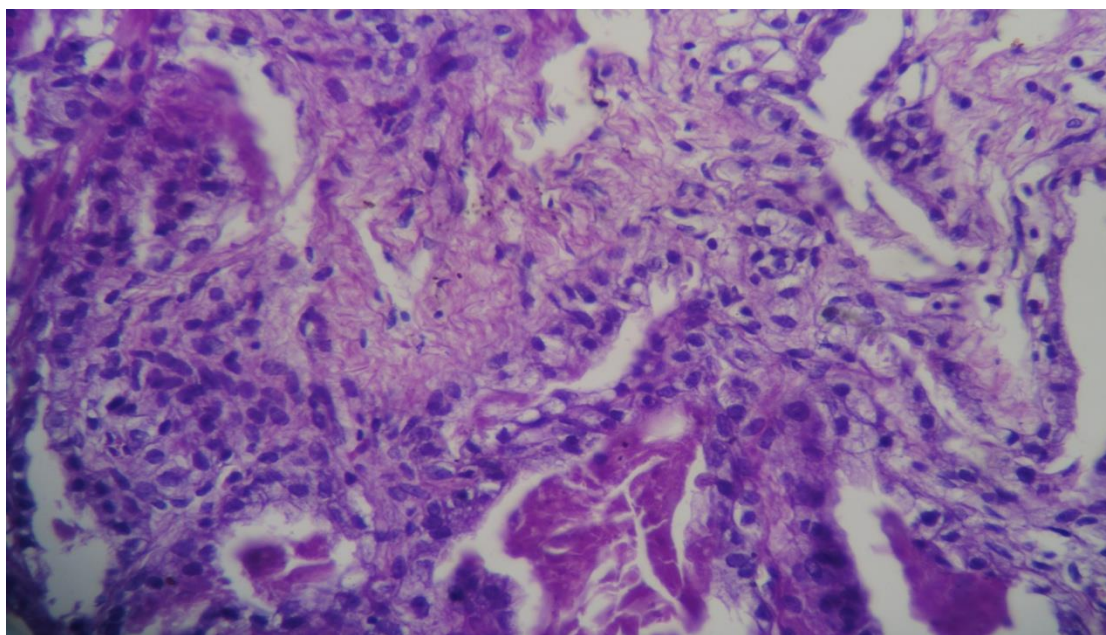


Fig 13. Chicken Lung (Foreign breed) (Male) exposed to cold environment. Shows severe fibrin network deposition in the tertiary bronchiole(Arrows) accompanied with heterophilic infiltration (arrows head). X200.H&E stain.

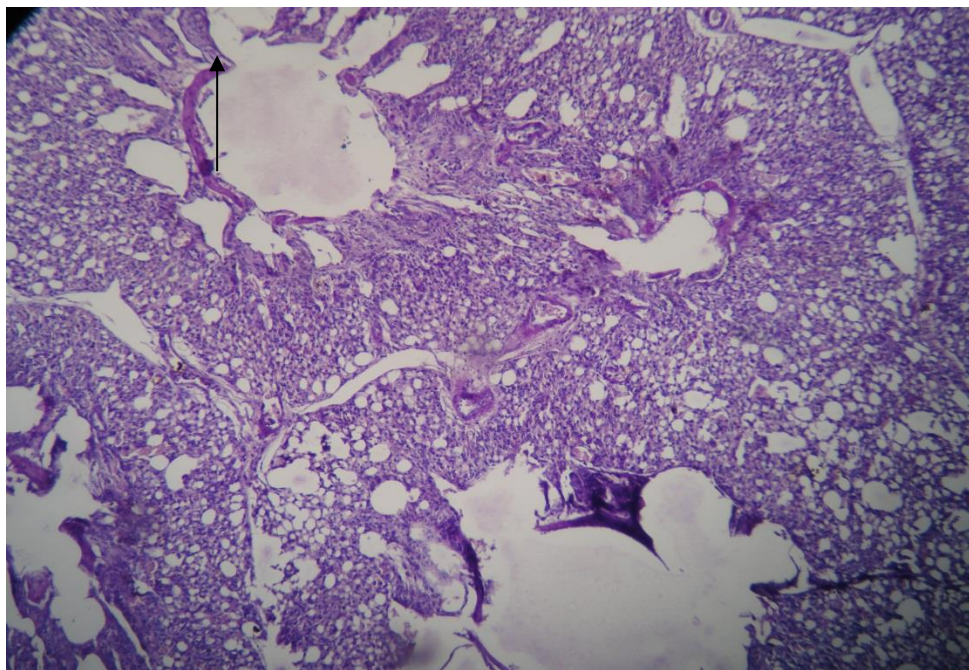


Fig 14. Chicken Lung (Foreign breed)(Female) exposed to cold environment. Show Destructive lesions of tertiary bronchioles (Long arrow).X 200 . H&E stain .

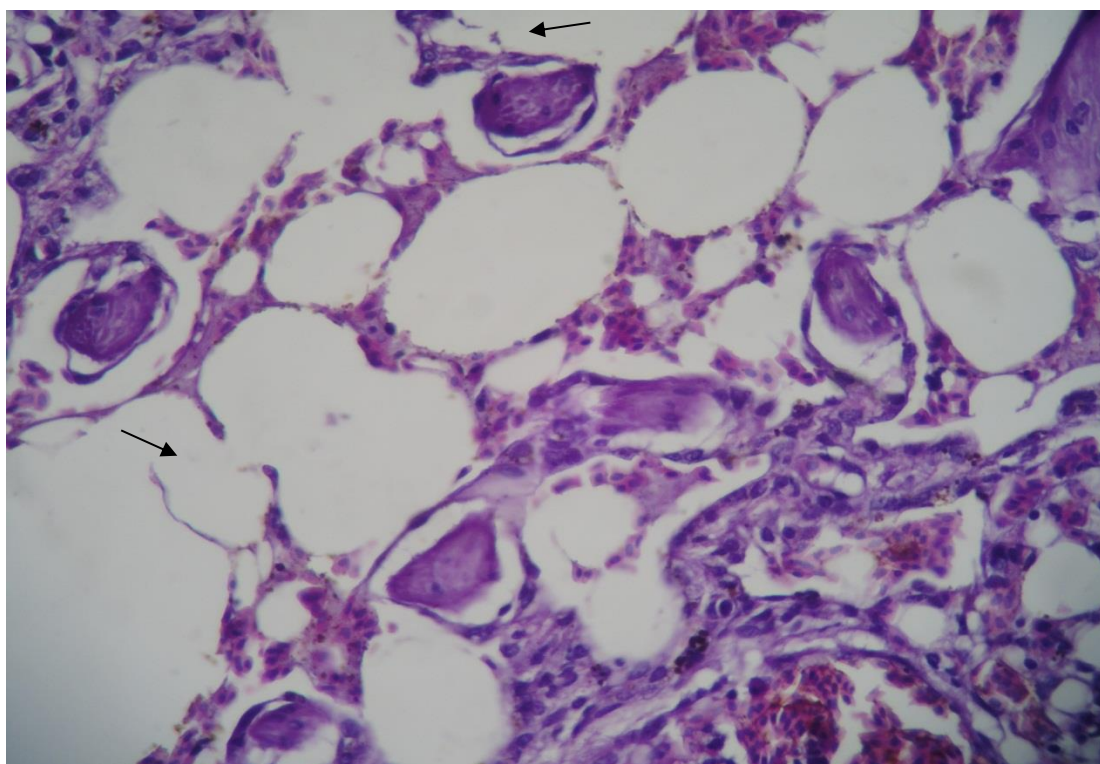


Fig 15.Chicken Lung (Foreign breed)(Female) exposed to cold environment. Shows serofibrinos exudate in the tertiary bronchi with wall destruction (Arrows). X400 . H&E stain.

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