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Anatomopathological And Histopathological Study Of The Respiratory System Of Local Chickens Of African Breeds (*Gallus Domesticus*) Following Natural Exposure To Avian Influenza Virus In Ivory Coast

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Abstract
Background: *There has been a lot of research on poultry around the world. But in Africa, and particularly in Ivory Coast, there are few data on chickens. This study is conducted on chickens of African breeds called «bicycle chickens» to compensate for the lack of data on this species. This work aims to combat avian influenza in Ivory Coast, by studying its impact on the respiratory system of farm chickens by histological technique.*
Materials and methods: *The sample consists of twenty local chickens of both sexes. Macroscopic observation with the naked eye as well as observation using an electronic magnifying glass device allowed to detect lesions and ranges of viruses. Histological treatments and observation under an optical microscope have identified histopathological lesions in the respiratory system.*
Results: *Numerous anatomopathological lesions are observed in the respiratory system of chickens. It is fibrinous sinusitis, aerosacculitis, fibrinous pneumonia and tracheitis.*
Histopathological lesions detected in the lungs were interstitial pneumonia, endothelioma and bronchial thrombosis. Tracheitis with deciliation, metaplasia of the epithelial glands were the lesions observed in the trachea.
Conclusion: *Finally, anatomopathological lesions are the consequence of the action of viruses at the tissue level. Histopathology therefore confirmed the pathological lesions. The anatomopathological study could be useful to poultry farmers because it would allow them to make an early diagnosis in order to prevent a possible outbreak of Avian Flu.*
Key words: *Chicken bicycle, respiratory system, histopathology, avian flu.*

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I. Introduction

Poultry farming is practiced throughout in Ivory Coast with a predominance in the East, South and North[1]. Chicken farming, as everywhere in the world, is today an important source of animal protein in Ivory Coast. It is an income-generating activity for rural and urban populations with at least 2,200 farms for more than 200,000 jobs, including 70,000 direct [2, 3], and a turnover of more than 250 billion CFA francs[4]. However, Ivorian poultry still remains a secondary economic activity with a 2% contribution to total GDP because the production of the poultry sector remains insufficient [2, 3]. This state of affairs is due to several constraints that hinder its development. Among these constraints, the most striking in recent decades is the outbreak of Highly Pathogenic Avian Influenza (HPAI) or avian influenza, which appeared in Ivory Coast in 2006 [5, 6]. Avian influenza mainly affects the respiratory system of poultry[7]. The peculiarity of this disease lies in the very high mortality of poultry, and its rapid spread to other farms; resulting in significant direct and indirect economic losses for the poultry sectors[6]. The main risk of highly pathogenic avian influenza is its possible transmission to humans[3]**.** In Ivory Coast, poultry farmers continue to face recurrences of avian influenza outbreaks such as those of 2015; 2017 and 2021 [8, 9, 10]. In addition, laboratory diagnosis of avian influenza virus involves several techniques, the most commonly used of which are hemagglutinin and RT-PCR inhibition techniques because of their reliability and speed[11, 12, 13, 14]**.** Several research studies have been conducted in many countries on other techniques for the diagnosis of avian influenza, namely histological techniques[15 ,16, 17] of which Germany[18] ; the USA[19] ; India[20] ; Korea[21] ; Egypt[22] ; Morocco[23]. Unfortunately, in Ivory Coast, no similar study is conducted[24].

Indeed, data on the histological and histopathological study of chickens are almost non-existent in Côte d'Ivoire.

In addition, breeders are constantly confronted with the plagues of avian influenza.In addition, breeders are constantly confronted with the plagues of avian influenza. It is from these findings that the present study was initiated in Ivory Coast by the Laboratory of Biology and Health of the University Félix Houphouët Boigny. The aim is to have a reliable database of the various organs of chickens *Gallus gallus domesticus*aand to make it available to poultry farmers. The present work consists in evaluating by histological method, the impact of the avian flu on the respiratory system of the chickens bicycles of the farms in Ivory Coast.

II. Material and Methods

Biological material

The biological material consists of chickens of the species *Gallus gallus domesticus* selected from several farms during the surveys from November 18, 2019 to February 25, 2020 in Odienné and Agnibilékrou, areas of high chicken production in Côte d'Ivoire. These are sick chickens with digestive, respiratory or nervous clinical signs and dead chickens. These chickens are local breeds with different characteristics.

Sampling methods

Sampling was conducted following an alert of suspected avian influenza on farms where high mortality and morbidity rates are observed. The sample consists of twenty (20) bicycle chickens or local chickens of which ten (10) are sick and ten (10) are dead.

Macroscopic methods:

The observation focused on the behavior, as well as the condition of the head, crest, barbs, feathers, legs and the condition of the body's epidermis.

Autopsy was performed only on dead chickens, directly dissected on the autopsy table. For sick poultry, dissection is preceded by euthanasia of birds. To do this, the poultry were introduced under an anesthesia bell containing previously cotton soaked in ether. After falling asleep, they were euthanized. The dissection consisted in the opening of the abdominal wall after the wetting of the feathers and the immobilization of each poultry. All isolated organs, mainly organs of the respiratory system, as well as those left on the carcass were examined for possible differences from the normal state.

After dissection of chickens, organs such as the trachea and lungs were identified and removed. Each organ was stored in jars containing 10% formalin for microscopic study.

The observation consisted in visualizing the foci of infection of the virus in the organs with visible foci likely to contain viral ranges. For viewing viral ranges, an electronic magnifying glass device was used. Indeed, the fresh and fixed organs are directly put in contact with the lens of the device previously connected to a computer in order to search for viral ranges.

III. Results

Anatomopathology of the respiratory system of sick or dead chickens

Necropsy examination revealed numerous lesions in the respiratory system of chickens **(Figure 2)**.

The lesions detected are varied and severe with several outbreaks of the virus **(Figures 2E and G)**. These affected the airways, trachea and lungs.

- In the nostrils and air sacs, considered as airways, fibrinous sinusitis **(Figure 2A)** and aerosacculitis were observed, respectively **(Figures 2C)**. At the nasal cavities congestions of the nasal mucosa are visible, with diffuse mucus **(Figure 2B)**.
- In the trachea, tracheitis was observed. Congestions with petechial hemorrhages and serous luminal exudation were visible on the surface of the tracheal wall **(Figure 2D)**.
-Lungs presented with fibrinous pneumonia, edema, congestion and pleurisy. The lungs also exhibited bilateral pulmonary consolidation, moderate to severe, focal extended to diffuse **(Figure 2F).**

Figure 1: Some local or

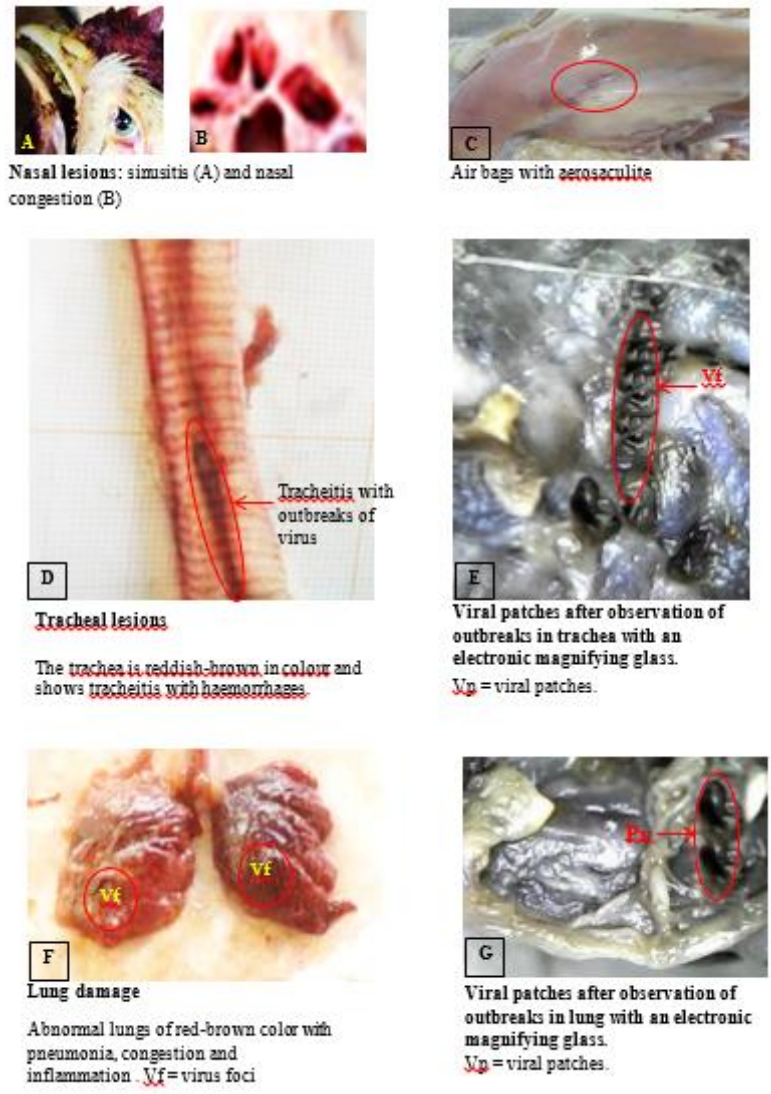

Figure 2 : Clinical signs and ranges of the avian influenza virus in the respiratory system of chickens.

Histopathology of the trachea

The lesions were observed in the trachea of chickens generally reflecting focal necrosis with lymphoplasmocyte inflammatory infiltrate in the mucosa, submucosa, hyalin cartilage and external tracheal striated muscle

(Figure 3). Indeed, the trachea showed a mucosa with an epithelium devoid of eyelashes and thickened by the hyperplastic intraepithelial glands; putting pressure on the chorion that integrates into the submucosa resulting in congestion of blood vessels in the submucosa. This is manifested by inflammatory lesions and edema **(Figure 3A and B).** The tracheal epithelium, in addition to edema, had an exudate of mucus inside the tracheal light **(Figure 3C).** In addition, hyaline cartilage showed necrosis and lymphoplasmocyte inflammatory infiltrates inducing a grouping of chondrocytes **(Figure 3D)**. As for the external tracheal muscle, it presents inflammation and necrosis of blood vessels **(Figure 3E).**

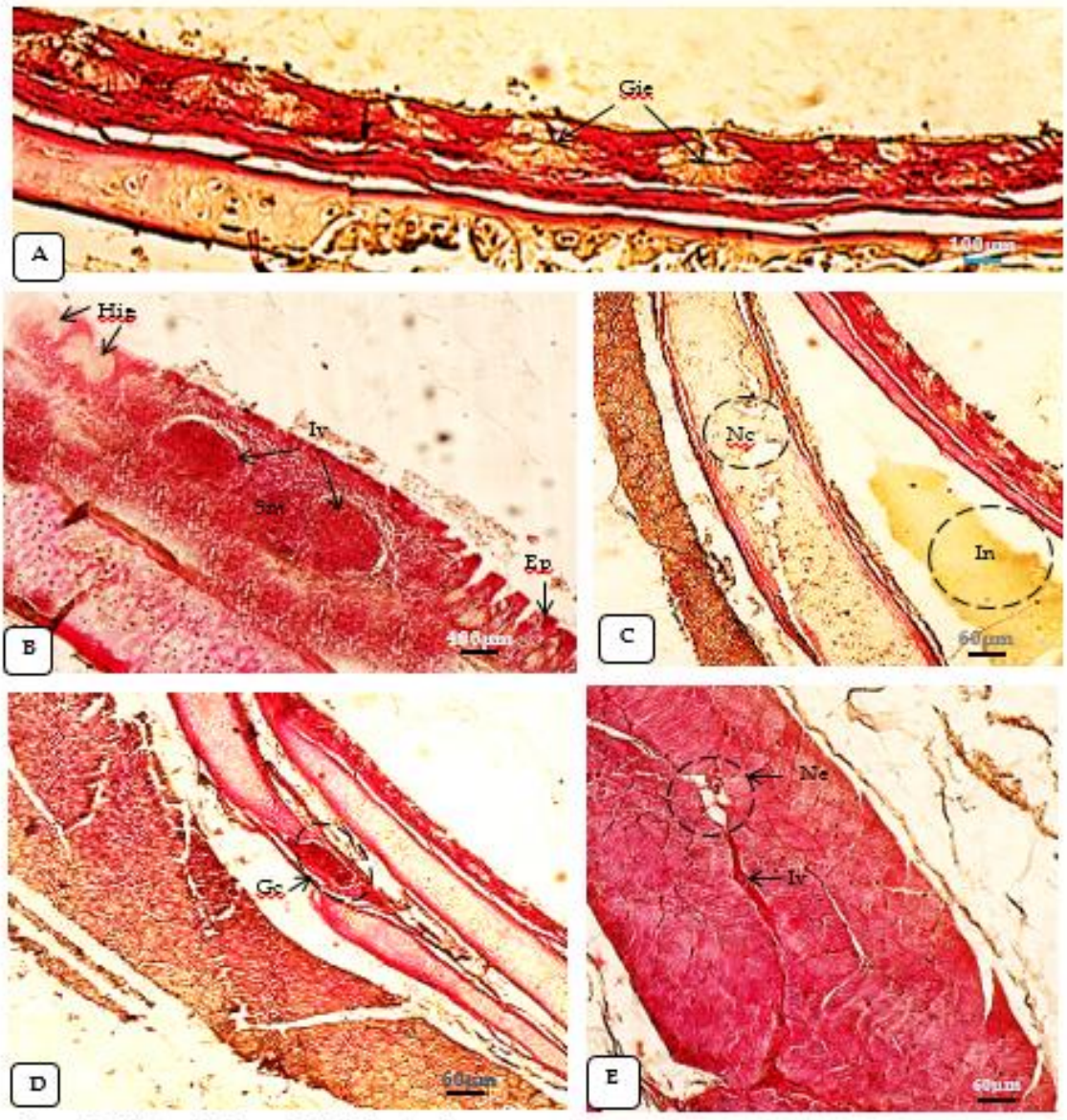

Figure 3 : Histopathology of the chicken trachea

Hie: hyperplastic intraepithelial glands; Iv: inflammation of blood vessels; **Sm**: submucosal undergoing pressure of mucosal thickening; **Ep**; thickened epithelium; **Ne**: necrosis; **In**: inflammatory infiltration; **Gc**: grouping of chondrocytes. **Hemalum- Eosin**

Histopathology of the lungs

The lungs of the chicken presented severe interstitial pneumonia, edema, congestions, necrosis, monocytic infiltrates in the epithelium of the aerial capillaries. Pneumonic areas are represented by intense leukocyte aggregation **(Figure 4A)**. Vasculitis of the pulmonary blood vessels was observed, sometimes with congested blood vessels and edema leading to endothelioma and thrombosis.

The bronchi presented a light containing molds of mucus mixed with heterophils and erythrocytes next to an intense necrosis of the bronchial mucosa. The bronchi and also the surrounding alveoli presented an infiltrated hyalinized vascular wall of heterophils with congestions and edema **(Figure 4B)**. The pulmonary capillaries also showed casts of mucus and intense necrosis leading to thrombosis **(Figure 4C).**

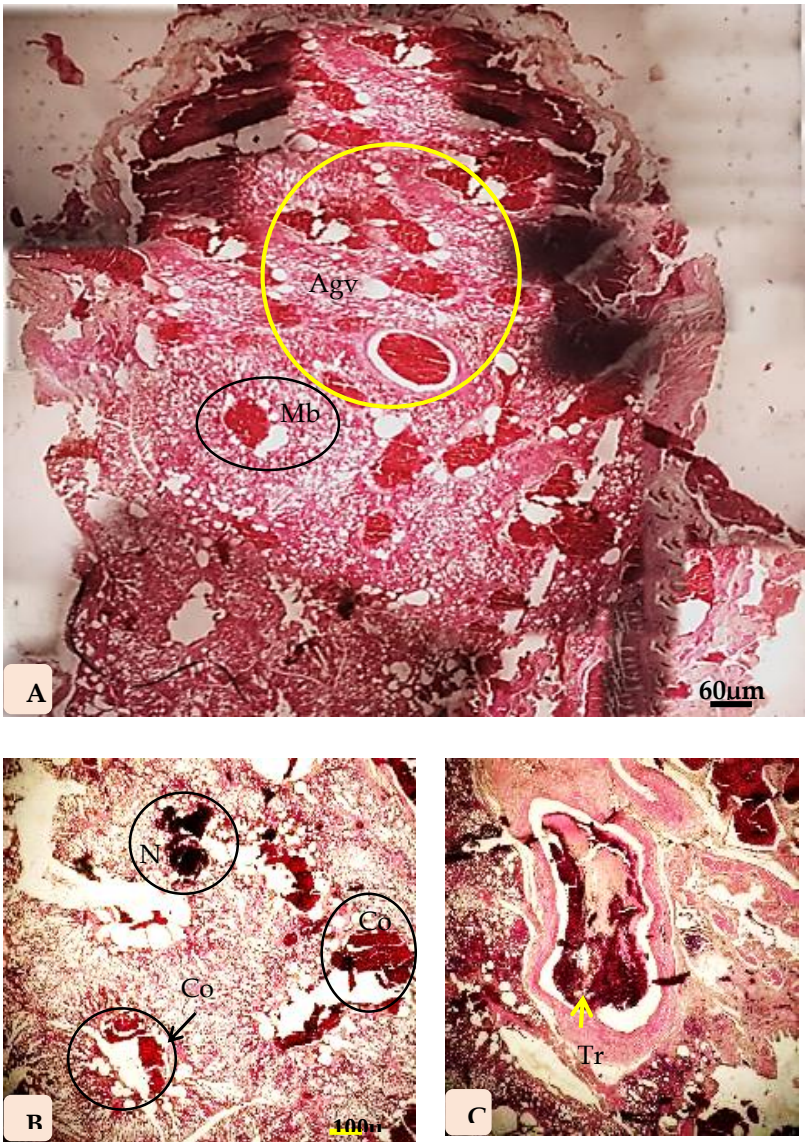

Figure 4 : Histopathology of chicken lung
Va: vessel aggregates; **Mb:** bronchial moulding with oedema; **Ne:** necrosis; **Cod:** congestion and oedema. **Hemalum- Eosin.**

IV. Discussion

The respiratory system of the chicken is in contact with the outside through the windpipe as well as other airways such as the nostrils and air bags. This contact of the respiratory system with the outside is a factor of possible contamination by infectious agents. In poultry with viral infection, macroscopic lesions are very common in the respiratory system. According to [25], the respiratory system, primarily the trachea, is the natural target of avian influenzavirus. For [26], viral infection of the avian respiratory system results in moderate and/or severe injury. At autopsy, more severe sinusitis, aerosaculitis, pulmonary hypertrophy and tracheal congestion are noted in these chickens. Widespread anatomopathological lesions in the respiratory system are considered one of the major features of the high pathogenicity of avian influenza in chickens according to [27]. These current results are consistent with[15, 22] where chickens affected with avian influenza showed similar signs of severe respiratory distress.

The study of histopathological lesions of the trachea revealed tracheitis characterized by mutifocal necrosis, deciliation, rarefaction of caliciform cells, with a variation in the severity of lesions depending on the condition of poultry, sick or dead. These characteristics of the trachea are the elements that promote the sensitivity of chickens to infectious agents. In the lungs, the histopathological lesions detected are, among others, severe interstitial pneumonia, multifocal necrosis. The histopathological results of this work are similar to several pathological data reported with avian influenza in chicken[28, 29].

The work of [30] reported similar results to those of this work. In addition, lesions observed in the various organs of the respiratory system were also noted by [19] where these lesions are detected in Virginia Quail, Ring-necked Pheasant and Japanese Quail following exposure to the H5N2 virus. The same is true for the Duck, the Guinea Fowl, and the Partridge exposed to the H5N8 virus. [22]observed the same lesions in chickens exposed to H5N1 viruses. These subtypes of avian influenza are highly pathogenic.

But the work of [23], on turkey exposed to the strain H9N2, a low pathogenic strain, allowed to observe histological lesions identical to those encountered especially in the respiratory system in study chickens. The same is true for [25]whose work focused on chickens, quails and ducks exposed to the low pathogenic strain H7N3.

Based on the pathogenicity of the virus in chickens, low pathogenic avian influenza (LPAI) viruses generally cause little to no clinical manifestation in birds, while highly pathogenic avian influenza (HPAI) causes serious clinical manifestations and/or high mortality. Indeed, LPAIV strains have a monobasic HA cleavage site, which is recognized and activated by tissue-restricted trypsin-like enzymes in the aerodigestive tract resulting in mild or non-existent clinical signs. Conversely, highly pathogenic avian influenza hemagglutinin (HPAI) includes patterns of polybasic cleavage sites, which are recognized and activated by ubiquitous furin-like enzymes. HPAI replicates systemically, causing organ dysfunction and high mortality[31, 32]. According to[33] and[3], emergency and preventive vaccinations are the only response measures against exposure to avian influenza viruses alongside biosecurity measures.

However, experimentally, whether avian influenza viruses are highly pathogenic or low pathogenic, vaccination has been shown to protect against clinical signs and mortality, reduce viral excretion and increase resistance to infection; it protects against various wild viruses with the same hemiglutinin subtype and thereby reduces the transmission of the test virus by contact[34]. However, the virus is still capable of infecting and replicating inapparently in vaccinated birds because long-term circulation of the virus in a vaccinated population can result in both antigenic and genetic changes to the virus[35]. In 2017, H7N9 virus, which has low pathogenicity in chickens, developed into a highly pathogenic strain for chickens and caused hundreds of cases of human infections in China [36, 37, 38]. In light of the above, poultry exposed to low pathogenic viruses would be true reservoirs for the unaccountable concentration and replication of avian influenza.

This replication following long-term exposures would induce the mutation of low pathogenic strains into highly pathogenic strains. Moreover, in farms, clinical signs are the only clues of suspicion allowing to use a diagnosis for the eradication of viruses. The pathological lesions observed in the respiratory system are the consequence of the action of viruses at the tissue level. The present work shows that histopathological lesions are almost identical both in poultry exposed to highly pathogenic influenza and in poultry exposed to low pathogenic influenza. Therefore, histological techniques may be necessary for the early diagnosis of avian influenza. Indeed, according to [39], histopathology plays a crucial role in the diagnosis of avian pathologies. It is performed as a first resort and helps to choose more specific diagnostic techniques to obtain a final diagnosis. By indicating the presence of neoplastic lesions, histopathology significantly contributes to an immediate diagnosis and reduces the subsequent differential diagnosis. [40, 39, 41] respectively used histopathological methods to identify avian influenzavirus in naturally infected chickens during an outbreak. The histopathological techniques used in this work are identical to those of [40, 39, 41]. These techniques complement hemagglutinin inhibition techniques and RT-PCR for the early diagnosis of avian influenza in strategies to combat avian pathologies.

V.Conclusion

The pathological lesions observed in the respiratory system are the consequence of the action of viruses at the tissue level. Histopathology has therefore confirmed these pathological lesions. The anatomopathological study could be useful to poultry farmers because it would allow them to make an early diagnosis in order to prevent a possible outbreak of Avian Flu.

Given the importance of chickens in terms of food and economy, It seems interesting to complete this work with a study of the evolution of avian influenza on the morphology and tissues of the different organs of chickens after experimental inoculation of influenzavirus to chickens. On the other hand, it will identify the subtypes of avian influenzavirus circulating in Côte d'Ivoire and make their respective genotyping.

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