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Overview of Aspergillosis in Poultry - A Review

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Aspergillosis is a respiratory disease, the infection that most usually found in turkey, chicken, human beings, and other mammals; it less frequently found in pigeons, ducks, geese, and other domestic and wild birds. It caused by a species of fungus from the *Aspergillus* genus. Upon those farms, the disease may be endemic in chicken and turkeys. In wild birds, it seems too random and frequently only affects one bird at a time. Usually, 7 to 40 day old immature birds would exhibit it. Because *Aspergillus spp.* are so common, the disease can found wherever that the environment is suitable for its growth. Aspergillosis frequently results from *Aspergillus fumigatus.* However, a number of different mould species, including *A. Flavus, A. niger, Rhizopus sp., Mucor sp,* and *Penicillium sp.* may be blamed. Infection generally carried out through inhalation of spore-filled dust from infected dusty range areas. The disease has two different forms: acute and chronic. Acute form affects birds who have ingested huge amounts of spores, while chronic form affects birds with suppressed immune systems. For the diagnosis of Aspergillosis in poultry, which requires a range of diagnostic methods, culturing is essential because the clinical signs are non-specific. Aspergillosis cannot effectively treated; instead, prevention is the key to keeping the condition under control. Good management techniques, such as sanitation, avoiding damp litter or dirt and moldy or dusty feeds, providing adequate ventilation, and sterilizing feed and water systems, should use to prevent and control the disease.

Keywords: Aspergillosis, Poultry, Epidemiology, Diagnosis, Treatment, Control

Introduction

Avian Aspergillosis is the major mycotic non-contagious disease in birds. It caused on by a species of fungus from the genus *Aspergillus*. When the host's immune system is weak or the bird exposed to an excessive amount of spores, the sickness develops [1]. The primary risk factor for the onset of the condition is stress [2]. Aspergillosis is the most frequent fungus that affects the bird respiratory system and results in severe morbidity and mortality. It is a typical case of poor management in both backyard and commercial poultry [3]. Lower respiratory system is the where the sickness mostly manifests itself [4]. Eggshells can penetrated by *Aspergillus* species, which can then infect the embryo, which may die or hatch with a lesion fully-grown. Large amounts of spores are emitted when contaminated eggs are ruptured, contaminating the

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hatchery equipment [5]. Depending on the bird's immunological condition, inhaling asexual spores (conidia) of Aspergillus species especially *A. fumigatus* might result in a wide range of clinical symptoms [6]. There are two different forms of Aspergillosis: acute and chronic. Young birds who contract acute aspergillosis experience severe morbidity and mortality rates [7]. The chronic variety typically affects older birds with weakened immune systems and is more sporadic, with lower mortality [8, 9]. Food contamination, a lack of proper sanitation, and inadequate ventilation all contribute to the growth of fungal spores, which increases the risk of airborne spore infection and invasion of the respiratory system in birds [10]. Because it most frequently found in newborn chickens and related with infection at approximately hatching, the illness known as "brooder pneumonia" in young poultry. Pneumomycosis, bronchomycosis, and colloquial terms like "asper" and "air sac" are further terms that can used to describe avian aspergillosis [11]. Eye, brain, skin, joint, bone, and visceral infections are less frequent symptoms [12]. Poultry **a**spergillosis caused by a type of fungus including *Aspergillus fumigates, A. niger, A. flavus, A. terreus and A. glaucus* [13]. The aspergillosis infection brought on by the inhalation of numerous tiny, hydrophobic fungus spores (conidia). Granulomas caused when infectious spores enter the nasal, tracheal, bronchial, and epithelium of air sac prior to entering respiratory tissue and developing [14]. Mycotoxins are poisonous secondary metabolites created by fungus-related organisms. When poultry swallows contaminated food, mycotoxins such as ochratoxin and aflatoxins, generated by *Aspergillus ochraecus*, *Aspergillus flavus*, and *Aspergillus parasiticus*, harm the poultry [15]. The most common diagnostic methods for aspergillosis in birds include a combination of clinical manifestations, epidemiological factors, and ultimately the isolation and identification of the causative agent; nevertheless, there are currently rare PCR assays or serological biomarkers for aspergillosis in birds [16]. The greatest method of controlling Aspergillosis is through prevention whereas the treatment is typically ineffective. Therefore, for control of the infection, it is important to employ good management techniques like sanitation, sufficient ventilation, and disinfection. Determining the control measures depends on the epidemiology and diagnostics of the aspergillosis infections [17]. Therefore, the the objectives of this study was to throw light on the poultry aspergillosis.

Origin

A fungus belonging to the genus Aspergillus, Family: Trichocomaceae, Order: Eurotiales Class: Eurotiomycetes, Division: Ascomycota, and belonging to the Kingdom: Fungi is what causes aspergillosis in chicken. There are over 250 species in the genus *Aspergillus*, however few well-known species were taken into consideration constituting significant opportunities for diseases in invertebrates and vertebrates [18]. Including *A. niger*, *Aspergillus fumigatus*, *A. terreus*, *A. flavus*, and *A. glaucus* among other species. *A. fumigatus* is the most frequent cause of sickness among all of these, though. These creatures are widespread saprophytic fungi that thrive in warm, humid environments (above 25 degrees Celsius) and on organic materials, including broken eggs in hatcheries. [19]. *A. fumigatus* is the main agent causing aspergillosis in poultry due to its very small spores and readily inhaled. *A. flavus* is less frequent but not rare in severely contaminated environments with high spore load (Fig. 1 a & b). Other species like *A niger*, *A. nidulans*, *A. terreus*, and *A. amstelodami* may also be isolated from avian cases of aspergillosis when the environment is

heavily contaminated with fungi. Mixed infections are possible [20], the majority of the *Aspergillus* species considered imperfect (asexual reproduction) fungus since they can reproduce by ascospores, which as they grow start hyphae and come together to create mycelia [21]. The superficial hyphae of vegetative mycelia create conidiophore, which formed end in vesicles and in foot cells. In this more restricted growth, one line produces a chain of conidia or spores. Conidia are always single-celled and can have one or more nuclei [22]. Conidia have the function of spreading the fungus. Because it can produce so many different enzymes, *Aspergillus* can used as food in a wide range of products. If organic matter and moisture are available, which are the two fundamental conditions for fungal growth, the fungus can flourish in almost any material [23]. Conidia can be globose, elliptical, or oval. They may also have surfaces that are smooth or slightly rough. Conidia's colour influences the colour of the conidial head, which determines the colony's color [24].

Epidemiology

Prevalence

The world is host to numerous *Aspergillus* species, which have evolved both parasitic and saprophytic lifestyles. Infection brought on by some *Aspergillus* species mostly affects humans and other animals' respiratory systems, with birds and mammals experiencing it more frequently than mammals [25]. Both domestic and wild birds can contract the fungus, which is also one of the most common causes of bird deaths in zoos, since there are many different species of *Aspergillus*, the disease can found wherever that the environmental conditions are right for fungus. These organisms, which are typical soil saprophytes, thrive in warm (>25°C), humid conditions where organic material is present, such as cracked eggs in hatcheries and insufficient ventilation growth [17]. The disease is more common in tropical nations. A severe outbreak linked to hatchery contamination may cause up to 15% of chickens to die during the first two weeks and alter survivors' growth rates In relation to the seasonal distribution of aspergillosis [26].

Transmission

Aspergillosis affects animals and humans and all birds are susceptible to aspergillosis. Both domestic and wild birds, including chicken, duck, and quails, have observed to affect. Aspergillosis brought on by respiratory tract contact with a large number of tiny, hydrophobic fungal spores (conidia) (Fig. 2). Conidia or spores from contaminated feed, feces, soil, and eggs in ova can be breathed and can infect the growing embryo [27]. After invading the nasal, tracheal, bronchial, and epithelium of air sac, infectious spores enter the respiratory tissue, to multiply by dividing into mycelia as tubular hyphae, and ultimately to begin granulomas here. Then they hematogenously distributed to the other tissues, including the kidney, pericardium, brain, and bone marrow. Hetrophils, lymphocytes, monocytes, and certain large cells infiltrate the lesion and develop the lesion as result of tissue invasion, which also causes an inflammatory condition and inflammatory response [28, 29].

Clinical signs

Acute aspergillosis indicated by a wide range of nonspecific clinical symptoms, such as anorexia, lethargy, ruffled feathers, respiratory symptoms, polydipsia, polyuria, stunting, or abrupt mortality. The illness, also known as "brooder pneumonia," causes severe respiratory distress in chicks that infected in the egg or after hatching and is extremely deadly in the first ten days of life. [30]. In poultry farms, the mortality rate (5–50%) may grow gradually or abruptly, reach a high for a few days, and then decline down to the previous level. Dyspnea, gasping, hyperpnoea with panting,

nonproductive coughing, wheezing, cyanosis, and occasionally nasal discharge are respiratory symptoms [31]. Symptoms of the chronic type include dyspnea, sadness, dehydration, emaciation, ataxia, tremor and opisthotonos. Nervous symptoms as, lateral recumbence, torticollis, seizures, convulsions, hind limb paresis may be involved [32].

Macroscopic Lesions

The lungs, air sacs, and other organs are the main locations. The involvement of the respiratory system typically seen before any clinical symptoms appear. White to yellowish granulomas that range in size from miliary (2 cm) and involve serosae and parenchyma of one or more organs are typical lesions. On sliced surfaces, a single necrotic region or several may seen. Lung parenchyma either exhibits localized granulomas of various sizes or consolidated. (Fig. 3 a & b) [11].

Granulomas develop cheesy caseous plaques on thicker membranes when consolidating in air sacs, where grey-greenish velvet, a sign of potential sporulation by fungi, may appear (Ceolin *et al.,* 2012). In the cerebrum and cerebellum of broiler breeders, abscesses are confined, whitish to grayish patches. Mycotic pod dermatitis, swollen and adherent eyelids with turbid discharge, clouded cornea, and cheesy yellow exudates within the conjunctival sac observed in footpads, along with keratinized epidermal disruption, encrustations, and acute inflammation. [27] (Fig. 4 a, b, c & d).

Histopathology

Microscopical findings show perivascular edema and pulmonary, peri-alveolar and parietal alveolar blood vessel congestion. Disseminated Pyo-granulomatous foci took the place of the lung's and air sacs' typical structural organization. Caseous necrosis and necrotic cellular debris were present in the granulomatous foci's Centre, which was encircled by inflammatory cells heterophils, lymphocytes, macrophages, and multinucleated giant cells (Fig. 5 a, b). The nodules have coagulative necrotic centres inside of them. On the pleura and underlying pulmonary lobules, there were a few, concentrated, more severe densification and inflammatory lesions [33].

Diagnosis

Despite the fact that aspergillosis symptoms are non-specific and difficult to identify, the diagnosis of aspergillosis most commonly based on clinical manifestations and gross lesions. The diagnosis is typically dependent on a collection of data from the history, clinical presentation, postmortem findings, hematology, biochemistry, serology, radiological alterations, endoscopy, and fungus culture; no single test can provide assurance [34].

Isolation and Identification of Causative Agent

Mold can detected microscopically in tissues from infected. There are several ways to obtain tissue samples for testing, involves removing some of the affected tissue, swabbing the lesion, and pulling one of the plaques away. The diagnostic samples you collect must carefully handle using aseptic techniques. Simply immersing a tiny portion of the nodule in 20% potassium hydroxide (KOH), separating the material, and placing it on a microscope slide, these samples can studied under a microscope. You can check for hyphae in the exudate with heating the slide over a flame (Fig. 6, a). Most commonly, samples placed on Sabouraud dextrose agar and incubating it for 24 hours at 37°C with a distinctive conidial head and colony, the pathogenic organism can be isolated [35]. Histopathological examination using a special fungal stain reveals granulomas containing mycelium of the tissue

samples, which include organs such as the lungs, trachea, throat, and thoracic air sacs, treated before embedded in pieces of paraffin and stained using the haematoxylin and eosin (HE) procedure. Other specialized stains, such as Periodic acid-Schiff (PAS), Bauer and Gridley's, Groote's, and Gomori Methanamine Silver stains, make it simple to identify the hyphae and mycelia of fungus (Fig. 6, b) [9].

Immunity and serologic tests

Serological assays not currently used in farms to look into aspergillosis epidemics and have not verified in poultry. Because the fungal antigens are generic, serologic testing have limited relevance. Enzyme linked immunosorbant assay (ELISA) and agar gel immunodiffusion measurements of the antibody response were inconsistent [36], but sandwich ELISA's early identification of circulating galactomannan is connected to high sensitivity and specificity (Fig. 7, a) [37]. Using monoclonal or polyclonal antibodies, immunohistochemistry is a potent and precise method for determining if lesions contain *A. fumigatus* (Fig. 7, b). Protein electrophoresis can be used to increase the sensitivity of the sero-diagnostic tests for Aspergillosis which carried out through increases in globulins with concurrent decreases in albumin, increases in alpha 2 globulins, and elevated levels of serum amyloid A [38].

Diagnostic imaging (Radiography and Endoscopy)

Radiographic examination is a quick and effective method for obtaining a preliminary assessment of the underlying disease in non-critical instances in birds. When examining the bird's lungs and air sacs for indications of inflammation or granulomas and to determine the general health of the bird's lower respiratory tract, non-invasive imaging modalities such as lateral and dorso-ventral radiography images of the bird may be helpful (Fig. 8, a). Although endoscopy is invasive, it does enable visual diagnosis of fungal development (Fig. 8, b), organs and abnormal growths found in the bird's trachea and coelomic cavity were sampled using biopsy, fine-needle aspiration, and culture techniques [39].

Molecular diagnosis

Ostriches, penguins, falcons, turkeys, and white storks are just a few of the avian species in which polymerase chain reaction (PCR) has employed to identify and detect fungal isolates. However, more study is required before PCR assays may eventually use in the work-up of avian practitioners. PCR assays have used in conjunction with other indicators for experimental goals in turkey models or on a variety of bodily fluids. PCR can be used to analyses fungal DNA from bodily fluids or serum, however although being extremely sensitive, it can produce erroneous positive results [40]. To identify the source of fungal isolates, molecular biological approaches have applied to the assessment of fungal contamination in poultry flocks. When examining frozen and fixed organ samples as well as in situations when culture is unable to identify the infectious agent, a*spergillus* DNA detection has value [41].

Differential diagnosis

The particular clinical signs of avian aspergillosis vary on the organ and systems affected. Early broiler chick mortality should screened out for Aspergillosis since mycotoxicosis, acute bacterial septicemia, or carbon monoxide poisoning may bring it on. Infectious laryngo-tracheitis, infectious bronchitis (coughing, gasping,and extension of the neck during inspiration), and Newcastle disease all cause dyspnea and watery, greenish diarrhea. mycobacteriosis, colibacillosis, dactylaria infection (nervous sign), other mycoses (such as ochroconosis, zygomycosis),

and nutritional encephalomalacia are among the differential diagnose [4]. The granulomatous lesions seen during necropsy typically used to distinguish pulmonary aspergillosis from other avian respiratory illnesses; however, *Staphylococcus aureus* pneumonia in newborn chicks can resemble it. In addition, pneumonia and exudative fibrinous or fibrinoheterophilic air sacculitis are frequently observed in mycoplasmosis, colibacillosis, poultry cholera, and chlamydophilosis patients. When granulomas predominate, mycobacteriosis and other mycoses must also take into consideration and Aspergillus ocular oedema resembles infectious coryza or vitamin A deficiency in chicks [42].

Treatment

Treatment for aspergillosis is ineffective, because the fungus walled off by the inflammatory reaction of the bird and consequently isolated from the blood stream. When there is widespread tissue infection and only systemic medications employed, the prognosis for the disease is poor. The finest benefits come from drying the granulomatuos lesion and applying topical medication alongside organized therapy. Use of one or more systemic antifungal medications is required for the treatment of aspergillosis. Itraconazole, ketoconazole, clotrimazole, miconazole, and fluconazole are some of the medicines that are frequently utilised [31]. Amphotericin B has traditionally been the medicine of choice for treating aspergillosis, although it has serious adverse effects and a high mortality rate. Take Itraconazole and voriconazole after amphotericin B for better results. Less toxic lipid versions of amphotericin are also efficient [43].

Prevention and control

There is no cure for aspergillosis, and vaccination is not a commercially sustainable method of prevention. If re-exposure to the mold avoided, a spontaneous recovery may take place. Major control measures include: 1) Minimizing exposure to the fungus and risk factors involve. 2) The removal of the birds from the polluted area. 3) Hatchery hygiene in young chickens through carefully cleaning and disinfection of the hatching equipment. 4) Contaminated material(s) removed to prevent additional exposure as removing the old litter and the mouldy feed. 5) To avoid an aspergillosis outbreak, resist using mouldy litter or feed. 6) Attempting to avoid disturbing the infected material(s) to reduce spore aerosolization further. 7) Higher air exchange rates or ventilation perhaps lessens the severity of the epidemic. 8) Eggs that are seriously infected or fractured should not incubated because they stimulate fungal growth and since they may rupture and release spores into the hatching machine [5, 44].

Public Health Significance

Aspergillosis is a significant occupational zoonotic mycosis, mostly affects people and employees who exposed to infected birds. The respiratory symptoms of aspergillosis in humans are often very severe, especially in chronic illnesses such diabetes, cancer, tuberculosis and immunocompromised people who are also receiving long-term antibiotic, antimetabolite, and corticosteroid treatment, and the current outbreak of aspergillosis in chicken has shocked and disturbed the chicken-eating community [45]. Man can contract aspergillosis through handling infected birds, inhaling in spores from contaminated feed and litter, abusing the environment's sanitation and hygiene standards, and eating contaminated poultry that has not properly cooked. There is no safe way to rescue food that has moulded because the majority of mycotoxins *Aspergillus* produces are not broken down by cooking temperatures. People who regularly exposed to materials (such as grain, hay,

cotton, wool, and other materials) that contaminated by fungus spores run a higher risk. When exposed to many conidia, healthy children may experience fever and dyspnea. Allergic broncho-pulmonary aspergillosis (ABPA) is a condition that affects patients with pre-existing asthma, eosinophlia, and intermittent bronchial obstruction [46].

Conclusion and Recommendations

The most common aspergillosis manifestation in young chickens or turkeys is a respiratory illness, although it can also appear as an ophthalmic or neurologic condition. Based on the observation of characteristic gross lesions, a diagnosis is formed, but it can also be supported by histopathology, infective agent isolation, and culture. There is no cure can succeeds; the key to control is limiting the amount of polluted materials that birds exposed to contaminated materials. To avoid aspergillosis-related economic losses in chicken farms, the following measures recommended; feeding and watering equipment must be thoroughly cleaned and disinfected. Prevent crowding in the poultry house, provide adequate ventilation for the coop, and avoid using feed that is dusty and moldy. Maintain the hatching apparatus's cleanliness; Use an antifungal substance to disinfect the chicken house and the litter. To stop the spread of disease, infected birds should remove, and feed treated with mold inhibitors when an epidemic suspected.

Conflicts of interest

The authors declared no competing interests.

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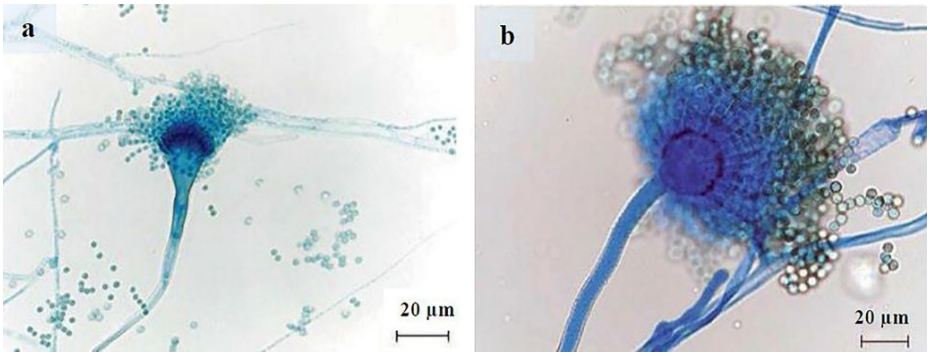

Fig. 1. *Aspergillus fumigatus,* conidiophore with flask-shaped vesicle, and small size spores (a) & Aspergillus *flavus* conidiophore with globose vesicle and large size spores (b). Lactophenol cotton blue stained, X400 [11].

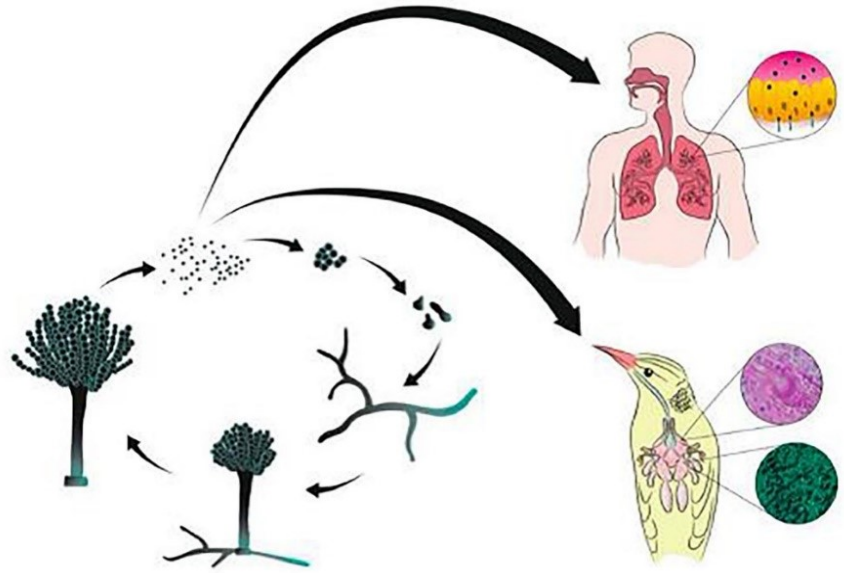

Fig. 2. Representation of vegetative cycle of *Aspergillus* spp. and infection of human and avian hosts by inhalation of conidia [28].

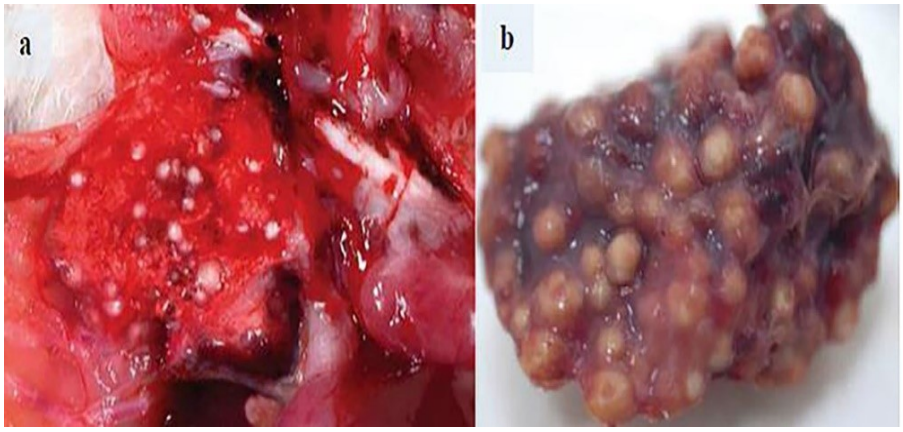

Fig. 3. Numerous caseous nodules in the lung in young chicks (a) and duck (b) are characteristic of acute respiratory aspergillosis (brooder pneumonia) [11].

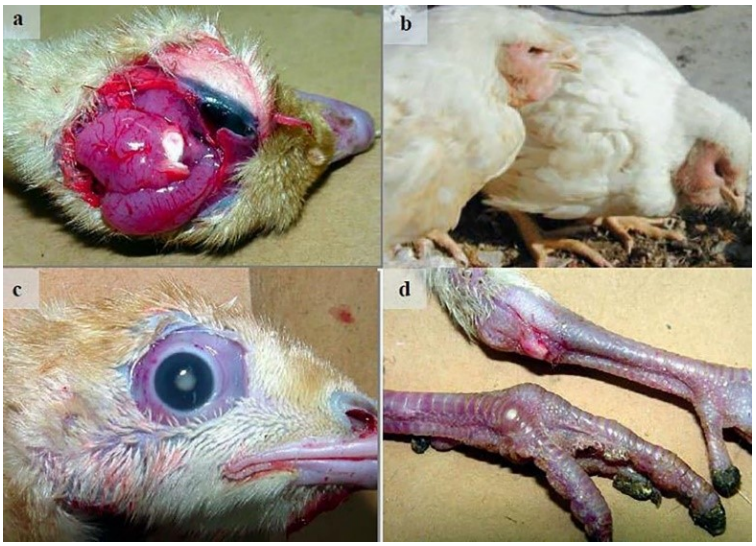

Fig. 4. ***Aspergillus*****,granulomatus méningitis (a), dermatitis (b), uveitis (c) and arthritis (d) [12].**

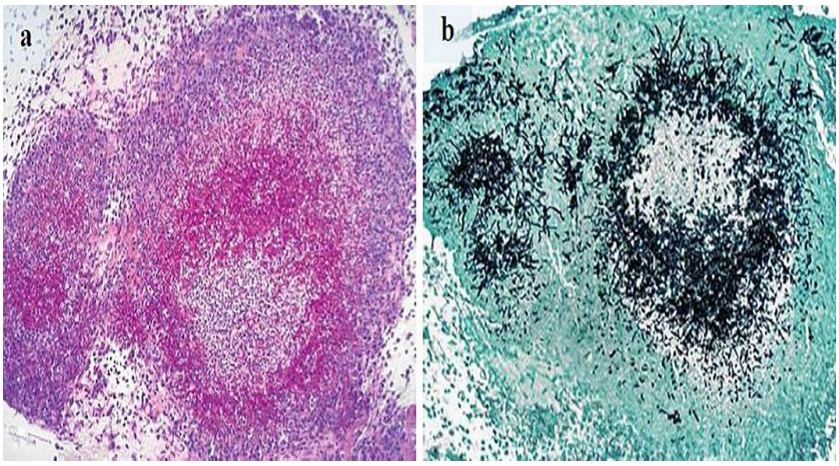

Fig. 5. ***Aspergillus*** **heterothallic granulomatous lesion in the air sac stained with Periodic acid–Schiff stain (a) and with Gomori methenamine sliver stain (b) ×90 [11].**

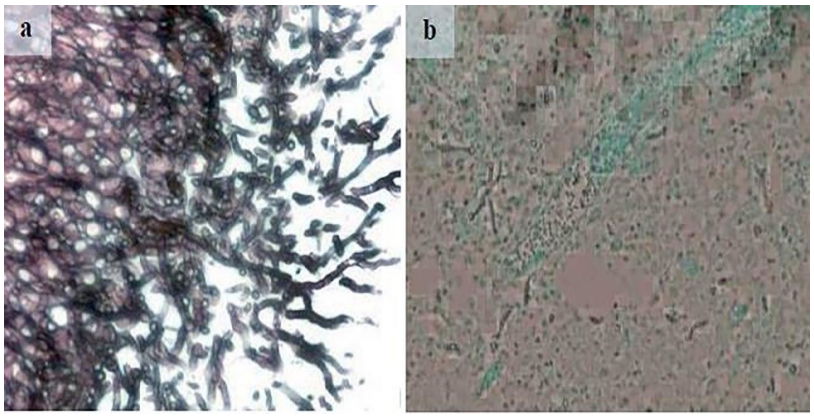

Fig. 6. ***Aspergillus*** **sp. hyphae consistent with in air sac wall (a) cerebellar tissue (b) of a chicken stained with Gomori methenamine sliver (GMS) stain.**

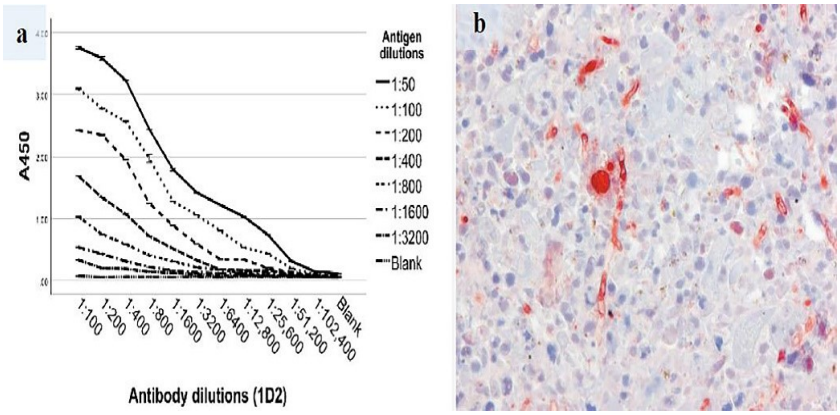

Fig. 7. Detection of *Aspergillus* sp. antigens by ELISA (a) and immunohistochemical staining (the fungal elements stained with anti-*Aspergillus* antibodies appear red) (b) ([37].

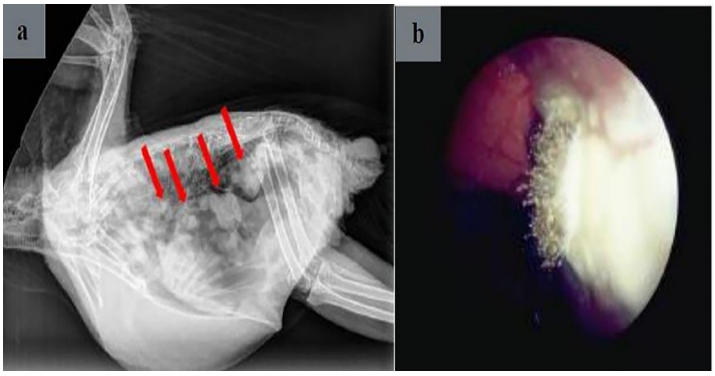

Fig. 8. Radiograph of bird with chronic aspergillosis, radio-opaque indicated by the red arrows (a), endoscopy of air sac with greenish-gray pigmented aspergilloma (b) [39].References

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نظرة عامة على داء الأسبرجلوسيس في الدواجن – بحث مرجعي

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داء الأسبرجلوسيس (الرشاشيات) هو مرض يصيب الجهاز التنفسي، وهي العدوى الأكثر شيوعًا في الدجاج والديك الرومي والإنسان والثدييات الأخرى ويتأثر بشكل أقل كل من البط والحمام والإوز وغيرها من الطيور البرية والمنزلية. ويسببه نوع من الفطريات من جنس الأسبرجلس وفي تلك المزارع، قد يكون المرض متوطنا في الدجاج والديوك الرومية وفي الطيور البرية، وتبدو الإصابة عشوائية للغاية وغالبًا ما تؤثر على طائر واحد فقط في كل مرة. وتظهر عادة في الطيور الصغيرة التي يتراوح عمرها بين 7-40 يومًا. لأن أجناس الرشاشيات شائعة جدًا، ويمكن أن يوجد المرض في أي مكان تكون فيه البيئة مناسبة لنموه. وينتج داء الرشاشيات في كثير من الأحيان عن جنس الرشاشيات الفيومجتس (الدخناء). ومع ذلك، هناك عدد من أنواع العفن المختلفة، بما في ذلك الفلبفس ، النيجر ، الريزوبس ، الميكوروانواع فطر البنسيسليوم . وتتم العدوى عمومًا من خلال استنشاق الغبار المليء بالأبواغ من مناطق المراعي المتربة المصابة. والمرض له شكلين مختلفين: حاد ومزمن. وقد يصيب الشكل الحاد الطيور التي تناولت كميات كبيرة من الجراثيم، بينما يصيب الشكل المزمن الطيور التي تعاني من ضعف جهاز المناعة. ولتشخيص داء الرشاشيات في الدواجن، يتطلب مجموعة من طرق التشخيص، فتعتبر الزراعة النسيجية للفطر ضرورية لأن العلامات السريرية غير محددة. ولا يمكن علاج داء الرشاشيات بشكل فعال. وبدلاً من ذلك، فإن الوقاية هي المفتاح لإبقاء الحالة تحت السيطرة. للوقاية من المرض ومكافحته، ويجب استخدام ممارسات الممارسات الصحية الجيدة مثل الصرف الصحي، وتجنب القمامة الرطبة أو التربة والأعلاف المتعفنة أو المتربة، وتوفير التهوية المناسبة، وتعقيم أنظمة الأعلاف والمياه.

الكلمات الدالة: الرشاشيات، الدواجن، علم الأوبئة، التشخيص، العلاج، السيطرة.