Mba. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (2): 172 - 181 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Apr 2025; Vol.26 No.2
AJCEM/2384. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Pathogenicity of filamentous fungi isolated from poultry farms on gastrointestinal system of day-old chicks in Anambra State, Nigeria

Mba, Anthonia Nkiruka

International Institute for Infectious Diseases, Biosafety and Biosecurity Research, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria
Correspondence to: tonia762013@gmail.com; +234 8035082191; ORCID: https://orcid.org/0000-0001-8409-8559

Abstract:

Background: The pervasive presence of filamentous fungi in poultry farming environments has a profound impact on the bird health, leading to reduced growth rates, increased mortality, and decreased egg production. The objective of this study was to use an avian model to determine the pathogenicity of some filamentous fungal isolates from litter, feed, and fecal samples on day-old birds.
Methodology: A total of 300 environmental samples (feed, litter, and droppings) were collected from 10 selected poultry farms in six local government areas (LGAs) in Anambra State, and cultured for fungi isolation by phenotypic (macroscopic and microscopic) and genotypic methods. Five of the fungi isolates were used for pathogenicity study on day-old chicks. A total of 25 day-old-chicks, randomly divided into 5 groups of 5 each, were orally infected with 1 ml of the different fungal conidia. A 6^{th} group served as the control and was inoculated with the same volume of phosphate-buffered saline. All the birds were observed twice daily for appearance of clinical signs for 10 days. Histological examination of the intestinal tissues and stomach of infected birds was done. Data were analysed using one-way ANOVA on SPSS version 21.0, with $p<0.05$ considered significant
Results: The pathogenic effects of the 5 fungi isolates (*Curvularia verruculosa, Aspergillus tubingensis, Cunninghamella bertholletiae, Fusarium oxysporum* and *Aspergillus fumigatus*) used for the pathogenicity study on the birds include symptoms such as; (i) reduction in food and water intake, fraying of feathers and loss of reflex within 5 days of exposure to *C. verruculosa,* and (ii) discolored and enlarged bursa of Fabricius, obstructed, dark, and slightly sloughed intestine, reduction in the sizes of livers and gizzards, and swelling of the rectum with exposure to *F. oxysporum* and *A. fumigatus.* Histological examination revealed severe destruction of the intestinal villi, distortion of the intestinal mucosa, and haemorrhage in all the groups of birds exposed to the 5 fungi isolates. Mortality in the groups of birds infected with *C. verruculosa* and *C. bertholletiae* (80.0%, 4/5) was significantly higher ($p<0.001$) than in other groups.
Conclusion: This study showed that the poultry farms were highly laddened with a variety of filamentous fungi, which have a lot of detrimental effects on the health of bird and needs to be controlled through proper hygiene practices and the provision of biosecurity for the birds.

Keywords: Poultry, filamentous fungi, birds, gastrointestinal tract, pathogenicity

Received Jan 2, 2025; Revised Mar 27, 2025; Accepted Mar 29, 2025

Pathogénicité de champignons filamenteux isolés dans des élevages avicoles sur le système gastro-intestinal de poussins d'un jour dans l'État d'Anambra, au Nigéria

Mba, Anthonia Nkiruka

Institut International de Recherche sur les Maladies Infectieuses, la Biosécurité et la Biosûreté, Université Fédérale des Sciences de la Santé David Umahi, Uburu, État d'Ebonyi, Nigéria
Correspondance à: tonia762013@gmail.com; +234 8035082191; ORCID: https://orcid.org/0000-0001-8409-8559

Résumé:

Contexte: La présence généralisée de champignons filamenteux dans les élevages avicoles a un impact profond sur la santé des oiseaux, entraînant une réduction des taux de croissance, une augmentation de la mortalité et une diminution de la ponte. L'objectif de cette étude était d'utiliser un modèle aviaire pour déterminer la pathogénicité de certains isolats de champignons filamenteux provenant d'échantillons de litière, d'aliments et de matières fécales chez des oiseaux d'un jour.

Méthodologie: Un total de 300 échantillons environnementaux (aliments, litière et fientes) ont été collectés dans 10 fermes avicoles sélectionnées dans six zones de gouvernement local (LGA) de l'État d'Anambra, et cultivés pour l'isolement des champignons par des méthodes phénotypiques (macroscopiques et microscopiques) et génotypiques. Cinq des isolats de champignons ont été utilisés pour des études de pathogénicité sur des poussins d'un jour. Un total de 25 poussins d'un jour, répartis aléatoirement en 5 groupes de 5 chacun, ont été infectés par voie orale avec 1 ml des différentes conidies fongiques. Un 6e groupe a servi de témoin et a été inoculé avec le même volume de solution saline tamponnée au phosphate. Tous les oiseaux ont été observés deux fois par jour pour l'apparition de signes cliniques pendant 10 jours. Un examen histologique des tissus intestinaux et de l'estomac des oiseaux infectés a été effectué. Les données ont été analysées à l'aide d'une ANOVA à un facteur sur SPSS version 21.0, avec $p<0,05$ considéré comme significatif.

Résultats: Les effets pathogènes des 5 isolats de champignons (*Curvularia verruculosa, Aspergillus tubingensis, Cunninghamella bertholletiae, Fusarium oxysporum* et *Aspergillus fumigatus*), utilisés pour l'étude de pathogénicité sur les oiseaux, comprennent des symptômes tels que; (i) réduction de la consommation de nourriture et d'eau, effilochage des plumes et perte de réflexe dans les 5 jours suivant l'exposition à *C. verruculosa*, et (ii) bourse de Fabricius décolorée et élargie, intestin obstrué, sombre et légèrement desquamé, réduction de la taille des foies et des gésiers, et gonflement du rectum avec exposition à *F. oxysporum* et *A. fumigatus*. L'examen histologique a révélé une destruction sévère des villosités intestinales, une distorsion de la muqueuse intestinale et une hémorragie dans tous les groupes d'oiseaux exposés aux 5 isolats de champignons. La mortalité dans les groupes d'oiseaux infectés par *C. verruculosa* et *C. bertholletiae* (80.0%, 4/5) était significativement plus élevée ($p<0,001$) que dans les autres groupes.

Conclusion: Cette étude a montré que les élevages avicoles étaient fortement contaminés par divers champignons filamenteux, qui ont de nombreux effets néfastes sur la santé des oiseaux et doivent être contrôlés par des pratiques d'hygiène appropriées et la mise en place de mesures de biosécurité pour les oiseaux.

Mots-clés: Volaille, champignons filamenteux, oiseaux, tractus gastro-intestinal, pathogénicité

Introduction:

Among the public health issues caused by microbes, fungal diseases are relatively neglected, owing to the low mortality rate of 1.5% globally (1). Filamentous fungi pose significant health risks to poultry, particularly through their insidious effects on the gastrointestinal tract (GIT) of young birds. These fungi impair gut health, reduce nutrient absorption, and increase susceptibility to secondary infections, severely affecting poultry production (2). Fungal pathogens have gained importance due to their wide-ranging impacts, causing infections that vary from superficial to life-threatening conditions in both animals and humans (3). According to the Global Action for Fungal Infections (GAFFI), fungal diseases claim approximately 150 lives per hour worldwide, underscoring the critical need to address their effects in both human and veterinary medicines (4).

Young birds, particularly day-old chicks, are highly vulnerable to fungal infections. Fungi are frequently associated with high morbidity and mortality in these birds, especially when they are exposed to contaminated environments early in life (5). The most common sources of fungal exposure in poultry include settled dust, bio-aerosols derived from soil, dust, mouldy feed, especially processed ones, contaminated litter, droppings and to a lesser extent, the birds themselves (6). These substrates, often found in poultry farms, create conducive environment for fungal growth and the subsequent spread of infection (7).

Fungal genera implicated in poultry farm diseases include *Acremonium*, *Fusarium, Lichtheimia, Aspergillus, Trichoderma, Penicillium*, *Scopulariopsis, Curvularia, Alternaria*, *Histoplasma Microsporium, Trichophyton,* and yeasts such as *Cryptococcus* and *Candida* spp (8,9,10,11). Among these, *Aspergillus, Penicillium* and *Fusarium* are particularly prevalent and have been linked to gastrointestinal infections in poultry (12). The avian gastrointestinal tract (GIT) plays a crucial role in nutrient absorption, and its health is vital for optimal poultry productivity. A healthy and efficiently functioning GIT is essential for sustainable animal production, influencing key performance indicators such as growth rates, feed efficiency, and the quality of meat and eggs (13). However, the GIT is constantly exposed to a range of microorganisms including bacteria, viruses, parasites and fungi. Although fungi make up a small portion (about 0.1%) of the gut microbiome, their presence can disrupt microbial balance, leading to the development of diseases such as mycosis, particularly when immunological defences are compromised (3, 14).

Mycotic infections are often contracted through contaminated feed or water, with fungal spores thriving in poultry litter and droppings, further increasing the risk of infection in farm environments (15). Despite the capacity of the avian immune system to combat many pathogens, filamentous fungi have evolved to exploit the vulnerabilities of young birds. Their thin skin, poor vascularised air sacs, and lack of sebaceous and sweat glands make birds more susceptible to fungal colonization compared to other vertebrates (16). Once the fungi bypass the physiological barriers and colonize the GIT, they can enter a commensal relationship with the host, sometimes remaining asymptomatic until microbial balance is disrupted (17).

Among the various genera of filamentous fungi, *Aspergillus* species are the most prevalent in poultry environments. Oral exposure to these fungi can lead to gastrointestinal symptoms such as diarrhoea, vomiting, and abdominal pain, and in severe cases, may result in mortality (18). Given the growing recognition of the impact of fungal infections on poultry health and the potential zoonotic risks, it is essential to study these pathogens more closely.

The primary objective of this study is to investigate the pathogenic impact of selected filamentous fungi isolated from litter, feed, and faecal samples in poultry farms located in Anambra State, Nigeria, on the gastrointestinal health of day-old chicks. By understanding the effects of these fungal isolates, the study aims to provide insights into their role in impairing avian GIT health and contribute to developing more effective management strategies for fungal infections in poultry farming.

Materials and method:

Study setting:

This study was conducted on 10 selected poultry farms in 6 local government areas (LGAs) of Anambra State, southeast Nigeria (latitude 6° 20'N and longitude 7° 00'E) from October 2014 to September 2015. The LGAs and farms include Idemili LGA (Apkaka farm in Umuoji, Jospan farm in Nkpor, Osakwe farm in Ogidi, Cyroby farm in Ogidi, Volant farm in Ogidi), Nnewi North LGA (Agroventures farm), Onitsha South LGA (Government farm), Awka South LGA (Takilita farm), Oyi LGA (EM farm), and Aguata L.G.A, (Eagle farm). The farms, housing approximately 227,200 laying birds, were examined. Six out of the 10 farms practiced open (floor) housing system, while the other four practiced closed (cage) housing system (19).

Ethical approval and informed consent:

Ethical approval was obtained from the university ethical committee before conducting the research. The permissions of the farm managements were obtained before sample collection.

Data collection:

A designed questionnaire was used to collect data from the poultry farm workers about the poultry and the clinical conditions of chicks.

Isolation and identification of fungi isolates:

A total of 300 samples (feeds, litters, and droppings) were collected from the selected poultry farms and cultured for isolation and identification of fungi isolates as previously described by Mba et al., (19), which involved comprehensive analyses of macroscopic, microscopic and genetic features of the isolates (20,21,22).

To confirm the identities of the isolates, a subset was sent to Macrogen, Europe (Meibergdreef Amsterdam, Netherlands) for further analysis, the findings of which have been published by Mba et al., (19). Five of the fungal species were selected for pathogenicity study; *Aspergillus tubingensis, Aspergillus fumigatus, Curvularia verruculosa, Fusarium oxysporum*, and *Cunninghamella bertholletiae*.

Pathogenicity testing of the fungal isolates:

Experimental and control chicks:

Thirty-one-day-old chicks were housed in cages (divided into 6 groups of 5 chicks each) in the animal facility of the Zoology Department, University of Nigeria, Nsukka, Nigeria. The chicks were provided with commercial feed and sterile water *ad libitum*. One of the 6 groups served as the control group.

Inoculum preparation:

To prepare the fungal inoculum, the isolates were grown on Sabouraud Dextrose Agar at 25°C for 6 days to induce asexual structures. Fungal spores were harvested by suspending the cultures in phosphate-buffered saline (PBS) containing 0.1% Tween-80. The suspension was filtered through two layers of sterile gauze to remove hyphal fragments. Tween-80 was removed by repeated washing with PBS, and the final spore suspension was adjusted to a concentration of approximately 1×10^6 spores/ml using haemocytometer (23).

Animal inoculation and clinical features:

Each experimental chick was administered 1ml of the fungal inoculum orally. The control group was administered an equivalent volume of PBS. The chicks were monitored twice daily for 10 days as previously described (11,24,25,26) for clinical signs such as diarrhea, vomiting, tremors, loss of balance, lethargy, weight loss, respiratory distress and other morphological and pathological changes including mortality (27,28,29)

Necropsy and histopathological examination:

Any dead bird was immediately subjected to necropsy, and sick birds were taken for further clinical examination. Detailed case histories and observed clinical signs were considered before conducting necropsy. Gross lesions in organs and tissues were carefully recorded. Tissue samples from the intestines and stomachs of infected birds were collected postmortem and fixed in 10% neutral buffered formalin to prevent tissue autolysis. The fixed tissue samples were processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H & E) following standard protocols (30). Histopathological lesions were examined under a light microscope at 100× magnification. Photomicrographs were captured using a Motic camera, and pathological changes were documented.

Statistical analysis:

Data were analyzed using one-way ANOVA on SPSS version 21.0. Values were considered significant if $p<0.05$.

Results:

Clinical features of experimental (infected) and control birds:

All the birds exposed to fungal isolates exhibited common clinical signs, including diarrhea, vomiting, reduced food and water intake, and weight loss within 5 days of exposure. Additionally, chicks inoculated with *C. verrucalosa*, *A. fumigatus*, and *A. tubingensis* displayed signs of lethargy and dehydration, with mortality rates of 80.0% (4/5) for *C. verrucalosa* and 60.0% (3/5) for both *A. fumigatus* and *A. tubingensis*. Infection with *F. oxysporum* also led to tremors and reduced growth rate, although the mortality rate was relatively low at 20.0% (1/5).

Furthermore, infection with *C. bertholletiae* resulted in high mortality rate of 80.0% (4/5), accompanied by signs of lethargy, dehydration, and frayed feathers. Notably, chicks exposed to *C. verruculosa* also exhibited frayed feathers, loss of balance and reduced reflex within 5 days of exposure. In contrast, the control group administered with an equivalent volume of PBS, which were not exposed to any fungal isolate, exhibited normal, healthy morphological features with no clinical signs and symptoms.

Mortality rate in fungi infected birds:

Fig 1 shows mortality rate of day-old chicks orally exposed to 1 ml inoculum suspension of the different fungal isolates. The group infected with *C. verruculosa* and *C. bertholletiae* recorded the highest mortality rate of 80.0% (4/5) while the group exposed to *F. oxysporum* had the lowest mortality rate of 20% (1/5).

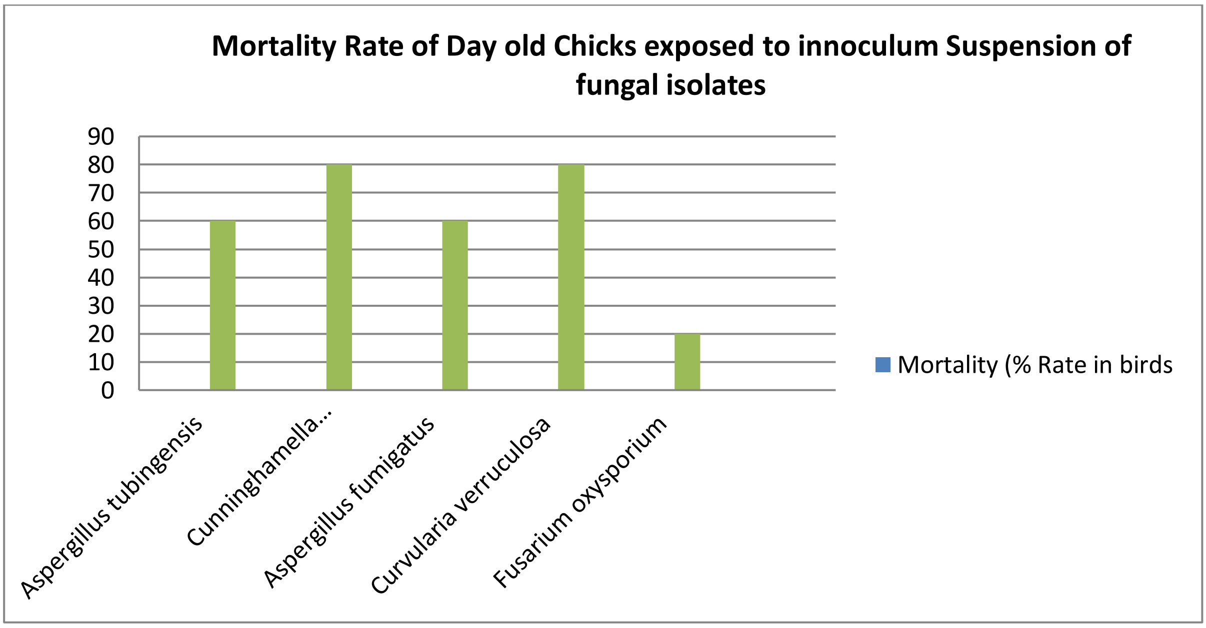
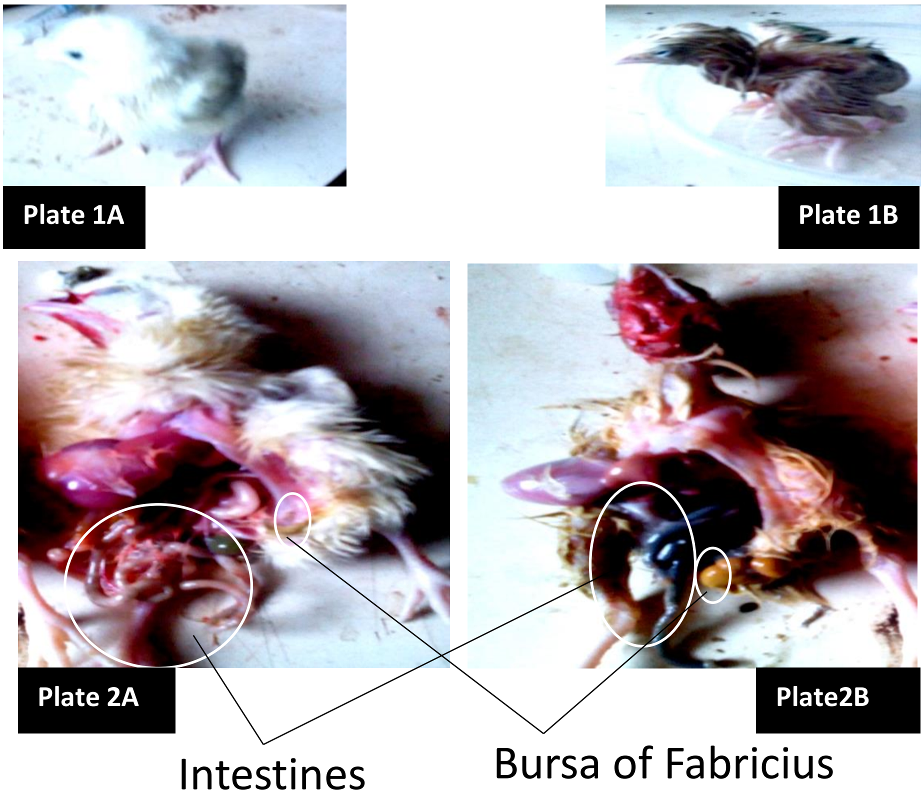


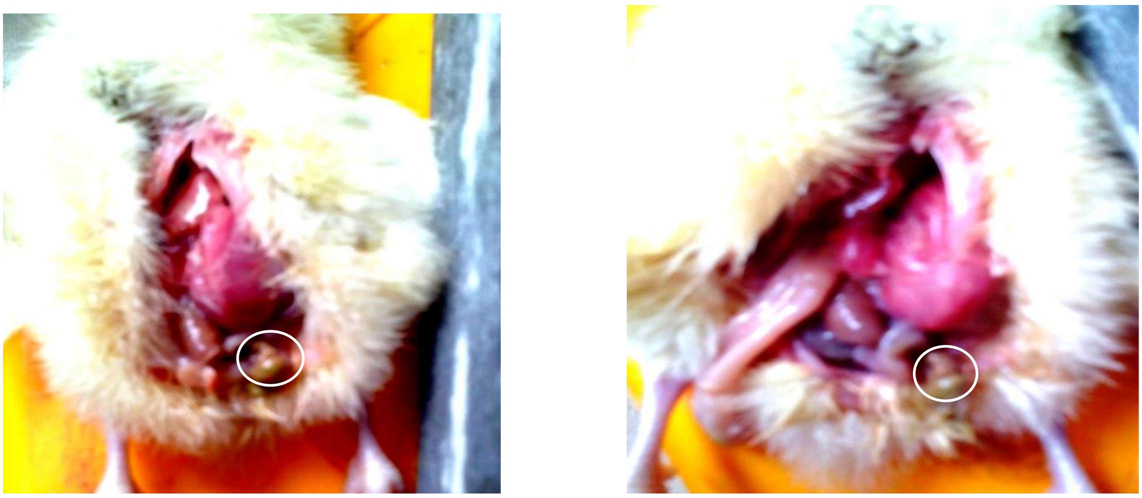
Fig 1: Mortality rate in infected birds

Morphological changes in the intestine of fungi infected birds:

Gross morphological changes were observed in the internal organs of the birds orally infected with different fungal isolates (Plates 1B, 2B, 3 and 4). In contrast, control birds, which were not exposed to any fungal isolate, exhibited normal, healthy morphological features (Plates 1A and 2A).



Plates 1A/2A and 1B/2B: Dissected bird showing various morphological changes in the internal organs, notably discoloured bursa of Fabricius (encircled 2B) and intestinal obstruction (encircled 2B) following oral exposure to *Curvularia verruculosa*



(A) (B)

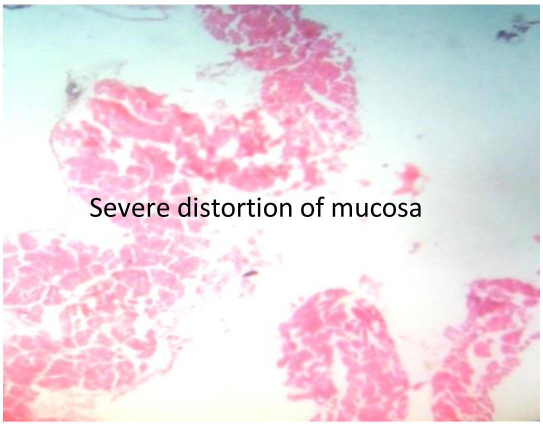
Plate 3: Dissected bird showing different morphological changes in the internal organs especially discoloured bursa of Fabricius (encircled) after oral exposure to; (A) *Aspergillus tubingensis* and (B) *Cunninghamella bertholletiae*

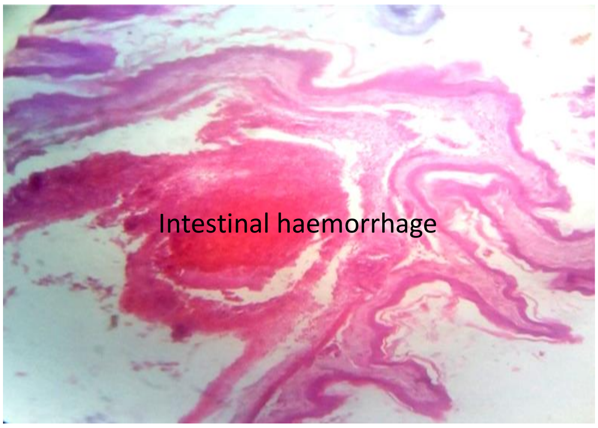
(A) (B)

Plate 4: Dissected bird showing different morphological changes in the internal organs [discoloured bursa of Fabricius (1) and swollen rectum (2)] after oral exposure to; (A) *Fusarium oxysporum*, and (B) *Aspergillus fumigatus*

Histopathological examination of experimental (infected) birds:

Histological analysis of the stomach and intestines of the infected birds revealed pathological changes (Plates 5–8), ranging from severe distortion of the intestinal mucosa to haemorrhages, villi destruction, and ulceration.





(A) (B)

Plate 5: Photomicrograph of orally infected chick intestine with; (A) *Aspergillus tubingensis* showing severe distortion of the mucosa, and (B) *Cunninghamella bertholletiae* showing severe intestinal haemorrhage at the luminal surface (H & E 100x).

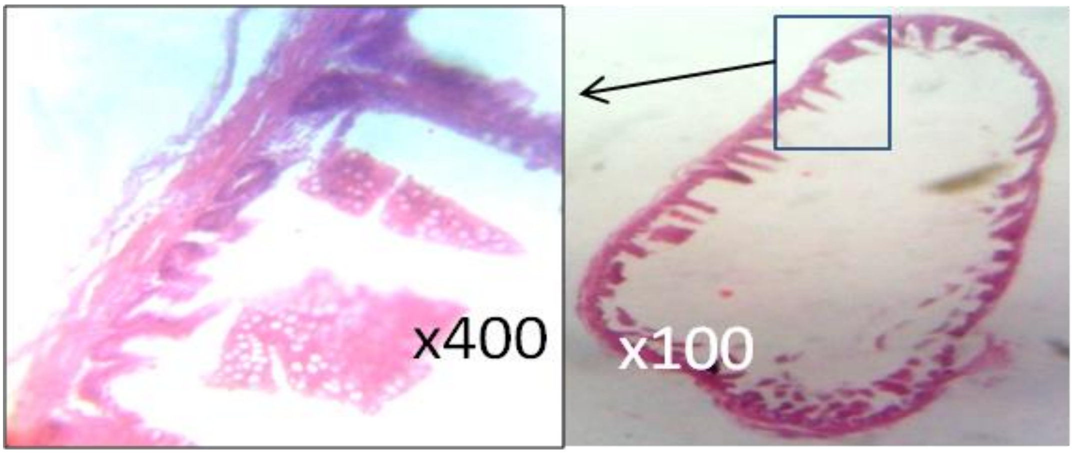
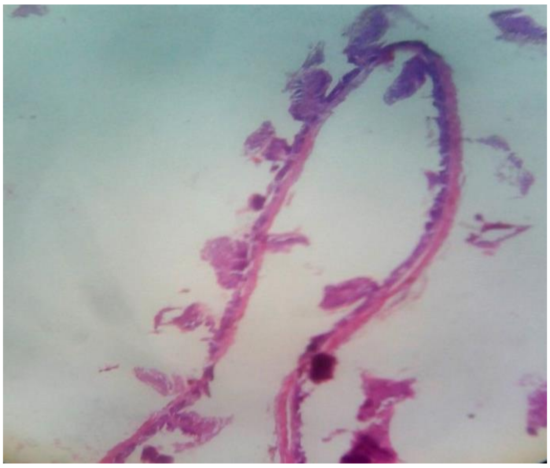


Plate 6: Photomicrograph of chick intestine orally exposed to *Curvularia verruculosa* showing severe destruction and reduction of the fused intestinal villi indicating mucosal atrophy (H & E 100x and X400x)

(A) (B)

Plate 7: Photomicrograph of chick small intestine orally exposed to; (A) *Curvularia verruculosa* showing minor disintegration of the intestinal villi from the base, and (B) *Fusarium oxysporum*, showing severe ulceration through the muscularis mucosa and severe destruction of the villi. (H & E 100x)



(A) (B)

Plate 8: Photomicrograph of *Gallus domesticus* small intestine orally exposed to; (A) *Fusarium oxysporum* showing predeveloped intestinal walls although the mucosal walls were all intact and no damage observed, and (B) *Aspergillus fumigatus*, showing severe destruction of the villi (H & E 100x)

Discussion:

Animal models are invaluable in enhancing our understanding of pathogen virulence, disease pathogenesis, and the feasibility of various therapies (31). Previous studies have shown that approximately 50.0% of birds can serve as reservoirs for fungi, which can be pathogenic to both humans and birds (32). The present study aimed to assess the health risks associated with the oral exposure of day-old chicks to spores from common filamentous fungi; *C. verruculosa, A. tubingensis, C. bertholletiae, F. oxysporum* and *A. fumigatus*. The results revealed varied clinical signs, symptoms, morphological changes, and mortality rates among the exposed birds.

Pathogenicity of *Curvularia verruculosa*

Historically, *Curvularia* species were considered non-pathogenic, however, recent reports suggest their increasing involvement in fungal diseases (33,34). In this study, chicks exposed to *C. verruculosa* exhibited symptoms such as loss of balance, frayed feathers, and reduced reflexes within five days. Additionally, necropsy findings showed intestinal obstruction and sloughing with oral ingestion of *C. verruculosa* (Plate 2B) consistent with Johnston et al., (35), who reported that fungi can disintegrate and obstruct the intestines. The high mortality rate (80.0%) observed in the infected bird supports previous reports that *C. verruculosa* can cause significant morbidity in birds (36).

The immune response in birds plays a crucial role in fighting infections, as their elevated body temperature (>40°C) and specific white blood cells help defend against pathogens (37). However as noted by Lowenthal et al., (38), the immature immune systems of day-old chicks may have contributed to their susceptibility to *C. verruculosa* infection. While previous studies suggest immunity in birds is linked to their innate and acquired systems (39), the present findings highlight the vulnerability of chicks, particularly through the observed morphological changes and mortality.

Pathogenicity of *Aspergillus fumigatus* and *Aspergillus tubingensis*:

Aspergillus fumigatus is the most commonly isolated fungal pathogen from both wild and domesticated birds (40), with known pathogenicity in respiratory and gastrointestinal systems. In this study, birds exposed to *A. fumigatus* and *A. tubingensis* displayed signs such as lethargy, diarrhoea, and dehydration, with mortality rate of 60.0% (3/5). This aligns with previous findings that *A. fumigatus* can cause significant morbidity and mortality in young birds (41). While fungal infections of the gastrointestinal tract are generally rare (42), the results suggest that these fungi can indeed induce gastrointestinal pathology, as evidenced by the clinical signs and histopathological findings of severe mucosal damage produced by *A. tubingensis* (Plate 5A) and *A. fumigatus* (Plate 8B).

Although study on mycotoxin was not carried out in our study, research studies have demonstrated that aflatoxin exposure is linked to various grades of gastrointestinal disorders and reproductive toxicity (6,42). The observed pathologies may therefore be associated with the presence of mycotoxin and further studies on this need to be carried out.

Pathogenicity of *Fusarium oxysporum* and *Cunninghamella bertholletiae:*

Infection with *F. oxysporum* led to notable pathologies, including rectal swelling (Plate 4A) and severe intestinal ulceration (Plates 7B and 8A). Despite these pathologies, the birds exposed to *F. oxysporum* had the highest survival rate of 80.0% (4/5) among the fungal isolates tested, indicating that while the infection was severe, it was less lethal compared to *C. verruculosa* and *C. bertholletiae.*

Necropsy findings of predeveloped intestinal walls suggest that the severity of the infection may have varied across individual birds. Similarly, *C. bertholletiae* caused extensive damage, as evidenced by severe intestinal haemorrhage and vessel invasion observed in the infected birds (Plate 5B). The high mortality rate (80.0%) agrees with the study by De Lucca (43), who reported that mucormycosis, often caused by *Cunninghamella* spp, carries a high mortality risk, particularly in cases of gastrointestinal involvement.

Mortality rate of day-old chicks:

The one-way ANOVA revealed a statistically significant difference in mortality rates between the different groups ($p<0.001$). Post-hoc comparisons indicated that the control group had a significantly lower mortality rate compared to all other groups. *C. verruculosa* and *C. bertholletiae* exhibited significantly higher mortality rates compared to *F. oxysporum. A. fumigatus* and *A. tubingensis* had mortality rates that were not significantly different from each other, but were significantly higher than *F. oxysporum*. These findings suggest that exposure to different fungal isolates resulted in varying levels of mortality in day-old chicks, with *C. verruculosa* and *Cunninghamella* spp. being the most pathogenic.

Role of the bursa of Fabricius:

Discolouration and enlargement of the bursa of Fabricius in birds exposed to *A. tubingensis, C. bertholletiae,* and *F. oxysporum* (Plates 3A, 3B, 4A and 4B) indicate its involvement in the immune response to these fungi. The bursa of Fabricius is crucial for the development of B-lymphocytes, essential for antibody production (44,45). Discolouration and enlargement of this organ suggest impaired immune function in the infected birds, which could have exacerbated the effects of the fungal infections. Contrary to these findings, El-Sharkawy et al., (11) reported no significant impact on the bursa of Fabricius in their study of fungal infections in broilers, suggesting that the roles of this organ may vary depending on the developmental stage of the bird or the fungal species involved.

Histopathological findings:

Histological analysis revealed significant pathological changes in the intestinal tissues of birds exposed to the fungal isolates. These ranged from mucosal distortion with *A. tubingensis* exposure (Plate 5A) to severe destruction of the villi with *A. fumigatus* (Plate 8B) and intestinal haemorrhage with *C. bertholletiae* (Plate 5B). These results are in contrast to earlier reports suggesting that the high body temperature of birds (>42°C) and their macrophage cells provide effective defence against fungal invasion (35,37). Findings of the study suggest that the fungi tested were able to bypass these defence mechanisms, leading to severe gastrointestinal pathology.

Conclusion:

This study highlights the pathogenic potential of several fungal species on day-old chicks, with *C. verruculosa* and *C. bertholletiae* posing the highest mortality risks. The observed discoloration and enlargement of the bursa of Fabricius suggest compromised immune function, exacerbating the severity of these infections. The high mortality rates and significant intestinal damage underscore the need for improved management of fungal contamination in poultry environments. These findings not only reveal the potential for substantial economic losses in poultry farming but also suggest possible zoonotic risks.

Further research is necessary to understand the role of fungal toxins in the observed pathology and to explore potential mitigation strategies, including vaccines, antifungal treatments, and biosecurity measures. This knowledge is essential for developing targeted interventions to safeguard poultry health and productivity.

Acknowledgements:

The author wishes to appreciate Dr Ikele Bright of Zoology Department, University of Nigeria Nsukka, who assisted with the pathogenicity study and histological analysis. The author also extends her appreciation to Macrogen, Europe, Meibergdreef Amsterdam, The Netherlands, for proper identification and confirmation of some of the recovered isolates. The author is equally grateful to Prof. Laura Okpala of Department of Nutrition and Dietetics, DUFUHS, Uburu, Ebonyi State and Dr. Ifeoma Prisca Udeozo of Department of Industrial Chemistry, ESUT, for their insightful comments on the manuscript.

Contributions of author:

The author conceived and conducted the study, and wrote the manuscript.

Source of funding:

The author received no external funding for this research. All costs associated with this study were borne personally by the author

Conflict of interest:

The author declares no conflicts of interest regarding the publication of this paper

References:

1. Mahalingam, S.S., Jayaraman, S., and Pandiyan, P. Fungal Colonization and Infections – Interactions with Other Human Diseases. Pathogens. 2022; 11 (2): 212. https://doi.org/10.3390/pathogens11020212
2. Aruwa, C. E., Pillay, C., Nyaga, M. M., and Sabiu, S. Poultry gut health-microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. J Anim Sci Biotechnol. 2021; 12 (1): 119. doi: 10.1186/s40104-021-00640-9.
3. Chao, A., and Vazquez, J.A. Fungal infections of the gastrointestinal tract. Gastroenterol Clin North Am. 2021; 50 (2): 243-260. doi: 10.1016/j.gtc.2021.02.009
4. Carpouron, J. E., de Hoog, S., Gentekaki, E., and Hyde, K.D. Emerging Animal-Associated Fungal Diseases. J Fungi (Basel). 2022; 8 (6): 611. doi: 10.3390/jof8060611.
5. Radwan, I. A., Abed, A. H., and Abdallah, A. S. Prevalence of fungal pathogens in broiler chickens and their environment. J Vet Med Res. 2018; 25 (2): 174-181. doi:10.21608/jvmr.2017.43316
6. Mba, A. N., Ekwealor, C. C., Ogbukagu, C. M., Ekwealor, I. A., and Chukwuezi, F. O. Occurrence and Distribution of Fungal Isolates from Sputum, Ears and Nose Samples of Poultry Farm Workers in Anambra State, Nigeria. Am J Infect Dis Microbiol. 2020;8 (4):139-145. doi: 10.12691/ajidm-8-4-4
7. Gomes, B., Pena, P., Cervantes, R., Dias, M., and Viegas, C. Microbial Contamination of Bedding Material: One Health in Poultry Production. Int J Environ Res Publ Health. 2022; 19 (24): 16508. doi: 10.3390/ijerph192416508.
8. Ekwealor, C. C., Mba, A. N., Anakwenze, V. N., Anaukwu, C. G., and Ekwealor, A. I. Efficacy of methanol extracts of *Lawsonia inermis* L. and *Cymbopogon citratus* Stapf. against fungal isolates from poultry farms in Anambra State, Nigeria. J Pharm Biores. 2024; 21 (3): 135-143 doi:10.4314/jpb.v21i3.5
9. Crameri, R., Weichel, M., Flückiger, S., Glaser, A. G., and Rhyner, C. Fungal allergies: yet unsolved problem. Chem Immunol Allergy. 2006; 91: 121–133. doi: 10.1159/000090276.
10. Hernandez, A. J. C. Poultry and avian diseases. Encyc Agric Food Syst. 2014; 504-520 doi: 10.1016/B978-0-444-52512-3.00183-2
11. El-Sharkawy, H., El-Salam, A. M. A., and Tahoun, A. Pathology and epidemiology of fungal infections in layer chicken flocks. Adv Gut Microb. Res. 2023; 1: 1-13. doi:10.1155/2023/9956074
12. Ghetas, A. M., Sedeek, D.M., Fedawy, H. S., et al. Detection of intestinal fungi in chickens naturally infected with infectious bursal disease. Adv Anim Vet Sci. 2022; 10 (3): 514-520. doi.org/10.17582/journal.aavs/2022/10.3.514.520
13. Celi, P., Cowieson, A. J., Fru-Nji, F., Steinert, R. E., Kluenter, A-M., and Verlhac, V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. Anim Feed Sci Technol. 2017; 234: 88-100, https://doi.org/10.1016/j.anifeedsci.2017.09.012.
14. Li, J., Chen, D., Yu, B., et al. Fungi in Gastrointestinal Tracts of Human and Mice: from Community to Functions. Microb Ecol. 2018; 75 (4): 821-829. doi: 10.1007/s00248-017-1105-9.
15. Mgbeahuruike, A. C., 1agina, O. A., 1anyanwu, M. U., et al. Microbial Contamination of Poultry Feed and the Effects on Birds' Performance. Anim Res Int. 2023; 20 (1): 4834 – 4861
16. Dulisz, B., Dynowska, M., and Nowakowski, J. J. Body condition and colonization by fungi of House Sparrows Passer domesticus in the urban and rural environment. Euro Zoolog J. 2021; 88 (1): 152-164. doi:10.1080/24750263.2020.1857447
17. Casadevall, A., and Pirofski, L. Host-Pathogen Interactions: Basic Concepts of Microbial Commensalism, Colonization, Infection, and Disease. Infect Immun. 2000; 68: 12. https://doi.org/10.1128/iai.68.12.6511-6518.2000
18. Lamps, L.W., Lai, K. K., and Milner, D. A. Jr. Fungal

infections of the gastrointestinal tract in the immunocompromised host: an update. Adv Anat Pathol. 2014; 21 (4):217-227.

19. Mba, A. N., Ekwealor, C. C., and Ekwealor, I. A. Fungal Contaminants of Feed, Litter and Faecal Dropping Samples of Poultry Birds and their Occurrence and Distribution in Selected Poultry Farms in Anambra State, Nigeria. Nig J Microbiol. 2024; 38 (1): 6859-6870
20. De Hoog, G. S., Guarro, J., Gene, J. and Figueras, M. J. Atlas of Clinical Fungi. 2000; 2 (1). Centraal bureau voor Schimmel cultures, Utrecht, The Netherlands.
21. Diba, K., Kordbacheh, P., Mirhendi, S. H., Rezaie, S., and Mahmoudi, M. Identification of *Aspergillus* species using morphological characteristics. Pak J Med Sci. 2007; 23 (6): 867- 872.
22. Nadia, E. A., Ibtissem, B. S., Mohamed, B. K., Mahmoud, M., and Naima, B. Isolation and identification of fungal communities in organic and conventional soils. Int J Curr Microbiol Appl Sci. (2017); 6 (4): 1111-1123 doi:10.20546/ijcmas.2017.604.138
23. Chhabra, D., and Dhakad, N. K. Study on Pathogenicity of the *Aspergillus* species in experimentally immunosuppressed mice. Vet World. 2008; 1(3): 69-70
24. Dhama, K., Chakraborty, S., Verma, K. A., et al. Fungal/mycotic diseases of poultry-diagnosis, treatment and control: A Review. Pak J Bio Sci. 2013; 16: 1626-1640 doi: 10.3923/pjbs.2013.1626.1640
25. Beernaert, L. A., Pasmans, F., Van Waeyenberghe, L., Haesebrouck, F., and Martel, A. *Aspergillus* infections in birds: a review. Avian Pathol. 2010; 39 (5): 325-331 doi: 10.1080/03079457.2010.506210.
26. Oladele, O. O., Ameji, N. O., Odey, M., et al. Management of acute aspergillosis in newly hatched chicks: a case report. Nig Vet J. 2022; 43 (4): 64-73
27. Sharma, J. M., et al. Fungal Diseases of Poultry. In: Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., and Nair, V. (Eds.). Diseases of Poultry, 13th edn. Ames, IA: Wiley-Blackwell. 2013: 981-996.
28. McLelland, D. A., and Moffett, P. J. Avian Aspergillosis. In B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, & Y. M. Saif (Eds.), Diseases of Poultry. 10th edn. Ames, IA: Iowa State University Press 1994: 91- 802.
29. Morris A. J., Byrne, T. C, Madden, J, F. and Reller, L. B. Duration of incubation of fungal cultures. J Clin Microbiol. 1996; 34 (6): 1583-1585 doi:10.1128/jcm.34.6.1583-1585.1996.
30. Luna, L. G. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. New York, NY, USA: McGraw-Hill; 1968: 111-112.
31. Clemons, K. V., and Stevens, D. A. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med Mycol. 2005; 43 (Suppl 1): S101-110. doi:10.1080/13693780500051919.
32. Mirhosseini, Z., and Khosravi, A. Fungal Pathogens: Emerging Threats to Birds and Human Health, Assessment of the Relative Frequency of Pathogenic Fungi in Ornamental Bird Feces. J Poultry Sci Avian Dis. 2023; 1 (4): 20-24. http://dx.doi.org/10.61838/kman.jpsad.1.4.4
33. Carter, E., and Boudreaux, C. Fatal Cerebral Phaeohyphomycosis Due to *Curvularia lunata* in an Immunocompetent Patient. J Clin Microbiol. 2004; 42: https://doi.org/10.1128/jcm.42.11.5419-5423.2004
34. Krizsán, K., Papp, T., Manikandan, P., et al. Clinical Importance of the Genus *Curvularia.* In: Medical Mycology: Current Trends and Future Prospects. In Medical Mycology. 2016; 148-204
35. Johnston, S. A., Voelz, K., and Robin, C. M. *Cryptococcus neoformans* Thermotolerance to Avian Body Temperature Is Sufficient for Extracellular Growth but Not Intracellular Survival In Macrophages. Sci Rep. 2016; 6: 20977. doi: 10.1038/srep20977
36. Severo, C. B., Oliveira, F. M., Pilar, E. F. S., and Severo, L. C. Phaeohyphomycosis: a clinical-epidemiological and diagnostic study of eighteen cases in Rio Grande do Sul, Brazil. Mem Inst Oswaldo Cruz. 2012; 107 (7): 854-858. doi:10.1590/s0074-02762012000700003
37. Lowenthal, J. W., Connick, T. E., McWaters, P. G., and York, J. J. Development of T cell immune responsiveness in the chicken. Immun Cell Biol. 1994; 72 (2): 115-122. doi: 10.1038/icb.1994.18
38. Pavlidis, H. Avian Immunity 101: The basics. Poultry World. 2019. https://www.poultryworld.net/health-nutrition/avian-immunity-101-the-basics/
39. Arné, P., Risco-Castillo, V., Jouvion, G., and Guillot, J. Aspergillosis in wild birds. J Fungi (Basel). 2021; 7 (3): 241. doi: 10.3390/jof7030241
40. Ulloa-Avellán, O., Calderón-Hernández, A., Rubí Chacón, R., and Bernardo Vargas Leitón. *Aspergillus* spp Isolated from Lungs of Poultry (*Gallus gallus*) at the Mycology Laboratory, School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica between 2008 and 2021 and Associated Factors. J Fungi (Basel). 2023; 9 (1): 58. doi: 10.3390/jof9010058
41. Maragkoudakis, E., Realdi., G., and Dore, M. P. Fungal infections of the gastrointestinal tract. Recenti Prog Med. 2005; 96 (6): 311–317.
42. World Health Organization (WHO). Food Safety Digest: Aflatoxins. Department of Food Safety and Zoonoses, World Health Organization, Geneva. 2018
43. De Lucca, J. A. Harmful fungi in both agriculture and medicine. Rev Iberoam Micol. 2007; 24: 3-13
44. Gitlin, D. A., and Nussenzweig, M. C. Immunology: fifty years of B lymphocytes. Nature. 2015; 517 (7533):139–141 doi: 10.1038/517139a
45. Nera, K., Kyläniemi, M. K., and Lassila, O. Bursa of Fabricius. In: eLS. John Wiley and Sons Ltd, Chichester. doi: 10.1002/9780470015902.a0000506.pub4