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Prevalence of fungal isolates in selected poultry farms and outcomes of ocular fungal infections in experimental murine model

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Abstract:

Background: Poultry farm workers, often exposed to fungal-laden environments, are at heightened risk of ocular mycoses, which is a potentially blinding infection that warrants urgent attention and investigation. This study investigates the fungal load and pathogenicity of fungal isolates from ten selected poultry farms in Anambra State, southeast Nigeria, and their potential to cause ocular mycoses in a murine model, simulating occupational exposure.
Methodology: A total of 300 environmental samples (droppings, feed, and litter) from 10 randomly selected poultry farms in 6 local government areas of Anambra State, southeast Nigeria, were examined. Fungal loads were determined, and isolates were characterized macroscopically, microscopically using needle mount and slide culture technique, and genetically using DNA sequencing and Basic Local Alignment Search Tool (BLAST) analysis. Pathogenicity testing of the characterized isolates was conducted in a murine model, wherein 30 albino mice, randomly divided into 6 groups of 5 mice each (with one group as uninfected control), were topically inoculated with 1ml of fungal inoculum (approximately $1x10^6$ spores/ml) directly into the eyes, and monitored twice daily for clinical signs and histopathological changes in the eyes for a period of 10 days. Data analysis was done using ANOVA in SPSS software version 21.0.
Results: The mean fungal loads in the environmental samples significantly differs ($p<0.05$) between dry and rainy seasons. The mean fungal loads (spore forming unit/g) during the rainy season ranged from $1.5x10^4$ - $2.0x10^5$ sfu/g in droppings, $1.1x10^4$ – 'too numerous to count' (TNTC) in litter samples, and $9.5x10^3$ - $2.10x10^5$ sfu/g in feed samples. The mean fungal loads during the dry season in droppings, litter, and feed samples ranged from $1.2x10^4$ - TNTC, $2.2x10^4$ -$9.5x10^4$, and $6.0x10^3$ -$1.7x10^5$ sfu/g, respectively. Fifteen genera of fungi were identified, with yeast being the most prevalent during the dry season and *Aspergillus* species during the rainy season. Pathogenicity testing showed significant differences ($p<0.05$) between the different fungi-induced ocular lesions in the experimental mice, with *Aspergillus tubingensis* and *Curvularia verruculosa* causing the most frequent and severe histopathological changes that included mild to severe erythema, blood congestion, distorted cornea, iris, and ciliary body, and sloughing off of the sclera.
Conclusion: The prevalence of fungi isolates is high in the farms. Due to the pathological changes produced in the eyes of infected experimental mice, ocular exposure to fungi may pose significant health risks to poultry farm workers in Anambra State. This highlights the need for personal protective equipment, improved hygiene practices, proper ventilation, and sanitation in these farms to prevent ocular exposure and potential risks to poultry farm workers.

Keywords: Ocular mycoses, poultry farming, fungal infections, murine model, pathogenicity.

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Prévalence des isolats fongiques dans des élevages avicoles sélectionnés et évolution des infections fongiques oculaires dans un modèle murin expérimental

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Résumé:

Contexte: Les travailleurs des élevages avicoles, souvent exposés à des environnements contaminés par des champignons, présentent un risque accru de mycoses oculaires, une infection potentiellement cécitante qui nécessite une attention et des investigations urgentes. Cette étude examine la charge fongique et la pathogénicité d'isolats fongiques provenant de dix élevages avicoles sélectionnés dans l'État d'Anambra, au sud-est du Nigéria, et leur potentiel à provoquer des mycoses oculaires dans un modèle murin simulant une exposition professionnelle.
Méthodologie: Au total, 300 échantillons environnementaux (fientes, aliments et litière) provenant de dix élevages avicoles sélectionnés au hasard dans six collectivités locales de l'État d'Anambra, au sud-est du Nigéria, ont été examinés. Les charges fongiques ont été déterminées et les isolats ont été caractérisés macroscopiquement, microscopiquement par la technique de culture sur lame et par montage à l'aiguille, et génétiquement par séquençage de l'ADN et analyse BLAST ((Outil de recherche d'alignement local de base). Des tests de pathogénicité des isolats caractérisés ont été réalisés sur un modèle murin. Trente souris albinos, réparties aléatoirement en six groupes de cinq souris chacun (dont un groupe témoin non infecté), ont reçu une inoculation topique de 1ml d'inoculum fongique (environ $1x10^6$ spores/ml) directement dans les yeux. Les signes cliniques et les modifications histopathologiques oculaires ont été surveillés deux fois par jour pendant dix jours. L'analyse des données a été réalisée par ANOVA avec le logiciel SPSS version 21.0.
Résultats: Les charges fongiques moyennes dans les échantillons environnementaux diffèrent significativement (p<0,05) entre la saison sèche et la saison des pluies. Français Les charges fongiques moyennes (unité formant des spores/g) pendant la saison des pluies variaient de $1,5x10^4$ -$2,0x10^5$ sfu/g dans les excréments, $1,1x10^4$– « trop nombreux pour être comptés » (TNTC) dans les échantillons de litière, et $9,5x10^3$ - $2,10x10^5$ sfu/g dans les échantillons d'aliments. Les charges fongiques moyennes pendant la saison sèche dans les échantillons d'excréments, de litière et d'aliments variaient respectivement de $1,2x10^4$ - TNTC, $2,2x10^4$ -$9,5x10^4$ et $6,0x10^3$ -$1,7x10^5$ sfu/g. Quinze genres de champignons ont été identifiés, les levures étant les plus répandues pendant la saison sèche et les espèces d'*Aspergillus* pendant la saison des pluies. Les tests de pathogénicité ont montré des différences significatives (p<0,05) entre les différentes lésions oculaires induites par des champignons chez les souris expérimentales. *Aspergillus tubingensis* et *Curvularia verruculosa* étaient responsables des modifications histopathologiques les plus fréquentes et les plus graves, incluant un érythème léger à sévère, une congestion sanguine, une déformation de la cornée, de l'iris et du corps ciliaire, ainsi qu'une desquamation de la sclère.
Conclusion: La prévalence des isolats de champignons est élevée dans les élevages. En raison des modifications pathologiques produites dans les yeux des souris expérimentales infectées, l'exposition oculaire aux champignons peut présenter des risques sanitaires importants pour les travailleurs des élevages avicoles de l'État d'Anambra. Ceci souligne la nécessité de porter des équipements de protection individuelle, d'améliorer les pratiques d'hygiène, d'assurer une ventilation et un assainissement adéquats dans ces élevages afin de prévenir l'exposition oculaire et les risques potentiels pour les travailleurs.

Mots-clés: Mycoses oculaires, élevage avicole, infections fongiques, modèle murin, pathogénicité.

Introduction:

Poultry farming is a crucial sector of agriculture, providing a significant source of protein for millions worldwide. However, this occupation poses substantial health risks to farmers, particularly ocular mycoses caused by filamentous fungi (1). Fungal keratitis, also known as mycotic keratitis, is a severe corneal infection that can lead to blindness and eye loss. Ocular mycoses can cause severe complications, including corneal ulcers, endophthalmitis, and vision loss (2). Globally, over one million people are affected by fungal keratitis annually, with 8-11% of patients losing their eyes (3). The tedious nature of poultry farming increases the risk of ocular mycoses, as farmers often work long hours in contaminated environments and handle infected birds, feed, and bedding materials (4).

Filamentous fungi, such as *Aspergillus*, *Fusarium*, and *Curvularia*, thrive in these environments, contaminating dust, water, feed, litter, and organic matter. *Aspergillus* and *Fusarium* species are the most common causative agents of fungal keratitis, followed by *Curvularia* and other dematiaceous fungi (5). Among *Aspergillus* species, *Aspergillus fumigatus* and *Aspergillus flavus* are frequently isolated from poultry farms and are also implicated in ocular mycoses (6). *Fusarium* species such as *Fusarium oxysporum* and *Fusarium solani*, have also been reported as significant causes of ocular mycoses among poultry farmers. Additionally, rare cases of fungal keratitis caused by *Cunninghamella bertholletiae* and *Aspergillus tubingensis* have been reported, highlighting the diversity of fungal pathogens involved in ocular infections

(6). *Cunninghamella bertholletiae* has been implicated in a rare case of fungal corneal ulcer and is associated with trauma and immunosuppression (7).

Clinical manifestations of ocular mycoses include blurred vision, eye pain, redness, and discharge (8). Diagnosis remains challenging due to similarities with bacterial and viral infections. This study aims to inve- stigate the prevalence of fungal isolates within poultry farms and the clinical outcomes experimental ocular infections caused by some of the filamentous isolates using a mouse model

Materials and method:

Description of study area:

A large-scale study was carried out on poultry droppings, feed and litter samples in 10 selected poultry farms (AK, JO, OS, CY, AV, GT, VO, TA, EM, EG) from six local government areas (Aguata, Awka South, Idemili North, Nnewi North, Onitsha North, Oyi) of Anambra State, southeast Nigeria (Latitude 6° 20'N and Longitude 7° 00'E). The farms were selected using random sampling method.

Ethical approval, sample and data collection:

Ethical approval was obtained from the university ethical committee before conducting the research. Sample collection and filling of questionnaires were voluntarily done on the site of the farm by the workers, with the permission of the management of the farms.

Sample collection and transportation:

A total of 300 samples (100 samples each of poultry droppings, feeds and litters) were aseptically collected from the selected poultry farms, labeled with the appropriate information (name and location of farm, source of sample, collection time and date), placed in sampling packets and transferred to the Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, for analysis.

Enumeration and isolation of fungi:

A 10-fold serial dilution of the environmental sample was prepared in sterile test tubes. One milliliter (1ml) of 10^{-4} dilution of the sample was inoculated on Sabouraud Dextrose Agar (SDA) plates, supplemented with 0.05mg/ml of chloramphenicol. After 3 to 6 days incubation at 25°C, the plates were observed for fungal growth (9,10). The heterotrophic fungal count was estimated in spore forming unit/ml (sfu/ml). Pure colonies of each isolate were sub-cultured on SDA slant and stored at 4°C for further analysis.

Identification of fungal isolates:

The fungal isolates were identified by detailed studies of their macroscopic, microscopic and genetic features using DNA sequencing and BLAST analysis (11).

Pathogenicity testing of fungal isolates:

Experimental and control mice:

Thirty 3-month-old adult albino mice, weighing 28-30g, were used for the experimental study. The mice were housed in cages (divided into 6 groups of 5 mice each) in the animal facility of the Zoology Department, University of Nigeria, Nsukka, Nigeria. The mice were provided with commercial feed and sterile water *ad libitum.* One group 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 (12).

Animal inoculation:

Each experimental mouse was topically inoculated with 1ml of the fungal inoculum directly into the eyes. The control group was administered an equivalent volume of phosphate-buffered saline. The mice were monitored twice daily for 10 days for clinical signs, including gross morphological changes, pathological symptoms, and mortality rate.

Necropsy and histopathological examination:

Detailed case histories and observed clinical signs were considered before conducting necropsy. Gross lesions in the eyes of the experimental animals were carefully recorded. Tissue samples from the eyes of infected mice were collected postmortem and fixed in 10% neutral buffered formalin to prevent autolysis.

The fixed tissue samples were processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H & E) following standard protocols (13). 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:

The results and data obtained were statistically analyzed using One-way Analysis of Variance (ANOVA) with SPSS Software version of 21.0.

Results:

Enumeration of fungal isolates from environmental samples:

The mean fungal loads in the environmental samples for each selected poultry farm is shown in Table 1 for raining season and Table 2 for dry season. The mean fungal loads during the rainy season ranged from $1.5x10^4$ - $2.0x10^5$ sfu/g in droppings, $1.1x10^4$ to 'Too Numerous To Count' (TNTC) in litter samples, and 9.5×10^3 - 2.10×10^5 sfu/g in feed samples. The mean fungal loads during the dry season in droppings, litter, and feed samples ranged from 1.2×10^4 - TNTC, 2.2×10^4 - 9.5×10^4, and $6.0x10^3$ - 1.7×10^5 spore forming unit (sfu)/g, respectively.

Identification of fungal isolates from the environmental samples:

A total of 15 genera of fungal isolates were recovered from droppings, feed and litter samples from the poultry farms in both seasons (Table 3). The morphological and microscopic features of some of the isolates are presented in Table 4 and Plates 1-5.

Table 1: Mean fungal loads (spore forming unit/g) of environmental samples from selected poultry farms during rainy season

Farm code	Town/Local Government Area	Feed	Litter	Droppings	System
AK	Umuoji/ Idemili North	1.0×10^5	TNTC	1.2×10^4	Floor
JO	Nkpor/ Idemili North	1.2×10^5	1.1×10^4	1.7×10^4	Floor
OS	Ogidi/ Idemili North	4.8×10^4	1.2×10^5	2.0×10^5	Floor
CY	Ogidi/ Idemili North	4.6×10^4	6.8×10^4	2.3×10^4	Floor
AV	Nnewi / Nnewi North	3.6×10^4	1.3×10^5	4.0×10^4	Floor
GT	Onitsha/ Onitsha North	2.6×10^4	-	1.9×10^4	Floor
VO	Ogidi/ Idemili North	2.2×10^4	NA	4.5×10^4	Cage
TA	Awka/Awka South	9.5×10^3	NA	1.6×10^4	Cage
EM	Nteje /Oyi	4.7×10^4	NA	2.0×10^4	Cage
EG	Uga/Aguata	2.1 x 105	NA	1.5×10^4	Cage

TNCT = Too numerous to count; NA = Not available

Table 2: Mean fungal load (spore forming unit/g) of environmental samples from selected poultry farms during dry season

Farm code	Town/Local Government Area	Feed	Litter	Droppings	System
AK	Umuoji/ Idemili North	1.7×10^5	9.7×10^4	2.4×10^5	Floor
JO	Nkpor/ Idemili North	1.1×10^4	2.2×10^4	TNTC	Floor
OS	Ogidi/ Idemili North	2.2×10^4	8.9×10^4	1.9×10^5	Floor
CY	Ogidi/ Idemili North	3.5×10^4	8.1×10^4	1.6×10^5	Floor
AV	Nnewi / Nnewi North	1.7×10^5	6.4×10^4	TNTC	Floor
GT	Onitsha/ Onitsha North	8.5×10^3	-	1.2×10^4	Floor
VO	Ogidi/ Idemili North	1.1×10^5	NA	1.2×10^4	Cage
TA	Awka/Awka South	6.0×10^3	NA	1.5×10^4	Cage
EM	Nteje /Oyi	8.1×10^4	NA	1.7×10^4	Cage
EG	Uga/Aguata	7.6×10^4	NA	1.1×10^4	Cage

TNCT = Too numerous to count; NA = Not available

Table 3: Fungal genera isolated from feed, litter and droppings during rain and dry seasons

Fungal genus	**Isolation**	
	Rain season	**Dry season**
Aspergillus spp	+	+
Yeast	+	+
Penicillium spp	+	+
Fusarium spp	+	+
Lichtheimia spp	+	-
Paecelomyces spp	+	-
Acremonium spp	+	-
Chrysonilia spp	+	-
Curvularia spp	-	+
Cunninghamella spp	-	+
Syncephalis spp	-	+
Aureobasidium spp	-	+
Nathrasia spp	-	+
Trichoderma spp	-	+
Scopulariopsis spp	-	+

+ = isolated, - = not isolated

Table 4: Morphological and microscopic features of some of the fungal isolates observed in the poultry farms

Species of fungi	**Source of isolate**	**Morphological features of isolate**			**Microscopic features of isolate**
		Texture of growth	**Colour on surface**	**Colour on reverse**	
Aspergillus tubingensis	Droppings, Feed and Litter	Fluffy and wrinkled with age	Yellow and white, appear black with age	Yellow	Produced sclerotia
Curvularia verruculosa	Feed, Litter	Lanose	Brown	Dark brown	Branched, simple conidiophores, geniculate near the apex and brown in colour.
Cunninghamella bertholletiae	Feed, Litter	Cottony	Initially white, later turns grey	Pale buff	Broad and septate hyphae, long and branched sporangia, ending in swollen vesicles. Vesicles on lateral branches are generally smaller and are covered with spine-like denticles for supporting a single round to oval sporangium.
Aspergillus fumigatus	Droppings	Dense felt of conidiophore	Initially white then blue-green	Cream	Columnar conidial heads, subclavate vesicles
Fusarium oxysporum	Feed	Velvety	White	Hyaline	Short, single conidiophores lateral monophialides in the aerial mycelium, later arranged in densely branched clusters, slightly curved fusiform macroconidia, pointed at the tips and mostly septate

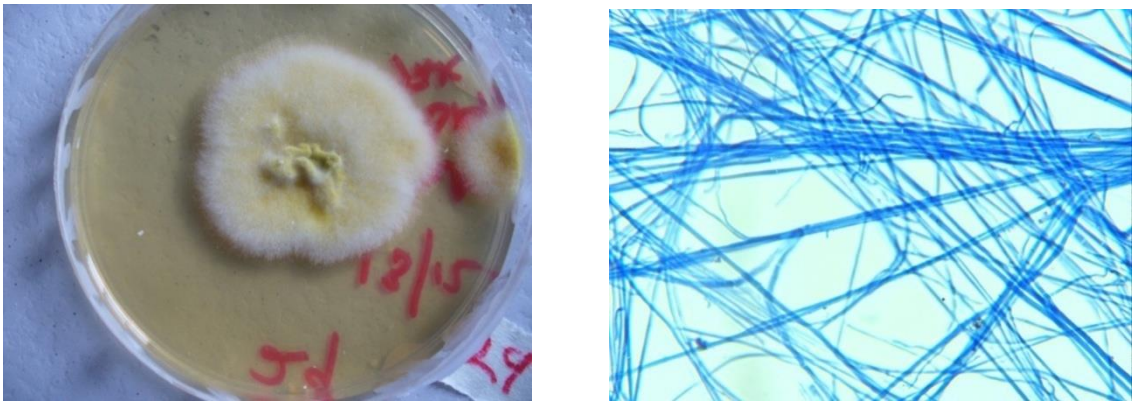

Plate 1: Morphological and microscopic features of *Aspergillus tubingensis* isolated from litter sample (7thday on SDA)

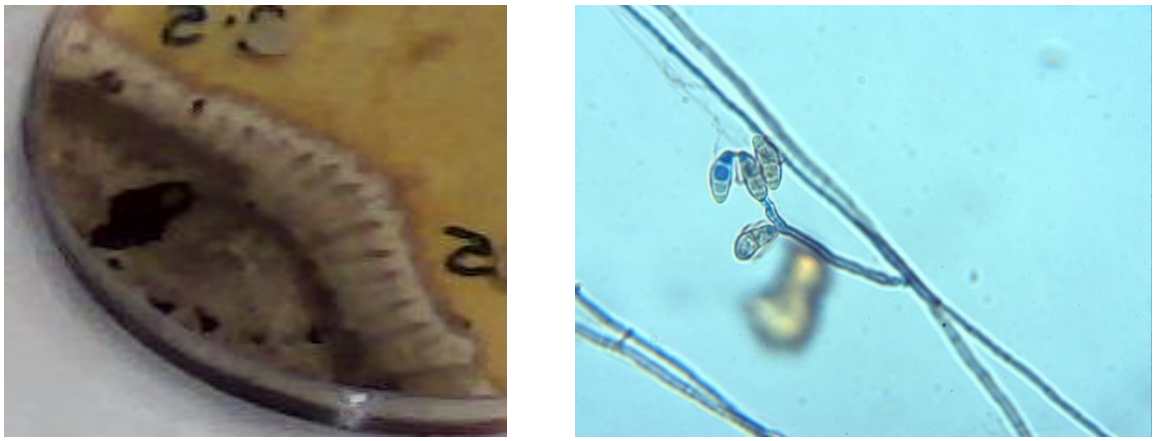

Plate 2: Morphological (2months on SDA) and microscopic (5 days on SDA) features of *Curvularia verruculosa* isolated from birds' droppings

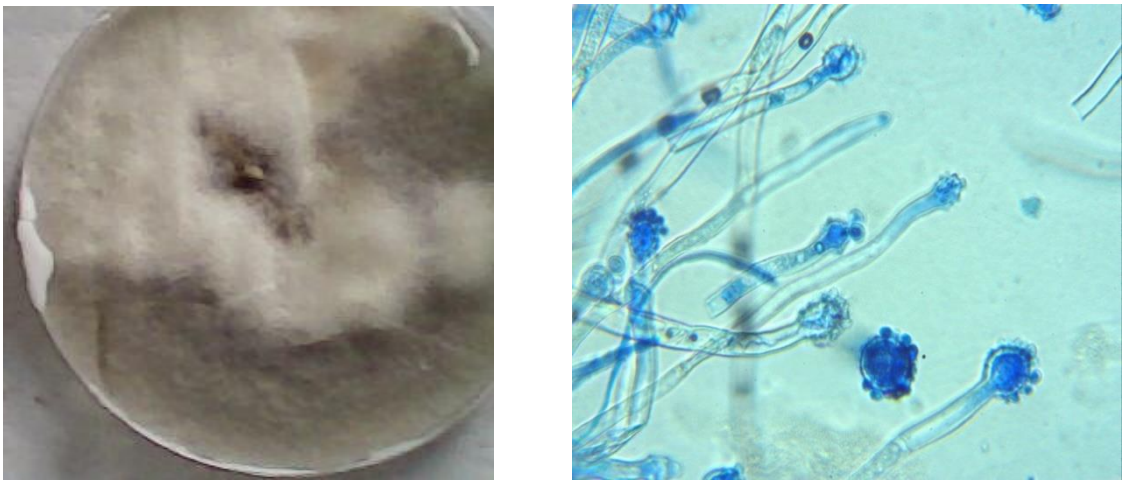

Plate 3: Morphological and microscopic features of *Cunninghamella bertholletiae* isolated from feed (5th day on SDA).

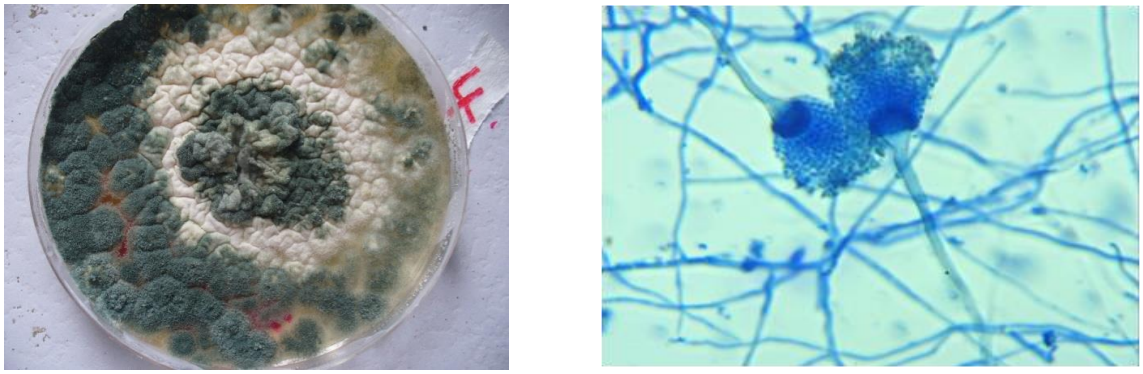

Plate 4: Morphological and microscopic features of *Aspergillus fumigatus* isolated from a bird's droppings (5th day on SDA).

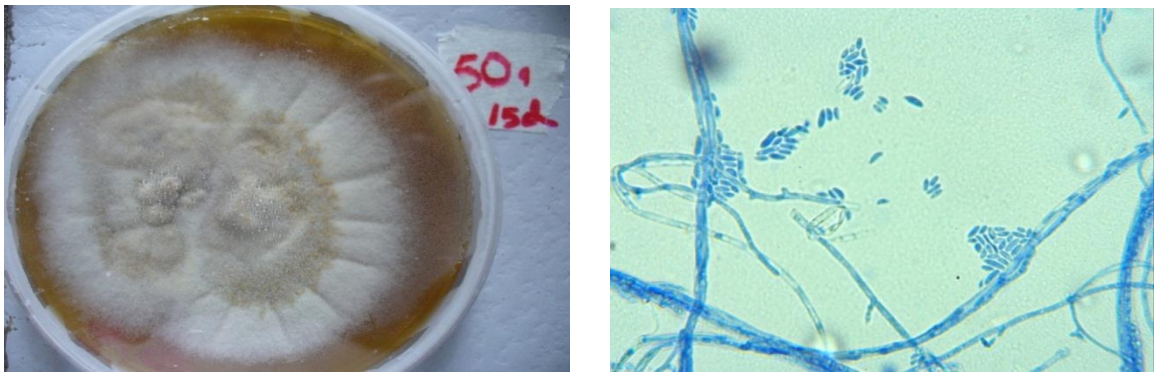

Plate 5: Morphological and microscopic features of *Fusarium oxysporum* isolated from feed (10th day on SDA)

Frequency distribution of fungal isolates during dry and rainy Seasons:

The frequency of fungal isolates in droppings, feed and litter samples from the poultry farms is presented in Fig 1 and Fig 2.

During the dry season, the most prevalent organism is yeast (Fig. 1), while *Aspergillus* species are the most prevalent during the rainy season (Fig 2).

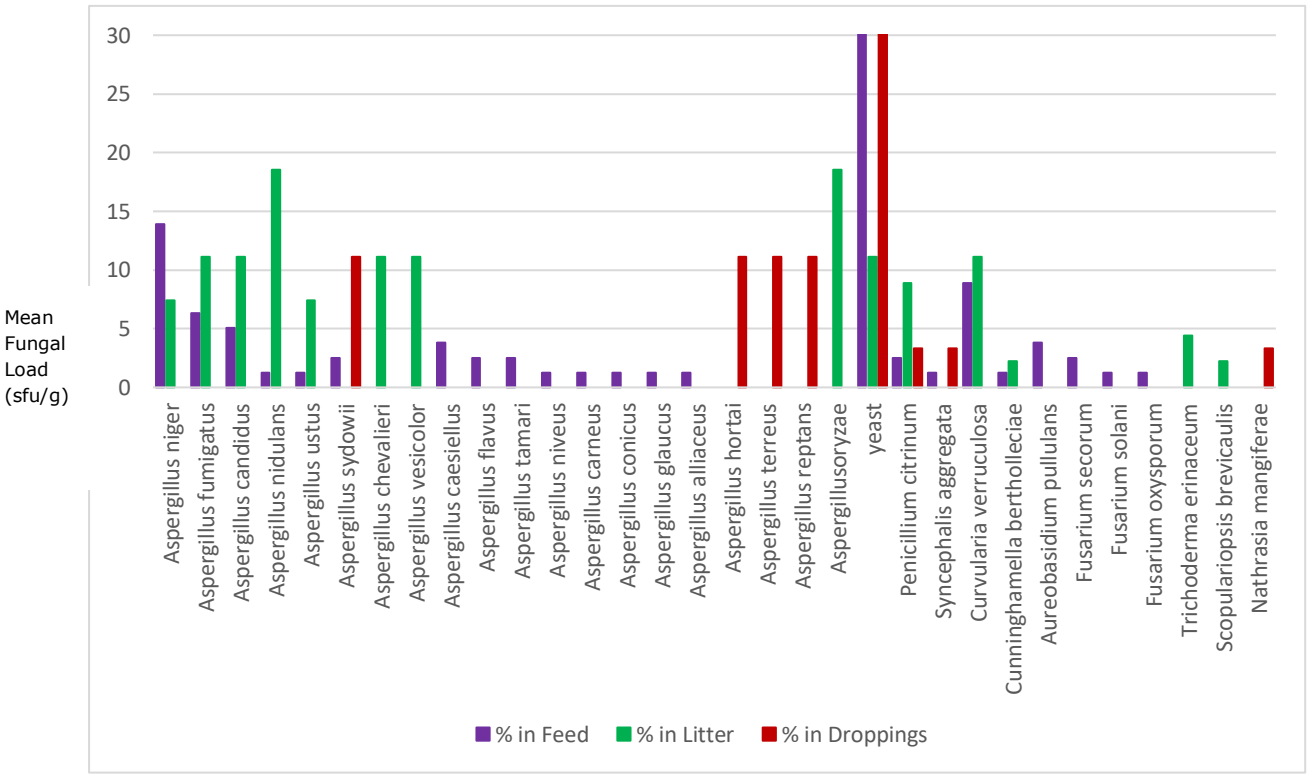

Fig 1. Frequency distribution of fungal isolates in environmental samples during dry season (14)

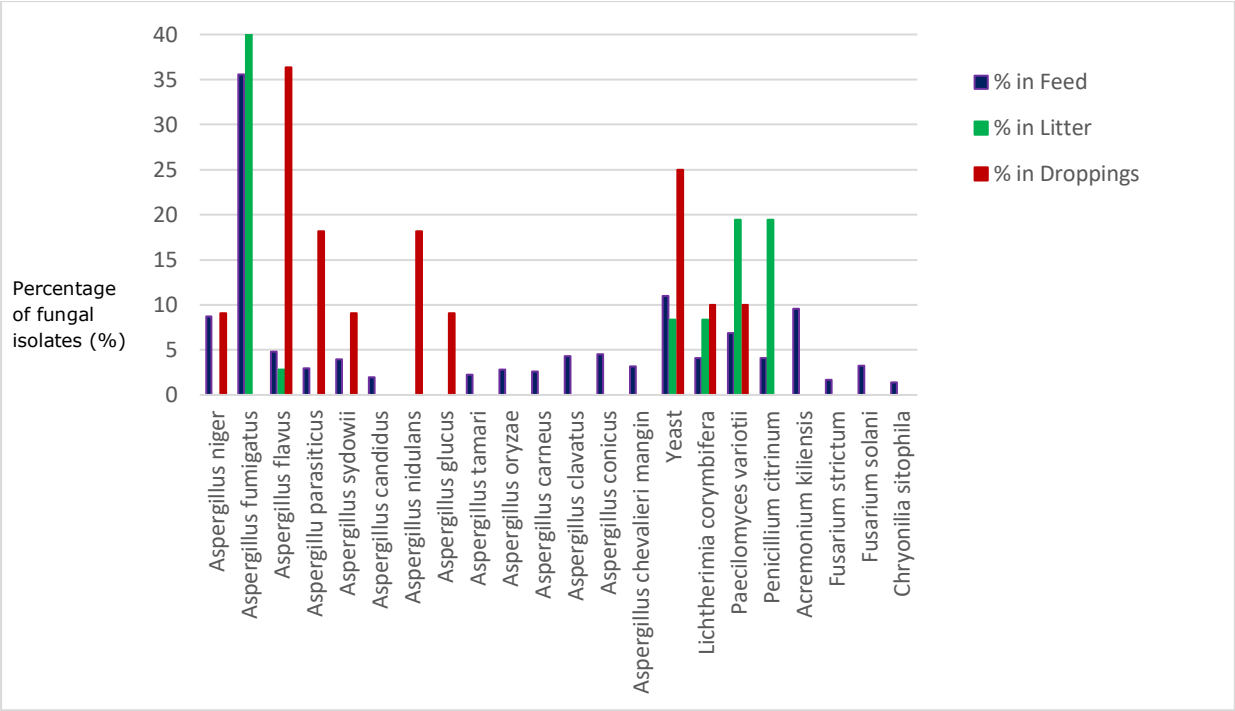

Fig 2: Frequency distribution of fungal isolates in environmental samples during rainy season (14)

Morphological changes in the eyes of fungal-infected mice:

Varying degrees of morphological and histopathological changes were observed in the eyes of mice topically infected with different fungal isolates. Mice infected with *A. tubingensis* showed severe erythema in the ocular region (6A) and histopathological changes including blood congestion, sloughing off of the sclera, distorted cornea, iris, and ciliary body, while the retina and optic nerves remained intact (6B). In contrast, mice infected with *C. bertholletiae* exhibited mild erythema in the cornea (7A) and histopathological changes include blood congestion, loosened strands of retina muscles, and distorted cornea, iris, and ciliary body (7B).

Mice infected with *Fusarium oxysporum* showed mild erythema and swollen cornea (8A), with severe blood congestion observed in histopathological examination (8B). *Curvularia verruculosa* infection resulted in severe erythema in the cornea (9A), damaged cornea and blood congestion (9B). Finally, mice infected with *Aspergillus fumigatus* exhibited slacked lower lid, swollen sclera, mild erythema in the cornea, (10A) and intact iris, with damaged lens, distorted cornea, sclera, retina, and pupil observed in histopathological examination (10B).

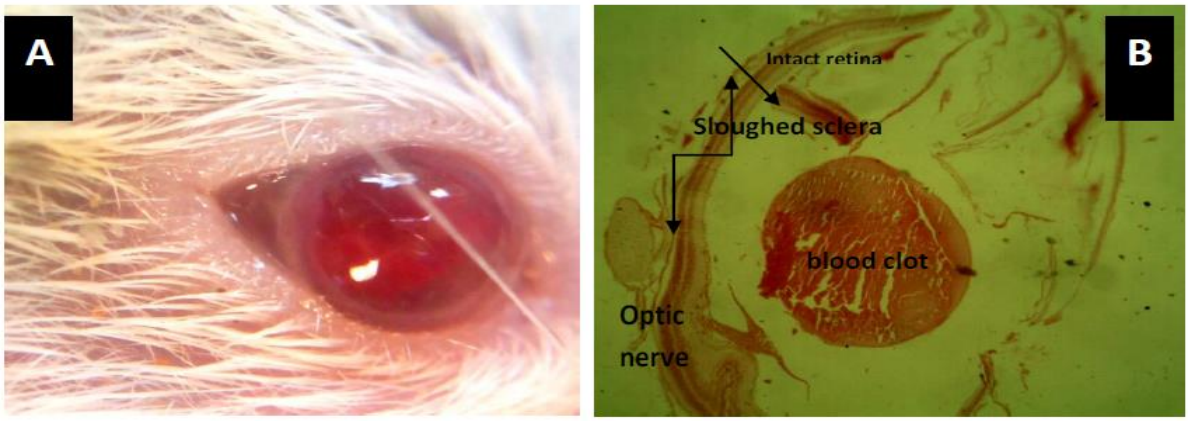

Plate 6: Morphological and histopathological changes in *Aspergillus tubingensis* ocular infection of a mouse
6A: Adult mouse showing severe erythema under steromicroscope and 6B: Photomicrograph of eye of adult mouse showing blood congestion with sloughing off of the sclera, distorted cornea, iris and ciliary body. The retina and optic nerves were intact. (H & E magnification 100x)

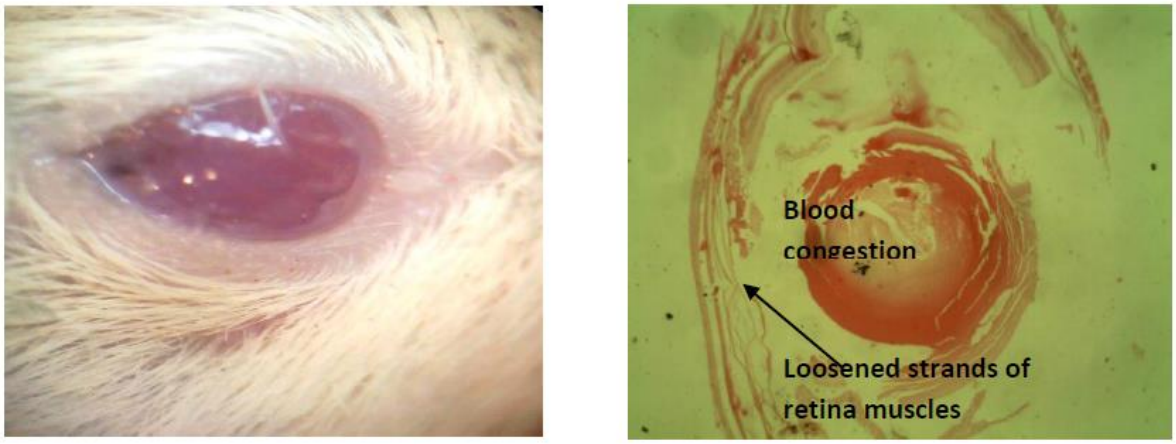

Plate 7: Morphological and histopathological changes in *Cunninghamella bertholletiae* ocular infection of a mouse
7A: Infected eye showing mild erythema in the cornea under steromicroscope. 7B: Photomicrograph of infected eye showing blood congestion and loosened strands of retina muscles. The cornea, iris and ciliary body appeared distorted. (H & E magnification 100x)

Plate 8: Morphological and histopathological changes in *Fusarium oxysporum* ocular infection of a mouse
8A: Infected mouse eye showing mild erythema and swollen cornea under stereomicroscope. 8B: Photomicrograph of infected mouse eye showing severe blood congestion (H & E magnification 100x)

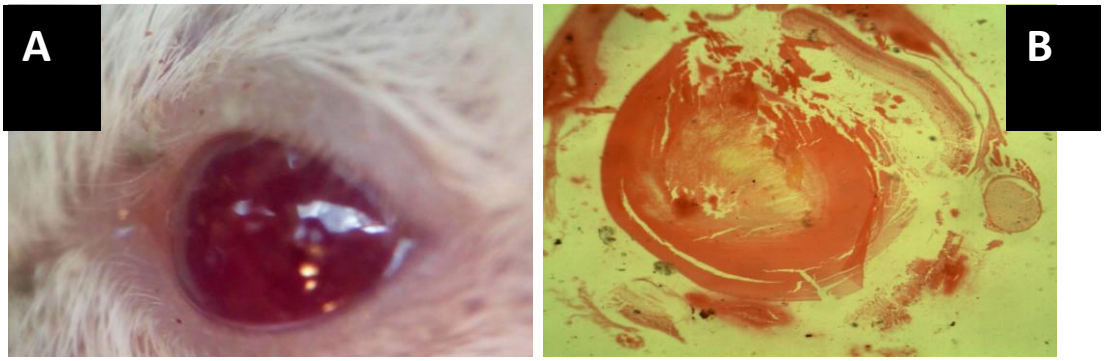

Plate 9: Morphological and histopathological changes in *Curvularia verruculosa* ocular infection of a mouse
9A: Infected mouse showing severe erythema in the cornea under stereomicroscope. 9B: Photomicrograph of infected mouse showing damages and blood congestion in the lens (H & E magnification 100x)

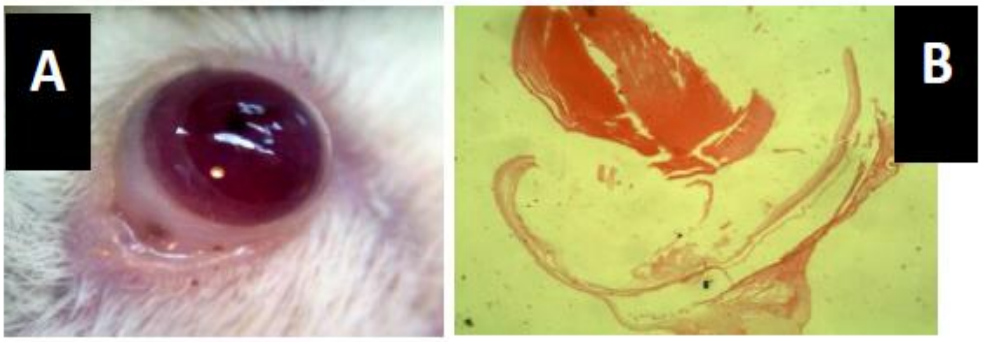

Plate 10: Morphological and histopathological changes in *Aspergillus fumigatus* ocular infection of a mouse
10A: Infected mouse eye under stereomicroscope showing slacked lower lid, swollen sclera, mild erythema in the cornea and intact iris. 10B: Photomicrograph of infected mouse eye showing damaged lens, distorted cornea, sclera, retina and pupil (H & E magnification 100x)

Discussion:

The occurrence and growth of fungi (moulds) in poultry farms is one of the major threats to poultry workers, poultry economy and health (14). In this study, we observed that all the environmental samples were overly contaminated by fungi, although to a variable extent as previously reported by Mba et al., (14). The frequency and distribution of fungal isolates in the environmental samples in this study showed presence of different fungal isolates with *Aspergillus* species having the highest frequency in the litter samples during rainy season and yeast showing the highest frequency in both droppings and feed samples in dry seasons. Contrary to our finding, Cruciani et al., (15) and Ostović et al., (16) reported *Aspergillus* species as the most frequent isolates in litter samples during dry season. However, they also observed *Aspergillus* species as the most frequent in litter samples in the rainy season which is in line with the result obtained in this study.

The unique structure of the eye as well as its exposure directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by fungi. Once the anatomical barrier is breached, host defenses directed against these microorganisms are often insufficient to prevent loss of vision. Filamentous fungal infections of the eye, known as mycotic keratitis, has been reported in many different parts of the world, particularly tropical area, where it may account for 50% of all ocular mycoses (2) or of all cases of microbial keratitis (4).

Filamentous fungi such as *A. fumigatus, A. flavus, Fusarium* species*, Curvularia, Alternaria* and *Acremonium* as well as yeasts such as *Candida* species have been reported to be implicated in fungal keratitis (17,18). Among these organisms, *Fusarium* species have been reported as the most common cause of keratitis followed by *Aspergillus* species and thirdly, the dematiaceous fungi such as *Curvularia*, with *Fusarium solani* and *Aspergillus* species constituting up to one-third of all cases of traumatic infectious keratitis (19). This agrees with the findings in this study as shown in Plate 6–10, in which topical ocular inoculation of *A. tubingensis* caused severe erythema (Plate 6A). Our findings in mice also agree with that observed by Kredics et al., (6), who reported pain, redness in the eyes, hypopyon and necrosis of the cornea in their patients, but contrary to our result, hypopyon was not observed.

It was also noted that of all the *Aspergillus* species implicated in keratitis, *A. tubingensis* had not been previously reported to cause mycotic keratitis. Studies however, showed that the paucity of information on the pathogenicity of *A. tubingensis* is possibly as a result of mis-leading identification results based on microscopy, morphology and culture, in which isolates of *A. tubingensis* were identified as *A. niger* because of similarities in their morphological and microscopic features (20).

Topical ocular inoculation of *C. bertholletiae* resulted in slight erythema and blurred cornea, with a discharge from the infected eyes. Ribes et al., (21) reported pain, blurring and loss of vision as the manifestations of ocular infection by *C. bertholletiae* which supports our finding however, pain and loss of vision were not observed. Spellberg et al., (8) reported symptoms of ocular infection by *Mucorales* as invasion of blood vessels, which resulted in formation of blood clots and death of surrounding tissues due to loss of blood supply. In our study, infected eye by *C. bertholletiae* showed formation of blood clot. This observation is supported by the study of Spellberg et al., (8) although loss of blood supply was not determined. Other pathological signs such as loosened retina walls, distorted cornea, iris and ciliary body were also reported.

Fusarium species have been reported by Liesegang and Forster (19) and Richard et al., (22) as one of the agents of fungal keratitis and the most common cause of corneal infection in the Southern United States (45-75%). Haynes et al., (1) described *F. oxysporum* as the most common isolate in fungal keratitis. Mikami and Stemmermann (23) in their study observed that keratitis due to *Fusarium* species, especially *F. solani* and *F. oxysporum* resulted in ulceration of the cornea which was progressive and destructive. Ocular infections by *F. oxysporum* in our study resulting in severe erythema, swelling, opacification of the cornea and blood congestion within 7 days (and clearing over 10 days of infection), were not observed by Mikami and Stemmermann (23), but were in line with the study of Kirk et al., (24), who also reported swollen eyes within one-half hour of infection, opacification and presence of edema in the cornea. However, their observation of the whiteness of the lower cornea, superficial ulcer which involved the cornea within 10 days and pus production, presence of pain by the 24th day, perforation of the limbus by the 28th day, with granular mass and mucopurulent discharge along the perforated area, denudation, necrosis, fragmentation and lysis of the cornea were not seen in our study. This may be as a result of non-extension of this study beyond 10days.

Mikami and Stemmermann (23) in their study reported that fungal keratitis by *Curvularia* species is characterized by presence of ulcer which became deep and lead to corneal perforation. Zhong et al., (25) reported superficial, feathery infiltrates of the central cornea, suppurative ulceration of the peripheral cornea and hypopyon in some cases, which resulted in poor vision. These observations by the researchers are at variance with that obtained in this study, in which only destruction of the various parts of the eyes with presence of erythema was observed. Feathery infiltrates, ulceration, perforation of the cornea and hypopyon were not observed.

As observed in our study, the ocular topical inoculation of *A. fumigatus* resulted in slacked lower lid, swollen cornea, mild erythema with intact iris (Plate 10A) while photomicrograph of the same eye showed damaged lens and distortion of the cornea, sclera, retina and pupil (Plate 10B). These findings are similar to the study by Darlene et al., (26) and Walther et al., (27) in which clinical manifestations such as opacification of the cornea, irregularity of the cornea indicating corneal damage were observed. Darlene et al., (26) also reported infiltration of inflammatory cells into the cornea which rapidly increased during the early stage of infection playing dual role of fighting the fungal pathogen and destroying the infected tissue.

Conclusion:

Our study reported a high prevalence of fungi isolates in selected poultry farms in Anambra State, southeast Nigeria. Experimental infections of mice demonstrate morphological and histopathological changes caused by different fungi in the eyes of the mice, providing valuable insights into the pathogenesis of fungal infections of the eyes. Ocular exposure to fungi may pose significant health risks to poultry farm workers.

Contributions of authors:

MAN conceptualize and performed the study, curate the data, and prepared the original manuscript; MAN and EAI reviewed and edited the manuscript; Both authors read and agreed to the published version of the manuscript.

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References:

1. Haynes, K. A., Westerneng, T. J., Fell, J. W., and Moens, W. Rapid detention and identification of pathogenic fungi by polymerase chain reaction amplification of large subunits ribosomal DNA. J Med Vet Mycol. 1995: 33 (5): 319-325 doi: 10.1080/02681219580000641
2. Srinivasan, M., Gonzales, C. A., and George, C. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. Br J Ophthalmol. 1997; 81:965–971 doi: 10.1136/bjo.81.11.965
3. Brown, L., Leck, A. K., Gichangi, M., Burton, M. J., and Denning, D.W. The global incidence and diagnosis of fungal keratitis. Lancet Infect Dis. 2021; 21: e49–e57. doi: 10.1016/S1473-3099(20)30448-5.
4. Sharma., S., Srinivasan, M., and George, C. The current status of *Fusarium* species in mycotic keratitis in south India. Indian J Med Microbiol. 1993; 11: 140–147
5. Ahmadikia, K., Aghaei Gharehbolagh, S., Fallah, B., et al. Distribution, Prevalence, and Causative Agents of Fungal Keratitis: A Systematic Review and Meta-Analysis (1990 to 2020). Front Cell Infect Microbiol. 2021; 11: 698780. doi: 10.3389/fcimb.2021.698780
6. Kredics, L. I., Varga, J., Kocsubé, S., et al. Infectious keratitis caused by *Aspergillus tubingensis*. Cornea. 2009; 28 (8): 951-954 doi: 10.1097/ICO.0b013e3181967098
7. Sagar, D., DedwL, A., Bhamare, S., and Karyakarte, R. *Cunninghamella bertholletiae* Fungal Corneal Ulcer: A Case Report. JETIR. 2019; 6 (6): 616-620

8. Spellberg, B., Edwards, J., and Ibrahim, A. Novel perspective on mucormycosis: Pathophysiology, presentation and management. Clin Microbiol Rev. 2005; 18: 556-569 doi: 10.1128/CMR.18.3.556-569.2005.
9. Krnjaja, V., Paviovski, Z., Lukie, M., et al. Fungal contamination and natural occurrence of ochratoxin A (OTA) in poultry feed. Biotechnol Anim Husbandry. 2014; 30 (3): 481-488 doi:10.2298/BAH1403481K
10. Pavan, R., and Manjunath, K. Qualitative analysis of indoor and outdoor airborne fungi in cowshed. J Mycol. 2014; 1: 8 doi:10.1155/2014/985921
11. 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 https://doi.org/10.20546/ijcmas.2017.604.138
12. Chhabra, D., and Dhakad, N. K. Study on pathogenicity of the *Aspergillus* species in experimentally immunosuppressed mice. Vet World. 2008; 1(3): 69-70
13. Luna, L. G. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill, New York. 1968: 4–8.
14. 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
15. Cruciani, D., Crotti, S., Maresca, C., et al. Preliminary Investigation about *Aspergillus* spp. Spread in Umbrian Avian Farms. J Fungi (Basel). 2022; 8 (11): 1213. doi: 10.3390/jof8111213.
16. Ostović, M., Ravić, I., Kovačić, M., et al. Differences in fungal contamination of broiler litter between summer and winter fattening periods. Arh Hig Rada Toksikol. 2021; 28; 72 (3):140-147. doi: 10.2478/aiht-2021-72-3508.
17. Centers for Disease Control and Prevention. *Fusarium* keratitis - Multiple States. Morb Mortal Wkly Rep. 2006: 55 (14): 400-401
18. Singh, D. Fungal keratitis. Medscape. 2015. http://emedicine.medscape.com/article/1194167-overview
19. Liesegang, T. J., and Forster, R. K. Spectrum of microbial keratitis in South Florida. Am J Ophthalmol. 1980: 90:38–47
20. Balajee, S. A., Kano, R., John, W., B., et al. Molecular identification of *Aspergillus* Species collected for the transplant-associated infection surveillance network. J Clin Microbiol. 2009; 47 (10): 3138-3141 doi: 10.1128/JCM.01070-09
21. Ribes, J, A., Vanover-Sams, C. L., and Baker, D. J. Zygomycetes in human disease. Clin Microbiol Rev. 2000; 13 (2): 236-301. doi: 10.1128/CMR.13.2.236
22. Richard, K. F., Gerbert, R., and Louis, A. W. Dematiaceous fungal keratitis clinical isolates and management. Br J Ophthalmol. 1975; 59: 372-376. doi: 10.1136/bjo.59.7.372
23. Mikami, B. S., and Stemmermann, G. N. Keratomycosis caused by *Fusarium oxysporum.* Am J Clin Pathol. 1958; 29: 257-262 doi: 10.1093/ajcp/29.3.257.
24. Kirk, R., Wilhelmus, M. D., and Dan, B. J. *Curvularia* keratitis. Trans Am Ophthalmol Soc. 2001; 99: 111-132
25. Zhong, W., Hongmei, Y., and Lixin, X. Expression and potential role of major inflammatory cytokines in experimental keratomycosis. Molecular Vision. 2009; 15:1303-1311
26. Darlene, A., Dartt, R. D., and Patricia, D. Immunology, inflammation and disease of the eye. 2011. https://books.google.com/books?isbn=012381975X.
27. Walther, G., Zimmermann, A., Theuersbacher, J., et al. Eye Infections Caused by Filamentous Fungi: and Antifungal Susceptibility of the Prevailing Agents in Germany. J Fungi (Basel). 2021; 26; 7 (7): 511. doi: 10.3390/jof7070511.