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Prevalence of Pulmonary Mucormycosis among Poultry Farmers in Barkin Ladi Local Government Area of Plateau State

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Abstract*— This research titled Incidence of Pulmonary Mucormycosis among Poultry Farmers in BarkinLadi Local Government Area of Plateau State was aimed at determining the number of Poultry Farmers who are infected with pulmonary mucormycosis. Sputum from 100 Poultry Farmers was investigated by cultivation on Sabouraud's Dextrose Agar containing antibiotics and examined for fungi. Fungal identification was carried out by direct microscopy and colony morphology using the Papanicolau stain, standard mycological stains and reagents. Consent was obtained from the participants and questionnaires were used to obtain their Bio-data. Statistical Packages for the Social Sciences (SPSS) version-25 software was used for Chi-Square and t-test analyses of the research findings. A total of 6 (6.0%) persons were infected with Mucor, other fungi were isolated in 14 (28%) of the farmers. Abnormal squamous epithelial cells were seen in 24 (48%) and inflammatory cells in 11 (22%) of farmers. Mucor was not isolated from the sputum of Persons with mean duration in years of poultry farming activities of 2.5 years and 6.5 years while those with 18.5 and 22.5 years had 2 (%) rate of infection each. Infection with mucor in relation to years of poultry farming was statistically significant, $x2(5) = 16.31$, $p \leq 0.05$. There was however no significant difference in abnormal substances found in the sputum of males (M = 4.61, SD = 2.05) and the sputum obtained from females (M = 4.33, SD = 1.83); t (109) = 0.742, p = 0.460. There is need for farmers to regularly use face mask to avoid inhalation of dust. Environmental sanitation should be regularly carried out so as to reduce wastes which serve as breeding ground for fungi. There is also the need for Farmers to also engage in routine medical checkups.

***Index Terms*— Pulmonary, Mucormycosis, Fungi, Poultry, Environment, Sputum, Farmers.**

I. INTRODUCTION

Mucormycosis is the term used to describe fungal infections caused by fungi of the order mucorales which include Rhizopus and Mucor (James & Berger, 2006). It is a fatal, opportunist condition with acute infectious and rapid progress nature. Although this aggressive fungal infection is rare, it is common among patients whose immune system of their body has been disturbed for different reasons. During recent decades, a high percent of infected patients by this infection has been observed significantly among diabetic patients, malnutrition children, patients with severe burning, people affected by hematologic malignancy and during therapy with immunosuppressive, cytotoxins, corticosteroids or after surgery and trauma (Noorifard*et al*., 2015). Though widely spread in nature especially in soil and decaying matter, these fungi rarely cause human disease, because the human immune system is effective at eliminating them (Yamin*et al*., 2017). Poultry is the category of domesticated birds that people keep for the purpose of collecting their eggs or killing for meat and or feathers, the term also refers to the flesh of such birds (Poultry guide, 2009).

According to the International Agency for Research on Cancer (IARC), fine particles of poultry feed mill dust when inhaled into nasal passages or lungs could lead to allergy and also cause cancer (IARC, 2000). Contact with decaying plants, moldy environments and other organic matter places persons at high risk of contacting various forms of respiratory disorders including mucormycosis (Centre for Disease Control, 2008).

In debilitated individuals**,** it often begins at the upper respiratory tract or lungs from which the mycelial growths metastasis to other organs (Dorlands Medical Dictionary for Health Consumers, 2007).

Mucoraceae are found worldwide and are responsible for initiating and decaying most organic material in the environment. Most fungi are identified by their unique morphological appearance usually viewed microscopically.

II. AIM OF THE STUDY

The aim of the study is to determine the prevalence of pulmonary mucormycosis among Poultry Farmers in BarkinLadi Local Government Area of Plateau State

Objectives

The Objectives of this work are:

i. To determine prevalence of pulmonary mucormycosis among Poultry Farmers.
ii. To determine the relationship between duration of exposure and possible infection.

III. HYPOTHESIS

$H0_1$: There is no relationship between duration of poultry farming to possible infection with pathological fungi

$H0_2$: There is no significant difference in the distribution of abnormal substances in sputum of males and females

Signs and Symptoms of Pulmonary Mucormycosis

Most patients with the pulmonary form of the disease are being treated for leukemia. The fungus enters the patient's

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lungs where it eventually invades a major blood artery or vessel causing the patient to cough up blood or hemorrhage into the lungs. Early symptoms include fever, difficulty in breathing with eventual bleeding from the lungs (Wolf, Gill &Leides, 2004). It is also the second most common form of mucormycosis next only to rhino cerebral disease and accounts for more than 30% of the disease (Spellberg, Edwards & Ibrahim, 2005). Its clinical presentation in the lungs is defined as being acute if symptoms are present for less than 30 days, where it is usually localized. It is found among a wide age range but with male predominance. Invasion of the blood vessel by the fungi hyphae results in necrosis of tissue parenchyma which may ultimately lead to cavitation and hemoptysis. Most common predisposing conditions in mucormycosis are uncontrolled diabetes mellitus, malignancy, chronic illness and transplant (Marr, Carter, Faripa& Corey, 2002). Fordiagnosis of the infectionswabs of tissue and discharge could be used. A biopsy specimen of the tissue could also be done.

If mucormycosis is suspected, prompt amphotericin B therapy is administered. Posaconazole has been shown to be effective against mucormycosis. After administration of either amphotericin B or Posaconazole, surgical removal of the "fungus ball" is indicated. The disease must be monitored carefully for any signs of reemergence (Rebecca & Frey, 2011).

Prognosis

In most cases, the prognosis of rnucormycosis is poor and has varied mortality rates depending on it's form and severity. In the rhino cerebral form, the mortality rate is between 30% and 70%, whereas the disseminated mucormycosis presents with the highest rate of mortality rate of up to 90% (Spellberg*et al.*, 2005). Patients with AIDS have a mortality rate of almost 100% (Rebecca, 2011). Possible complications of mucormycosis include the partial loss of neurological functions, blindness and clotting of brain or lung vessels (Medline Plus Encyclopedia, 2011).

Epidemiology

Pulmonary mucormycosis is a very rare infection and as such it is hard to note histories of patients and incidence of infection (Nancy and Crum-cionflone, 2011). The disease affects both sex and is somewhat more common in men than in women, it may develop in any age group including the newborn.

Prevention

Prevention depends on protecting high-risk persons from contacts with sugary foods, decaying plants, moldy bread and other breeding grounds for fungi. In addition, healthcare professionals treating hospital inpatients should be careful to change dressings frequently and check the underlying skin for signs of possible fungal infection (Centre for Disease Control, 2008).

IV. MATERIALS AND METHODS

Sample Size

A total of 100 sputum samples were collected for this work from Poultry farmers. The sample size was calculated using the formula according to Daniel, (1999).

$$n = \frac{p\,(1-P)\,Z^2}{d^2}$$

Where n = sample size,

Z = statistic for a level of confidence (1.96 at 95% level of confidence).

P = expected prevalence or proportion based on a previous study at 7.2% (Aliyu, Olajevwo&Iweriebor, 2010)

d = precision (6%)

Minimum sample is 72 but 100 samples were collected

Sample Collection

Consent was formally obtained from the participants and questionnaires used to obtain theirBiodata. All sputum samples were collected into sterile screw caped plastic containers which were given out to participants a day prior to sample collection. Each sputum sample collected was shared into two portions; the first portion was cultured on Sabouraud Dextrose Agar (SDA) while the second portion was transferred into another plastic container having 2mls of 70% alcohol, these were transported to the Microbiology Laboratory of the Plateau State Polytechnic. Statistical Packages for the Social Sciences (SPSS) version-25 software was used for Chi-Square and t-test analyses of the research findings.

Cultural Studies

Sterile Sabouraud Dextrose Agar (SDA) was prepared (according to manufacturers instruction), sterilized by autoclaving at 121psi for 15mins, allowed to cool and while in molten form, modified by addition of 0.4g of chloramphenicol, 20,000 units of penicillin and 4,000 units of streptomycin sulphate each added to a litre of molten agar which was then dispensed into sterile Petri dishes, allowed to set and stored in the refrigerator. With the aid of a sterile wire loop, the sputum samples were inoculated on the SDA and streaked out to obtain discrete colonies. These plates were then incubated at both room temperature and at 37^{o}C in the incubator to aid growth of dimorphic fungi i.e. those that grow as yeasts (parasitic phase) at 37^{o}C and as moulds (saprophytic phase) at room temperature. Cultures were observed daily for growth for 2 weeks. Gross colonial morphology, colour and texture of the colony surface and underside which can be very distinctive were observed.

Direct Microscopy

A drop of sodium hydroxide (20% w/v) solution was placed on a slide. An aliquot of each sputum sample was transferred to the drop of sodium hydroxide on the side and this was covered with a glass cover slip. This was then allowed to stay for 5-10 mins to enable it to clear, after which it was examined microscopically using X40 objective. Characteristic morphological elements such as nature and form of microconidia, macrocondia, spherule, fruiting bodies, sporangium, encapsulated cells, budding cells and broad based cyst, spherical fish like yeast cells were observed. Direct microscopy of culture isolates was also done. A drop of lactophenol cotton blue was placed on grease free slide and a fraction of culture was transferred to drop of lactophenol cotton blue on the slide and covered with a glass cover slip.

This was then examined microscopically using the X10 and X40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast. Structures such as

spherules, microconidia, pseudohyphae, encapsulated cells, sporangia were noted.

GermTube Test

Human Serum (0.5ml) was pipetted into a small test tube. Using a sterile wire loop, the serum was inoculated with a yeast colony from the culture. The tube was placed in an incubator at 37^{o}C for 2-3 hrs. With the aid of a Pasteur pipette, a drop of the serum yeast culture was transferred to a glass slide and covered with a cover slip. This was then examined using the X10 and X40 objectives.

Formation of Pseudomycelia and Chlamydospores

Certain yeasts develop special fruiting forms (called chlamydospores) when grown in glucose free medium like Rice Agar. Isolates identified as yeasts were inoculated onto prepared sterile rice agar plates and incubated at room temperature for 24-48 hours and observed for pseudomycelia and chlamydospore development.

To the second portions of sputum, equal volumes of 8% Hydrochloric acid was added to each sample and allowed to stand overnight. They were centrifuged at 5000 rpm for 5 minutes after which the supernatant was discarded. For each sample 2 smears were made on clean grease-free albuminized glass slides which were then placed in 70% alcohol while still wet. The smears were stained using the Papanicolaou staining technique according to Carleton, in Drury, Wallington &Camerron, (1967). These were then microscopically examined using x10 and x40 magnifications.

V. RESULTS

[1] A total of 100 Poultry Farmers were examined for Pulmonary mucormycosis, out of which 6 (6.0%) were infected while 94 (94.0%) were negative for infection with *mucor*.

[2] Table 1 presents the duration of exposure to fungi due to poultry farming activities, 46 (46%) persons had mean farming duration of 2.5 years, while 7 (7%) had mean farming duration of 22.5 years. Persons with the mean duration of 2.5 and 6.5 years showed no infection with mucor. On the contrary persons with mean duration of 10.5 and 14.5 years had 1 (1%) rate of infection each, while those with mean duration of poultry farming of 18.5 and 22.5 years showed infection rates of 2 (2%) each. There was a statistically significant difference between duration of farming activities in years and possible infections with pathologic fungi $x^2(5) = 16.31$, $p \leq 0.05$.

Table 2 presents the cytological findings of test subjects. It showed that the males were more infected with 4 (4%) mucor compared to the females who had 2 (2%) mucor isolated from their sputum. It also showed that 10 (20%) of the males had abnormal squamous epithelial cells compared to the females with 14 (28%) distribution. There was no significant difference in the distribution of abnormal substances in sputum from males (M=4.61, SD=2.05) and the sputum from females (M=4.33, SD=1.85): t (109) = 0.742, p = 0.460.

TABLE 1.0: Infection with Mucor in Relation to Years of Poultry Farming

Duration (Years)	Mean Duration (Years)	Number of Persons Individuals (%)	Infectedwith Mucor (%)	x^2	p-value
1-4	2.5	46 (46)	0(0)	16.31	0.006
5-8	6.5	19 (19)	0 (0)		
9-12	10.5	12 (12)	1 (1)		
13-16	14.5	8 (8)	1 (1)		
17-20	18.5	8 (8)	2 (2)		
21-24	22.5	7 (7)	2 (2)		
TOTAL	**12.5**	**100**	**6 (6)**		

Table 2.0: Cytological Findings of Test Subjects.

Findings	Male(s)	Female(s)	Total	t	p-value
Mucor	4 (4%)	2 (2%)	6(6%)	0.742	0.460
Other fungi hyphae	6 (16%)	8 (12%)	14 (28%)		
Bacteria	6 (12%)	13 (26%)	19 (38%)		
Fungi spores	6 (12%)	9 (18%)	15 (30%)		
ASEC	10 (20%)	14 (28%)	24 (48%)		
Dust cells	12 (24%)	6 (12%)	18 (36%)		
Ferruginous bodies	1 (2%)	3 (6%)	4 (8%)		
Inflammatory cells	6 (12%)	5 (10%)	11 (22%)		

Key:

ASEC => Abnormal Squamous Epithelial Cells

VI. DISCUSSION

The infection rate with mucor showed male predominance. The commonest changes observed was metaplasia which could be due to the inhalation of various dust particles as similarly stated by Milham (2000).

The findings of this study revealed a prevalence rate of 6 (6%) in agreement with outcome of a meta analysis done in India with a 6% prevalence (Chakraborty& Singh, 2014). Oladele and Denning (2014) revealed that 11.2% of Nigerians suffer from various fungal infections each year. In a similar study carried out in Edo State Nigeria, 7.2% of Mucor was isolated from patients (Aliyu*et al.*, 2010). Out of the 100 poultry farmers, 46 (46%) had mean farming duration of 2.5 years, while 7 (7%) had farming duration of 22.5 years. Persons with the mean duration of 2.5 and 6.5 years had no mucor isolated from their sputum. Numerous Abnormal Squamous Epithelial Cells (ASEC) were observed in 14(28%) of the female samples compared to 10 (20%) in males. The presence of these infections could be attributed to the Inhalation of organic dusts which provide good breeding environment for the fungi (Spier, 2005). Ventilation increases susceptibility of the lungs to different types of pulmonary infections as these fungal respiratory infections are important causes of morbidity and mortality among HIV positive individuals (Muhammad et al., 2017). Pulmonary mycoses remains an under diagnosed problem because it is neither given close attention nor investigated like other opportunistic infections such as tuberculosis and HIV/AIDS.

VII. CONCLUSION

Findings from this study revealed a low prevalence of pulmonary mucormycosis among Poultry Farmers; it however also showed a male predominance. There were remarkably obvious metaplastic changes and other mycotic infections, among the poultry farmers. It therefore becomes necessary for poultry and non poultry farmers to take adequate protective measures to guard against mycotic infections of the respiratory tract.

VIII. RECOMMENDATIONS

The following recommendations are hereby made:

i. Poultry farmers should regularly use face masks so as to minimize the inhalation of organic dusts and fungi spores while on the farms.
ii. Regular sanitation of the poultry farms is necessary since various fungi thrive in damp and untidy environments.
iii. Medical checkups should be routinely undertaken, by poultry farmers.
iv. Farmers with HIV/A.I.D.S, diabetes, renal failure, energy malnutrition cirrhosis and conditions which suppress the immune system are at high risk of the disease and should avoid sugary (sweetened) foods and moldy bread, as they can promote the growth of these organisms in the body.
v. Government and non Governmental organizations should be involved in sensitizing farmers with regards to other ways of minimizing health risks due to poultry farming.
vi. A more detailed longitudinal (cohort) study may should be embarked upon on this seemingly rare opportunistic infection

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