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Distribution of *Aspergillus* species among apparently healthy birds in poultry farms in Kaduna state, Nigeria

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ABSTRACT

The study was conducted to determine the prevalence and distribution of *Aspergilllus* species among apparently healthy birds in poultry farms. Trachea swabs from 1500 birds in 52 commercial (10% of birds in each poultry farm visited) poultry farms were collected for this study. Six species of *Aspergillus* were isolated was isolated from 718 (47.87%) of the birds viz: *A. fumigatus* made up 52.37% (376) of the *Aspergillus* isolates followed by *A. flavus* 21.87% (157), *A. niger* 11.42% (82), *A. terreus* 8.64% (62), *A. restrictus* 2.79% (20) and *A. ochraceous* 2.92% (21). *Aspergillus* species was found to occur throughout the year in the farms though with a higher incidence during the rainy season compared to the dry season.

1. Introduction

Fungi species belonging to the genus *Aspergillus* represent one of the most important among the fungi that affect poultry. *Aspergillus* species especially *Aspergillus fumigatus* is responsible for the disease called Aspergillosis in both poultry and humans (Hashempour et al., 2011). Other species of the genus such as *A. flavus, A. niger* and *A. nidulans* have also been reported to cause the disease as well as been responsible for mycotoxicosis (aflatoxicosis) in poultry (Beernaert et al., 2010). The warm and humid environment of the poultry house favors the growth of

Aspergillus spp. *Aspergillus* are ubiquitous and opportunistic pathogens capable of growing on most substrates and producing spores that may contaminates the environment and air of the poultry house. The spores may get inhaled by birds and causing disease especially in immunocompromised birds or birds that inhale a high dose of the spores (Beernaert et al., 2010). The disease represents an important threat to poultry production worldwide causing significant morbidity and mortality in both young and adult birds (Khosravinia et al., 2009). The disease is characterized by non specific clinical signs affecting the respiratory system (dyspeonea, gasping, cyanosis) and also causes weight loss, loss of appetite. Disseminated forms (via the hematogenous routes) of the disease affecting other organs can also occur (Beernaert et al., 2010). The study was therefore carried out to determine the prevalence and distribution of *Aspergillus* species among apparently healthy birds (layers) in light of the threat they may pose to the birds and the poultry attendants.

2. Materials and methods

2.1. Study area

The study area was farms located in Zaria and Kaduna of Kaduna State, Nigeria. Kaduna State is located between latitudes $11^0$32' and $9^0$20'N and longitude 8^o50' and 6^o51'E.the State is positioned in the Northern Guinea Savannah zone of Nigeria. The area is characterized by a cold dry season (November-February), hot-dry season (March-April) (both dry seasons) and the wet/rainy season (May-October) (Ayo *et al.,* 1999). The annual rainfall peaks in the month of August with the average of 146 mm. The average humidity is highest in August with 75.6 mm/Hg and lowest at the months of December- January with 38.2 mm/Hg. The mean temperatures for the zone are 10.7^0C and 38.75^0C minimum and maximum respectively (Agbogu et al., 2006).

2.2. Study population and sampling

The study population comprised of 10% (1,500) of the total population of birds (15,000), in 52 poultry farms visited. These farms were selected based on clinical records of the farms from the Poultry Ambulatory Unit of the Veterinary Teaching Hospital, Ahmadu Bello University Zaria.

A total number of 1,500 chicken tracheal swabs were obtained from apparently healthy layers. In each of the farm visited, trachea swabs were collected randomly from 10% of the chickens. Sampling was conducted all year round (January- December) to observe for seasonal variation in *Aspergillus* species isolation. All the samples were labeled and taken to the laboratory for culture and isolation of fungi.

2.3. Fungal isolation and identification

The fungi isolation was carried out using Sabouraud's Dextrose Agar (SDA) (Oxoid UK) medium, prepared according to the manufacturer's specification (Oxoid UK) with addition of 0.05 mg/ml of chloramphenicol to control bacterial contamination (Ainstworth and Austwick, 1973). Each swab was inoculated on the SDA slant in 10ml universal bottles, labeled, incubated at room temperature and at 37°C with daily observation for a period of 7 days. All the samples that had no fungal growth were discarded after seven days (Ainstworth and Austwick, 1973).

The growths from the cultures were observed for the colonial morphologies i.e. size, color, topography and aerial growth and these characteristics were used to identify the genus and species. Growths were prepared for microscopic observation by staining them on glass slides using lactophenol cotton blue stain. The stained slides were observed under ×10 magnification using a light microscope (Olympus USA). Characteristics of fungi such as hyphae, conidial heads and the arrangement of the conidia where also observed and used for the fungi identification.

3. Results

Aspergillus species were isolated from 718 (47.87%) of the 1500 apparently healthy birds sampled. Six *Aspergillus* isolates were identified with *A. fumigatus* presenting as the major species isolated 376 (52.37%) followed by *A. flavus* 157 (21.87%), *A. niger* 82 (11.42%), *A. terreus* 62 (8.64%), *A. restrictus* 20 (2.79%) and *A. ochraceous* 21 (2.92%) (Table 1).

Aspergillus species was also isolated both in the rainy and dry season. *A. fumigatus* had the highest rate of occurrence/isolation in both the rainy 262 (59.95%) and dry seasons 114 (40.57%). This is followed by *A. flavus* 72 (16.48%) and 85 (30.25%) and *A. niger* 38 (8.70%) and 44 (15.66%) in the rainy and dry seasons respectively (Table 2).

Table 1
Distribution of *Aspergillus* species isolated from apparently healthy chickens in 52 poultry farms across Kaduna State, Nigeria.

***Aspergillus* species**	**No. of isolates (%)**
A. fumigates	376 (52.37)
A. flavus	157 (21.87)
A. niger	82 (11.42)
A. terreus	62 (8.64)
A. restrictus	20 (2.79)
A. ochraceous	21 (2.92)
Total	718

Table 2
Distribution of *Aspergillus* species isolated from apparently healthy chickens during the rainy and dry seasons in 52 poultry farms across Kaduna State, Nigeria.

***Aspergillus* species**	**No of isolates (%)**	
	Rainy (May – October)	**Dry Season (November – April)**
A. fumigatus	262 (59.95)	114 (40.57)
A. flavus	72 (16.48)	85 (30.25)
A. niger	38 (8.70)	44 (15.66)
A. terreus	29 (6.64)	33 (11.74)
A. restrictus	15 (3.43)	5 (1.78)
A. ochraceous	21 (4.84)	0
Total	437	281

4. Discussion

The results of this study have shown that aspergillus species are present among apparently healthy birds. This species of fungi has been reported to be one of the most common fungal infections of poultry (Greenacre et al., 1992). The spores of *Aspergillus* especially *A. fumigatus* has been reported to be highly thermotolerant growing at temperatures between 15°C - 53°C (Greenacre et al., 1992) which is consistent with the average minimum (10.7^0C) and maximum (38.75^0C) temperature for this zone (Agbogu et al., 2006). This may likely be the reason for the presence of the fungi all year round. *A. fumigatus* accounts for about 95% of aspergillus cases in birds followed by *A. flavus* (Deem, 2003). Aspergillus species have been reported to produce toxic metabolites which are responsible for the clinicopathologic manifestations seen in cases of aspergillosis. *A. fumigatus* produces gliotoxin, *A. flavus* produces aflatoxin B1 while *A. niger* produces oxalic acid (Joseph, 2000). The high prevalence of these species is of economic importance as is an indication of possibility of aspergillosis which is associated with a high morbidity and mortality (Abdu *et al.,* 1995). This is the first time of isolating *A. ochraceus* from apparently healthy chickens in this environment this species is highly important in aflatoxicosis and of public health concern. *A. terreus* and *A. niger* though not commonly isolated are pathogens of birds and man (Redig *et al*., 1980). Some of these fungi especially those that produce mycotoxins can contaminate the feed of the birds which they may subsequently consume resulting in mycotoxicosis.

5. Conclusion

The study has shown that *Aspergillus* spp are present among apparently healthy birds with a high prevalence. The presence of these fungi may pose a potential threat to the health of the birds and source of economic lose to the farmer in the event of an outbreak. Fungal infection in poultry is very difficult and expensive to treat with a

guarded prognosis (Joseph, 2000). Therefore, prevention is the most effective way of combating this disease. Proper ventilation and regular cleaning with fungicides especially after removal of fecal or decaying matter is very important. Stressors which have been shown to play an important role in the development of fungal disease should be minimized.

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