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With 1 table and 2 figures in the text

Isolation and Identification of Water-borne Fungi From Poultry Farms in Khartoum State, Sudan

Wisal G. Abdalla

Central Veterinary Research Laboratory P.O.Box 8067, Alamarat, Khartoum, Sudan
Corresponding author: wisalgafar8@yahoo.com

الملخص

جُمعت 60 عينة مياه من صنابير و خزانات و مشارب عشرة مزارع للدجاج في ولاية الخرطوم فى الفترة من مايو الى سبتمبر 2014 في قوارير عينات معقمة. عُزلت 9 أجناس من الفطريات: الرشاشية و البنسليوم و الميوكر و الكلفليريا و الكلادوسبوريم و الرايزوميوكر و أوريوبلازيديوم و الأرترنيريا و إكسيروهايلم من مياه الصنبور و البراميل و المشارب. عُزلت الكريبتوكوكس البيدس و الكريبتوكوكس لورنتاي من البراميل في حين تم عزل المبيضة الروقوسا من المشارب.

Summary

Sixty water samples were collected from ten poultry farms in Khartoum State during May to September 2014. The samples were collected from tap water, tanks and drinkers in sterile sample bottles. Nine genera of moulds were isolated: *Aspergillus, Penicillium, Mucor, Curvularia, Cladosporium, Rhizomucor, Aureobasidium, Alternaria* and *Exserohilum* were isolated from tap water, tanks, and drinkers. *Cryptococcus albidus* and *Cr. Laurentii* from tanks and *Candida rugosa* from drinkers.

Introduction

Hygienic water supply system is one of the most important key factors for good health and growth of poultry. It is vitally important that water should be hygienic all the way until it reaches the birds, and so water and drinkers lines hygiene must be a focus of attention for the farm (Hageskal *et al*., 2009). One of the greatest concerns for water consumers, with respect to the quality of drinking water, is contamination with pathogenic microorganisms. Certain micro-organisms, including bacteria, viruses, and parasites, are well-known as water contaminants, of which several may lead to water-borne diseases and epidemics. Bacteria are probably the most frequently studied group of microorganisms with respect to the quality of drinking water. Pathogenic viruses in water are also of great importance, as viruses are the most common cause of gastrointestinal infections worldwide (Mara and Horan, 2006).

In the past, fungi were infrequently considered when discussing pathogenic microorganisms in water. However, there is a significant range of zoonotic yeasts and moulds which include *Trichophyton*, *Cryptococcus*, and *Coccidiodes* species. They are transmitted by contact, ingestion, or inhalation; credible waterborne routes may at some stage be demonstrated (Hageskal *et al*., 2006). Fungi have been isolated from all types of water, from untreated water to treated water, from heavily polluted water to distilled or ultrapure water and bottled drinking water (Fujikawa *et al*., 1997; Cabral and Fernandez, 2002; Ribeiro *et al*., 2006).

This study was carried out to identify fungi that contaminate water in poultry farms in Khartoum State, Sudan.

Materials and Methods

Sterilization and precaution

Materials (sample bottles, medium containing agar) used in this study were sterilized by autoclaving at 121°C for 15 min. During sample collection taps were washed, flushed several times and allowed to run for 5 min. Sample bottles were then opened and water was quickly collected making sure that the bottles did not touch the taps before, during and after collection.

Collection of samples

Sixty water samples were collected from ten poultry farms from tap water, tanks and drinkers in sterile sample bottles (400ml). Samples were kept in the fridge till usage.

Fungal isolation and identification

Fungi were isolated by plating method. 500 µl of samples were plated were onto Sabouraud's dextrose agar plates (Frankova´ and Horecka, 1995; Go¨ ttlich *et al.,* 2002). Each colony from the primary plates was subcultured onto same fresh meduim. It was further incubated at room temperature for seven days. Fungal colonies were isolated upon formation, stained with lactophenol cotton blue and examined under the microscope. The observed fungi were identified using appropriate taxonomic guides (Watanabe, 1994; Larone, 1995; Doggett, 2000). Yeast species were stained by Gram's stain which was applied on the direct preparation. Identification of the yeast isolate was done by formation of pseudohyphae and chlamydospores (Evans and Richardson, 1989), formation of germ tube (Cheesbrough, 1984), fermentation of sugars (Wickerham, 1951), assimilation test (auxanographic method; Lodder and Kreger-Van Rij, 1952), urease test (Evans and Richardson, 1989) and the results were confirmed by the vitek2-compact.

Results

A summary of mould species isolated in this study is presented in Table 1. *Aspergillus nigar* (20.9%), *A.flavus* (19.4%), *A.terreus* (17.9%), and *Penicillium* spp (11.8%) were the most frequently isolated. *Rhizomucor* spp (3%), *Aureobasidium* spp (3%), *Alternaria* spp (3%) and *Exserohilum* spp (1.5%) were present (1.5%; Fig. 1) in low frequencies. *Curvularia* spp. (Fig. 2) were also isolated.

Aspergillus nigar, *A.flavus* and *A. terreus* were isolated from tap water, tanks and drinkers while the other species of fungi were restricted to tanks and drinkers.

Yeast species isolated from poultry farm are shown in Table 1. *Crypto-coccus albidus* and *Cr. Laurentii* were isolated from tanks while *Candida rugosa* was isolated from drinkers.

Table 1: Mould and yeast species isolated from poultry farms.

Mould	Number	%
Aspergillus nigar	14	20.9
Aspergillus flavus	13	19.4
Aspergillus terreus	12	17.9
Penicillium spp.	8	11.8
Mucor spp.	6	9
Curvularia spp.	4	6
Cladosporium spp.	3	4.5
Rhizomucor spp.	2	3
Aureobasidium spp.	2	3
Alternaria spp.	2	3
Exserohilum sp.	1	1.5
Total	**67**	**100**

Yeast	Number	%
Cryptococcus laurentii	2	40
Cryptococcus albidus	2	40
Candida rugosa	1	20
Total	**5**	**100**

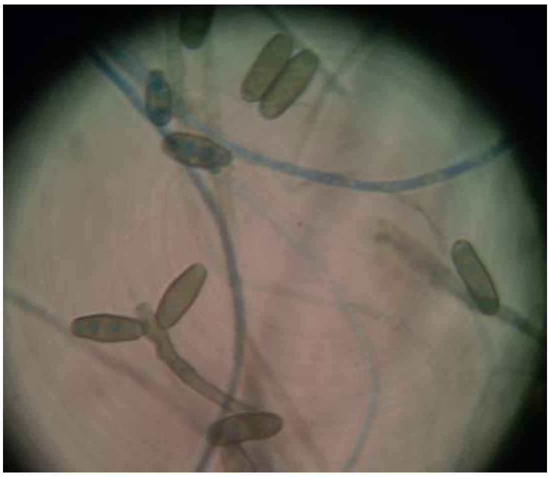

Fig. 1: Conidiophores and conidia of *Exserohilum* spp.

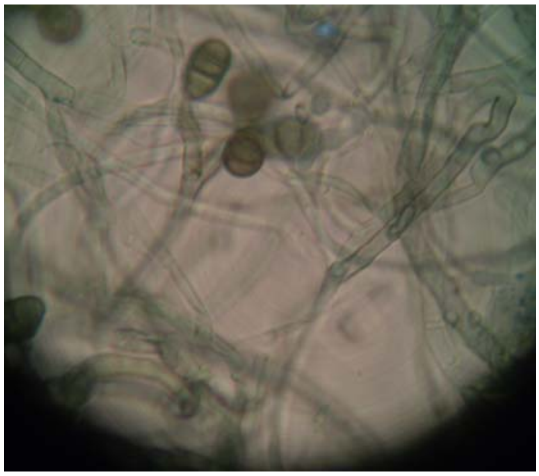

Fig 2.: Conidia of *Curvularia* spp.

Discussion

The isolation of fungi from water has demonstrated a common presence of fungi in water distribution systems of poultry farms. Isolation of *Aspergillus flavus* from tanks in poultry farms was in accordance with Paterson *et al.* (1997) who detected aflatoxins, produced by *A. flavus* in water from a cold water storage tank. In addition, an *in vitro* study concluded that mycotoxins and other metabolites can be produced by fungi in water (Kelley *et al*., 2003). Mycotoxins produced in water will, of course, be extremely diluted, and are perhaps of minor concern. Nevertheless, water is occasionally stored in tanks for prolonged periods without cleaning of these tanks. In such cases, the concentrations of mycotoxins may increase. Large amounts of water are daily consumed and daily intake of even small amounts of mycotoxins over many years may be hazardous to human health.

Isolation of *Aureobasidium* spp. from water in poultry farms is in agreement with Metzger *et al.* (1976) who had indicated that elevated levels of this fungus in water from a home sauna caused hypersensitivity pneumonia. Likewise, *Pencillium* and *Cladosporium* spp. were isolated from Terkos Lake water (Asan *et al.,* 2003), while *Aspergillus*, *Cladosporium* and *Mucor* species were predominant in treated and untreated water (Kinsey and Paterson, 1999).

If the microbiological quality of drinking water is to include fungi, implementing fungal parameters in the water regulations shall be required. Fungi are a difficult group to examine, and fungal water studies require experience and caution. However, this should not mean that fungal contamination of drinking water can be ignored. As fungi may influence water quality in several ways, the mycobiota of drinking water should be considered when the microbiological safety and quality of drinking water are assessed.

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