First record of respiratory mycosis in chicks due to *Aspergillus fumigatus* in Debre Zeit, Ethiopia

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SHORT COMMUNICATION

First record of respiratory mycosis in chicks due to *Aspergillus fumigatus* in Debre Zeit, Ethiopia

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Aspergillosis, principally caused by *Aspergillus fumigatus*, is an important infectious fungal disease of chicks. This paper delineates the first isolation and identification of *A. fumigatus* from the affected lung tissues of chicks in Debre Zeit, Ethiopia. The pathogen was detected in the lung tissues of four chicks by direct microscopy in potassium hydroxide as well as by isolation from clinical materials in pure and luxuriant growth on APRM medium. Epidemiological investigation established the source of infection in the immediate environment of chicks as evidenced by isolation of *A.fumigatus* from litter, feed, and water, besides its demonstration in the air of chick pen. Since *A.fumigatus* is a medically important opportunistic pathogen, the poultry workers with weak immune system, must take precautions to prevent the inhalation of the highly infectious spores of the fungus by applying face mask during work. To the author's best of knowledge, this appears to be the first report of isolation and identification of *A.fumigatus* from the affected lungs of chicks in Debre Zeit, Ethiopia.

Key words: APRM medium, *Aspergillus fumigatus*, Chicks, Ethiopia, feed, litter, Narayan stain

Poultry industries are developing in many countries, including Ethiopia. According to CSA (2015), the poultry population in Ethiopia is estimated to be 56.87 million. Poultry sector has the potential to provide relatively cheap animal protein to the people, create employment, and generate income at time of economic hardship (EARO,2000). Some people keep small number of chicks in the house, and maintain them on kitchen household wastes.

Like mammals, birds are also susceptible to many types of infectious diseases, which carry high morbidity and mortality (Pal,1992; Pal and Lee,1994; Jordan *et al,* 2002; Kahn,2005). Among these diseases, aspergillosis (brooder pneumonia, mycotic pneumonia, pneumomycosis) is one of the highly infectious important respiratory mycoses of chicks and turkey poults, and less frequently of other birds (Pal *et al*,1992; Kunkle and Rimler,1996;Pal, 2007). The disease can occur in sporadic as well as in epidemic form, resulting into high morbidity and mortality (Pal *et al,*2012). It is primarily caused *by A.fumigatus*, though other species such as *A.flavus, A.niger, A.terreus* are also implicated in the etiology of disease (Pal *et al.,*1990; Pal,1991; Pal, 2003; Pal, 2007). The genus *Aspergillus* con-

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tains over 600 species, of which 27 are implicated in the etiology of disease both in humans and animals including birds (Pal *et al,* 2014). *Aspergillus* is a Gram positive, aerobic, filamentous, ubiquitous fungus, which is found in a wide variety of natural substrates such as soil, litter, plant debris, water, and feed (Smith, 1989; Pal, 2007). As clinical signs are not pathognomonic, the laboratory help is pertinent to confirm the diagnosis of aspergillosis (Forbes,1992; Jensen *et al*,1997; Pal, 2003; Pal, 2007; Pal *et al,* 2012).The possible available literature scan did not indicate any information on aspergillosis in chicks in Ethiopia. Hence, the present investigation has been aimed to elucidate the etiologic role of *Aspergillus fumigatus* in respiratory mycosis of chicks in Debre Zeit, Ethiopia. In addition, the efficacy of newly introduced APRM medium for the isolation of *A.fumigatus* from clinical as well as environmental materials is also reported.

In all four moribund/dead chicks about four week old brought by the poultry farmer constituted the material for this investigation. The autopsy was conducted, and the affected lung tissues from all the four chicks were collected aseptically into the sterilized disposable Petri dishes. Six samples comprised of 2 feed, 2 litter, and 2 water were also collected from the chick pen. In addition, four plates of APRM [Anubha Pratibha, Raj and Mahendra, containing 2.0g of dried marigold flowers, 2.0g agar, 50 mg of chloramphenicol and 100 ml of distilled water] medium were also exposed in the pen, where the chicks died. The affected lung tissues were digested in 10% potassium hydroxide for direct microscopic demonstration of the pathogen. A small piece of affected lung was directly implanted on the plates of APRM medium, and incubated at 25^0C for the fungal growth (Dave and Pal, 2015).The new medium contains 2.0 g of dried marigold flowers, 2.0 g of agar, 50 mg of chloramphenicol, and 100 ml of water. The detailed morphological identification of the isolates was done in Narayan stain, which contained 0.5 ml of methylene blue 93% aqueous solution), 6.0 ml of dimethyl sulfoxide and 4.0 ml of glycerine (Pal, 2004).

The poultry farmer narrated that 34 chicks died due to respiratory distress. However, the mycological investigation was carried out only on four moribund/dead chicks. All the autopsied chicks showed pinhead to large size white to yellowish caseous nodules, especially in the lungs, and also on the air sacs. The direct microscopical examination of the affected lungs from four chicks in 10 % potassium hydroxide solution revealed hyaline, thin, branched hyphae morphologically resembling to *Aspergillus*. On APRM medium, smoky grey color colonies grew in abundant and pure form from the affected lungs of all the four chicks. The pathogen was also recovered from the all the samples of feed, litter, and water on APRM medium. In addition, high concentration of *A.fumigatus* in the air of chick pen was demonstrated by recovering the fungus on the Petri dishes of APRM medium. All the isolates when examined in Narayan stain revealed thin branched, septate hyphae with conidiophores and phialids consistent with the description of *A.fumigatus* (Pal, 2007).

Aspergillosis is an economically important fungal disease of chicks as it may cause heavy financial loss to the poultry industry (Pal *et al*, 2012). The isolation and identification of *A.fumigatus* from the lungs of affected chicks by direct microscopy and cultural isolation conclusively proved the etiology of this thermotolrant fungus in the respiratory mycosis of chicks in Debre Zeit, Ethiopia. The epidemiological investigation established that chicks acquired infection from their immediate environment as revealed by high concentration of *A.fumigatus* in litter, feed, water and air. Litter is usually the source of *Aspergillus* infection to birds (Dyar *et al*,1984; Pal *et al*,2012).It is important to mention that an increase concentration of fungal spores in the environment may predispose a bird to aspergillosis. Furthermore, a warm environment, humidity, ill ventilation, poor sanitation, and long storage of feed may increase the amount of spores in the air (Beernaert *et al,* 2010).This preliminary investigation suggests that comprehensive studies on the causative role of *A.fumigatus* in various clinical disorders of avians, and mammals should be undertaken in Ethiopia. Aspergillosis should be differentiated from other avian diseases such as zygomycosis, dictalariosis, colibacillosis, mycobacteriosis, New Castle disease, infectious larayngotracheitis, and infectious bronchitis (Pal, 2007; Pal *et al*, 2012). As far as could be ascertained, this seems to be first record of mycologically confirmed record of pulmonary mycosis due to *A.fumigatus* in chicks in Debre Zeit, Ethiopia. The recovery of *A.fumigatus* from clinical and environmental s amples on APRM medium emphasize its usefulness as a less expensive medium for the primary isolation of fungi, particularly in the

laboratory of poor resource countries who can hardly afford expensive media for the study of fungi.

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