Detection of Fungi in Faecal Samples of Domestic Chicken (*Gallus gallus domesticus*) in Selected Poultry Farm

By

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DETECTION OF FUNGI IN FAECAL SAMPLES OF DOMESTIC CHICKEN (*GALLUS GALLUS DOMESTICUS*) IN SELECTED POULTRY FARM

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT55204 – Research Project.

ABSTRACT : Domestic chickens may act as carriers of human pathogenic fungi, however, there is limited information on the occurrence of fungal genera and species in domestic chicken poultry farms in Malaysia. This knowledge gap hinders the development of targeted interventions and preventive measures to mitigate the spread of the disease. Therefore, this study aims to determine the common fungi presence in faecal samples of domestic chickens. A total of 50 faecal samples were collected and cultured on Sabouraud Dextrose Agar for up to 14 days. Fungi and yeast were identified by colony morphology and Lactophenol Blue staining. Different fungal genera were identified including *Aspergillus* spp. (19 out of 50), *Syncephalastrum* spp. (13 out of 50), *Penicillium* spp. (11 out of 50) and remaining seven samples are yeast. The results from the present study will provide fungi background genera in poultry faecal samples and will play crucial role in mitigating potential spread of disease
***Keywords**: Fungal, Poultry, Aspergillus, Penicillium, Syncephalastrum*

ABSTRAK : Ayam ternakan boleh bertindak sebagai pembawa kulat patogen manusia, walau bagaimanapun, terdapat maklumat yang terhad mengenai genera dan spesies kulat dalam ternakan ayam di Malaysia. Jurang pengetahuan ini menghalang pembangunan intervensi yang disasarkan dan langkah pencegahan untuk mengurangkan penyebaran penyakit. Kajian ini bertujuan untuk mengetahui latar belakang genera kulat dalam sampel najis ayam ternakan. Sebanyak 50 sampel najis telah dikultur pada Sabouraud Dextrose Agar sehingga 14 hari. Kulat dan yis dikenalpasti berdasarkan morfologi koloni dan pewarnaan Laktofenol biru. Beberapa kulat yang berbeza telah dikenal pasti termasuk *Aspergillus* spp. (19 daripada 50), *Syncephalastrum* spp. (13 daripada 50), *Penicillium* spp. (11 daripada 50) dan sampel yang tinggal adalah kulat dengan genera yang tidak diketahui. Hasil daripada kajian ini akan menyediakan latar belakang kulat biasa dalam sampel najis ayam dan akan memainkan peranan penting dalam mengurangkan potensi penyebaran penyakit.
***Kata kunci:** Kulat, Ayam, Aspergillus, Penicillium, Syncephalastrum*

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LIST OF ABBREVIATIONS

SDA Saboraud Dextrose Agar

PDA Potato Dextrose Agar

LPCB Lactophenol cotton blue

LIST OF SYMBOLS

% Percentage

°C Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Background

Chicken, scientifically known as *Gallus gallus domesticus* is a domesticated fowl, within the subspecies of the Red Jungle fowl (*Gallus gallus)*. It is one of the most populated and commonly domesticated birds. In 2003 the total population of birds was more than 24 billion worldwide and out of this population, chickens were the majority species of birds (Kemoi *et al*., 2012). All varieties of poultry birds including domestic chickens are susceptible to fungal infections, though they are less common than bacterial and viral infections. Fungi are eukaryotic organisms that consist of yeast and moulds. Aspergillosis, candidiasis, dactylariosis, mucormycosis, favus, rhodotorulosis, torulopsis, histoplasmosis and cryptococcosis are among the fungal disease that can infect poultry. According to Dhama *et al.* (2013) the first two diseases, candidasis and aspergillosis have a significant impact on poultry farming, whereas the last two, histoplasmosis and cryptococcosis, have some zoonotic significance. Domestic chicken's fungal flora served as a representative sample of potential human infection sources (Al-Temimay & Hasan, 2016). Thus, poultry farms, specifically those in Malaysia, may serve as potential reservoirs for the pathogenic fungi leading to the dissemination of the pathogen and potential transmission to humans. Understanding the fungi background genera in poultry faecal samples from Malaysian poultry farms is crucial for the preparation of effective disease control and prevention.

1.2 Problem Statement

Fungal infection poses a significant public health concern, and domestic chickens may play a role in the transmission of zoonotic fungal species to humans. However, there is limited information on the common fungi that can be found in domestic chickens in Malaysia. This knowledge gap hinders the development of targeted interventions and preventive measures to mitigate the spread of the disease.

1.3 Research Questions

What are the fungal genera in faecal samples obtained from domestic chicken in Malaysia?

1.4 Hypothesis

Different fungal genera can be detected in the faecal samples obtained from domestic chicken through fungal culture.

1.5 Objectives

To determine the fungal genera in faecal samples collected from domestic chicken in selected farms in Bachok and Pasir Mas, Kelantan.

CHAPTER 2

LITERATURE REVIEW

2.1 Prevalence of Fungi in Poultry

Numerous studies have reported on the frequency of fungi in faecal samples from domestic chicken. *Aspergillus* and *Penicillium* are two of the most prevalent genera found in the ceca of commercial poultry (Byrd *et al.*, 2017). According to Mohammed and Abdel-Latef (2021), *Aspergillus*, *Candida*, and *Penicillium* were the genera that could be identified from the chicken faecal sample. Conversely, in a 2016 study conducted by Al-Temimay & Hasan, *Candida* spp. and *Aspergillus* spp. are the fungi that are most frequently isolated from chicken faeces. *Rhodotorula* spp., *Mucor* spp., and *Penicillium* spp. also were isolated in the same study. These results show that the faecal samples of domestic chickens contained a wide range of fungal species, which could have an impact on the health of the birds and the safety of poultry products.

2.2 Laboratory Cultivation of Fungal Samples

Accurate detection of fungal infections relies on effective laboratory cultivation methods. One of the conventional and economic cultivation techniques is through Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) culture. The PDA is composed of potato infusion and dextrose, while SDA is composed of peptone, dextrose, and agar. Peptone provides carbon, nitrogen, and micronutrients, while potato infusion provides a rich source of nutrients for fungal growth (Chang *et al*., 1999). The growth of fungi in PDA and SDA can vary, but in general, most fungi that grow on SDA also grow on PDA (Chang *et al*., 1999). The SDA is often considered more selective for fungi than PDA due to its relatively low pH, which inhibits

the growth of bacteria and favours the growth of fungi (Black, 2020). The SDA was introduced by Raymond Sabouraud in the late 1800s and remains the medium of choice for fungi isolation (Odds, 1991). It acts as selective media for fungal culture and mainly used for the isolation of various pathogenic and non-pathogenic fungi. SDA has a low pH (5.6), which is advantageous for fungi and helps in their identification. Because of its acidic properties, the medium is selective for fungi and helps prevent the growth of bacteria (Smithee *et al*., 2014). In contrast to the low pH which prevents bacterial growth, the mycological peptone and dextrose in SDA give fungi a rich supply of nutrition and energy for their growth. For the isolation and cultivation of a broad variety of fungi, including those that need lengthy incubation times, SDA are often used (Hare, 2008).

2.3 Impact of Fungal Infection on Domestic Chicken and Public Health.

Fungal infections can have significant negative effects on poultry health and welfare. Aspergillosis in poultry can lead to respiratory issues, reduced weight gain, and high mortality rates in affected young animals (Lorenzoni, 2023). Certain pathogenic fungi found in poultry have also been known to be transmissible to humans, which are concerning for public health. Among the fungi that can infect poultry, aspergillosis and candidiasis have great importance and impact in poultry farming while histoplasmosis and cryptococcosis, are fungi that have some zoonotic significance (Dhama *et al.*, 2013). Al-Temimay and Hasan (2016) state the two circumstances that pose the greatest risk to human health are direct contact with infectious organisms or inhalation of fungal spores. These organisms grow in nutrient-rich bird dropping accumulations and easily disperse their spores through wind, making them airborne and capable of infecting humans. This highlights the importance of monitoring and controlling fungal infections in poultry farms for production and public health.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Area

This research will be carried out in Kelantan, Malaysia.

3.2 Study Design

The study design selected for this study is cross-sectional study.

3.3 Source Population

The source population in this study is the avian species in selected poultry farms in Kelantan, Malaysia

3.4 Study Population

All species of broiler chickens in selected poultry farms in Kelantan, Malaysia

3.5 Selection Criteria

3.4.1 Inclusion Criteria

All species of broiler chickens in selected poultry farms in Kelantan, Malaysia

3.6 Sample Collection

All samples that will be used are available archived samples

3.7 Data Collection Tools

1. Cloacal swab samples (n= 50)
2. Sabouraud Dextrose Agar (SDA)
3. Incubator
4. Glass slide

5. Lactophenol cotton blue stain
6. Light compound microscope

3.8 Data Collection

A total of 50 samples of cloacal swabs were collected from domestic chicken from local poultry farms in Kelantan.

Table 1. Number of samples collected from local poultry farms in Kelantan.

Poultry Farm	No. of Samples
PROKA Farm, Telong, Bachok	20
UMK Teaching Farm, Bachok	16
Shurim's Farm, Salor Pasir Mas	14

All samples were inoculated onto SDA and incubated at 36°C for up to 14 days. The growth on the SDA media were observed daily and recorded. Slide for microscopic examination was prepared via tape preparation method with lactophenol cotton blue used as staining agent. All details of macroscopic and microscopic features of the fungi isolates were tabulated. Identification of fungal isolates was done based on colony morphology and microscopy.

CHAPTER 4

FINDINGS AND DISCUSSION

4.1 Fungal Identification

In this study, *Aspergillus* spp. was the most frequently isolated (38%), followed by *Syncephalastrum* spp. (26%) *Penicillium* spp. (22%), with the remaining percentage are unidentified yeast-like colonies (14%).

Figure 1 shows the colony morphology and microscopic characteristics for *Aspergillus* spp. The colony morphology of *Aspergillus* spp. is white fluffy cottony growth, became bluish green in colour. Conidiophores are moderate in length, have 'foot cell' at base. Fruiting head is biseriate, dome shaped vesicle has metulae that bear phialides from which chains of globose conidia borne for the microscopic characteristic.

Figure 2 shows the colony morphology and microscopic characteristics for *Syncephalastrum* spp. The colony morphology of *Syncephalastrum* spp. is fast growing, white cottony growth that became greyish with age. Short branched sporangiophore that have swollen tips bearing chain of spore enclosed in tubular sporangia for the microscopic characteristic.

Figure 3 shows the colony morphology and microscopic characteristic for *Penicillium* spp.

The colony morphology of *Penicillium* spp. is whitish velvety growth. Brush-like arrangement of fruiting head, conidiophores have secondary branches (metulae) bearing whorls of phialides from which rough conidia are borne. For the unidentified yeast-like colony, the colony morphology shows whitish umbonate colony. No clear definite structure was identified for microscopic characteristic.

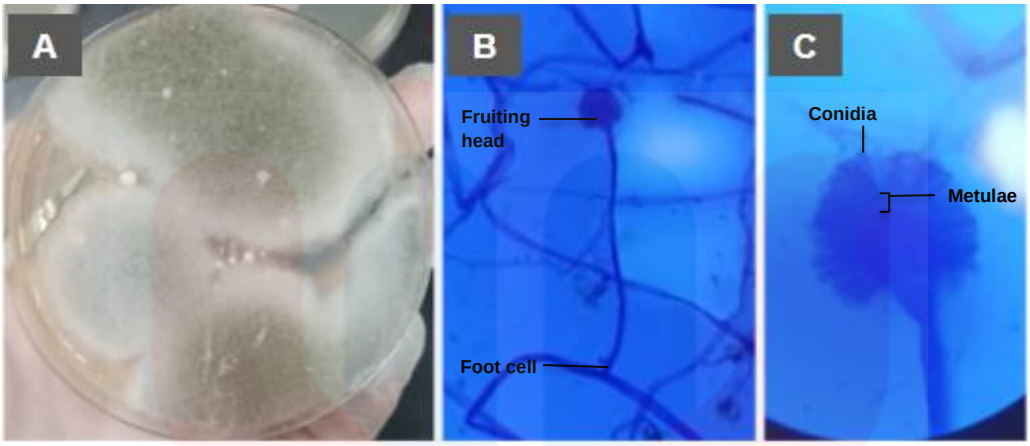

Figure 1. Morphological characteristics of *Aspergillus* spp. **A)** Gross colony morphology of *Aspergillus* spp. on SDA. B**)** and **C)** show microscopic image of *Aspergillus* spp.

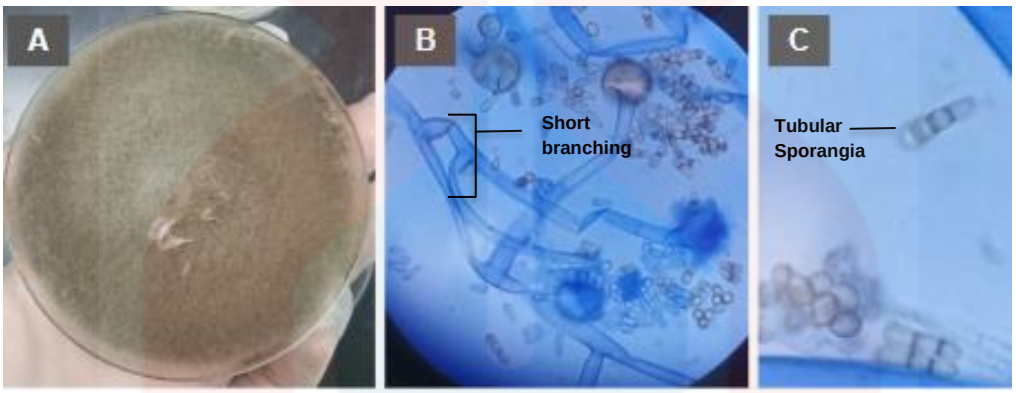

Figure 2. Morphological characteristic of *Syncephalastrum* spp. **A)** Gross colony morphology of *Syncephalastrum* spp. on SDA. **B)** and **C)** show microscopic images of *Syncephalastrum* spp.

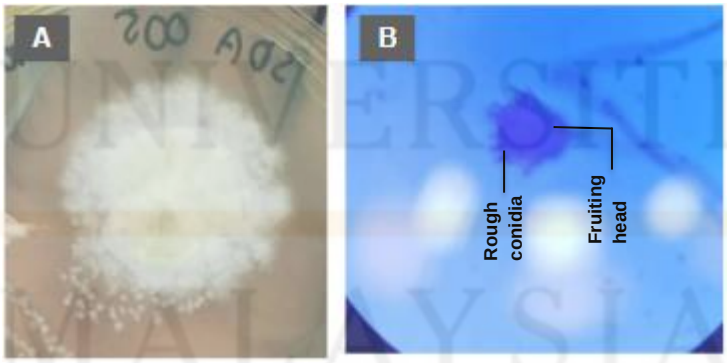

Figure 3. Morphological characteristic of *Penicillium* spp. **A)** Gross colony morphology of *Penicillium* spp. on SDA. **B)** Microscopic images of *Penicillium* spp.

4.2 Discussion

In this study, *Aspergillus*, *Syncephalastrum*, *Penicillium* were isolated with the highest percentage of fungi isolates was *Aspergillus* spp (38%). *Aspergillus*, *Penicillium*, and *Syncephalastrum* are commonly found in domestic chicken fecal samples due to their ubiquitous nature. *Aspergillus* is a widespread fungus found in various environments, including soil and organic matter and often the most prevalent due to its high ubiquity and pathogenic potential (Girma *et al.*, 2016). It can easily spread throughout poultry farms due to its ability to grow on a wide range of substrates, including feed, litter, and feathers (Arné *et al.*, 2011). The result of this study is consistent with Byrd *et al.* (2017), in which they found 88 different fungal isolates, including *Aspergillus* spp., *Penicillium* spp. and *Syncephalastrum* spp., with *Aspergillus* was the most frequently isolated (22.5%), followed by *Penicillium* (19.08%). Additionally, research on the mycoflora of chicken hatcheries reported the presence of *Aspergillus* and *Penicillium* species (Seifi *et al.*, 2018). These findings support the presence of *Aspergillus*, *Penicillium*, and *Syncephalastrum* in poultry-related environments, emphasizing the need for targeted interventions to mitigate the potential spread of disease.

According to Leach and Cowen (2013), the ability to grow at high temperature are essential for pathogenesis of fungi. Quinn et al. (1994) state that high-temperature will inhibit growth of many non-pathogenic fungi. Thus, the ability of some fungus such as *Aspergillus* spp. to grow in high temperature signifying it ability to invade animal tissue and causing disease. Aspergillus is a genus of fungi that includes several species that can cause serious diseases in humans and animals. According to Byrd et al. (2017) there are over 167 species of *Aspergillus* which 16 species have been found to cause disease. *Aspergillus. fumigatus* is the primary causative agent of human infections, followed by *A. flavus, A. terreus, A. niger, and A. nidulans* (Dagenais & Keller, 2009). The most common pathogenic species are *A. fumigatus and A. flavus*, which

produce aflatoxin, a toxin and carcinogen that can contaminate foods such as nuts (Dagenais & Keller, 2009).

Although the results of the current study were in accordance with other studies, fungal identification through morphological characteristics on SDA and microscopic examination has some challenges and limitations. This includes the presumptive nature of the method itself, which required clinical signs and history to support the preliminary diagnosis. In line with Hussain *et al.,* (2019), the process of identifying fungi based on their morphological traits on SDA is frequently a presumptive identification since certain fungal species might be difficult to differentiate from one another. Another limitation in this study is, although microscopic examination can yield more comprehensive insight into fungal morphology, it may not always be possible to observe all relevant structures in a given sample (Ehgartner *et al*., 2017). Additionally, interpretation of microscopic images can be subjective and dependent on the experience and expertise of the observer. This was demonstrated by the fact that seven of the fifty fungal isolates in this study were categorised as unidentified yeast because they only produced a typical yeast-like colony on SDA, but no distinct or identifiable yeast or other fungal structure could be seen during microscopy, which may also be contributed by structural damage during sampling (Gu *et al*., 2022). Molecular identification technique, on the other hand, can be used to get around this restriction. Molecular methods are recommended as a confirmatory diagnosis for fungal identification due to their reliability and accuracy (Tsui *et al*., 2011). These techniques, which go beyond the constraints of morphological identification, include sequence-based identification and DNA barcoding, which make it possible to accurately identify the genus and species of fungi (Raja *et al.*, 2017).

CHAPTER 5

CONCLUSION

In summary, *Aspergillus* spp., *Syncephalastrum* spp., and *Penicillium* spp. were isolated in the faecal samples that were obtained from poultry farms and the most common fungi to be isolated are *Aspergillus* spp. The background information on fungal genera derived from faecal samples is essential for preventing the possible spread of illness. To achieve a more accurate and precise identification down to the species level, however, more sophisticated molecular identification techniques can be done.

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