Occurrence of Aspergillosis Infection Among Poultry in Northern Region of Malaysia from the Year 2014-2018

Azima Laili, H*[1], Humairak, S.[2], Mohd Azizul, O.[1], Zakirah, S.[2], Saira Banu, M.R.[2] and Mastura, Y.[3]

[1]Veterinary Research Institute, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak.
[2]Regional Veterinary Laboratory Bukit Tengah 14007 Bukit Mertajam, Penang.
[3]Regional Veterinary Laboratory Kota Bharu, Kubang Kerian, 16150 Kota Bharu, Kelantan

*Corresponding author: azimalaili@gmail.com

Received: 09 December 2021
Accepted: 04 January 2022

Abstract

Aspergillosis is an assertive fungal infection spread through the air by a common mold known as Aspergillus sp. The disease develops when the host's immune system is impaired or when birds are revealed to have an excessive amount of spores through the inbreathing of conidia or spores from adulterated feed, faeces or soil. This study was conducted to determine positive cases of aspergillosis infection among poultry in the Northern Region of Malaysia based on the poultry cases received by Regional Veterinary Laboratory Bukit Tengah (RVL-Bukit Tengah), Penang from 2014 to 2018. Over the 5 years, a total of 1,268 samples of sick poultry were reported under Bacteriology Section with 123 cases were reported positive with aspergillosis. Although these cases were accounted for only 9.7% of the total cases reported, the analysis is deemed important since information is scarce concerning the occurrence of aspergillosis among poultry especially in the Northern Region of Malaysia. Analysis of these data revealed that 58.5% of the poultry were infected with *A. niger* followed by *A. flavus* (24.4%) and *A. fumigatus* (17.1%). This study showed that aspergillosis due to *A. niger* is prevalent among the poultry in Northern Region and the infection occurs throughout the year at a low rate. In conclusion, necessary preventive measures should be undertaken to control aspergillosis among poultry in the Northern Region of Malaysia even though the infection rate is low as the infection could lead to high mortality among young birds which may induce significant production and economic losses.

Keywords: Occurrence, aspergillosis, poultry, Northern Region

Introduction

Aspergillosis is an assertive fungal infection spread through the air by a common mold known as *Aspergillus sp.* (Desoubeaux et al., 2014). The disease develops when the host's immune system is impaired or when birds are revealed to have an excessive amount of spores through the inbreathing of conidia or spores from adulterated feed, faeces or soil. Eggs that were contaminated with *Aspergillus spp* can lead to eggs shell penetration and will infect the embryo (Shoukat et al., 2018). This *Aspergillus spp.* in chicken eggs have killed the embryo or will be hatched with well-established lesion (Bauk, 1994). This lesion forms like white caseous nodules (1-2mm) in the lungs and granuloma

in the air sacs (1-5mm) (5m Editor, 2005). When contaminated eggs are ruptured, spores are expelled in vast numbers, contaminating hatchery equipment (Bauk, 1994). Apart from filthy surroundings, overpopulation, malnutrition, vitamin A and abuse of certain medications (corticosteroids) along with respiratory irritants (disinfectant fumes and aerosol sprays) (Girma et al., 2016), stress is the key exacerbating factor for the development of the disease (Saif et al., 1999).

In young birds, aspergillosis can become an acute or peracute ailment, leading to severe morbidity and mortality (Jordon et al., 2002). It usually takes less than a week for the acute form to appear. Difficulty breathing, anorexia, polydipsia, cyanosis, foetid diarrhoea, and emaciation are some of the clinical symptoms. Birds can die unexpectedly without displaying any clinical signs. A post-mortem examination is usually used to make a diagnosis (Leishangthem et al., 2015). The chronic type of aspergillosis can take weeks or months to develop, and it is more common in older birds (Leishangthem et al., 2015). Chronic forms in older birds are usually under immune suppression due to poor husbandry conditions and it causes lesser mortality (Jordon et al., 2002). Clinical symptoms differ depending on where the infection is located. It involves indications of nerve involvement, inappetence, emaciation, dyspnea, gasping, increased thirst, fever, and diarrhoea (Jensen et al., 1997).

Aspergillosis has been reported in both domestic and wild birds, including chickens, ducks, and quails (Jordon et al., 2002). *A. fumigatus* was the most often isolated species from the affected organ, followed by *A. flavus*, *A. niger*, *A. galucus* and *A. terreus*. *A. fumigatus* is a prevalent source of sickness among these species (Salem and Ali, 2014) and it can cause serious and life-threatening illness in birds (Khosravi et al., 2008). Conidia of an aspergillus can be found practically anywhere and are easily spread through the air. Conidia vary in size according on the species, and aspergillus conidia can invade the entire respiratory tree. Because of the tiny size of the conidia and other virulence characteristics associated with this species, *A. fumigatus* is one of the most commonly connected with respiratory symptoms (Viegas et al. 2017). Aspergillosis treatment is ineffective, there are no systemic antifungal medicines approved for use in food-producing birds in many countries. Due to the poor safety margin of these medications, treating aspergillosis in other birds with systemic antifungal medicines can be problematic (Wlaź et al., 2015). In addition, antifungal resistance is also becoming more common in human medicine. *A. fumigatus* strains obtained from birds have been found to be resistant to antifungal medications (Nawrot et al., 2019), so the most important prevention strategy against aspergillosis is keeping the infection pressure low by adequate ventilation as well as cleaning and disinfection (Beckman et al. 1994). To prevent and control the disease, good management techniques like sanitation, avoiding moist litter or soil, mouldy or dusty feeds, providing proper ventilation, and sanitising feed and water lines should be implemented (Girma et al., 2016). Mouldy bedding material and feed are common causes of infection, and the amount of conidia produced increases as the temperature and humidity of the environment rises. (Ruediger et al. 2020). Based on the poultry cases received by Regional Veterinary Laboratory Bukit Tengah (RVL-Bukit Tengah), Penang from 2014 to 2018, the goal of this study is to determine the positive instances of aspergillosis infection among poultry in Malaysia's Northern Region.

Materials and Methods

Source of data

The evaluated, summarized, and analyzed laboratory records over five years (2014 -

2018) from the Bacteriology Section of the RVL-Bukit Tengah, Penang, Malaysia, were used in the study. The records included information on the chicken's age, breed, and sex, as well as sample types and registration numbers (for identification).

Isolation and identification of the fungus

Isolation of the fungus

Using sterile scissors, the organ or tissue was sliced into minute pieces or macerated. A little amount of sample was then transferred six to seven places onto one plate of Sabouraud's Dextrose Agar (SBA) with antibiotics and one plate of SAB without antibiotics using a sterile hard needle or hook. Then samples were gently pressed into the agar. Samples were incubated for 3 to 5 days at 25°C-29°C.

Identification of the fungus

Lactophenol cotton blue wet mount

In the centre of a clean glass slide, a small drop of lactophenol cotton blue was placed. Using a bacteriologic needle, a small portion of fungal culture were removed and teased apart in a drop of lactophenol cotton blue stain. Then samples were covered with a coverslip and examined under a compound microscope (Saipul et al., 2017).

Statistical analysis

The data were analyzed using IBM SPSS Statistics Version 21.0 USA by descriptive and cross-tabulation statistics.

Results and Discussion

The analysis of the data from the year 2014 to 2018 revealed that there were a total of 1268 samples of sick poultry were submitted to Regional Veterinary Laboratory, Bukit Tengah in Penang. Out of these, 123 (9.70%) samples were positive with aspergillosis. Over the sixteen (16) breeds of poultry animals represented in this study (Figure 1) the highest samples were received from Cobb breed followed by village chicken, Ross breed, Kelly and AA+ while other breeds had less number of samples received.

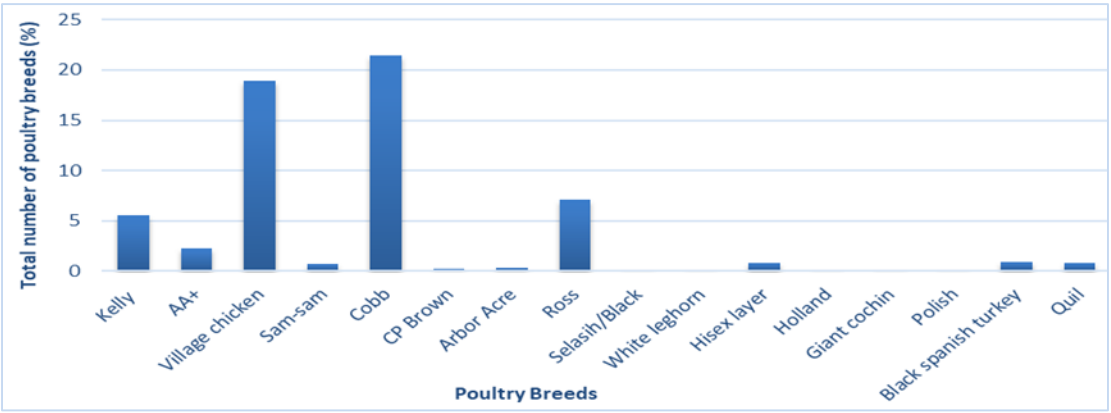

Figure 1. Percentage of total number of poultry breed

Ninety per cent (90%) of the sample tested were from lung samples while the rest were from air sacs, heart, embryo, all egg, all samples, intestine, proventriculus which represents only 1 to 2% of the sample types only. The highest percentage of aspergillosis isolation was *A. niger* (n = 72; 58.5%)

followed by *A. flavus* and *A. fumigatus* with 24.4% and 17.1% respectively (Table 1).

Table 1. The number and percentage of samples with positive for Aspergillosis

Aspergillosis species	Number	Percentage (%)
A. niger	72	58.5
A. flavus	30	24.4
A. fumigatus	21	17.1
Total	123	100

Figure 2 shows that five of the sixteen (16) breeds studied have more than 10% aspergillus infection, whereas the remaining breeds have less than 10% infection. *A. niger* infection was highest in Cobb breeds (50%) followed by Ross breed (15.3%) and village chicken (13.9%). As for *A. flavus* infection, the highest infection was also in the Cobb breed (46.70%) followed by village chicken (13.80%) and Kelly breed as well as AA+ breed (10.30%). Meanwhile, *A. fumigatus* infects 20% of Cobb breeds and 15% of Kelly, Ross and AA+ breeds. The poultry age in this study was mostly between 0 days to 17 months except for the Kelly breed reached the age up to 41 months (3 years 5 months).

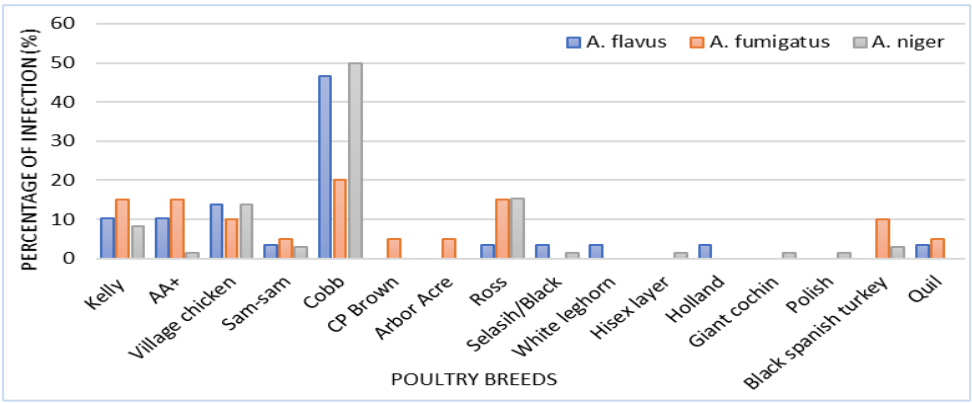

Figure 2. Percentage of aspergillosis species infection in poultry animal breeds

In this study, the most prevalent species isolated from chickens was *A. niger*. In a study involving chickens by Kwanashie et al. (2012) found that 6.70% of *A. niger* was isolated from 60samples, while Salem and Ali (2014) reported that *A. niger* was isolated (8%) from 100 lung tissues samples of chicken. However, this study contradicts the findings by Lim et al. (2020) who found that 5% mortality in 3-week-old ducklings from 900 muscovy ducks in Kota Bharu, Kelantan, Malaysia, which was caused by *A. fumigatus*. However, *A. niger* is still a fungus that can infect poultry, albeit it is less common than *A. fumigatus* and *A. flavus* (Kunkle, 2003; Martin et al., 2007). Tomic et al. (2021) found that *A. niger* was substantially more commonly discovered ($p<0.001$) in the house air and broiler trachea in the summer, which they linked to the poultry house's environment (air temperature °C (22.6-32.10); relative humidity % (54.00-74.50) in the Republic of Croatia while none of *A. fumigatus* was isolated compared during winter. This suggests that seasonal changes and microclimate conditions in the poultry house also play a significant role in fungal infections of different species. It is quite likely that *A. niger* infection is higher than *A. fumigatus* in this study due to the ambient temperature and

relative humidity in the country (Malaysia) which are more or less the same as the study conducted by Tomic et al. (2021) at that time. According to the Department of Environment Malaysia, the average daily temperature in Malaysia is around 32°C during the day and 24°C at night while relative humidity is around 74%. According to Zaki et al. (2016), heavy exposure of poultry to materials such as fallen leaves, stored grain, compost piles, and other decaying vegetation, which is a common living habitat of *A. niger* might have contributed to the increase of *A. niger* infection.

Several researchers reported that *A. fumigatus* was a common cause of aspergillosis among poultry (Salem and Ali, 2014; Tell, 2005). Meanwhile, Tell (2005) reported that *A. flavus* is the second most common organism associated with avian infection.

In this study as well, a high rate of aspergillus infection occurred between poultry ages from 0 to 37 days. This is similar to a study carried out by Kromm and Lighty (2020) who stated that, in birds, major outbreaks usually last 7 to 40 days. The highest infection in this study was at the age of 20–23 days, this is in contrast to Sultana et al. (2014) who reported the highest infection was when the poultry was 6–10 days old. Age only play a role, in certain breed young chick is usually infected while other breeds cause chronic infection.

A comparative study of the resistance of different species of poultry to *A. fumigatus* infection revealed that turkeys are more vulnerable than chickens (Kureljusic et. al., 2012). In contrast to this study, turkeys have an almost similar infection rate (12.8%) compared with other poultry, this may be because fewer samples were received from this breed. Meanwhile, substantial samples from other breeds including Cobb, Ross, Native chicken, and AA+ have resulted in high infection levels in these varieties.

Conclusion

In this study, although Aspergillus infection is not very high among poultry in the Northern Region, precautions should be taken as aspergillosis infection can lead to high mortality rates among young birds. Although fungal diseases are less common in poultry as compared with bacterial and viral diseases, they should not be neglected because when occurring, they can cause considerable economic losses either by direct infestation or via mycotoxin production. However, it is still unclear the reason for the high infection of *A. niger* in this study since most researchers found that *A. fumigatus* is the species that infects poultry the most. Therefore, further studies need to be conducted to identify the factors that cause high infection by *A. niger* compared to *A. fumigatus* and *A. flavus* as found by previous researchers.

Acknowledgement

The authors wish to thank the Director-General of DVS, Director of Regional Veterinary Laboratory Bukit Tengah, Penang and Director of Veterinary Research Institute, Ipoh Perak for permission to publish this article. We would also like to express our gratitude to every one of the study's participants for their assistance and support.

References

Beckman, B.J., Howe, C.W., Trampel, D.W., DeBey, M.C., Richard, J.L. and Niyo, Y. 1994. Aspergillus fumigatus keratitis with intraocular invasion in 15-day-old chicks. Avian Dis. 38: 660–665.

Bauk, L. 1994. Mycosis. In: Ritchie, B.W., Harrison, G.J. and Harrison, L.R. (eds). Avian Medicine Principle and Application. pp. 174-180. Wingers Publishing Inc., Lake Worth, USA.

Desoubeaux, G., Bailly, E. and Chandenier, J. 2014. Diagnosis of invasive pulmonary aspergillosis: Updates and recommendations. Med Mal Infect. 44:89-101.

Girma, G., Abebaw, M., Mebrie Zemene. M., Yergashewa Mamuye, Y., Gashaw Getaneh, G. 2016. A Review on Aspergillosis in Poultry. J. Vet. Sci. Tech. 7(6): 1-5.

Jensen, H.E., Christensen, J.P., Bisgaard, M. and Nielsen, O.L. 1997. Immunohistochemistry for the diagnosis of aspergillosis in turkey poults. Avian Path. 26(1): 5-18.

Jordon, F., Pattisson, M., Alexander, D. and Faragher, T. 2002. Poultry Disease. 5th Edition. pp: 387-390. Elsevier Limited Sannders, UK.

Khosravi, A.R., Shokri, H., Ziglari, T., Naeini, A.R., Mousavi, Z. and Hashemi, H. 2008. Outbreak of severe disseminated aspergillosis in a flock of ostrich (*Struthiocamelus*). Mycoses. 51(6): 557-559.

Kromm, M. and Lighty, M. 2020. Aspergillosis in poultry (Brooder Pneumonia, Mycotic Pneumonia, Pneumonycosis). http://www.msdvetmanual.com/poultry.

Kunkle, R.A. 2003. Aspergillosis. In:. Saif Y.M., Barnes H. J., Glisson J. R. et al. (11th Eds.). Diseases of poultry, pp: 883–895.Iowa State University Press, Ames, Iowa, USA.

Kureljušic, B., Radanović, O., Kureljušić, J., Jezdimirović, N., Maslić-Strižak, D., Prodanović, R. and Ivetić, V. 2012. The occurrence of aspergillosis in flock of turkey poults. Biotechnol. Anim. Husb. 28(1): 129-136.

Kwanashie, C.N., Paul, A.A., Jarlath, U.U., Haruna, M. and Kazeem. 2012. Retrospective study of Aspergillosis and other fungi in poultry (1980-2008). International Journal Livestock Research. 2(3): 84-88.

Leishangthem, G.D., Singh, N.D., Brar, R.S. and Banga, H.S. 2015. Aspergillosis in Avian Species: A Review. J. Poult. Sc. Technol. 3(1): 1-14.

Lim, E.T.C., Reduan, M.F.H., Nordin, M.L., Abdullah, F.F.J., Mohd Zairi, N.H., Mohd Rajdi, N.Z.I., Intan Kamaruzaman, N.A. and Shaharulnizam, N. 2020. A case of aspergillosis outbreak in a broiler duck farm in Kelantan, Malaysia. J. Adv. Vet. Anim. Res. 7(4): 692-697.

Martin, M.P., Bouck, K.P., Helm, J., Dykstra, M.J., Wages, D.P. and Barnes, H.J. 2007. Disseminated Aspergillus flavus infection in broiler breeder pullets. Avian Dis. 51(2): 626–631.

Nawrot, U., Wieliczko, A., Włodarczyk, K., Kurzyk, E. and Brillowska-Dąbrowska, A. 2019. Low frequency of itraconazole resistance found among Aspergillus fumigatus originating from poultry farms in Southwest Poland. J. Mycol. Med. 29: 24–27.

Richard, J.L. and Thurston, J.R. 1983. Rapid hematogenous dissemination of Aspergillus fumigatus and A. flavus spores in turkey poults following aerosol exposure. Avian Dis. 27:1025-1033.

Ruediger Hauck, Carolyn Cray and Monique Franca. 2020. Spotlight on avian pathology: Aspergillosis. Avian Pathol. 49(2): 115-118.

Saif, Y. M, Fadly, A.M., Glisson, J.R., McDougald, L.R. and Nolan, L.K., 1999. Disease of poultry. 12th Edition. pp: 542-550.Iowa State University Press, Ames, London.

Saipul Bahari, A.R., Norinar, L., et al. 2017. Isolation and identification of fungus. In: Manual of Veterinary Laboratory Testing for Bacteriology. pp: 171-176. Department of Veterinary Services, Ministry of Agriculture and Agro-based Industry Malaysia.

Salem, L.M.A. and Ali, A.F. 2014. Epidemiological study of Aspergillosis in chickens and human contacts in chicken farms at Kalyoubia Governorate. J. Agric. Vet. Sci. 7(7):20-24.

Shoukat, S., Wani, H., Jeelani, R., Ali, U. and Ali, M. 2018. An overview of avian aspergillosis. Int. J. Avian Wildl. Biol. 3(3): 215-216.

Sultana, S., Harun-ur-Rashid, S.M., Islam, M.N. and Ali, M.Z. 2014. Pathological investigation of avian aspergillosis in commercial broiler chicken at Chittagong district. Int. J. Adv. Res. Biol. Sci. 1(8): 74-85.

Tell, L.A. 2005. Aspergillosis in mammals and birds: Impact on veterinary medicine. Medical Mycology. 1(43): 71-73.

Tomic, D.H., Ivica Ravic, I., Kabalin, A.E., Kovacic, M., Gottstein, Z. and Ostovic, M. 2021. Effects of Season and House Microclimate on Fungal Flora in Air and Broiler Trachea. Atmosphere. 12(459): 1-10.

Viegas, C., Faria, T., Aranha Caetano, L., Carolino, E., Quintal Gomes, A., Viegas, S. 2017. Aspergillus spp. prevalence in different occupational settings. J Occup Environ Hyg. (14): 771–785.

Wlaź, P., Knaga, S., Kasperek, K., Wlaź, A., Poleszak, E., Jeżewska-Witkowska, G., Winiarczyk, S., Wyska, E., Heinekamp, T. and Rundfeldt, C. 2015. Activity and safety of inhaled itraconazole nanosuspension in a model pulmonary Aspergillus fumigatus infection in inoculated young quails. Mycopathologia. 180: 35–42.

Zaki, M.S., Mustafa, S.O., Rabie, N. 2016. Aspergillosis in chickens and human contacts. Der Pharm. Lett. 8(12): 216-220.