



Identification and Prevalence of *Aspergillus* sp Isolated from Bali Dog's Skin

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Abstract

Aspergillus sp are fungi that commonly caused systemic respiratory infection in humans and dogs. In Bali, most of the dog are free-roaming dogs and has a poor skin health condition. Some of them are suspected with fungal infections. *Aspergillus* sp has high risk to infect dog, because they are available everywhere in environment. Therefore, this research is conducted for adding primary data on *Aspergillus* sp isolated from dog's skin and their prevalence. The samples are 15 free-roaming Bali dogs with suspected fungal infection. Identification was carried out by using microscopic and macroscopic observation methods, skin swab was taken from the dog's skin using the Mackenzie method, and the sterile toothbrush swab gently from the dog's skin to the Sabouraud Dextrose Agar (SDA) media. The fungi were cultured on SDA media for macroscopic identification for approximately 3-4 days, mostly to observe how the fungal colony grew and its color. Microscopic identification are using adhesive tape, the tape was gently pressed into the fungal colony, the tape was stuck into the surface of the object-glass with 3-4 drops of Methylene Blue staining, and the object glass was examined by using a light microscope, the findings of *Aspergillus* sp fungi are recorded and the data are descriptively presented. The result shows that most *Aspergillus* species that were found are *Aspergillus fumigatus* and *Aspergillus niger* according to their colonies growth and microscopic appearance, and the prevalence from 15 samples was 53.33%.

Keywords: Aspergillus sp, fungi, Bali Dogs, Identification, Prevalence

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Introduction

The fungal infection in dogs is a well-known zoonotic disease but it is sometimes underestimated. Lots of fungal species were identified infecting dogs and humans, such as Dermatophytes, *Curvularia*, and *Aspergillus*. In Bali, the dog population touched 540.000 (Duana et al., 2011). The proportion of dogs that are housed is only about 30% or even less. The rest are stray dogs that roam the streets, public places, ceremonial sites, trash cans, and bushes. (Mahardika, 2010). The density of the dog population and the pattern of dog maintenance in Bali, raise the risk for transmission of zoonotic disease. One of the diseases is Aspergillosis fungal infection that is caused by *Aspergillus* sp.

Aspergillus can grow as a saprophyte on decaying plants and is also present in the soil, organic dust, water, or on the surface of food including fruit and vegetables (Hayani & Darniati, 2017). Although there are hundred species in the *Aspergillus* genus, there are only a few species that have considerable impacts on human or animal health. Infections are typically caused by *Aspergillus flavus*, *Aspergillus Fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus* (Enoch et al., 2006). Most of the infection caused by *Aspergillus* was systemic infection, such as *A. fumigatus*, which has been shown to secrete mycotoxins like gliotoxin, verruculogen, fumagillin, and pelvicanic acid, which can affect the ciliary beat frequency and promote colonization of lung epithelium (Khoufache et al., 2007). This fungi is also responsible for causing canine sino-nasal aspergillosis (SNA) which is clinicopathological similar to chronic erosive non-invasive fungal sinusitis in humans (Sharman & Mansfield, 2012). The next *Aspergillus* is *Aspergillus terreus* and *Aspergillus deflexus*, it was major species found in patients with disseminated sickness (Day, 2006) and associated pathology frequently involves several tissues, such as the kidneys, intervertebral discs, bones, and central apprehensive system (Schultz et al. 2008).

Aspergillus can result in different allergies and infectious diseases in immunocompromised people. This can develop into an invasive and deadly infection of the respiration system, often accompanied by dissemination to different organs, a circumstance known as invasive aspergillosis. A regionally invasive model of the ailment, continual necrotizing pulmonary aspergillosis, is particularly located in human beings with slight immunodeficiency or with a continual lung sickness. Non-invasive *Aspergillus*-caused lung sickness includes aspergilloma and allergic bronchopulmonary aspergillosis (ABPA) (Kosmidis and Denning, 2015a,b). In dogs, the three major forms of aspergillosis are nasal, bronchopulmonary, and disseminated infections. The nasal form, frequently accompanied by invasive sinusitis, occurs most commonly in medium to large, dolichocephalic or mesocephalic breeds (Peeters & Clercx, 2007). The most common agent is *A. fumigatus*, *A. flavus*, and *A. niger* (Sharp & Mathews KG, 2006). *Aspergillus* is very dangerous if it infects systemically, but their abilities to infect skin are questionable. Because Bali dogs mostly has poor skin health and some of them are infected with fungi. *Aspergillus* are available everywhere, and has risk to infect them. Some fungi did infect the dog's skin, for example, *Microsporum* molds and *Trichophyton* which consists of three species that cause most cases of dermatophytosis in dogs and cats, namely *M. canis*, *M. gypseum*, and *T. mentagrophytes* (Miller et al. 2013). This research is trying to find the primary data to identify and give information about the prevalence of *Aspergillus* that is isolated from a dog's skin suspected of fungi infection.

Materials and Methods

Sample

The samples are 15 free-roaming Bali dogs with suspected fungal infections.

Sample Collecting Method

Identification was carried out by using microscopic and macroscopic observation methods, skin swab was taken from the dog's skin using the Mackenzie method, and the sterile toothbrush swab gently from the dog's skin to the Sabouraud Dextrose Agar (SDA) media (Abdalla and Wisal, 2018). Samples were taken by rubbing the area of the skin that was suspected of being infected with a fungus with a tooth-brush technique (Moriello 2001). The toothbrush is combed over the suspected area and then pressed onto the surface of an SDA media plate, after that the media is incubated at room temperature for 3-4 days.

Macroscopic identification

The fungi were cultured on SDA media for macroscopic identification for approximately 3-4 days, mostly to observe how the fungal colony grew and its color.

Microscopic identification

After the colony grows the identification continues with microscopic examination by using the adhesive tape preparation (Scotch tape prep or cellophane tape prep) for microscopic examination of fungal colonies, which is a standard technique found in manuals of medical mycology (Forbes et al. 2002). After the tape was gently pressed into the fungal colony, the tape was stuck into the surface of object-glass with 3-4 drops of Methylene Blue staining, and the object glass was examined by using a light microscope. Microscopic examination of *Aspergillus* showed the presence of short smooth greenish conidia (conidiophores), conidia heads (vesicles) were clavate and round, and became oval (columnar) with increasing age of the colony. Sterigmata appear to cover the upper half of the vesicle. The spores/conidia are round, green in color, and have an echinulate surface (Redig, 2005). All the *Aspergillus* sp findings are recorded.

Data Presentation

Data was descriptively presented.

Results and Discussion

Data from Table 1. shows that from 15 samples, 8 (53.33%) are positive and confirmed with the growth of *Aspergillus* sp in the SDA, and the other 7 (46.67%) dogs are negative. The macroscopic appearance in Figure 1 is a green or mild green border, with powder and cotton-like consistency and in Figure 2 appears with black-colored colonies.

Table 1. Result of *Aspergillus* sp Identification

Dog's code	Positive	Negative
Dog 1	-	1
Dog 2	-	1
Dog 3	-	1
Dog 4	1	-
Dog 5	1	-
Dog 6	-	1
Dog 7	-	1
Dog 8	-	1
Dog 9	1	-

Dog 10	1	-
Dog 11	1	-
Dog 12	1	-
Dog 13	-	1
Dog 14	1	-
Dog 15	1	-
Total	8	7
%	53.33%	46.67%

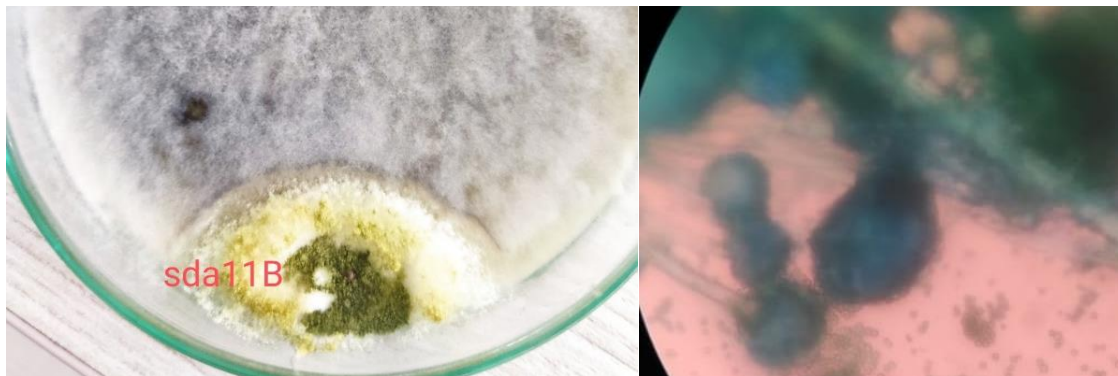


Figure 1. Macroscopic appearance (black arrow, Left), and microscopic appearance (right) of *Aspergillus sp.* with a green colored colony.

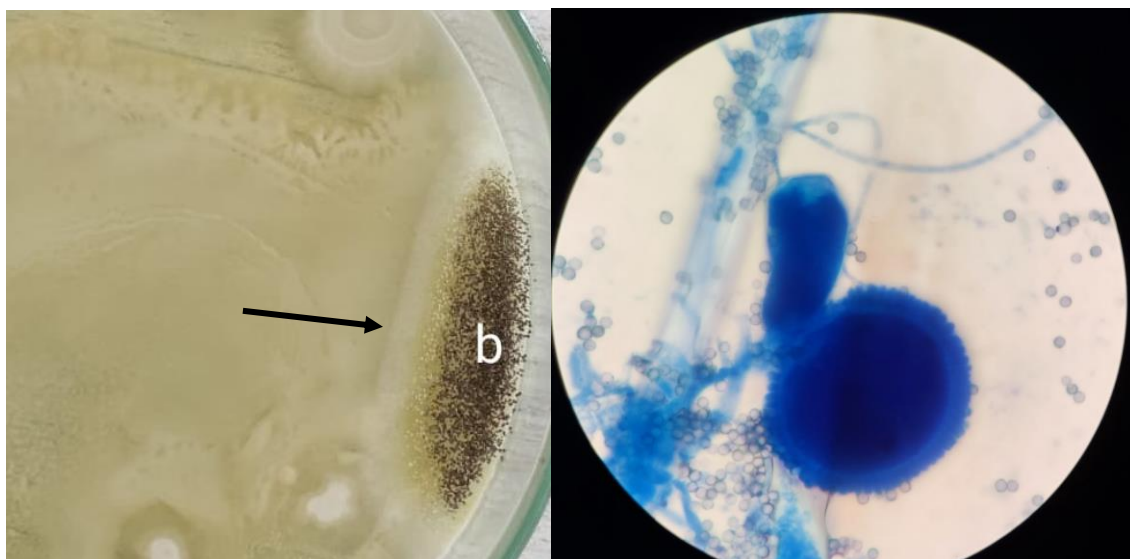


Figure 2. Macroscopic appearance (black arrow, Left), and microscopic appearance (right) of *Aspergillus sp.* with a black-colored colony.

The microscopic examination resulted in an image of a circle-shaped conidiophore at the end of a non-septated hypha with a lot of conidia spread around it. This characteristic is in accordance to Kidd et al. (2016), that said *Aspergillus sp.* colonies are usually fast-growing, white, yellow, yellow brown, brown to black or green gradations, mostly consisting of erect conidiophores. Conidia are single-celled, smooth or rough-walled, hyaline or pigmented, produced in long dry chains that may diverge or coalesce in compact columns.

In this research probably the most *Aspergillus* species that were found is *Aspergillus fumigatus* and *Aspergillus niger* according to their colonies growth and microscopic appearance. Although, the conventional morphological and microscopic characterization are most popular classical methods but, it is believed that these are time consuming and are not sufficient to characterize the different fungal species, due to their intra and inter specific morphological divergences (Klich and Pitt, 1988; Samson et al., 2004). According to Hartana (2014) *Aspergillus niger* looks black with white edges. *Aspergillus fumigatus* is dark green with white edges. The colony is light in color with a cottony mycelium. Initially, colonies appeared as white filaments and changed color depending on the species. *Aspergillus* colonies were also characterized by spreading conidia. Microscopic examination of *Aspergillus* showed the presence of short smooth greenish conidia (conidiophores), conidia heads (vesicles) were clavate and round, and became oval (columnar) with increasing age of the colony. Sterigmata appear to cover the upper half of the vesicle. The spores/conidia are round, green in color, and have an echinulate surface (Redig, 2005).

Aspergillus are easy to find according to Lee's (1998) study where they both found from skin and hair, *Aspergillus* was the most common genus, followed by *Cladosporium* and *Penicillium*. The ratio of fungal isolates belonging to this genera was 25, 23, 20.5%, respectively. These results indicate that *Aspergillus*, *Cladosporium*, and *Penicillium* are the most common fungal groups in domesticated dogs and they are fungi that are commonly found in the indoor air of homes and offices. de Hoog et al (2000) added that although there are more than 200 known species in the genus, only a small percentage are associated with infections. Among them, *A. fumigatus* (subgenus *Fumigati*, section *Fumigati*), *A. flavus* (subgenus *Circumdati*, section *Flavi*), and *A. niger* (subgenus *Circumdati*, section *Nigri*) are the most frequently encountered species.

As mentioned before, *Aspergillus* in dogs only has 3 major forms of infection, they are nasal, bronchopulmonary, and disseminated infections. The nasal form, frequently accompanied by invasive sinusitis, occurs most commonly in medium to large, dolichocephalic or mesaticephalic breeds (Peeters and Clercx, 2007). The primary etiologic agent is *A. fumigatus*, followed by *A. flavus* and *A. niger* (Sharp and Mathews, 2006). *Aspergillus* is available in environments and close to dogs living areas. In humans, the cutaneous form of *Aspergillosis* is a rare manifestation, usually the result of the spread of primary pulmonary infection in immunocompromised patients. However, cases of primary cutaneous *aspergillosis* also occur, usually as a result of trauma or colonization (Hasanah, 2017). But in dogs, cases have not been widely reported. Hypothetically, the spores may attach to the skin because the free-roaming dogs are laying and playing mostly in outside areas that are unclean, for example, roads, abandoned houses, and garbage center areas. Plus, fungi infections are related to immunosuppression conditions, caused by lack of food and infection from other diseases. As we know, the free-roaming Bali's dog health conditions are poorly maintained, which is why fungi infection can rapidly grow and spread among dogs. The skin infection from *Aspergillosis* needs to be studied and researched more, to confirm that one of the causes of fungi infection in dogs is them, not other fungi, parasites, or even bacteria.

Conclusion

The most *Aspergillus* species that were found are *Aspergillus fumigatus* and *Aspergillus niger* according to their colonies growth and microscopic appearance, and the prevalence from 15 samples were 53.33%.

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