



Prevalence and Types of Fungi in Kintamani Bali Dogs

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ABSTRACT

Indonesia, located in the tropics with high humidity, is a suitable area for the growth of various types of fungi. Kintamani Bali dogs have thick and beautiful fur, so it is unfortunate to get a fungal infection. There is a high risk for fungal infection, and no research data for Kintamani Bali Dog make this research very important. Samples were taken randomly from Kintamani Bali dogs with fungal disease characteristics using the toothbrush technique. The samples were identified by colony identification and microscopic examination through slide culture using *Methylene blue* staining. The results showed that from 15 samples, identified 31 fungi. Among these, commonly found were *Aspergillus sp.* 54.84% and each of *Curvularia sp.* *Trichophyton sp.* *Candida sp.* 12.9% while *Penicillium sp.* 6.45%. Based on sex, generally, the female Kintamani had a higher (61.29%) fungal infection than male Kintamani Bali dogs (38.71%). In young age dogs, more infection was found (70.97%) than adult dogs (29.03%).

Key words: Age, Fungi, Kintamani Bali dogs, Sex.

INTRODUCTION

Fungal diseases are very common in all animal species and can also be found in humans or zoonotic (Imran et al. 2020; Abuowarda et al. 2020). In dogs, disorders of the skin can interfere with the beauty of appearance and if not treated immediately can spread throughout the body and have an impact on a more widespread infection. At this time the Kintamani Bali Dog enthusiasts are increasing, because the Bali Kintamani Dog has a brave nature, has an attractive appearance, and is the first breed of dog in Indonesia which was inaugurated by PERKIN (Indonesian Kinology Association). Currently, the Bali Kintamani Dog is being prepared for submission to the FCI (Federation Cynologique Internationale) which is an international dog organization, to be designated as a world dog breed (Evayana et al. 2017). The Kintamani Bali Dog is the name for a local mountain dog group that lives around Sukawana Village, Kintamani District, Bangli Regency, Bali. This local mountain-type dog has a very beautiful appearance that is different from the street dog in Bali (Puja 2007). Kintamani Bali dogs have thick and beautiful fur, so it is very unfortunate if they get a fungal infection.

Fungal infections in dogs are dominated by several agents, including dermatophytes or *Microsporum* 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). Indonesia has a tropics season with high humidity, which is a suitable area for the growth of various types of fungi. Thick and long fur dogs are a suitable predilection for the growth of fungi (Pohan 2007). Dermatophytes are often found in hair and the keratin layer of the skin because they can feed on the protein keratin (Outerbridge 2006). Disease mortality is low; however, economic losses can occur due to skin and hair damage or loss of body weight because the animal becomes restless and there is a zoonotic risk posed by *M. canis* (Kotnik 2007). Then there is *Ptyriasis versicolor* (PV) or also known as tinea versicolor, is a skin disease caused by the genus *Malassezia* (Gupta and Foley 2015), then there is Yeast *Candida spp.* and the genus *Aspergillus*. Opportunistic fungi have a preferred habitat independent from the living host and cause infection after accidentally penetration of intact skin barriers, or when immunologic defects or other debilitating conditions exist in the host (Casadevall and Pirofski 2000). Based on Sudipa et al. (2021) research in Bali dogs, the results of dogs that have skin diseases and are infected *Curvularia* were 5 dogs (19%) from 26 dogs, but there is no data of fungal infection in Kintamani Bali Dog, therefore, it is necessary to do research to see the prevalence of fungal infections and what types of fungi infect the Kintamani Bali Dog for preliminary data.

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MATERIALS AND METHODS

Sample Collection

Sample were taken from random Kintamani Bali Dogs that has characteristic of fungal disease, like a regional or generalized alopecia with erythema (exfoliative erythroderma), scaly, waxy, or greasy seborrhea (yellow or slate gray), crusts or papulocrustous lesions resembling superficial staphylococcal infection, lichenification and/or hyperpigmentation (leathery or elephant-like skin), paronychia with dark brown nail bed discoloration, with or without obsessive paw chewing, lip margin hypotrichosis and/or crusting; and intertrigo (Bajwa 2017). The sample were taken from various location in Bali, and the gender and age of the Kintamani Bali dogs were noted.

Fungi Identification

Samples were taken by rubbing the skin that suspected 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 a culture plate. The fungi was cultured on *Sabouraud Dextrose Agar* media for colony identification and 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 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.

Data Presentation

The data was tabulated by fungi type, sex, age and the data descriptively presented.

RESULTS

Fungal Prevalence

The results of the identification of fungal colonies growing on *Sabouraud Dextrose Agar* (SDA) media macroscopically and microscopically using a light microscope showed the results where 31 fungi from 5 species were identified from 15 Kintamani Bali dogs with skin disease (Table 1) dominated by *Aspergillus sp.* fungi with a total of 17 (54.84%), followed by *Curvularia sp.* with 4 (12.9%), *Trichophyton sp.* with 4 (12.9%), *Candida sp.* with 4 (12.9%), then the least found is *Penicillium sp.* with 2 (6.45%).

Fungal Prevalence Based on Sex

The fungal prevalence based on sex shown in Table 2, where generally the female had bigger percentage (61.29%) of fungal infection than male Kintamani Bali dogs (38.71%), *Aspergillus sp.* was found more in female (52.94%) than male Kintamani Bali dogs (47.06%), *Curvularia sp.* was found both in male (50%) and female dogs (50%), *Trichophyton sp.* not found in male dogs but found in female, the same cases with *Candida Sp.* they only found in female Kintamani Bali dogs, in the other hand, *Penicillium sp.* was found only in male Kintamani Bali dogs.

Table 1: Fungal prevalence in Kintamani Bali dogs

Fungi	Qty	Percentage
<i>Aspergillus sp.</i>	17	54.84
<i>Curvularia sp.</i>	4	12.90
<i>Trichophyton sp.</i>	4	12.90
<i>Candida sp.</i>	4	12.90
<i>Penicillium sp.</i>	2	6.45
Total	31	100.00

Table 2: Fungal prevalence based on sex in Kintamani Bali dogs

Fungi	Male	Percentage	Female	Percentage
<i>Aspergillus sp.</i>	8	47.06	9	52.94
<i>Curvularia sp.</i>	2	50	2	50
<i>Trichophyton sp.</i>	0	0	4	100
<i>Candida sp.</i>	0	0	4	100
<i>Penicillium sp.</i>	2	100	0	0
Total	12	38.71	19	61.29

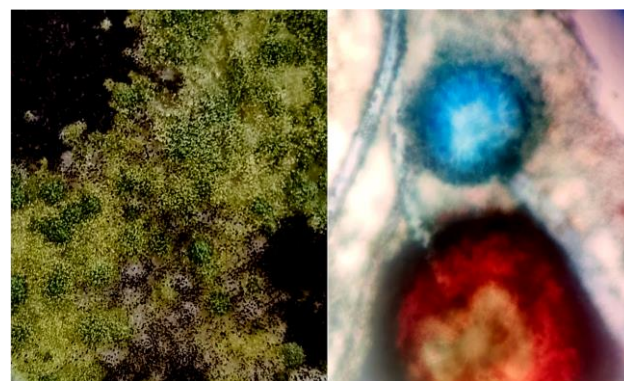


Fig. 1: *Aspergillus sp.* colony growth on Sabouraud Dextrose Agar macroscopically (left) and microscopically (Right).

Fungal Prevalence Based on Age

Generally, more fungi were found in young age dogs (70.97%) than adult dogs (29.03%), the most fungi found was in age of 3 month (48.39%), and the second was in age of 2 month (16.13%), followed up by age of 12 month (12.9%), and age of 48 month (9.68%), and age of 4 month and 24 months showed the same number (6.45%) as also shown in Table 3.

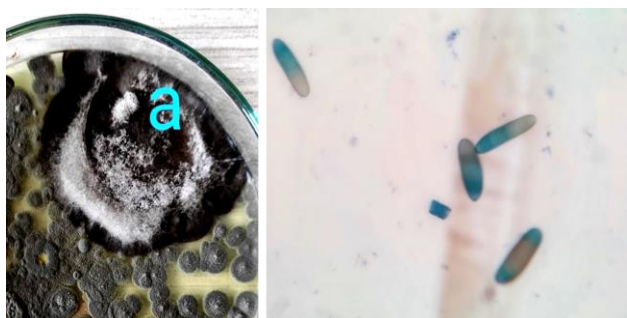
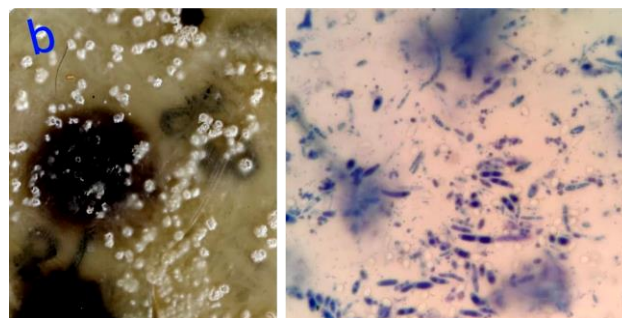
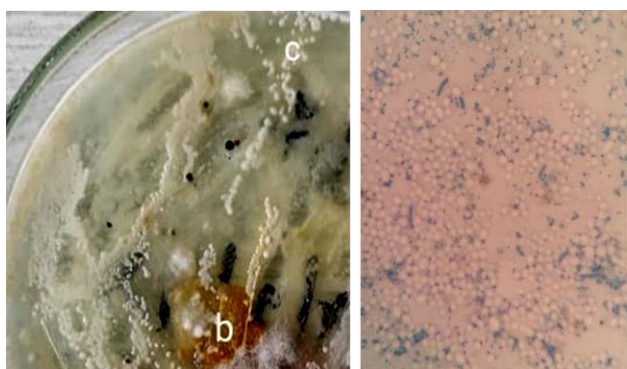
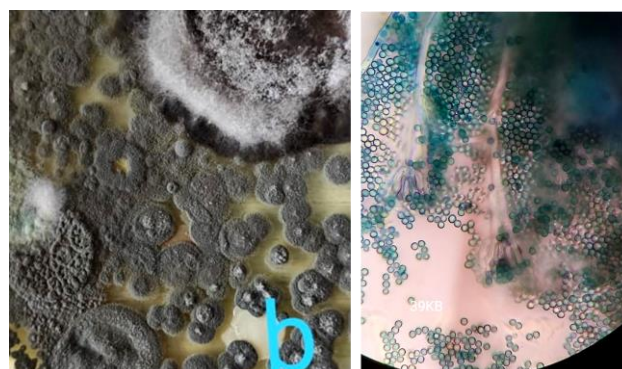
DISCUSSION

Aspergillus Species

Aspergillus sp. can cause mycosis in dogs and cats (Pitt 1994) and is responsible for most cases of sino-nasal aspergillosis in dogs (Peeters and Clercx 2007). And this disease is clinically pathologically similar to non-invasive chronic erosion of sinusitis in humans (Sharman and Mansfield 2012). *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 (Kidd et al. 2016; Saleemi et al. 2017). *Aspergillus* is one of the molds from the *Ascomycota* class. It can be recognized by the presence of conidia that are oval, semi-spherical, or shaped round. The results obtained (Fig. 1) are in accordance with the above characteristics, the macroscopic colonies was found with green gradations, and microscopically there are oval or shaped round conidia heads.

Table 3: Fungal prevalence based on age

Age (Month)	<i>Aspergillus sp</i>	<i>Curvularia sp</i>	<i>Penicillium sp</i>	<i>Candida sp</i>	<i>Trycophyton sp</i>	Total	%	
2	4	0	0	1	0	5	16.13	Puppy
3	9	2	0	2	2	15	48.39	70.97
4	1	0	1	0	0	2	6.45	
12	2	1	1	0	0	4	12.9	Adult
24	0	1	0	0	1	2	6.45	29.03
48	1	0	0	1	1	3	9.68	
Total	17	4	2	4	4	31	100	

**Fig. 2:** *Curvularia sp* colony growth on Sabouraud Dextrose Agar macroscopically (left) and microscopically (right).**Fig. 3:** *Trycophyton sp* colony growth on Sabouraud Dextrose Agar macroscopically (left) and microscopically (right).**Fig. 4:** *Candida sp* colony growth on Sabouraud Dextrose Agar macroscopically (left) and microscopically (right).**Fig. 5:** *Penicillium sp* colony growth on Sabouraud Dextrose Agar macroscopically (left) and microscopically (right).

Curvularia Species

Fungi are widespread and have been the cause of several infections in humans and animals. The main routes of infection usually occur through inhalation and skin inoculation (Balla et al. 2016). Infection is also usually caused by piercing or penetrating wounds (Thomas et al. 1988) or through exposure to environmental sources containing fungal spores such as plants, dirt, metals and water. For eye and skin infections, trauma with spore-containing material is an important risk factor. (Paterson and Lima 2015). Species identification was based on morphological characteristics of cultured specimens including (1) conidia size, (2) conidia cell count and color, and (3) conidia bending level (Pimentel et al. 2005). Macroscopically, colonies on *Sabouraud Dextrose Agar* were initially grayish which later became dark brown (Ponnusamy et al. 2018). Colonies were found to develop within 2 to 3 days after inoculation. The color of mature *Curvularia* colonies looks dark brown to black. On the reverse side of the petri dish, the colonies looked black while the texture of the colonies was hairy like cotton and slightly protruding (Subapriya et al. 2015). *Curvularia* microscopically shows the characteristics of brown hyphae, brown conidiophores, and produces branched

septa. The width of the *Curvularia* hyphae is 2.55 μ m, the length of the conidia is 12.01 μ m, and the width of the conidia is 6.87 μ m (Natalia et al. 2019). As the *Curvularia* fungus matures, one of the central cells in the conidia grows larger and darker than the lateral cells which produces a characteristic curvature and gives it a croissant-like appearance (Paterson and Lima 2015). The results of the growth of *Curvularia Sp* in this study (Fig.2) are in accordance with the characteristics of Subapriya et al. (2015) and Paterson and Lima (2015), where the colonies were macroscopically black, like cotton and microscopically one of the diconidia cells was enlarged and darker than the other colony.

Trichophyton Species

Trycophyton sp is one of the causes of dermatophytosis, the research of Indarjulianto et al. (2014) showed that 34% of dogs in Yogyakarta, Indonesia, were positive for dermatophytes. The genus *Trichophyton* is morphologically characterized by the development of fine-walled macro and microconidia. The macroconidia are pencil-shaped and consist of few cells, while the microconidia are oval and thin-walled (Saputra 2017). The results of *Trycophyton sp* (Fig. 3) show irregular and

elongated macroconidia, with white colonies, but not so distinctive, the number of *Trychophyton* species with different shapes makes it difficult to identify macroscopically and microscopically. According to Rebell and Taplin (1970) the surface of *Trychophyton* is folded, rising in a convex shape, the bottom of the colony is united with the media, making it difficult to separate, the consistency is dense, hard. Colony color is grayish white, smooth (glabrous). However, the nature of dermatophytes is pleomorphic, often changes shape, and this is mainly related to the composition of the media such as colonies that turn into a cotton-like shape (floccose) when the medium contains glucose, so repeated culturing of newly isolated fungi is important in the laboratory until a stable form is reached (Ainsworth and Austwick 1973; Ainsworth 1986).

Candida Species

Several reports indicate that *Candida* is an important pathogen in dogs, associated with: urinary tract infections (Ozawa et al. 2005), endophthalmitis (Linek 2004), skin lesions (Moretti et al. 2004), and systemic infections (Brown et al. 2005). Macroscopically *Candida* is tentatively identified based on its morphology, namely: colonies with white to cream color, smooth, glabrous, and shaped like yeast (Hamid et al. 2014). According to Tjampakasari (2006) *Candida* generally round in shape with a size of (3.5-6) x (6-10) μ with surface slightly convex, smooth, slippery, sometimes slightly doubled, especially in colonies that is old. Colony size influenced by the age of the culture. Color *Candida* colonies yellowish white (cream soft) and has a distinctive smell. The results obtained (Fig.4) are in accordance with the description of the study by Hamid et al. (2014) where the colonies are white, smooth, slippery and microscopically the cells are round in shape like yeast.

Penicillium Species

Penicillium is a ubiquitous genus of saprobic fungi with >300 known species, but few of which act as canine or human pathogens (Pitt 1994 and Watt et al. 1995). *Penicillium marneffeii*, the only member of the dimorphic genus, is an emerging pathogen in immunocompromised humans, and infections in immunocompetent patients have also been reported (Duong, 1996 and Hu et al. 2013). Dogs are a potential reservoir of *P. marneffeii* in some areas (Chaiwun et al. 2011). Infections caused by other species are very rare in humans. Specific characteristics of *Penicillium* are insulated or septate hyphae, branched mycelium, usually colorless, conidiophores insulated or septate and appear above the surface originating from hyphae below the surface of branched or unbranched hyphae, the head of the hyphae carrying spores is shaped like a broom, with Sterigmata appear in clusters, conidia are chain-shaped because they arise one by one from the sterigmata. Conidium when young is green, then turns bluish or brown (Fardiaz 1992). Meanwhile, according to Ristiari et al. (2018), showing *Penicillium sp.* on SDA medium had septate hyphae. Conidia are spherical in shape. *Penicillium sp.* initially white, then changed to blue-green, greenish gray and the opposite color is usually pale yellow, while the microscopic form of the fungus *Penicillium sp.* which has hyphae, round conidia, and has a collection of

phialids. The growth results of *Penicillium sp.* in this study (Fig. 5) the color of the colonies was green and then brownish gray, while microscopically it showed the shape of the hyphae head like a broom, in accordance with Fardiaz's (1992) statement.

Aspergillus sp. was the most fungi found in this research with 17 fungi (54.48%) out of 31 fungi that were identified. Mostly found in female Kintamani Bali dogs (52.94%) and at age of 3 month with 9 findings. *Aspergillus* species is fungus that widely distributed in environment and has capacity of growing in different substrates. There are different species cause disease in dogs including *Aspergillus fumigatus*, *A. flavus*, *A. terreus*, *A. niger* and *A. deflexus*. Several studies reported that aspergillosis affects every age, but it primarily affects young to middle-aged animals (Kelly et al. 1995). The numerous members of this genus are saprobic filamentous fungi commonly found in soil, decaying vegetation, and on seeds and grains, with an occasional potential to infect living animal hosts including insects, birds, and mammals (Heitman 2011). These results are in accordance with Lee (1998) study where both from skin and hair, *Aspergillus* was the most common genus, followed by *Cladosporium* and *Penicillium*. The ratio of fungal isolates belonging to these genera was 25, 23, 20.5%, respectively. These results indicate that *Aspergillus*, *Cladosporium* and *Penicillium* are the most common fungal groups in domesticated dogs. These findings indicate that *Aspergillus*, *Cladosporium*, and *Penicillium* are fungi that are commonly found in the indoor air of homes and offices. Several studies also showed that dermatophytosis occurs more often in female animals than in males (Almuzaini et al. 2016; Haggag et al. 2017). Thus, sex and age play important roles in the prevalence of dermatophytosis in animals (Paryuni et al. 2020). The prevalence findings based on sex was not very significant, maybe it need more sample and data analysis. Because according Conkova et al. (2011), male dogs tend to be more susceptible to *Malassezia* infection than female dogs. The cause is the presence of androgen hormones in male dogs that increase sebum production, so the risk of fungal infection will rise. The prevalence based on age show high number of fungal infections on puppy stage dogs, maybe because the younger Kintamani Bali dogs love to play with other dogs and in contaminated environment when they were releases from cages so the infection will spread and all the puppy were put in cages with bad circulation and high humidity. Susceptibility to dermatophyte contamination is depending on animal age, sex, and species. The host immune system and virulence from the agent also are essential for infection (Paryuni et al. 2020).

Conclusions

According to the macroscopic and microscopic identification from Kintamani Bali dogs skin sample, *Aspergillus sp* is the most fungi that was found in the sample. Sex factor not significantly affect the infection of fungi, and based on age, the younger dogs intend to be more likely infected by fungi.

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Author's Contribution

All the authors contribute for this manuscript, from collecting sample, identifying sample and complete the manuscripts.

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