Scopulariopsis AND *Penicillium* IN AFRICAN PYGMY HEDGEHOGS (*Atelerix albiventris*) AT DHONJE GOLDEN FARM, BOGOR

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BOGOR
2024

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ABSTRACT

SATRIA TEGAR RAHMADANI. *Scopulariopsis* and *Penicillium* in African Pygmy Hedgehogs (*Atelerix albiventris*) at Dhonje Golden Farm, Bogor. Supervised by NOVERICKO GINGER BUDIONO and NURHIDAYAT.

This research detected the presence of *Scopulariopsis* and *Penicillium* in African pygmy hedgehogs (*Atelerix albiventris*) bred at Golden Dhonje Farm, Bogor District. Twenty hedgehogs were sampled to detect the presence of *Scopulariopsis* and *Penicillium*. The signs found include alopecia, crusty, and dermatitis. The presence of the fungus was confirmed by taking samples from the skin and then culturing on Potato Dextrose Agar (PDA) supplemented with chloramphenicol and cycloheximide. Macroscopic and microscopic observations were carried out to identify the two fungal genera. The results showed that eight hedgehogs were infected with *Scopulariopsis* (40%), six were infected with *Penicillium* (30%), and four animals were infected with co-infection (20%). The total prevalence of hedgehogs affected by this fungus is 90%. Further research is needed to expand the sample area, identify other types of fungi, and improve the understanding of fungal diseases in African pygmy hedgehogs to ensure the safety of hedgehogs in Indonesia.

keyword: African pygmy hedgehogs, *Scopulariopsis, Penicillium,* dermatomycosis

ABSTRAK

SATRIA TEGAR RAHMADANI. *Scopulariopsis* dan *Penicillium* pada Landak Mini Afrika (*Atelerix albiventris*) di Dhonje Golden Farm, Bogor. Dibawah bimbingan NOVERICKO GINGER BUDIONO dan NURHIDAYAT.

Penelitian ini mendeteksi keberadaan *Scopulariopsis* dan *Penicillium* pada landak mini Afrika (*Atelerix albiventris*) yang diternakan di Golden Dhonje Farm, Kabupaten Bogor. Dua puluh ekor landak diambil sampelnya untuk deteksi keberadaan *Scopulariopsis* dan *Penicillium*. Tanda-tanda yang ditemukan berupa *alopecia, crusty*, dan dermatitis. Keberadaan cendawan tersebut dikonfirmasi melalui pengambilan sampel dari kulit dan selanjutnya dikulturkan pada *Potato Dextrose Agar* (PDA) yang disuplementasi dengan chloramphenicol dan cycloheximide. Pengamatan makroskopis dan mikroskopis dilakukan untuk mengidentifikasi kedua genus jamur tersebut. Hasil penelitian menunjukkan terdapat delapan hewan yang terkena *Scopulariopsis* (40%), enam *Penicillium* (30%), dan empat ekor yang terkena koinfeksi (20%). Total prevalensi landak yang terkena jamur tersebut sebesar 90%. Penelitian lebih lanjut diperlukan untuk memperluas area *sampling*, mengidentifikasi jenis jamur lain, dan meningkatkan pemahaman tentang penyakit cendawan pada landak mini Afrika untuk memastikan keamanan landak di Indonesia.

Keywords: Landak mini Afrika, *Scopulariopsis, Penicillium,* dermatomikosis

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Bogor, July 2024

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I INTRODUCTION

1.1. Background

African pygmy hedgehog (*Atelerix albiventris*), also known as the four-toed hedgehog, is a species of hedgehog commonly kept as a pet by humans. African pygmy hedgehogs originate from the central Africa. The African pygmy hedgehog has a small quill and soft white fur on its stomach, which are their characteristics. African pygmy hedgehog is indigenous to equatorial Africa and can be found in savannas, steppes, agricultural fields, grasslands from Senegal to Ethiopia, and the Zambezi River. They are commonly used in biomedical research and are also popular as pets (Santana *et al.* 2010).

Recently, these animals have often become pets among Indonesian people. They are usually found in offline and online pet shops. Its small size, ranging from 17–25 cm (Santana *et al.* 2010), and lighter weight (Wissink-Argilaga 2020a) than cats and dogs are why this animal is kept. This animal is also suitable for pet owners who are busy during the day because it is active at night or nocturnal (Heatley 2009). However, they are quiet animals that do not take up ample space and do not need much attention. Plus, they can be interesting and entertaining companions. Their size allows owners to bring them anywhere outside to accompany the keeper.

Even though they are popular as pets because they are cute and can be carried everywhere, these animals can be agents of zoonotic. Hedgehogs can be asymptomatic and symptomatic carriers of zoonotic diseases (Le Barzic *et al.* 2021). The typical symptoms of dermatomycosis in hedgehogs are crusty lesions around the ears and face and hair loss. When they are in the external environment, especially outside their cage, these animals are at risk of being exposed to external diseases, both non-infectious and infectious diseases. Zoonotic diseases can be transmitted indirectly, through intermediaries or contaminated biological objects, or through direct contact, such as encountering animals infected with zoonotic skin diseases.

Fungal infections can be a serious threat to animals and humans. They can cause direct and indirect losses in terms of health, economics, and aesthetics in animals and humans (Lindahl and Grace 2015; Samanta 2015). Fungi can cause infections in several organs, such as the skin, respiratory system, nervous system, liver, and eyes (Xiao *et al.* 2020; Howell 2023). Every year, there are more than 150 million people suffer from severe cases of fungal infections worldwide, which lead to around 1.7 million deaths (Kainz *et al.* 2020). The most frequent fungal infections in humans are dermatomycoses, which involve skin, nail, and hair infections. These infections are frequently found in humans all around the world and may have significant impacts on public health and the economy. The infections can occur in humans but most commonly in livestock, pets, and wild animals. The causative agents include *Candida*, *Malassezia*, dermatophytes, and non-dermatophyte moulds (Gugnani 2022).

Non-dermatophyte fungi can infect and cause problems in humans and animals, including the genera *Scopulariopsis* and *Penicillium*. *Scopulariopsis* and *Penicillium* are two fungi commonly found in the environment, such as plants and soil, and are often considered contaminants (Rodríguez-Andrade *et al.* 2021; Torres-Garcia *et al.* 2022; Westblade *et al.* 2023).

Scopulariopsis is an emerging opportunistic fungus with high antifungal resistance. It is mainly associated with superficial infection of keratinised tissues in humans, namely onychomycosis (tinea unguium) with 10% of reported cases (Macura and Skóra 2015; Paredes *et al.* 2016; Fitzpatrick *et al.* 2018). Iwen *et al.* (2012) reported that invasive infections of *Scopulariopsis* in humans can affect various organs, including the skin, heart, brain, sinuses, lungs, and blood. Additionally, cases of disseminated disease have been reported, indicating that multiple organs throughout the body can be affected. In addition to humans, cases of infection in animals have also been reported. *Scopulariopsis* has been reported as the cause of infections in various animals such as goats (Ozturk *et al.* 2009), Japanese black calves (Ogawa *et al.* 2008), dogs (Sri-Jayantha *et al.* 2019), cats (Costa *et al.* 2019), and horses (Apprich *et al.* 2010).

Penicillium is a fungi genera that can be found all over the world, that is an occasional agent of human and animal mycoses (Guevara-Suarez *et al.* 2016). Fifteen species of *Penicillium* are known to cause opportunistic mycoses in immunocompromised humans (Zanatta *et al.* 2006). According to Westblade *et al.* (2023), this fungus can cause allergies and infections of the cutaneous, corneal, external ear, urinary and respiratory tract. Currently, there is no available information about these two fungal genera that can potentially cause zoonotic diseases from African pygmy hedgehogs in Indonesia. Therefore, further research must be conducted to identify and detect these fungi from African pygmy hedgehogs in Indonesia.

1.2. Problem Statement

The risk of spreading zoonotic diseases from African pygmy hedgehogs is a serious problem abroad. The lack of research on fungal infections in hedgehogs in Indonesia is one of the reasons this research was conducted. Hence, research is needed to determine the type of fungi that can be found on the skin of African pygmy hedgehog and their prevalence to achieve good maintenance management and prevention of spread.

1.3. Objectives of the Study

This study aims to determine the presence of *Scopulariopsis* and *Penicillium* in African pygmy hedgehogs (*Atelerix albiventris*) skin and their prevalence in Dhonje Golden Farm, Bogor.

1.4. Benefits of the Study

This study is expected to be helpful for researchers, to give valuable additional and supporting information regarding fungal infection in African pygmy hedgehogs' skin, and as the basis for future studies. It is also hoped that it will be useful for hedgehog owners, as a reference for animal care management, as well as increasing their awareness of fungal zoonotic diseases that can be transmitted from African pygmy hedgehogs.

II LITERATURE REVIEW

2.1 African Pygmy Hedgehogs (*Atelerix albiventris*)

African pygmy hedgehogs originate from Africa and are currently popular as exotic pets. The African pygmy hedgehog is a fearful, nocturnal, and insectivorous mammal species. This species, when frightened, curls up in a protective posture and shows its quills (Maguire *et al.* 2021). African pygmy hedgehogs are easily recognised by the small quills on their backs and the fine hairs on their bellies. African pygmy hedgehogs are characterised by a white colour on their stomach and soles of their feet (Ramadita and Paramita 2019). Their body size is small, ranging from 17–25 cm. In addition to being employed in scientific research, African pygmy hedgehogs are also gaining popularity as unusual pets (Santana *et al.* 2010). The taxonomy classification of the African pygmy hedgehog is as follows:

Order	: Erinaceomorpha
Family	: Erinaceidae
Subfamily	: Erinaceinae
Genus	: *Atelerix*
Species	: *Atelerix albiventris* (Santana *et al.* 2010).

Figure 2.1 White-bellied four-toed hedgehog (*Atelerix albiventris)* in a curled (left) and supine (right) position (Simone-Freilicher and Hoefer 2004; Frantz *et al.* 2020)

2.2 Diseases that Commonly Occur in African Pygmy Hedgehogs

According to Hedley (2014), various diseases commonly occur in African pygmy hedgehogs, including skin, gastrointestinal, respiratory, and miscellaneous diseases such as neoplasia. Hedley (2014), reported that tumour is common in pet hedgehogs (29– 51.5%), especially in epithelial tumours. Pet hedgehogs can get gastrointestinal infections from bacteria or fungi such as *Salmonella*, *Candida,* and *Cryptosporidium*. Respiratory infections in upper and lower respiratory tracts caused by *Pasteurella multocida, Bordetella bronchiseptica*, and *Corynebacterium* have also been reported. Dermatitis that occurs in four-toed hedgehogs is commonly caused by fungi and parasites such as fleas, ticks, and mites (*Otodectes cynotis*) (Hedley 2014; Frantz *et al.* 2020; Patel and Mukherjee 2023). The presence of hedgehogs near humans and its numerous pathogens indicate that this species should be considered a significant element of the epidemiology of various zoonotic infections (Ruszkowski *et al.* 2021).

2.3 Dermatomycosis

Dermatomycosis is a skin infection caused by fungi that can occur in humans and animals. Fungi consist of moulds and yeasts. Yeasts are fungi composed of individual cells that reproduce through a process called budding. Conversely, moulds have thread-like structures called hyphae, which extend by apical extension. Hyphae originate from non-germinating yeast cells, have no constriction at the neck of the parent cell and have parallel sides along their length (Chen *et al.* 2020). A typical yeast that can infect both animals and humans is *Candida albicans*. *Candida albicans* is a yeast-like fungus that grows as both yeast and elongated cells resembling hyphae that form pseudo mycelium (R and Rafiq 2023).

Some mycoses, known as zoonoses, can be transmitted between animals and humans (Cabañes 2021). Mould can pose serious health risks both to humans and animals. Some types of moulds can produce mycotoxin, which can cause several side effects (Greeff-Laubscher *et al.* 2020). Moulds are a type of multicellular fungus whose life requires nutrients from the environment or its host, for example, the skin of both animals and humans. Hedgehogs can be reservoirs or carriers of fungi without showing symptoms, posing a risk of transmission to humans (Abarca *et al.* 2017).

2.4 *Scopulariopsis*

Scopulariopsis spp. are hyalohypomycetes moulds that have a structure that looks like *Penicillium* and usually can be found in the plant materials, soil, air, feathers, insects, food, and indoor environments (Sandoval-Denis *et al.* 2016; Westblade *et al.* 2023; Pérez-Cantero and Guarro 2019). Macroscopic characteristics of the colonies present a varied texture, including flat, velvety, or powdery, with colours ranging from white, tan, and dark brown. The fungus had microscopic features including hyaline and septate hyphae. Its conidiophores resembled a finger structure, from which annelids produced chains of conidia (Tunç *et al.* 2022). The taxonomy classification of the *Scopulariopsis* genus is as follows:

Kingdom	: Fungi
Phylum	: Ascomycota
Class	: Euascomycetes
Order	: Microascales
Family	: Microascaceae
Genus	: *Scopulariopsis* (Sandoval-Denis *et al.* 2016)

Figure 2.2 Macroscopic morphology of *Scopulariopsis* surface (left) and reverse (right) (Walsh *et al.* 2018)

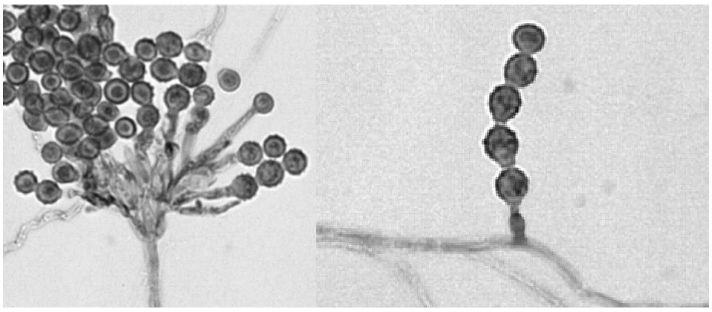

Figure 2.3 *Scopulariopsis brevicaulis* (left) and *S. brumptii* (right) under the microscope (Walsh *et al.* 2018)

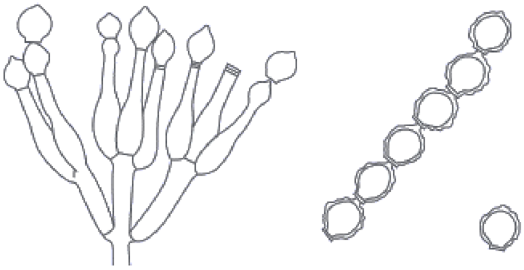

Figure 2.4 Schematic morphology of *Scopulariopsis*, penicillate-like structure and conidia in chains (Campbell *et al.* 2013)

There are several species of *Scopulariopsis* known, including *S. Asperula*, *S. flava*, *S. alboflavescens*, and *S. acremonium*, with the most common species are *S. brevicaulis* and *S. brumptii* (Westblade *et al.* 2023; Pérez-Cantero and Guarro 2019). *S. brevicaulis* produces rapidly growing powdery colonies, and tan to beige in colour. The reverse side of the colony is usually tan with a brown centre. According to Westblade *et al.* (2023), the difference between *S. brevicaulis* and *S. brumptii* morphology are in the colony colour, shape of annelids, and conidia, which are shown in the table below:

Table 2.1 Morphological difference of *Scopulariopsis brevicaulis* and *Scopulariopsis brumptii* (Walsh *et al.* 2018)

Species	Colony	Annellides			Conidia	
		Shape	Size (µm)	Arrangement	Diameter (µm)	Colour
S. brevicaulis	Light brown	Fairly cylindrical	2.5–3.5 × 9.0–25.0	Most in brushlike groups, some single	5.0-8.0	Hyaline or pale brown
S. brumptii	Dark grey or greyish brown	Like tenpins, narrowing at the apex	2.5–3.5 (at base) × 5.0–10.0	Most single, some in groups	3.5-5.5	Dark brown to blackish

Scopulariopsis spp. tend to show resistance to various antifungal medications such as fluconazole, flucytosine, and itraconazole. They somewhat susceptible to miconazole, ketoconazole, and amphotericin B (Nucci and Anaissie 2009). *Scopulariopsis* is a type of fungi found to cause various health issues in animals. For instance, it has been linked to hair loss in goats (Ozturk *et al.* 2009), hyperkeratosis in Japanese black calves (Ogawa *et al.* 2008), rhinosinusitis in dogs (Sri-Jayantha *et al.* 2019), sino-orbital infection in cats (Costa *et al.* 2019), and hoof infections in horses (Apprich *et al.* 2010). Furthermore, *Scopulariopsis* has been isolated from the beak and lungs of canaries (Tunç *et al.* 2022).

Figure 2.5 Hyperkeratosis on the hindlimb of Japanese black calf (left) and hair loss in goats (right) caused by *Scopulariopsis brevicaulis* (Ogawa *et al.* 2008; Ozturk *et al.* 2009)

2.5 *Penicillium*

Penicillium is a commonly observed genus of fungi worldwide, has a significant economic impact, and is an occasional agent of human and animal mycoses (Guevara-Suarez *et al.* 2016). They are usually found in the environment including plant debris, soil, air, and food products (Torres-Garcia *et al.* 2022). According to Visagie *et al.* (2014), There are at least 354 accepted species of the *Penicillium* genus. The taxonomy classification of *Penicillium* genus is as follows:

Kingdom : Fungi
Division : Ascomycota
Class : Eurotiomycetes
Order : Eurotiales
Family : Trichocomaceae
Genus : *Penicillium* (Prayuda *et al.* 2023)

A floccose texture and bluish-green to green colouration with white edges characterise the macroscopic appearance of *Penicillium* colonies. Clear exudate droplets are usually observed. The colony's reverse colour is beige to beige-brown on various agar media such as Malt Extract Agar (MEA) and Czapek Yeast Autolysate agar (CYA) (Demjanová *et al.* 2020).

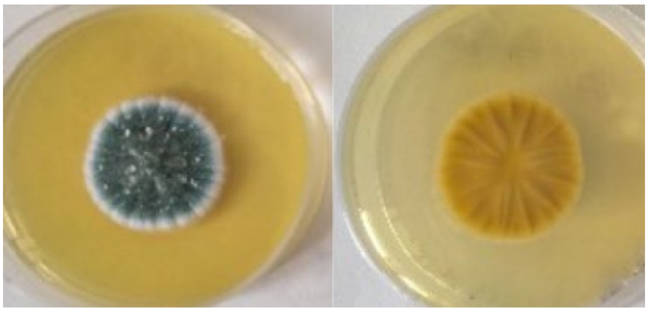

Figure 2.6 Macroscopic morphology of *Penicillium* surface (left) and reverse (right) (Demjanová *et al.* 2020)

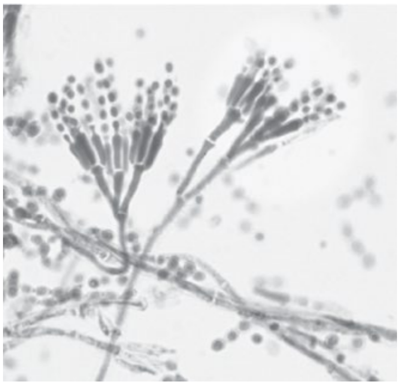

Figure 2.7 Morphology of *Penicillium* under microscope (Westblade *et al.* 2023)

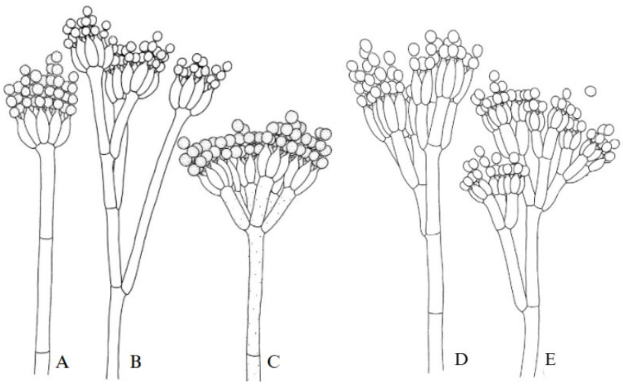

Figure 2.8 Type of conidiophore branching in *Penicillium*: monoverticillate (A), divaricate (B), biverticillate (C), terverticillate (D), and quarterverticillate (E) (Visagie *et al.* 2014a).

Penicillium itself has morphological characteristics, namely in the form of septate hyphae, branched mycelium, usually colourless, septate conidiophores, the head of the spore-bearing hyphae is shaped like a broom, with Sterigmata or phialides appearing in groups, the conidia are chain-shaped (Sudipa and Gelgel 2022). The morphology of *Penicillium* can be divided based on the degree of branching of the conidiophores within the genus. There are several branching type of classification: monoverticillate, divaricate, biverticillate, terverticillate, and quarterverticillate (Visagie *et al.* 2014a). The differences in each morphological branching type are determined based on the growth structure patterns of the conidiophore, metulae, and phialide (Figure 2.3).

2.6 Fungal Culture

Fungal culture is necessary to explore existence, biology, and morphology of fungi. It can be done in various agar media such as potato dextrose agar (PDA), potato flake agar, rice starch agar, Sabouraud dextrose agar, and dermatophyte test medium. PDA is a media commonly used to isolate fungi in the laboratory and is categorised as a semi-synthetic medium. PDA contains potatoes as a natural component and dextrose and agar as synthetic (Nurdin and Nurdin 2020). Potatoes are a source of carbohydrates, vitamins, and sugar that promote microbial growth. On the other hand, dextrose is an energy source and solidifies the PDA medium. These three components, potato, dextrose, and agar, are necessary for the growth and reproduction of microorganisms, especially fungi (Arifah 2019).

Subculturing can be done to obtain pure cultures from the initial agar (Mayser 2007). Subculturing fungi is used to evaluate their viability, morphology, and dimorphic ability, with preservation methods influencing these traits (Lima and Borba 2001). It involves transferring a small amount of fungal material, such as mycelium or spores, from one culture to another.

Antibiotics in the media aim to reduce bacterial contamination (Cardoso *et al.* 2015). Chloramphenicol is primarily an antibiotic used to inhibit bacterial contamination. This antibiotic may also prevent the growth of aerobic actinomycetes (Walsh *et al.* 2018). Cycloheximide can also be used on agar media with chloramphenicol (Weitzman and Summerbell 1995). Cycloheximide, also known as actidione, is used in selective media to inhibit the fast-growing growing saprobic fungi that may cover the slow-growing primary pathogens. Several fungi are inhibited by cycloheximide, including most *mucormycetes*, many *Candida* species, *Cryptococcus neoformans*, *Neoscytalidium dimidiatum*, *Aspergillus* spp., *Fusarium* spp., *Lomentospora prolificans*, and *Talaromyces marneffei* (Westblade *et al.* 2023).

2.7 Animal Care Management

African pygmy hedgehog (*Atelerix albiventris*) is a species kept by researchers, pet traders, pet owners, and breeders for different purposes. Keepers must know how to provide appropriate housing and care for hedgehogs, as they are sensitive animals that can easily become stressed. Stressed animals have higher maintenance requirements, lower production, and increased risk of disease and poor health (Collier *et al.* 2017).

Smooth floors and walls are required for housing so it can be cleaned thoroughly. Wire cages should be avoided because African pygmy hedgehogs can be injured by them. Hedgehogs are solitary and active animals, so they should be placed independently to prevent aggressive behaviour with others (Hedley 2014). A hedgehog cage is recommended to have a minimum size of 0.6 m × 0.9 m (Wissink-Argilaga 2020a). Stress is common among this animal, so a small shelter or box is recommended inside the housing as a hiding place. Deep bedding, such as wood shavings, should be provided to prevent urine and faeces contamination (Santana *et al.* 2010). Hedgehogs are messy in the cage, so bedding should be cleaned routinely. Fresh water should continuously be available inside the cage (Wissink-Argilaga 2020a).

Hedgehogs often travel long distances in the wild, so it is essential to exercise to avoid captivity problems such as being overweight. It is also related to the importance of the food offered volume limitation. The night is their feeding time, and in the early morning, leftover food should be thrown away (Hedley 2014). The hedgehog environmental temperature had better be sustained at 24–30 °C to keep them comfortable at adequate temperatures and to avoid heatstroke (Ramadita and Paramita 2019; Wissink-Argilaga 2020b). Radiant heat bulbs or thermostatically controlled heat mats can be used to gain the required temperature (Wissink-Argilaga 2020a)

III METHODS

3.1 Time and Place

The samples were collected from Dhonje Golden Farm located in Bogor District. The research was carried out at the Mycology Laboratory of the School of Veterinary Medicine and Biomedical Sciences, IPB University, from December 2023 to April 2023. The research work began with proposal preparation and ended with the completion of the study.

3.2 Methodology

3.2.1 Sample Size and Code

The sample size used in this research was 20 samples, of which these samples are the entire population on the farm. Each sample obtained from pygmy hedgehogs was marked with a different code label. The code consists of numbers and letters, and the numbers define the identity of the first animal to the last (20th animal). Meanwhile, the letters indicate the sampling technique used. Three letters are used as the code: A and B. Letter A means sampling the skin scraping technique, and B uses the McKenzie technique. For example, code 20B means that the number indicates the 20th animal sampled, and the letter B indicates the technique used; skin scraping.

3.2.2 Clinical Symptom Record

The clinical lesions of the hedgehog skin, including alopecia, scaling, crusting, and dermatitis were assessed and scored following the previous report (Bexton and Nelson 2016).

Table 3.1 Scoring method for dermatological lesions clinical examination in hedgehogs with fungal infection.

Clinical condition	Score
Alopecia	
Absent	0
Small focal solitary lesion	1
More significant focal solitary lesion or mild multifocal lesion	2
Moderate multifocal or scattered alopecia	3
Severe multifocal or scattered alopecia	4
Scaling/Crusting	
Absent	0
Small focal area	1
Mild multifocal areas	2
Moderate multifocal areas	3
Severe crusting, exudate	4
Dermatitis	
Absent	0
Minimal skin inflammation/erythema	1
Moderate to severe skin inflammation, open wound	2

Modified from (Bexton and Nelson 2016) *Vet Dermatol*. 27(6): 500.

3.2.3 Anamneses and Identity Record

The identity of the hedgehogs was recorded, starting from age, sex, and body weight or body condition score (BCS). Apart from that, external factors such as where the animal was obtained (animal origin), daily activities such as eating patterns, sleeping/bedding patterns, and history of maintenance and medication administration are also recorded. Pictures of the animal sample are taken, which could be the lesion or the characteristic finding that can be found on them.

3.2.4 Sample Collection

The sample collection procedures conducted in this research were aligned with ethical standards and good veterinary practices. However, ethical clearance was waived because the study did not include any experimental procedures that might cause pain, suffering, distress, or long-term harm equal to or greater than that caused by needle use.

3.2.4.1 Handling technique

Handling hedgehogs can be difficult as they tend to curl up into a ball when stressed or frightened. The examination should be conducted in a quiet and dimly lit room to help the animal relax. Since they are nocturnal, handling them in the evening is recommended so as not to interfere with their habits (Wissink-Argilaga 2020b).

Uncurling a hedgehog is a difficult task, and numerous ways have already been attempted, including slowly elevating the rear legs and wheelbarrowing, as well as immersing the hedgehog in shallow water (Bexton and Couper 2019; Wissink-Argilaga 2020b). Cupping the hedgehog’s hands inward and gently swinging the hedgehog can also relax the hedgehog so that it releases its spines or quills backwards.

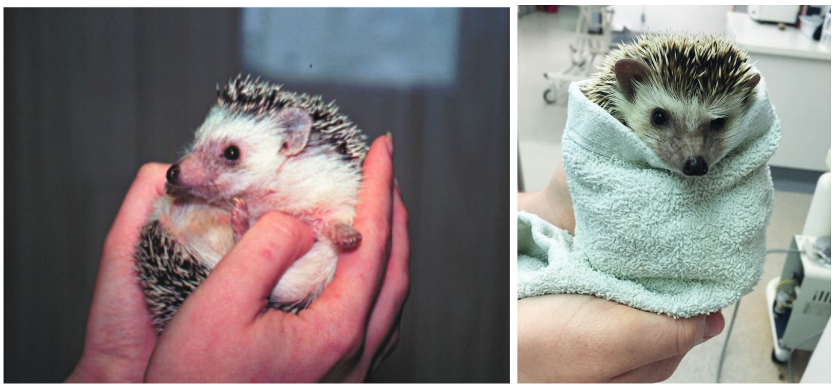

Figure 3.1 Procedure of how to handle a tame hedgehog that does not feel discomfort (Heatley 2009; Wissink-Argilaga 2020b)

Figure 3.2 Wheelbarrow posture of a hedgehog (Hedley 2014)

Some hedgehogs will readily unroll if socialised from a young age, yet many people will be more cautious in strange situations. It takes time and patience for a hedgehog to gain trust and investigate its surroundings. If the hands-off method fails, firmly stroking the vertebral column from head to tail may cause unrolling. Grabbing and extending the hind limbs in a ‘wheelbarrow’ stance (Figure 3.2) can be performed after partially unrolling for a short examination (Hedley 2014).

3.2.4.2 Skin scrap technique

The skin scrap technique used in this study used small tweezers. The tweezers to be used are sterilised first in an autoclave. Skin suspected to have crusty lesions is taken by scratching or swiping with the tip of sterilised tweezers. Meanwhile, flaky textured skin is taken by pinching it with a tweezer. Both techniques are done by opening the space between its spines or quills. According to Kurade *et al.* (2006), in the skin scrap technique, scraping should be moved across the edge of the lesion while pulling up the skin above the site with the other hand. Scrapings must be taken from the outside edge of the lesion at right angles to the skin. Areas adjacent to papules, crusting, or lesions are good to sample. Sufficient skin samples should be taken based on the research conducted. Insufficient or inadequate samples can produce inaccurate results that may show false negatives (Gautam and Bhatia 2020). Samples should be placed into a sterile container, delivered to the laboratory, and can be stored at room temperature (25 °C).

3.2.4.3 McKenzie brush technique

A sterilised toothbrush is carefully brushed over the suspected area, including the skin and hair edges of alopecic or crusty areas. The sampling process using the toothbrush technique is carried out at least 10 times or for 1 minute (Mozes *et al.* 2017). In this study, this process was carried out for 3 minutes. The unaffected area is brushed first, then the lesions second to avoid dispersal of the spores to unaffected areas and to avoid losing spores from affected areas. Once the samples were collected, each toothbrush was carefully placed inside its plastic bag (Santana *et al.* 2020).

3.2.5 Direct Examination

Examination under the microscope using potassium hydroxide (KOH 10%) is a rapid diagnostic method but has low sensitivity and specificity (Wijaya *et al.* 2021). Direct microscopic examination is carried out by preparing preparations by placing a skin scraping sample on an object glass. After that, the sample was dripped with 10% KOH, and covered with a cover glass. The microscope is used to examine samples for fungal elements, including hyphae and spores, at 10× and 40× magnification.

3.2.6 Fungal Culture

The fungal culture samples used were obtained by using the McKenzie toothbrush technique. The toothbrush is touched to potato dextrose agar (PDA) supplemented with chloramphenicol and cycloheximide, in a circular pattern starting from the middle area to the outside and then incubated at room temperature (25 °C) for up to 14–28 days.

Once the fungal colony has grown, subculturing can be done to obtain pure culture if more than one type of fungus is observed macroscopically. This procedure involves transferring a small amount of the colony onto a fresh agar plate. The plates may need to be sealed or covered to prevent contamination from airborne spores (Mayser 2007). After obtaining pure cultures, further identification techniques, such as microscopy examination can be carried out to determine the species of the cultured fungi.

3.2.7 Macroscopic Examination

Macroscopic examination was carried out on each sample cultured on agar media. The appearance of isolates, such as colour, texture and shape, can provide valuable information for identification, especially characteristics that lead to *Scopulariopsis* and *Penicillium*.

Scopulariopsis isolates can be recognized by their typical appearance of white, glabrous or powdery light brown with a light tan periphery on the surface and a tan colour on the reverse side. In contrast, *Penicillium* can be identified by its powdery bluish-green surface, with or without a white margin, and its reverse side can vary in colour, such as white, yellow, orange, or red (Walsh *et al.* 2018).

3.2.8 Microscopic Examination

Identification of isolates suspected to be *Scopulariopsis* and *Penicillium* was carried out by examining their morphological characteristics under the microscope using Lactophenol Cotton Blue (LPCB) stain (Kaur *et al.* 2015). Microscopic examination was done by touching the sample grown on agar media using tape attached to the tip of the inoculation needle on a glass object. The tape was then dripped with

96% alcohol and attached to a slide dripped with LPCB. The object glass was then dripped with LPCB one more time before finally being covered with a cover glass. Under the microscope, the characteristics or elements of *Scopulariopsis* or *Penicillium*, such as conidia and hyphae are observed in 10x, 40x, or higher objective magnification.

3.3 Data Analysis

The resulting data from the procedure is processed and analysed descriptively by identifying each sample from macroscopic morphology such as colour, texture, size or diameter on the media, and growth speed of the fungus inoculated daily for 14–28 days. Analysis was also carried out on morphological data and microscopic characteristics of fungi, such as the number and size of conidia and hyphae for each sample. Isolated fungi were identified using previously published textbooks (Campbell *et al.* 2013; Walsh *et al.* 2018). The prevalence of each fungus is calculated after the type of each fungus is identified using the prevalence formula.

IV RESULT AND DISCUSSION

4.1 African Pygmy Hedgehog

A total of twenty hedgehogs were used in this study with various physical conditions. Most hedgehogs were obtained from outside the farm, but there were also four baby hedgehogs (APH6, APH7, APH8, APH9) born from breeding hedgehogs on the farm. Table 4.1 shows the status present and the results of three parameters for assessing the skin condition, namely alopecia, crusty, and dermatitis, of each hedgehog subjectively, along with data on sex, body weight, and origin.

Table 4.1 Data of cage, status presents, lesions, and severity of each African pygmy hedgehog in the farm

Cage	Code of Animal	Sex	Weight (gram)	Origin	Length of Stay	Lesion		
						A	C	D
L1	APH1	Female	281	n/a	±2 months	2	2	0
L2	APH2	Female	202	n/a	±2 months	1	3	2
L2	APH3	Male	204	n/a	±2 months	1	2	2
L3	APH4	Female	395	n/a	±2 months	1	2	0
L3	APH5	Male	262	n/a	±2 months	1	3	1
L4	APH6	Male	18	Breeding	±1 week	0	1	0
L4	APH7	Female	14	Breeding	±1 week	0	1	0
L4	APH8	Male	16	Breeding	±1 week	1	2	0
L4	APH9	Female	18	Breeding	±1 week	1	1	0
L4	APH10	Female	202	n/a	±2 months	0	1	0
L5	APH11	Male	88	n/a	±1 month	1	1	1
L6	APH12	Male	238	n/a	±2 months	0	2	0
L6	APH13	Female	314	n/a	±2 months	2	2	0
L7	APH14	Female	228	n/a	±2 months	0	2	1
L8	APH15	Female	416	n/a	±2 months	1	2	0
L8	APH16	Male	305	n/a	±2 months	1	1	0
L9	APH17	Male	89	n/a	±1 month	0	1	0
L9	APH18	Female	109	n/a	±1 month	0	1	0
L9	APH19	Male	88	n/a	±1 month	1	1	0
L9	APH20	Female	274	n/a	±2 months	0	2	0

Note: APH1 until APH 20 means: 1st African pygmy hedgehog until 20th African pygmy hedgehog; n/a: not available; A: Alopecia; C: Crusty; D: dermatitis

Based on the data above, 9 male and 11 female hedgehogs are stated in this study. The hedgehog population consists of 12 adult hedgehogs (lived > 2 months, BW > 200 g), and 8 young hedgehogs consisting of 4 baby hedgehogs (1 week, BW < 20 g) and 4 juvenile hedgehogs (1 month, BW < 110 g). The average weight of adult hedgehogs is 276 grams, while young hedgehogs are 55 grams.

There were twelve hedgehogs (60%) who experienced alopecia, two (10%) of them with a severity level of 2, and ten (50%) of them with a severity level of 1 or the lowest (Figure 4.1.A). All hedgehogs (100%) had crusty, flaky skin. There were two hedgehogs (10%) who experienced crustiness with a severity level of 3, nine hedgehogs (45%) with a severity level of 2, and nine hedgehogs (45%) with the lowest severity level (Figure 4.1.B and Figure 4.1.C). Dermatitis was only experienced by five hedgehogs (25%), consisting of three hedgehogs (15%) with severity level 1 and two hedgehogs (10%) with severity level 2 as open wounds (Figure 4.1.D). Apart from that, four hedgehogs (20%) were also found to have open wounds that had hardened or dried and swelling on the feet, the area around the face, and the tips of the earlobes.

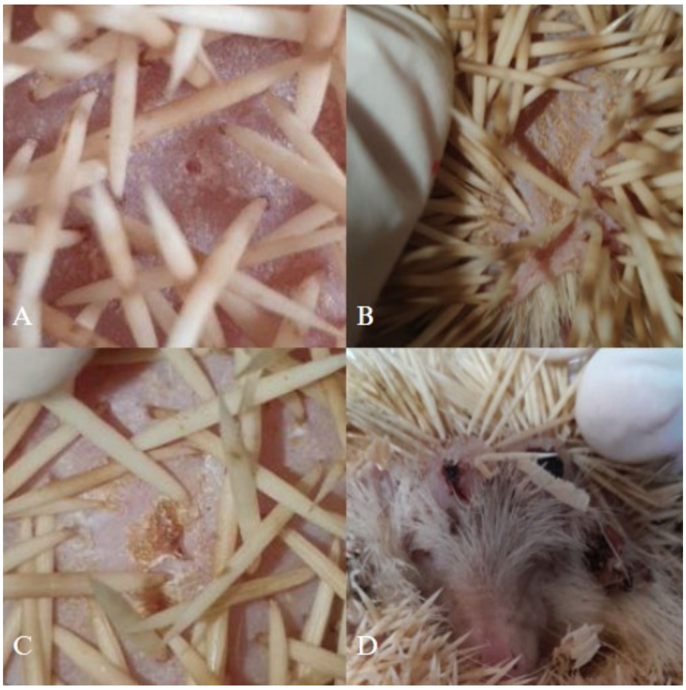

Figure 4.1 Skin condition of African pygmy hedgehogs sampled. (A) Level 2 alopecia in APH13. (B, C) Level 2 crusty in APH12 and APH13. (D) Level 2 dermatitis in APH2.

All male and female hedgehogs exhibited clinical symptoms such as alopecia, crusty, or dermatitis, both young and adults. These clinical symptoms are not always caused by fungi alone (Fehr 2015), but can also be caused by including mites (Budiono *et al.* 2023), bacteria (Blume *et al.* 2021), and nutritional deficiencies (Wissink-Argilaga 2020b).

In this study, crusty was characterised by the appearance of dry, rough-textured skin with flaky skin. Crusty in African pygmy hedgehogs are mainly caused by fungi such as dermatophyte and mite infections caused by *Caparina tripilis* (Bexton and Couper 2019). However, according to Heatley (2009), mild crusty which looks flaky, is somewhat a normal skin condition of African pygmy hedgehog. No available report that mentioned *Scopulariopsis* or *Penicillium* can cause this lesion in hedgehogs. However, Ogawa *et al.* (2008) reported hyperkeratosis lesions due to *Scopulariopsis* infection in a calf.

Dermatitis is a skin inflammation due to internal or external factors, causing clinical disorders such as erythema (Ritonga 2016). Dermatitis is related to various factors and agents such as fungi, ectoparasites, bacteria, and metabolic disease (Ayukhaliza *et al.* 2019; Prayuda *et al.* 2023). This study showed dermatitis on the skin of hedgehogs with redness and open wounds. Erythema skin in African pygmy hedgehogs can occur due to traumatic injuries, irritation from cage conditions, or allergies. Traumatic injuries can cause open wounds, as reported in two hedgehogs in this study. According to the farm owner and keeper, open wounds on the hedgehogs on this farm usually occur due to the natural behaviour of the hedgehogs during the breeding season, when one hedgehog is incompatible with their cage mate, which may result in fighting. A similar statement was also made by Santana *et al.* (2010), captive African pygmy hedgehogs, cannot be housed together, especially males, as they will fight, causing serious injury with their teeth and quills.

Alopecia in African pygmy hedgehogs are characterised by quill loss which represents an unhealthy skin condition. In this study, alopecia can occur due to the handling or sampling techniques. The toothbrush' bristles caught the quills of several African pygmy hedgehogs easily. A healthy hedgehog's quills do not fall off easily (Wissink-Argilaga 2020a), so it should not fall out easily when sampling with a toothbrush. Alopecia can also be a sign of mite infection, bacterial infection, or due to diet (Wissink-Argilaga 2020b; Blume *et al.* 2021; Budiono *et al.* 2023). No available report that mentioned *Scopulariopsis* or *Penicillium* can cause this lesion in hedgehogs. However, studies reported that *Scopulariopsis* can cause hair loss in goats and rats (Ozturk *et al.* 2009; Yapicier *et al.* 2020).

Scopulariopsis and *Penicillium* are keratinophilic fungi which means able to invade and degrade keratinised tissues (Hamm *et al.* 2020; De Macedo dan Freitas 2021; Hamada *et al.* 2024). These keratinised tissues can be found in the skin, hair, and quill of African pygmy hedgehogs (Crofts and Stankowich 2021). Other than that, *Scopulariopsis* and *Penicillium* were the ones that grew the most in each agar plate in this study. According to Watanabe *et al.* (2022), cutaneous fungal infections are considered to have occurred and are suspected when five or more of the same colonies grow on a single agar plate medium. There is a possibility that *Scopulariopsis* and *Penicillium* are the ones that cause alopecia, crusty, or dermatitis in this study due to the number of the colonies and their characteristic that can grow in keratin tissue.

4.2 Direct Examination

Direct examination is done as a screening procedure to detect the presence of fungi on the skin. Thirteen samples were taken from 20 African pygmy hedgehogs in this study using the superficial skin scraping technique to conduct direct examination. All adult hedgehogs were sampled using the skin scraping technique, while only one young hedgehog represented a small hedgehog group was sampled due to their thin skin condition. Direct examination preparations are made from skin samples taken using the skin scraping technique.

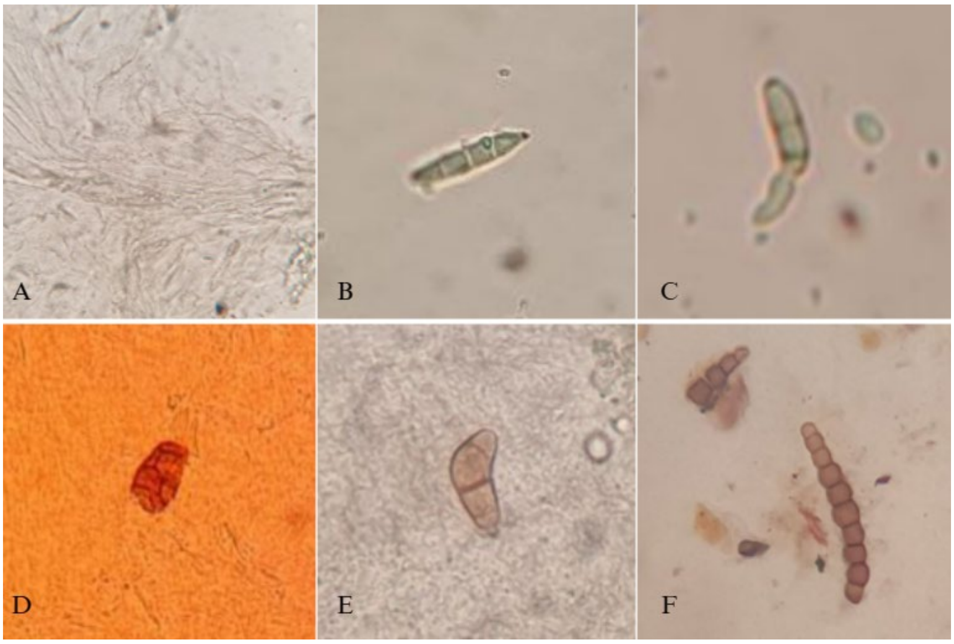

Figure 4.2 Fungal elements found in direct examination at 40× magnification. (A) Hyphal structure in sample 2A. (B, C) Three-celled macroconidia in sample 3A and 16A. (D) Transverse and longitudinal septate macroconidia in sample 4A suspected to be *Alternaria*. (E) Curved-shaped macroconidia in sample 13A suspected to be *Curvularia*. (F) Brown chlamydoconidia in chain in sample 15A.

In this study, hyphae were found in all thirteen skin scraping samples, and several samples were found to show microconidia, macroconidia, and chlamydoconidia structures (Figure 4.2). However, the type of fungus found could not be confirmed with certainty during this direct examination, especially the existence of the two genera to be studied. The results of the direct examination did not show specific *Scopulariopsis* and *Penicillium* fungal infections. According to previous identification textbooks, in this study, the fungal elements found in direct examination led to several fungal genera, namely *Altenaria*, *Curvularia*, *Candida*, and types of fungi that can produce macroconidia such as dermatophytes (Campbell *et al.* 2013; Walsh *et al.* 2018). Other agents than fungi like ectoparasite were not found in this procedure.

4.3 Identification of Isolated Fungi

Fungi were identified based on the results of macroscopic and microscopic examination. Table 4.2 shows the results of fungal growth on PDA with cycloheximide from 20 McKenzie toothbrush samples. This study revealed that 278 isolates were obtained from PDA supplemented with chloramphenicol and cycloheximide (BCC). The macroscopic morphology that leads to *Scopulariopsis* and *Penicillium* on BCC media was observed and subcultured on fresh agar containing chloramphenicol to obtain pure and uncontaminated isolates.

Table 4.2 Number of isolates grew on PDA supplemented with chloramphenicol and cycloheximide

Code of Animal	BCC	
	Code of Plate	Number of Isolate(s)
APH1	1BCC	16
APH2	2BCC	n/a
APH3	3BCC	11
APH4	4BCC	n/a
APH5	5BCC	5
APH6	6BCC	26
APH7	7BCC	41
APH8	8BCC	40
APH9	9BCC	27
APH10	10BCC	20
APH11	11BCC	20
APH12	12BCC	24
APH13	13BCC	24
APH14	14BCC	1
APH15	15bCC	11
APH16	16BCC	1
APH17	17BCC	2
APH18	18BCC	3
APH19	19BCC	4
APH20	20BCC	2
Total		278

Note: n/a; not available, BCC; Agar plates contain chloramphenicol and cycloheximide

Of the 278 fungal isolates that grew on media supplemented with chloramphenicol and cycloheximide, 41 were subcultured due to their morphology representing *Scopulariopsis* and *Penicillium*. There are 16 isolates identified as *Penicillium* and 25 isolates identified as *Scopulariopsis*. Both fungi grew widely on PDA agar media supplemented with cycloheximide, which showed resistance to the semi-selective agent, followed by subculturing onto new agar. The results of *Scopulariopsis* and *Penicillium* subculture isolates can be seen in Table 4.3.

This study used a combination of chloramphenicol and cycloheximide on the PDA media. Chloramphenicol is used to inhibit the growth of bacteria whereas cycloheximide is used to inhibit the growth of contaminant saprophytic fungi (Tariq *et al.* 2024). According to Hamm *et al.* (2020) and Hamada *et al.* (2024), keratinophilic fungi isolation, including *Scopulariopsis* and *Penicillium*, is difficult to grow on PDA because the growth rate is generally less than other indoor fungi. This study found that PDA with cycloheximide and chloramphenicol can be used to isolate *Scopulariopsis* and *Penicillium* from hedgehog skin samples. These results are similar to El-Said *et al.* (2009), who reported the growth of cycloheximide-resistant fungi, including *Scopulariopsis* and *Penicillium*. *Scopulariopsis* is highly resistant to antifungal therapies due to its intrinsic resistance. Some reports also described that several antifungal therapies have also been reported to show poor efficacy in experimental in vivo studies in murine models (Paredes *et al.* 2016; Pérez-Cantero and Guarro 2019).

Table 4.3 Macroscopic morphology of *Scopulariopsis* and *Penicillium* on subculture plates

Sample Code	Genus	Colony				
		Colour	Texture	Reverse Colour	Topography	Diameter (mm)
1bcc1	*Penicillium*	G	P	Y	Rf	16
1bcc8	*Penicillium*	G	P	Y	Fl	17
3bcc4	*Penicillium*	Bg	V	W	Fl	24
5bcc3	*Scopulariopsis*	Wt	Gl	T	Rg	12
5bcc5	*Scopulariopsis*	Wt	Lf	W	Rg	12
6bcc4	*Scopulariopsis*	B	Gl	T	Fl	17
7bcc1	*Scopulariopsis*	B	Gl	T	Fl	16
7bcc7	*Scopulariopsis*	Wt	Gl	Yt	Rg	13
7bcc14	*Scopulariopsis*	C	Gl	Wt	Rg	15
7bcc18	*Scopulariopsis*	Wt	Gl	T	Fl	15
7bcc19	*Scopulariopsis*	B	Gl	T	Fl	15
7bcc29	*Scopulariopsis*	Wt	Gl	W	Rg	13
7bcc31	*Scopulariopsis*	B	Gl	T	Fl	24
8bcc7	*Scopulariopsis*	Wt	Gl	T	Rg	14
8bcc14	*Scopulariopsis*	Wt	Gl	W	Rg	13
8bcc16	*Scopulariopsis*	B	Lf	T	Fl	15
8bcc26	*Scopulariopsis*	B	Lf	T	Fl	15
9bcc16	*Scopulariopsis*	B	Lf	T	Fl	14
10bcc1	*Penicillium*	Bg	P	W	Fl	22
10bcc2	*Penicillium*	Bg	P	W	Fl	20
10bcc3	*Penicillium*	G	P	W	Fl	15
10bcc12	*Scopulariopsis*	B	Lf	Tb	Fl	13
11bcc1	*Penicillium*	Bg	V	W	Fl	20
11bcc3	*Scopulariopsis*	B	Lf	Wt	Rg	15
12bcc8	*Penicillium*	G	P,	W	Fl	13
13bcc1	*Penicillium*	G	P	W	Rf	17
13bcc4	*Penicillium*	G	P, F	W	Fl	19
13bcc6	*Scopulariopsis*	Wt	Gl	W	Fd	13
13bcc13	*Scopulariopsis*	B	Lf	T	Fd	11
14bcc1	*Scopulariopsis*	B	Lf	T	Fd	10
15bcc1	*Penicillium*	G	P	Y	Fl	14
15bcc3	*Penicillium*	Bg	Lf	W	Fl	20
15bcc5	*Scopulariopsis*	W	Lf	W	Fd	12
16bcc1	*Penicillium*	Bg	P	Y	Rg	15
17bcc1	*Scopulariopsis*	B	Lf	T	Fl	13
17bcc2	*Scopulariopsis*	B	Lf	T	Fl	11
18bcc2	*Scopulariopsis*	W	Lf	W	Fd	11
18bcc3	*Scopulariopsis*	B	Lf	T	Fd	16
19bcc1	*Penicillium*	Bg	Lf	W	Fl	20
20bcc1	*Penicillium*	Bg	P, Lf	W	Fl	19
20bcc2	*Penicillium*	G	P	R	Fl	16

Note: **Colour:** B: Brown; Bg: Bluish-green; C: Cream; G: Green; R: Red; T: Tan; Tb: Tan-brown; W: White; Wt: White-tan; Y: Yellow; Yt; Yellow-tan: **Texture:** P: Powdery; V: Velvety; F: Floccose; Lf: Loosely floccose; **Topography:** Rf: Radial fold; Fl: Flat; Rg: Rugose; Gl: glabrous; Fd: folded in the middle

The macroscopic morphological characteristics of *Scopulariopsis* isolates observed in this study are represented by three isolate codes, namely 13BCC13, 15BCC5, and 18BCC3. Isolate 13BCC13 has a cream-brown surface and a loosely floccose texture with a light margin. Meanwhile, its reverse is light tan. Isolate 13BCC13 and 18BCC3 look similar, but isolate 18BCC13 has a darker surface and reverse colour with a glabrous-loosely floccose texture. On the other hand, 15BCC5 shows a significant difference with a white surface and loosely floccose texture. Figure 4.3 and Table 4.3 show that *Scopulariopsis* isolates have white to brown surfaces with light tan margins. The texture is glabrous to loosely floccose, and the topography is flat, rugose, or with a folded centre. The colour of the reverse fungi is varied, such as white, tan, and tan with a browner centre which is similarly described by other studies (Yapicier *et al.* 2020; Westblade *et al.* 2023). This mould produced two brown colour levels, such as light brown and dark brown. *Scopulariopsis* isolates in this study showed a surface colouration of white to brown, with a light tan margin, a flat with a folded centre topography, and a texture varying from glabrous to loosely floccose. The diameter growth of *Scopulariopsis* in the first week ranged from 10–17 mm with an average diameter of 13.56 mm. Studies reported that *Scopulariopsis* diameter on SDA and glucose peptone agar can grow up to 20 and 50 mm, respectively, after 7 days, at 30°C (Iwen *et al.* 2012; Campbell *et al.* 2013). This result shows that the diameter of the fungi varies depending on the media used and the temperature of the environment.

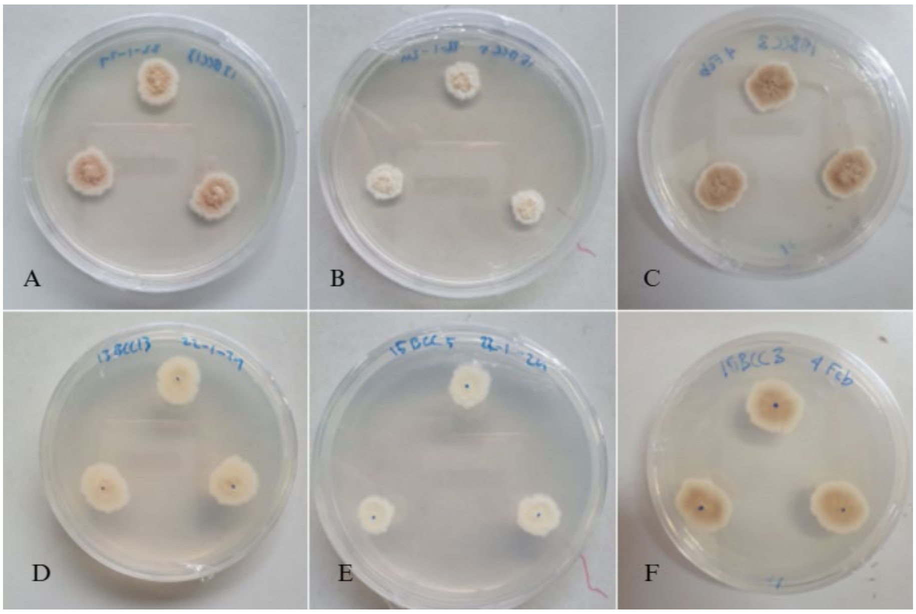

Figure 4.3 Macroscopic morphology of *Scopulariopsis* colonies after 7 days. Surface of 13BCC13 (A), 15BCC5 (B), and 18BCC3 (C). Reverse of 13BCC13 (D), 15BCC5 (E), and 18BCC3 (F)

Further microscopic observation was conducted using a lactophenol cotton blue (LPCB) stain. *Scopulariopsis* showed a microscopic structure that looks like a *Penicillium*. The structure consists of septate hyphae, branched annelids, and smooth- and rough-walled conidia in chains that are consistently found in most samples (Figure 4.4). The annelids may be solitary or in a group that forms a finger-like conidiophore structure.

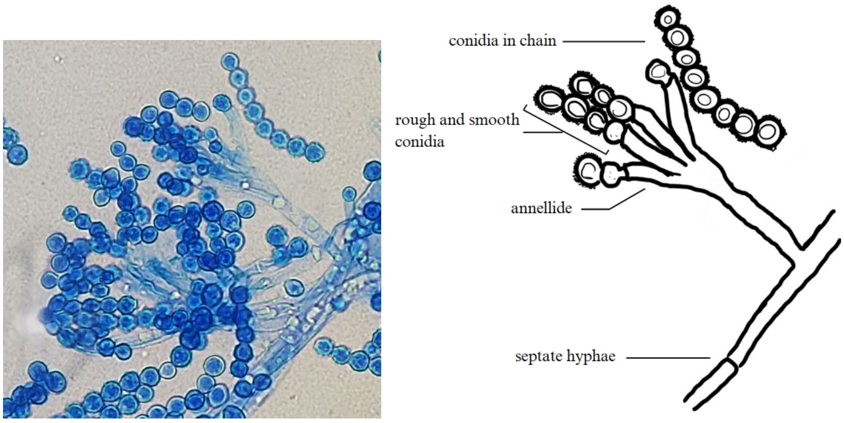

Figure 4.4 Observation of *Scopulariopsis* (7BCC18) under a microscope at 40× magnification using LPCB stain (left), and schematic representation of its morphology (right)

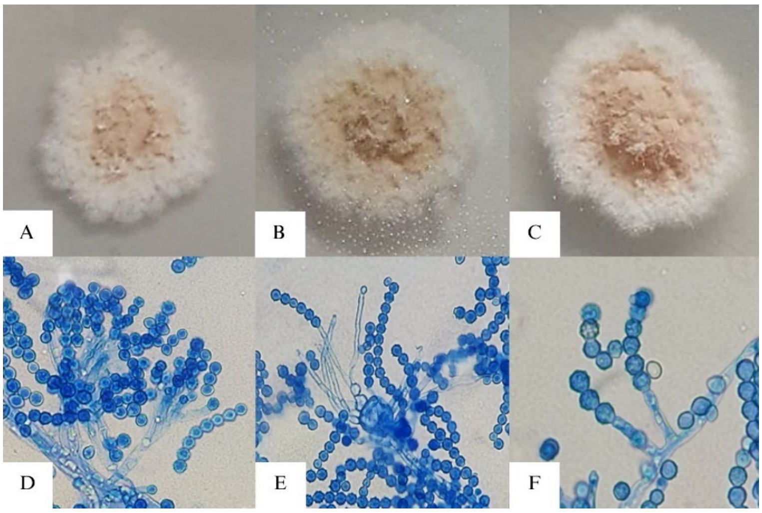

Figure 4.5 Macroscopic and microscopic morphology of *S. brevicaulis* in 7BCC18 (A, D), 8BCC26 (B, E), and 10BCC12 (C, F) on day 7 at 40× magnification

Based on the macro- and micro-morphological observations, all of the *Scopulariopsis* species observed in this study lead to *Scopulariopsis brevicaulis*. According to Yapicier *et al.* (2020), macroscopically, the surface of *S. brevicaulis* initially appears white, powdery, and smooth, then turns to a powdery light brown colour with a light tan periphery which has also been observed in this study. The reverse side of the fungus is tan and a brownish colour in the middle. Microscopically, *S. brevicaulis* showed hyaline septate hyphae with branched annelids, a structure similar to *Penicillium* that branches into finger-like shapes. The annelids of this species are straight and relatively long. The length and width of *Scopulariopsis* annelids in this study are varied. The length of the annelid depends on how many conidia have been produced. The annelid undergoes progressive elongation and narrowing during the formation of each conidium (Hospenthal and Rinaldi 2015). Apart from that, many spores or conidia formed in chains with a smooth and rough texture and relatively visible size which is one characteristic of *Scopulariopsis* (Figure 4.5).

Penicillium isolates in this study showed a flat powdery or floccose surface, predominantly having a green and bluish-green surface, with white margins on PDA. Similar surface appearance of *Penicillium* growth has also been reported by other studies (Demjanová *et al.* 2020; Westblade *et al.* 2023). The reverse side of the *Penicillium* isolates in this study exhibited pigmentation ranging from white, yellow, and red. Westblade *et al.* (2023) also reported similar reverse pigmentations. *Penicillium* ranged from 14–24 mm with an average diameter of 17.88 mm at room temperature. *Penicillium* species are recognized for their rapid growth rate, with colonies spreading quickly across the surface of the agar. The colour and diameter of *Penicillium* varied depending on the species and media used (Walsh *et al.* 2018). Elattaapy and Selim (2020) reported that *Penicillium* diameter ranged from 30–40 mm after 5 days on PDA at different temperatures (21, 24, 27, 30 and 33 °C). It can be concluded that temperature also influences the growth rate of a fungus.

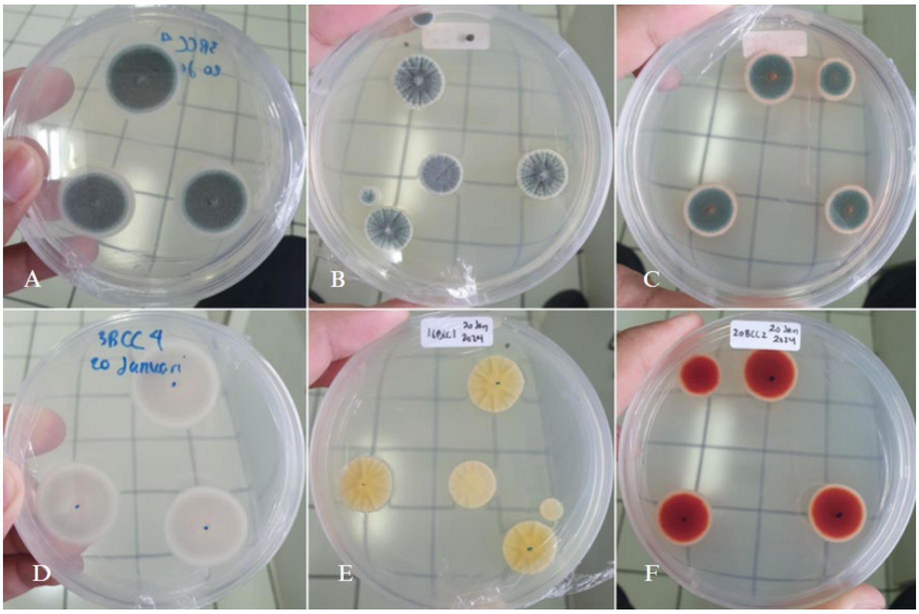

Figure 4.6 Macroscopic morphology of *Penicillium* colonies on day 7. Surface and reverse of 3BCC4 (A, D), 16BCC1 (B, E), and 20BCC2 (C, F)

The macroscopic morphological characteristics of *Penicillium* isolates observed in this study (Table 4.3) are represented by three isolate codes, namely 3BCC4, 16BCC1, and 20BCC2. Figure 4.7 shows that *Penicillium* isolates have green or bluish-green colour. The texture is powdery or floccose with flat or rugose topography. The colour of the reverse fungi is varied, such as white, yellow, or even red. Isolate 3BCC4 has wider sporulation than the other two and also has a bluish-green surface, a powdery texture with a light margin, and a white reverse. Isolate 16BCC1 shows a bluish-green surface, velvety texture, rugose topography, and yellowish reverse.

Microscopically, *Penicillium* samples showed a typical structure, sequentially; septate hyphae, stipe, branches like metulae, branched phialide, and conidia formed in a chain at the end of the phialide (Figure 4.7). Several branching types were found, such as divaricate, biverticillate, and terverticillate. The type of conidiophore branching pattern is varied among species (Samanta 2015). Almost all predominant conidiophores of the *Penicillium* samples observed have a biverticillate branching pattern. Meanwhile, sample 19BCC1 was the only one with a divaricate conidiophore pattern (Table 4.4). Figure 4.7 shows biverticillate branching indicated by the beginning of the branching at the end of the stipe, consisting of phialide and metulae.

Table 4.4 Predominant branching type in *Penicillium* isolates

Conidiophore Branching Type	Isolate(s)	Code of Animal
Monoverticilate	-	-
Divaricate	19BCC1	APH19
Biverticillate	1BCC1, 1BCC2, 3BCC4, 10BCC1, 10BCC2, 10BCC3, 11BCC1, 12BCC8, 13BCC1, 13BCC4, 15BCC1, 15BCC3, 16BCC1, 20BCC1, 20BCC2	APH1, APH10, APH11, APH12, APH13, APH15, APH16, APH20
Terverticillate	-	-
Quarterverticillate	-	-

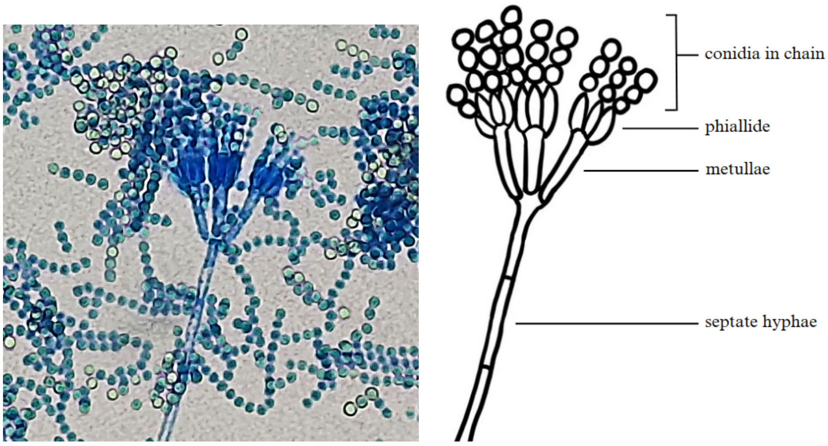

Figure 4.7 Microscopic (left) and schematic (right) morphology of biverticillate *Penicillium* in isolate 15BCC1 under the microscope at 40× magnification

Isolate 20BCC2 shows a significant reverse colour difference. It is the only isolate that shows red pigmentation on its reverse. Its surface shows a green powdery texture with flat topography. A green, flat powdery, with red reverse pigmentation, and the biverticillate conidiophore structure is characteristic of *P. marneffei* and *P. purpurogenum* (Vi and Kh 2017; Westblade *et al.* 2023). Isolate 20BCC2 shows these characteristics in its macro and microscopic morphology (Table 4.3 and Table 4.4). *P. marneffei* is a dimorphic fungus and can convert into the yeast phase at a temperature of 37 °C (Westblade *et al.* 2023). However, to confirm 20BCC2 as dimorphic fungi, the procedure of subculture and incubation at 37 °C for 7–14 days and further microscopic observation should be carried out to confirm yeast colony formation. Previous research stated that cycloheximide in agar media could inhibit the growth of *P. marneffei* and *P. purpurogenum* (Atalay *et al.* 2016; Walsh *et al.* 2018). On the other hand, Torres-Garcia *et al.* (2022) reported that *Penicillium* isolates cultured on PDA supplemented with cycloheximide were resistant to relatively high concentrations of cycloheximide. Nevertheless, further research is necessary to fully understand the resistance trait of *Penicillium* to cycloheximide and to confirm the species of the genus.

4.4 Prevalence of Single Infection and Coinfection

This study reported single and coinfection of *Scopulariopsis* and *Penicillium* in African pygmy hedgehogs. Among 20 sampled African pygmy hedgehogs, the single infection prevalence of *Scopulariopsis* and *Penicillium* is 40% (8/20) and 30% (6/20), respectively. *Scopulariopsis* and *Penicillium* co-infections were observed in 20% (4/20) of examined hedgehogs, namely APH10, APH11, APH13, and APH15. The total prevalence of *Scopulariopsis* and *Penicillium* infection in this study is 90% (18/20) (Table 4.5). As per the number of isolates on BCC media, two hedgehogs in samples APH2 and APH4 were uninfected by both genera. This report supported a previous study by Aftab *et al.* (2023) in Iran, that successfully isolated *Scopulariopsis* from 12.5% (6/48) of other hedgehog breeds. *Scopulariopsis* has also been reported with a prevalence of 5% (10/200) in canaries, 50% (16/32) in horses, and 10% in humans (Rosa *et al.* 2003; Fitzpatrick *et al.* 2018; Tunç *et al.* 2022). In addition, *Penicillium* has also been reported in Spain as the second-highest skin mycoflora of wild European hedgehogs, with a prevalence of 74.7% (76/102) (Molina-López *et al.* 2012), 13% (2/15) in Kintamani dogs (Sudipa and Gelgel 2022), and 9.45% (12/127) in Thailand cats (Sattasathuchana *et al.* 2020). According to several studies that have been mentioned, the prevalence of *Scopulariopsis* and *Penicillium* varies with each host and environmental conditions. The differences in prevalence in each animal species are influenced by their daily behaviour. African pygmy hedgehogs are nocturnal animals, and they like to bury their bodies during the day to get a dark environment. They also have the habit of digging to find food (Wissink-Argilaga 2020a). If there are spores on the ground or bedding, they will cover most of the hedgehog's body. Therefore, hedgehogs have a higher risk of contracting saprophytic fungi, including *Scopulariopsis* and *Penicillium*, than other animals because of their habit of burying themselves.

This study reported lower *Scopulariopsis* prevalence in males (55%; 5/9) compared to female hedgehogs (63%; 7/11). In contrast, the infection rate of *Penicillium* in male hedgehogs in this study was higher (55%; 5/9) compared to female ones (45%; 5/11) (Table 4.6). No previous study has reported a comparison of *Scopulariopsis* and *Penicillium* prevalences based on age and sex group in African pygmy hedgehogs. However, D'Ovidio *et al.* (2014) reported that female guinea pigs have a higher prevalence of *Scopulariopsis* (4.7%; 5/105) compared to male ones (2.1%; 2/95) from hair samples. They also reported that *Penicillium* infected more female guinea pigs (13%; 14/105) than males (12.6%; 12/95) from the same sample. In this study, the total prevalence in male hedgehogs (100%; 9/9) is higher than in female hedgehogs (72%; 8/11). The differences in reported prevalences in this study and others may be due to varying animal species and sample sizes. This result does not indicate whether male or female hedgehogs have a higher risk of fungal infection. Further studies are needed to assess sex as a risk factor for fungal infections in hedgehogs in larger populations or other regions.

Table 4.5 Prevalence of *Scopulariopsis* and *Penicillium* infection from 20 African pygmy hedgehogs

Genus of Fungi	Code of sample	Frequency	Prevalence (%)
Scopulariopsis	APH5, APH6, APH7, APH8, APH9, APH14, APH17, APH18	8	40
Penicillium	APH1, APH3, APH12, APH16, APH19, APH20	6	30
Coinfection	APH10, APH11, APH13, APH15	4	20
Total Positive	APH1, APH3, APH5, APH6, APH7, APH8, APH9, APH10, APH11, APH12, APH13, APH14, APH15, APH16, APH17, APH18, APH19, APH20	18	90
No infection	APH2, APH4	2	10

Table 4.6 Prevalence of *Scopulariopsis* and *Penicillium* based on their sex

Genus of Fungi	Male (9)			Female (11)		
	Code of Sample	Freq	%	Code of Sample	Freq	%
Scopulariopsis	APH5, APH6, APH8, APH11, APH17	5	55	APH7, APH9, APH10, APH13, APH14, APH15, APH18	7	63
Penicillium	APH3, APH11, APH12, APH16, APH19	5	55	APH1, APH10, APH13, APH15, APH20	5	45
Total Prevalence		9	100		8	72

Note: Freq: Frequency; %: Prevalence in percentage

Table 4.7 Prevalence of *Scopulariopsis* and *Penicillium* based on their age

Genus of Fungi	Young (8)			Adult (12)		
	Code of Sample	Freq	%	Code of sample	Freq	%
Scopulariopsis	APH6, APH7, APH8, APH9, APH11, APH17, APH18	7	87.5	APH5, APH10, APH13, APH14, APH15	5	41.6
Penicillium	APH11, APH19	2	25	APH1, APH3, APH10, APH12, APH13, APH14, APH15, APH16, APH20	9	75
Total Prevalence		8	100		10	83%

Note: Freq: Frequency; %: Prevalence in percentage

This study also presents the total prevalences of *Scopulariopsis* and *Penicillium* based on age groups of African pygmy hedgehogs sampled (Table 4.7). The prevalences of *Scopulariopsis* in the young and adult age groups were 87.5% (7/8) and 41.6% (5/12), respectively. The adult hedgehog group has a higher *Penicillium* infection rate of 75% (9/12) than the young hedgehog group (25%; 2/8). This study reported that all of the young hedgehogs (100%) had at least one genus either *Scopulariopsis*, *Penicillium*, or coinfection of both genera. Meanwhile, 83% of the hedgehogs in the adult group (9/12) were affected with the same condition. This result shows that hedgehogs in the young age group have a higher prevalence of *Scopulariopsis* and *Penicillium* infection than older hedgehogs. No available study reported the difference in *Scopulariopsis* prevalence based on the age group, but Sudipa and Gelgel (2022) reported that *Penicillium* infection in puppies has the same prevalence as adults (6%; 1/15). Still, these data do not clarify the risk in young hedgehogs is higher than in the adult group. Generally, animals that cannot produce a normal immune response have a higher risk of fungal infection (Seyedmousavi *et al.* 2018). So, more research is still needed to determine comparative risk levels between young and adult groups in African pygmy hedgehogs.

Several possibilities of these two fungal genera origin, *Scopulariopsis* and *Penicillium*, are from bedding like wood shaving (Gomes *et al.* 2022), dung (Woudenberg *et al.* 2017) feeds (Macura and Skóra 2015; Witaszak *et al.* 2020), drinks (Babič *et al.* 2017), caretakers, other hedgehogs, or from the surrounding environment, including air (Macura and Skóra 2015) and soil (Visagie *et al.* 2014b; Yapicier *et al.* 2020). According to the farm keeper, the African pygmy hedgehogs on the farm have never been bathed. Sanitation is only done on their cage, such as changing bedding every 1–2 weeks and giving water *ad libitum*. Dry feed is given regularly, twice every day. If the wood shaving used for bedding, feed, or a source of drinking water has been contaminated by fungi, it will most likely contaminate the animals. If the hedgehogs were taken from the wild, it could be that the animal has been contaminated since it was first kept. After the hedgehogs are infected, they can spread to other hedgehogs directly or through the air since those are airborne fungi. On the farm, the hedgehog cages are located in one indoor area with the cages of other species, such as sugar gliders, chickens, rabbits, and geckos. Even though each animal cage was still separated from the others, there is still a possibility that this animal can be communicated by the spores of these fungi through airborne. Therefore, the sanitation of the animals and their cage is necessary to prevent and overcome the fungi infection problem in the animals.

4.5 Limitations of the Study

This study has some limitations that need to be addressed. Firstly, the study is limited to a population of 20 African pygmy hedgehogs that were observed on a farm in Bogor District. This sample size is not representative of the prevalence of African pygmy hedgehogs in Indonesia, as they can be found in the wild, on other farms, and are also commonly kept as pets. However, this is the first study in Indonesia to report the presence of two fungal genera, namely *Scopulariopsis* and *Penicillium* in the skin of African pygmy hedgehogs. Secondly, the study was restricted by limited time and resources, and only two fungal genera were described, despite the presence of many other genera of fungi (Table 4.2). This emphasizes that further studies are still very much needed to identify the types of fungus that grow on media with and without cycloheximide. Lastly, the source of hedgehogs is unknown, hence, the source of *Scopulariopsis*, and *Penicillium* is unclear, and further investigation is needed to determine the infection origin.

V CONCLUSION AND SUGGESTION

5.1 Conclusion

African pygmy hedgehog is a species of hedgehog commonly kept as a pet by humans that can carry zoonotic diseases including fungal infections. This study revealed the significant presence of *Scopulariopsis* and *Penicillium* in Dhonje Golden Farm, Bogor, which can be a serious threat and cause losses to animals and humans. Identification is made based on the macro and micro morphology characteristics of the fungi. The prevalence of single *Scopulariopsis* and *Penicillium* infection in this study respectively, are 40% (8/20) and 30% (6/20), with 20% (4/20) of the sampled animals being coinfected with both fungal genera.

5.2 Suggestion

The results of this study may have implications for future research on African pygmy hedgehogs especially in Indonesia, to study fungal diseases that threaten human and animal health. Research on fungi related to African pygmy hedgehogs is highly recommended considering that not much research has been conducted in Indonesia. Further research is still needed to expand the sampling and study area in one area so that the data obtained is more abundant, informative, and can represent the area. Additionally, identifying specific fungal species and associated clinical symptoms may contribute to the development of more targeted diagnostic and therapeutic tools to prevent and treat fungal infections in hedgehogs. This study revealed more than a thousand isolates on the PDA. Therefore, further study is needed to uncover and identify other types of fungi in other colonies besides *Scopulariopsis* and *Penicillium*, as well as the pathogenicity level of each fungus and preventive measures and treatment of infected hedgehogs. Further investigation is necessary to determine whether these two fungi are the etiological agents behind the clinical manifestations observed in African pygmy hedgehogs. This may involve the application of Koch's postulates to ascertain their pathogenic potential. Additionally, it is essential to raise awareness among hedgehog keepers about the presence of pathogens in hedgehogs. Interested parties also need to educate and disseminate information about zoonotic diseases from wild animals, livestock, and pets, including African pygmy hedgehogs which have zoonotic potential and are rarely known by the public.

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