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Research Article

PREVALENCE OF DERMATOPHYTE INFECTION IN PIGS AMONG SMALLHOLDER FARMERS IN BAYBAY CITY, LEYTE, PHILIPPINES AND ASSOCIATED RISK FACTORS

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ABSTRACT: The province of Leyte accounts for at least half of the swine population in Eastern Visayas, Philippines and records the highest number of slaughtered pigs within the region. Given that the majority of pigs in the region are raised by smallholder farmers and that occupational exposure to ringworm has been recorded particularly in rural pig farmers, dermatophytosis could pose a significant public health risk. This study aimed to determine the prevalence of, and risk factors involved with dermatophyte infection in pigs raised by smallholder farmers in Baybay City, Leyte. Using actual culture procedures from 384 samples, our study confirmed the presence of dermatophytes among pigs which could be used as basis for prevention and control programs in the future. While the over-all prevalence is quite low (5.47%) with the *Microsporum nanum* being the most prevalent (3.91%) followed by *Trichophyton mentagrophytes* (1.04%) and finally *Microsporum canis* (0.52%), it is imperative that pig farmers should improve their management practices. In particular, the use of open pit for manure disposal and the non-specific medication of pigs should be avoided.

Key words: Associated risk factors, Backyard pigs, Epidemiology, Dermatophyte infection, Prevalence.

INTRODUCTION

Dermatophytosis is a contagious fungal disease commonly caused by a group of pathogenic fungi affecting a wide range of animal species including humans (White 2012, Moretti *et al.* 2013). It is considered among the high economic burden category of zoonotic diseases due to its highly contagious characteristic, persistent and relatively stable conidia, high cost of treatment, and the limited antifungal agents available for veterinary use (Chermette *et al.* 2008, Uddin Khan *et al.* 2013).The clinical forms of dermatophytosis (ringworm) in humans have been extensively discussed relative to the specific area of the body affected and keratinization of the affected site (Degreef 2008, Goldstein and Goldstein 2017). In pigs, lesions are commonly seen as a light to dark brown discoloration of the skin particularly behind the ears and on the back and flank which may progress as circular patches (Pittman and Roberts 2005). Moreover, while the prevalence and mortality due to dermatophytosis in pigs is low (Uddin Khan *et al.* 2013), earlier studies have reported potential outbreaks particularly in adult pigs (Pittman and Roberts 2005) and growers in contaminated conditions (White 2012). Economically, affected pigs could result in reduced marketability and unnecessary treatments costs.

The province of Leyte accounts for at least half (52.4%) of the swine population in Eastern Visayas (PSA 2019). It also records the highest number of slaughtered pigs (almost 60%, PSA 2018) within the region despite the region's very low swine inventory in the country (3.2%, PSA 2019). Given that the majority (95.3%) of pigs in the region are raised by smallholder raisers (backyard type, PSA 2019) and that occupational exposure to ringworm has been recorded particularly in rural pig farmers (Roller and Westblom 1986) including a butcher (Pal and Dave 2013), dermatophytosis poses a significant public health risk among pig farmers and those engaged in the pig processing business. The aim of this study was

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therefore to determine the prevalence of dermatophytosis in pigs raised by smallholder pig raisers in Baybay City, Leyte, including the associated risk factors. Baybay City, Leyte is one of the largest cities within Eastern Visayas in terms of population and land area, and has a long-standing ordinance for adopting a systematic and ecological waste management program for all pig raising projects (M.O. No. 004 2004). As zoonotic diseases continue to play a major public health significance, results of this study may serve as a warning and a baseline information useful for the control and prevention of dermatophytosis particularly among smallholder pig farmers.

MATERIALS AND METHODS

Survey location and sample size

The study was conducted in different barangays (villages) of Baybay City, Leyte, Philippines (10°39'50.3"N 124°50'55.3"E) in January to June 2019. Baybay City has a tropical climate with an average temperature of 27°C (± 1.9°C), about 80% humidity and a fairly significant amount of rainfall in the year. The City of Baybay is a small agricultural city composed of at least 92 barangays and a population of about 110,000 (PSA 2017). Smallholder pig farming is a dominant livestock activity with many pigs commonly reared in close proximity to humans. Following appropriate sampling procedures (Martin *et al.* 1987, Fosgate 2009), at least 384 pigs were required for this study at 95% level of confidence. Pigs were randomly selected following proportional allocation from an estimated population of pigs in each barangay after receiving consent of the Barangay Captains for collection of culture samples and conducting the interview of the pig farmers on this survey. The study was approved by the Student Research Committee of the College of Veterinary Medicine, Visayas State University, Baybay City, Leyte.

Sample collection, culture and identification of dermatophytes

Culture samples were collected from pigs regardless of weight, sex, breed and age in the absence of clinical lesions. Specimens were sampled from either the neck and/or behind the ears (Robert and Pihet 2008) using the brush technique (Mackenzie 1963, Goldberg 1965). This technique uses a new toothbrush each time for collecting a sample of keratinized, alopecic or scaly lesion with or without hairs from each pig. The new toothbrush was removed from its packaging and was rubbed gently over the target area to obtain the desired sample. Each brush was then secured in individual plastic bags and transported to the Microbiology Laboratory of the College of Veterinary Medicine, Visayas State University for processing. Pilot collection and culture was conducted using samples from neighboring areas prior to final collection and survey.

For culture procedures, a small amount of the collected samples were scattered over the surface of the Sabouraud's Dextrose agar, gently pressed down into the medium with sterile forceps and incubated at room temperature (25°C). After two days to a week, those samples that have yielded dermatophyte growth were examined under the microscope to examine the colonies using the sticky tape preparation. In this technique, the adhesive side of a cellophane tape strip was pressed gently on the suspected colony and mounted on a clean microscope slide with a drop of lactophenol cotton blue (Leck 1999). Final identification of the dermatophyte species was based on the characteristic growth and colour reaction of colonies on Sabouraud's Dextrose agar, and the characteristic morphology of macroconidia under the microscope.

Determination of risk factors

During sample collection, a one-on-one interview using a prepared survey questionnaire was conducted among pig owners to collect information that may lead to possible identification of risk factors associated with dermatophyte infection. The questions were grouped according to the individual pig characteristics, pig farmers' demographic background, as well as feeding, animal health and biosecurity management. The questionnaire was written

Table 1. Prevalence of dermatophyte infection of pigs raised in Baybay City, Leyte.

Dermatophytes	Obs.	Prevalence (%)	Upper and lower limits at 95% CI
Pooled over species	21	5.47% ± 0.0	3.50 - 8.37%
Microsporum nanum	15	3.91% ± 0.0	2.28 - 6.50%
Trichophyton mentagrophytes	4	1.04% ± 0.0	0.33 - 2.83%
Microsporum canis	2	0.52% ± 0.0	0.09- 2.08%

Sample size (n) = 384; CI= confidence interval.

Table 2. Logistic regression modeling for dermatophyte infection in pigs raised in Baybay city, Leyte.

Significant conditions associated with dermatophyte infection	Odds Ratio	Upper and lower limits at 95% C.I.		Coefficient	S.E.	Z-statistic	p-value
Nonspecific medication (Yes/No)	4.3514	1.2374	15.3014	1.4705	0.6416	2.2920	0.0219
Pit for waste disposal (Yes/No)	9.4706	1.7712	50.6394	2.2482	0.8554	2.6283	0.0086
Constant	*	*	*	-3.9828	0.5827	-6.8350	0.0000

Convergence:	Converged
Iterations:	6
Final -2*Log-Likelihood:	153.4145
Cases included:	384

Test	Statistic	df	p-value
Score	9.3128	2	0.0095
Likelihood Ratio	9.4726	2	0.0088

in English but was translated into the local dialect during the interview.

Data management and statistical analysis

All data were encoded using the Microsoft Excel and analyzed using EpiInfo™ version 7.2.2.2 (Centers for Disease Control and Prevention, Division of Health Informatics and Surveillance, USA). The prevalence of dermatophyte infection was determined as the proportion of positive reactors *i.e.*

$$\text{proportion positive (p)} = \frac{\text{number of positive pigs}}{\text{total number of pigs examined}} \times 100, \text{ with SE(p)} = \sqrt{\frac{p(1-p)}{n}}$$

where, SE is the standard error and n is the sample size.

Minimum effective sample size was determined for conducting the present epidemiological survey on prevalence of dermatophyte infection in pig as

$$n = (1.96)^2 \times \frac{p(1-p)}{var(p)} \text{ at 95\% confidence interval}$$

and the upper and lower limits of a 95% confidence interval are obtained as $p \pm 1.96 \times SE(p)$ (Martin *et al*. 1987).

Employing the EpiInfo™, the Chi-Square test was used to assess the unconditional association between dermatophyte positive (dependent variable) and the probable related independent variables, where variables demonstrating the attribute of unconditional association under $p \leq 0.20$ (Peña and Lañada 2019) were thereafter subjected to multiple logistic regression analysis on the EpiInfo™ software using the backward elimination approach and removing the least significant variable one after another until the p-value of the whole model and each remaining variables in the model was $p < 0.05$.

RESULTS AND DISCUSSION

Farmer demographics and pig characteristics

Smallholder pig farmer respondents under this investigation were between 21 to 82 years aged, with a mean of 46.26 years and a median of 47 years. More than half of the respondents were females (57.03%) and about 42.97% were males. Raising pigs (96.35%) occupationally serves as a primary source of income rather than a mere hobby of farmers (3.65%). While a proportion of farmers (16.93 %) were aware about ringworm infection in pigs, all claimed that their pigs were not infected.

From a total of 384 pigs sampled, 82.29% were females and only 17.71% were males. Large White was the predominant breed (76.56%), followed by Duroc (20.31%) and native pigs (3.13%). Our investigation covered all physiological stages *i.e*. suckling (3.65%), weaners (23.44%), growers (5.73%), finishers (3.13%), gilts (9.38%), and that of either sex: sow (48.44%) and boars (6.25%).

General management practices

Most of the pigs (96.35%) were kept in their own pens, while others were tethered (2.34%), in the shed (1.30%), and left to roam freely (0.52%). Farmers mostly used concrete (90.10%) flooring to facilitate cleaning while other pigs have direct access to the ground (5.99%) or rice hull as a bedding material (3.39%). While some of the pigs sampled (11.98%) were raised as the only animal, most of the pigs (88.02%) were raised along with a group of other animals such as chickens, goats, cattle, ducks, horses, monkey and fishes (62.24%) or, with chickens

Table 3. Logistic regression modeling for *Microsporum nanum* infection in pigs raised in Baybay City, Leyte.

Significant conditions associated with dermatophyte infection	Odds Ratio	Upper and lower limits at 95% C.I.		Coefficient	S.E.	Z-statistic	p-value
Non-specific medication (Yes/No)	3.8511	1.0695	13.8673	1.3483	0.6537	2.0627	0.0391
Constant	*	*	*	-4.0999	0.5819	-7.0453	0.0000

Convergence:	Converged
Iterations:	5
Final -2*Log-Likelihood:	121.4358
Cases included:	384

Test	Statistic	df.	p-value
Score	4.8746	1	0.0273
Likelihood Ratio	5.2483	1	0.0220

(4.95%), carabaos (3.13%), dogs (11.72%), and cats (5.99%) alone.

Piped water was the major source of water (74.48%) although some pig farmers utilized wells (14.32%), river (3.13%) and rain (3.13%) water. Most of the pig farmers practiced dry feeding (60.68%) using mostly commercial finished feeds (94.01%) and less on wet feeding (39.32%). Others gave kitchen leftovers or locally mixed feedstuffs (5.99%). More than half of the pigs sampled were fed twice (59.64%) or thrice (38.80%) daily. Most pig farmers practiced floor feeding (74.22%) while other farmers feed their pigs using a feed through (25.78%). Moreover, pig owners also provide their pigs with vitamins and mineral supplements (49.22%).

The majority of pig owners regularly clean their pig pens (84.38%) either with water only (60.16%) while some also used detergent (17.19%) or chlorine (7.03%) in addition to water. Pig manures were disposed either into a septic tank (32.29%), or a catchment area (28.13%) or an open pit (5.21%). However, some farmers just leave the manure either outside the pen (11.20%) or allow to dump to a nearby body of water (23.18%). Pigs were bathed almost once a day (83.07%). Deworming was commonly practiced (94.79%).

Prevalence and risk factors involved with dermatophyte infection

Our investigation revealed a relatively low 5.47% prevalence of dermatophyte infection (Table 1) in pigs raised by small-holder farmers in Baybay City, Leyte. This constitutes 21 pigs positive over 384 pigs sampled and was mainly concentrated within 14 barangays at a prevalence rate of 4.76% in 10 barangays, 9.52% in three barangays and 19.05% in one barangay, of about 44 barangays that were included in the study, respectively. It was also observed (Table 1) that *Microsporum nanum* was the most prevalent (3.91%) followed by *Trichophyton mentagrophytes* (1.04%) and *Microsporum canis* (0.52%). At least five independent variables were screened out to be unconditionally associated with dermatophyte infection in the pigs. These included the

Table 4. Logistic regression modeling for *Microsporum canis* infection in pigs raised in Baybay City, Leyte.

Significant conditions associated with dermatophyte infection	Odds Ratio	Upper and lower limits at 95% C.I.		Coefficient	S.E.	Z-statistic	p-value
Native Breed (Yes/No)	33.7272	1.9806	574.3348	3.5183	1.4464	2.4324	0.0150
Constant	*	*	*	-5.9162	1.0006	-5.9127	0.0000

Convergence:	Converged
Iterations:	9
Final -2*Log-Likelihood:	20.7192
Cases included:	384

Test	Statistic	df.	p-value
Score	14.5921	1	0.0001
Likelihood Ratio	4.3004	1	0.0381

Table 5. Logistic regression modelling for *Trichophyton mentagrophytes* infection in pigs raised in Baybay City, Leyte.

Significant conditions associated with dermatophyte infection	Odds Ratio	Upper and lower limits at 95% C.I.		Coefficient	S.E.	Z-statistic	p-value
Pit for waste disposal (Yes/No)	20.1111	2.6777	151.0439	3.0013	1.0287	2.9174	0.0035
Constant	*	*	*	-5.1985	0.7091	-7.3316	0.0000

Convergence:	Converged
Iterations:	9
Final -2*Log-Likelihood:	37.8083
Cases included:	384

Test	Statistic	df.	p-value
Score	16.4260	1	0.0001
Likelihood Ratio	6.6646	1	0.0098

factors: pig-sex, frequency of feeding, cleaning of pens using water only, using pit for manure disposal, and non-specific medication. In the present study, sex, frequency of waste removal and non-specific medication were found to be unconditionally associated with *Microsporum nanum* infection; native breed of pig, suckling, and twice a week bathing frequency were associated with *Microsporum canis* infection; and using an open pit for manure disposal, removal of waste only once a week, deworming and bathing of pigs twice every week were associated with *Trichophyton mentagrophytes* infection.

Multivariate analysis revealed that the use of an open pit (p= 0.0086) and the practice of non-specific medication (p= 0.0219) appeared to be the most contributing factors associated with dermatophyte infection (Table 2). At the individual level, non-specific medication was significantly associated in *Microsporum nanum* infection (p= 0.0391, Table 3), the use of native breed for *Microsporum canis* infection (p= 0.0150, Table 4), and the use of open pit for manure disposal for *Trichophyton mentagrophytes* infection (p= 0.0035, Table 4).

A common and often the most cited cause of fungal disease affecting the keratinized tissues of skin, hair, and nails by dermatophilic fungi could be a major concern both in humans and animals (Cabañes 2000). The incidence might accordingly be affected by geography, environmental conditions, human presence, and animal management practices (Kacinová *et al.* 2013, Uddin Khan *et al*. 2013, Valandro *et al.* 2017), with species belonging to *Microsporum* and *Trichophyton* causing most of the dermatophytosis in domestic animals (Cabañes 2000). Pigs normally harbor *Microsporum nanum*, however pigs might also contact other dermatophyte species like

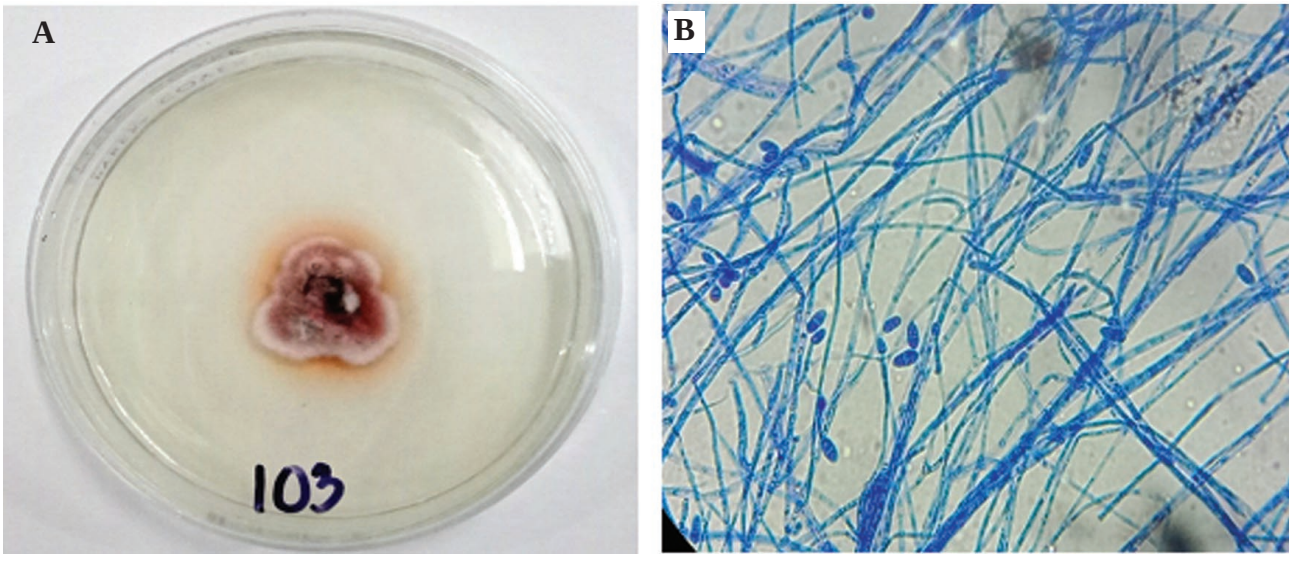

Fig. 1. (A) White colony of *Microsporum nanum* becomes granular and buff colored on Sabouraud's dextrose agar after 4 days incubation at 25°C; (B) Pear-shaped macroconidia stained with Lactophenol cotton blue.

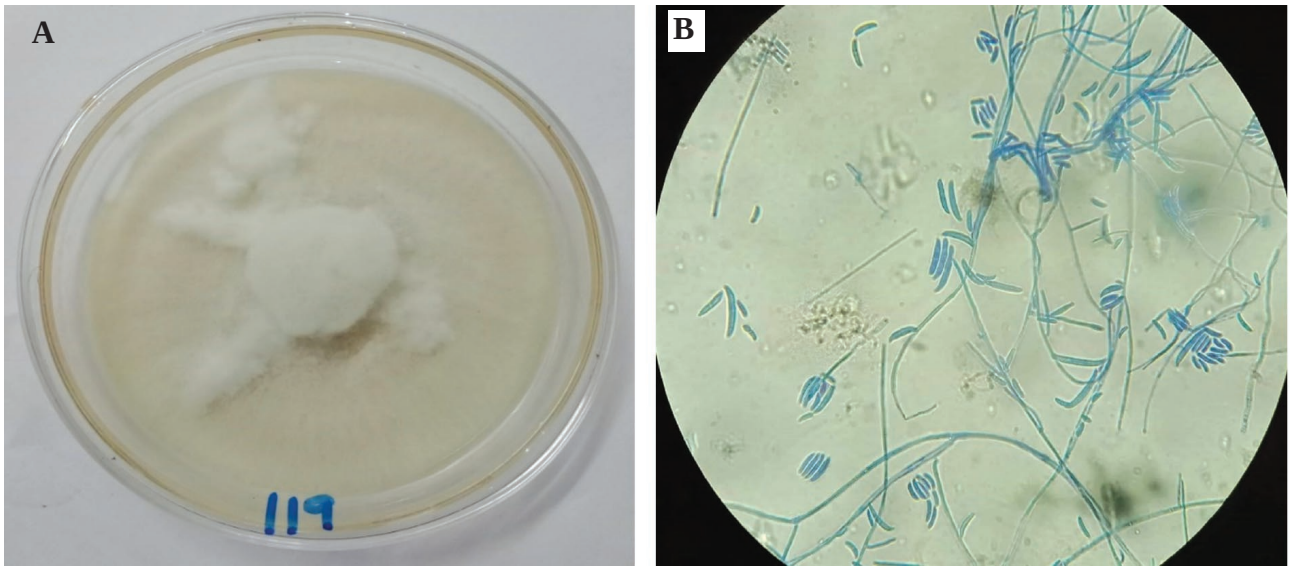

Fig. 2. (A) White colony of *Microsporum canis* on Sabouraud's dextrose agar after 5 days of incubation at 25°C; (B) Canoe-shaped macroconidia stained with Lactophenol cotton blue.

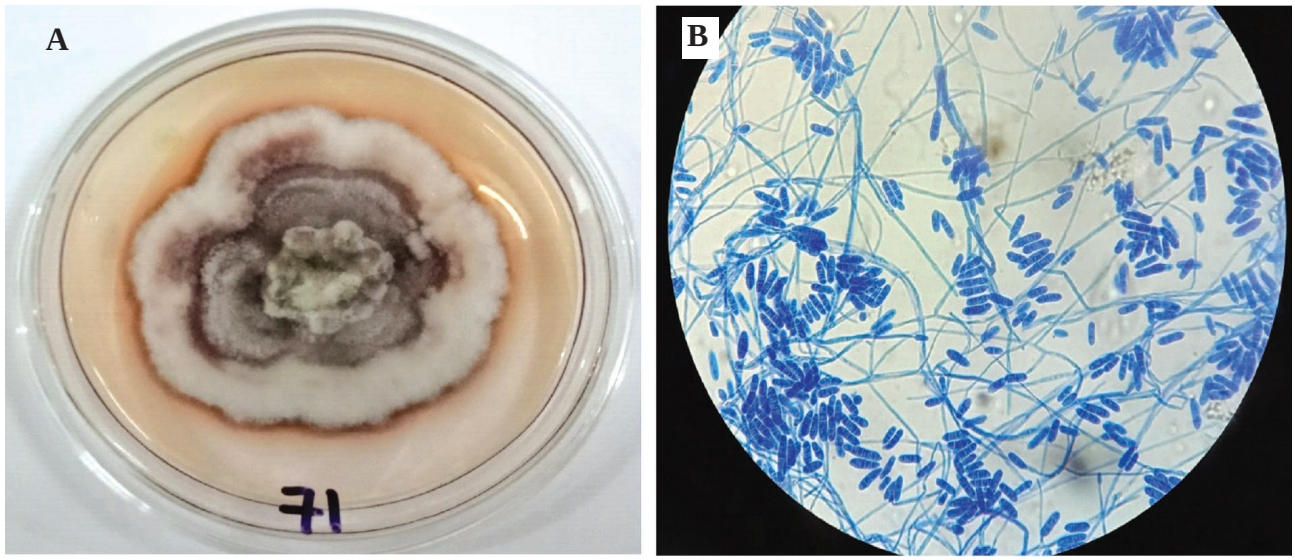

Fig. 3. (A) Cream colored colonies of *Trichophyton mentagrophytes* with a powdery to granular surface on Sabouraud's dextrose agar after 7 days of incubation at 25°C; (B) Characteristic cigar-shaped macroconidia stained with Lactophenol cotton blue.

Trichophyton mentagrophytes and *Microsporum canis* (Cabañes 2000), and could corroborate our findings of prevalence percentage in pigs for dermatophyte infection of different species.

The use of a man-made open pit for manure disposal appeared to be a significant predisposing factor to dermatophyte infection (p=0.0086, Odds Ratio = 9.4706). This was not surprising as *M. nanum* had been isolated from soil cultures where pigs were reared (Ajello *et al.* 1964, Roller and Westblom 1986). Keratinophilic fungi belonging to *Microsporum* along with *Trichophyton* and *Epidermophyton floccosum* normally make up the main dermatophyte fungi species (Hay 2015). An earlier study demonstrated that *M. nanum* could easily multiply in the soil and eventually might serve as a reservoir for re-infection (Long *et al.* 1972) in susceptible animals. Nevertheless, while many pig farmers practice floor feeding, the fact that piped water and concrete flooring is being used by the majority of pig raisers appears to correlate with the relatively low prevalence of dermatophyte infection.

We also found that non-specific medication might

increase the likelihood of pigs contracting dermatophytes (p = 0.0213, Odds Ratio = 4.3784). Non-specific medication in the context of this study refers to pig farmers treating their sick pigs without proper consultation to a pig veterinarian and/or using non-specific antibiotics or combinations thereof including those incorporated as feed supplements. The respondents were asked if they give such medications and interestingly, the use of antibiotics including feed additives in an off-label manner including dosing above the recommended concentrations were documented (Dewey *et al.* 1997). Unfortunately, such practice could have negative ramifications on the health and immunity of animals by increasing the risk of antibiotic resistance (Sørum and Sunde 2001), emergence of infection potentially caused by otherwise nontypical pathogens (Truong *et al.* 2019), and possible promotion of fungal growth and unexpected drug interactions (Azevedo *et al.* 2015). Ultimately, pig producers should consider proper medication including consulting a veterinarian before applying any kind of medications to their animals. To emphasize, this finding needs to be interpreted with caution since respondents were not specifically asked whether the medication was directed against a specific disease, what kind of medication was used, nor the exact time period by which certain medication was given relative to the time the interview was conducted. Nevertheless, the risks involved with non-specific medication cannot be overstated as such practice could easily lead to incorrect mode of drug administration, wrong dosage, and more importantly incorrect choice of therapeutic agents.

The presence of other animals in close proximity with pigs raised was the other potential factor. While this was not the case in general, close contact with dogs and cats is something worth considering as *Microsporum canis* has been found in high rates among dogs and cats (Brilhante *et al*. 2003). In fact, potential *M. nanum* infection of a dog used for hunting wild boars has been documented (Valandro *et al.* 2017). As we commonly observed, the type of housing used and the management practices for raising pigs by many smallholder farmers could easily expose pigs to pet dogs and cats both from those owned by the pig raisers themselves and/or from the neighbors. This could be the reason of the increased likelihood of infection among native pigs in our study (p = 0.0150, Odds Ratio = 33.7272). Native pigs were commonly raised in poor housing conditions with some tethered and therefore prone to increased likelihood of contact with other animals particularly dogs and cats.

Normally, the infective arthrospores of dermatophytes germinate by adhering and hydrolyzing keratin structures in the hairs and skin (Hay 2006). Continued hyphal growth causes an inflammatory response (Quinn *et al.* 2015) which eventually leads to damage in the stratum corneum, hair follicles, and hair shafts among others. As the hyphae grow centrifugally, lesions in the skin characteristically form in circular appearance commonly known as 'ringworm' (Markey *et al*. 2013, Quinn *et al.* 2015). While the virulence and the immunological response of the host animals affect the nature of the lesions, infection among pigs cannot be taken lightly. Infected pigs may reduce its marketing value while the severity of infection could aggravate secondary bacterial infection particularly in very young, old and/or immunocompromised animals. While treatment could help relieve symptoms and prevent further spread in human infections (Goldstein and Goldstein 2017), such might only add unnecessary burden to pig farmers.

CONCLUSION

In conclusion, this study confirmed the prevalence of pathogenic dermatophytes among smallholder pigs in Baybay city, Leyte, Philippines. While the prevalence was low, it is imperative that the pig farmers should improve their management practices. In particular, the use of open pit for manure disposal and the non-specific medication of pigs should be avoided.

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