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Candida albicans in backyard chicken flocks raised in a small community in Leyte, Philippines: Prevalence and risk factors associated

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ABSTRACT

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This study aimed to determine the prevalence of *Candida albicans* in backyard chickens flocks among smallholder raisers in selected villages of Baybay City, Leyte and to identify the risks factors associated with the prevalence of *C. albicans*. Using Sabouraud's dextrose agar, 185 (68.5%) out of 270 samples were found to be positive with yeast, and 94 (34.81%) out of those yeast isolates were positive for *C. albicans*. Multivariable logistic regression analysis results show unconditional association between the sex of the chicken with the isolation of *C. albicans*. The odds of roosters harboring *C. albicans* is 56% lower than hens (OR=0.44, 95% CI 0.2-0.7) with a *p*-value of 0.0047. Considering the possibility of contact between smallholder chicken flocks and the community, the isolation of *C. albicans* from apparently healthy chickens necessitates the implementation of proper hygiene and sanitation management especially in smallhold chicken flocks.

Keywords: candidiasis, fungal zoonosis, chicken, poultry candidiasis

INTRODUCTION

Candida albicans is a fungus that affects the digestive tract of chickens and turkeys, specifically their crop, resulting in mucosal thickening with whitish, raised pseudomembranes (Kahn 2010). Compared to other animal species (buffalo, cattle sheep), chickens have the highest prevalence of yeasts isolates (*C. albicans* and *Cryptococcus neoformans*) at 24.86% (Abou-Elmagd et al 2011). Prevalence of *C. albicans* in chickens varies among countries which ranges from 12.15% (Egypt), to 41.5% (India) (Abou-Elmagd et al 2011, Sharma et al 2017). Oral, esophageal or crop

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candidiasis are common and are associated with concurrent disease, nutritional deficiencies, immunosuppression or altered microflora (Jordan 2008).

In the Philippines, increased susceptibility of Filipinos to candidiasis was attributed to the warm tropical climate and its interaction with cultural practices, occupation and immune responsiveness (Handog and Dayrit 2005). Chickens including other animals are potential reservoirs of *Candida albicans* for human candidiasis (Edelmann et al 2005). *Candida albicans,* as an opportunistic fungi, is very able to cause candidiasis (thrush) and systemic mycoses in man with many predispositions including exposure to litter from poultry houses and game bird areas and waste and disposal areas contaminated with human waste (Dhama et al 2013). Notably, the transmission of *Candida* spp. among chickens mainly occurs via contaminated feed and water. Systemic infections, however, are commonly contracted by inhaling fungal spores present in soil or in droppings filled soil (Frey 2005).

This study aimed to determine the prevalence of *C. albicans* in free-range chicken flocks among smallhold raisers in selected barangays of Baybay City, Leyte and to identify the risk factors associated with the presence of *C. albicans*. Understanding the risk factors associated with the isolation of *Candida albicans* in chickens will be useful in considering efficient control and preventive measures to reduce the risk of candidiasis both in humans and chickens.

MATERIALS AND METHODS

Sample Collection

Opportunistic fungi such as *C. albicans* are commonly found in the digestive tracts of healthy chickens (Abbas et al 2017). Fecal samples were collected from 270 randomly selected healthy chicken flocks from the villages of Marcos, Gabas, Pangasugan, Guadalupe, and Patag. For the identified flocks, fresh feces were collected early in the morning from chickens by swabbing the cloaca using sterile cotton swabs (Kemoi et al 2013). Collected samples were placed in test tubes containing 0.9% physiologic saline solution (PSS) for transport to the laboratory.

Sample Design

This study followed the one-stage random sampling design with proportional allocation. Proportional allocation was done using the estimated population of chicken flocks in each barangay based on initial chicken population survey. The total number of samples was allocated to the 5 villages in proportion to their chicken population (Table 1).

Table 1. Number of samples collected per barangay based on proportional allocation

Barangays	Estimated Chicken Population	Number of Samples Collected
Pangasugan	2016	113
Patag	1177	65
Gabas	1151	64
Marcos	250	14
Guadalupe	242	14
Total	4836	270

Isolation and Identification of C. albicans

Prior to inoculation unto Sabouraud's dextrose agar (SDA), samples were added with 2mg streptomycin and 500IU penicillin per mL to exclude other contaminants such as bacteria and other fungi. A loopful of the supernatant was streaked onto SDA plates and incubated at 37°C aerobically for two days. To confirm the growths characteristic of *Candida sp.* as colonies of *C. albicans*, germ tube test was conducted. Cells of the colonies were incubated in sheep serum at 37°C for two hours, through microscopic examination, the presence of germ tubes were then identified (Abou-Elmagd et al 2011, Aryal 2015, Markey et al 2013, Quin 1994). Tubes were added with a drop of 10% formalin, sealed with paraffin, and stored in a refrigerator at 2-4°C overnight when microscopic examination was not performed immediately (Yeast Identification 2014).

Survey Questionnaire

A survey questionnaire was developed consisting of close-ended and open-ended questions. The questionnaire asked about flock owner's demography, flock information, and management and health practices employed by flock owners. It had farm-level and animal-specific questions aimed at gathering data about the variables related to the prevalence of *C. albicans*. It was in English, but translated into Cebuano during the interview.

Data Analysis

The prevalence of *C. albicans* was calculated by dividing the number of *C. albicans*-positive chickens over the total number of sampled chickens, multiplied by 100.

To assess the unconditional association between *C. albicans*-positive (dependent variable) and the possible related independent variable, Yates Corrected Chi-square test was used in the univariate analysis to determine statistical significance of categorical non-continuous variables and Kruskal-Wallis test for non-normal continuous variables. Variables with p value <0.20 at the univariable analysis were considered to have a significant association and were included in the multivariable analysis. In the multivariable logistic regression model, backward elimination approach was done, with p-value of 0.05 as the limit, removing the least significant variable one by one. Modelling was rerun until the variables in the final model had a p-value of <0.05. The analysis was done using Epi info™ 7.

RESULTS AND DISCUSSION

Prevalence of Candida albicans

The prevalence of *C. albicans* in this study is 34.81% ± 5.68%. This confirms the study of Abou-Elmagd et al (2011) that *C. albicans* can be isolated in fecal samples of healthy chickens in high percentages. In the study of Sharma et al (2017), the prevalence of *C. albicans* in chickens was 41.5%. In addition, it conforms to the study of Kemoi et al (2013) that domestic chicken excreta are possible reservoirs

and sources of infections to humans, basically by inhaling spores from chicken droppings. Fungal spores present in soil or in dropping-filled soil are likely to be inhaled when the soil is dug up or otherwise disturbed. This is the common way of contracting systemic fungal infections (Frey 2005).

Flock Owners' Demography

Majority of the respondents of the study were male (229, 84.81%). This data represents a different case than in sub-Saharan Africa, where the generation of income by poultry keeping is a role traditionally assigned to women (Sonaiya and Swan 2004). In the Philippines, however, cockfighting or "sabong" is a popular recreational activity and an instant source of additional money particularly in the provinces, which incline men to raising chickens (Almendral 2013, Cabadsan 2019). Owner's age ranged from 16-77 years old with a mean of 44.3. Most of the flock owners were young adults (age 20-35) at 32.22%, married (65.56%), and head of the family (66.67%). Most of the flock owner's family members also handled or had close contact with the chickens (85.19%).

Flock Information and Management

Of the 270 sampled chickens, 184 (68.15%) were mixed breed and the rest were Philippine-native breed. Two hundred and three (75.19%) were males and 67 (24.81%) were females. The flocks were composed of 157 (58.15%) roosters, 46 (17.04%) cockerels, (13.33%) pullets 36, and 31 (11.48%) hens.

Family poultry or small-scale poultry raising involves flocks less than 100 birds (Sonaiya and Swan 2004). The chicken population in the sampled households ranged from 1 to 107, with a mean of 21 chickens. Flock owners typically caged their chickens (92.59%) or allowed free roaming (18.89%), although there were some who had combinations. With caged chickens, 74.44% had contact to other chickens through partition of cages. Other animals had also association to the chickens including dogs (70%), cats (50.37%), pigs (5.93%) and other avian species (ducks, geese, etc.) (5.19%). It was common for the flock owners to slaughter chickens at home (91.85%), considering that one of the objectives in keeping chickens is for home consumption (Sonaiya and Swan 2004).

Most diets used in feeding poultry are nutritionally "complete" diets that are commercially mixed or prepared by manufacturing companies with trained nutritionists (Kahn 2010). This may be the reason why most flock owners in this study opted to feed their chickens with commercial feeds (253 or 93.70%). Some raisers fed their chickens with left overs or just allowed the chickens to find their own food by free roaming (18 or 6.67%). Common source of water provided to the chickens were rivers and streams (164 or 60.74%) in the study site; only about 39.26% (106) used piped water provided by the municipality. The advantage of piped water from the water district is that, it has undergone filtration and chlorination processes. It was known that one effective and economical method to control *Candida* is through chlorination of drinking water using chlorox or sodium hypochlorite at a concentration of 5ppm (Candidiasis et al 2000). Water is provided more than two times a day by 46.30% (125) of the raisers usually with supplements (210 or 77.78%). Supplementation of chickens with vitamins and electrolytes

prevent occurrence of nutritional deficiencies (Vitamin Deficiencies in Backyard Chicks and Chickens 2014).

Flock Health Management

Practically, the most economical and ideal method to control disease is through prevention, which could be achieved by proper management, good sanitation, and having an effective vaccination program (Tips on Poultry Raising 2013). Table 1 shows the health management adopted by the flock owners. Two hundred and twenty (81.48%) owners practiced handwashing before handling feeds. This practice is important especially that the transmission of *C. albicans* involves contamination of feed (Dhama et al 2013, Jordan 2008). Although *C. albicans* is resistant to many disinfectants (Candidiasis, Moniliasis, Thrush, 2000), 134 (49.63%) of the flock owners used disinfectants (detergent, bleach) in cleaning the cages. Most flock owners infrequently removed dung from chicken houses or cages (145, 53.70%). Chicken manure is composed of feed residue, intestinal bacteria, digestive juices, mineral by-products from metabolic processes, and water. These components lead to issues in humidity and odor, thus, manure should be managed, and that is, subjecting it to composting and use it as fertilizer (Weidland and Nolden 2015). Raisers disposed manure as direct fertilizer to plants (104, 38.52%), and or other methods. Some did not practice manure disposal (102, 37.78%) because their chickens were free ranging.

Of the 270 chickens sampled, only 60 (22.22%) were not dewormed, in contrast to vaccination, only 16 (5.93%) were said to be vaccinated. When chickens got sick, majority of the raisers treated their own animals (267, 98.89%) instead of consulting a veterinarian or an animal technician (1.11%). Majority of the flock owners buried the dead chickens (263, 97.41%), while others disposed them at the back of the animal shelter (1.85%) or burned them (1.48%). Dead poultry on farms can cause nuisance, release of unpleasant odor, aesthetic problems, surface and groundwater pollution, and diseases; insect, rodent, and predator problems may also occur if the birds are not disposed of properly.

Table 1. Flock health management provided to chicken flocks in selected villages of Baybay City, Leyte

Variable	Description	N	% (95% CI)
Wash hands before handling of feeds	Yes	220	81.48 (76.33-85.93)
	No	50	18.52 (14.07-23.67)
Disinfect shelter	Yes	134	49.63 (43.51-55.75)
	No	136	50.37 (44.25-56.49)
Dung Removal	Low frequency	145	53.70 (47.56-59.77)
	High frequency	125	46.30 (40.23-52.44)
Manure disposal			
In pits	Yes	61	22.59 (17.74-28.05)
	No	209	77.41 (71.95-82.26)
Into bodies of water	Yes	2	0.74 (0.09-2.65)
	No	268	99.26 (97.35-99.91)
As fertilizer	Yes	104	38.52 (32.68-44.61)
	No	166	61.48 (55.39-67.32)
No disposal	Yes	102	37.78 (31.97-43.85)
	No	168	62.22 (56.15-68.03)

Table 1. continued

Variable	Description	N	% (95% CI)
Deworming	Yes	210	77.78 (72.34-82.59)
	No	60	22.22 (17.41-27.66)
Vaccination	Yes	16	5.93 (3.42-9.45)
	No	254	94.07 (90.55-96.58)
Treatment of sick animal	Yes	267	98.89 (96.79-99.77)
	No	3	1.11 (0.23-3.21)
Management of dead animals			
Thrown away	Yes	5	1.85 (0.60-4.27)
	No	265	98.15 (95.73-99.40)
Burned	Yes	4	1.48 (0.41-3.75)
	No	266	98.52 (96.25-99.59)
Buried	Yes	263	97.41 (94.73-98.95)
	No	7	2.59 (1.05-5.27)

Risk Factors Associated with the Prevalence of C. albicans in Chicken Flocks

Table 2 shows eight independent variables identified to be unconditionally associated (*p* value <0.20) with the presence of *Candida albicans* in chicken flocks. The most important variables include sex of chicken (male X^2=7.36 *p*=0.0067), washing of hands (X^2=4.02 *p*=0.0451), and manure disposal (direct fertilizer X^2=4.74 *p*=0.0295).

Table 2. Factors unconditionally associated with *Candida albicans* infection in chickens

Variable	Response	*C. albicans* +		*C. albicans* -		Odds ratio (95% CI)	*p*-value
		n	%	n	%		
Sex of chicken							
Male	Yes	61	30.05	142	69.95	0.4426	0.0067
Female	No	33	49.25	34	50.75	(0.2515-0.7789)	
Chicken type							
Hen		16	51.61	15	48.39	2.3510 (1.0765-5.1344)	0.0320
Contact with other animals							
Dog	Yes	60	31.75	129	68.25	0.6430	0.1395
	No	34	41.98	47	58.02	(0.3757-1.1002)	
Wash hands before handling of feeds	Yes	70	31.82	150	68.18	0.5056	0.0451
	No	24	48.0	26	52.0	(0.2711-0.9428)	
Manure disposal							
In pits	Yes	16	26.23	45	73.77	0.5972	0.1479
	No	78	37.32	131	62.68	(0.3162-1.1276)	
Direct fertilizer	Yes	45	43.27	59	56.73	1.8212 (1.0919-3.0376)	0.0295
	No	49	29.52	117	70.48		
Management of dead animals							
Thrown away	Yes	4	80.0	1	20.0	7.7778	0.0955
	No	90	33.96	175	66.04	(0.8566-70.6202)	
Buried	Yes	89	33.84	174	66.16	0.2046	0.0972
	No	5	71.43	2	28.57	(0.0389-1.0756)	

Factors unconditionally associated with *Candida albicans* infection were included in the multivariable logistic regression analysis. The final model determined that sex of the chicken was found to be associated with the presence of *Candida albicans*.

Table 3 shows the final model identifying the associated factor to the presence of *C. albicans*. The odds of *C. albicans* occurring in a male chicken is 0.44 with a *p*-value of 0.0047 and a coefficient of - 0.8151. This means that the odds of male chickens to have the infection is 56% less than the female chickens. Despite the fact of acquiring more samples from male chickens, the prevalence of *C. albicans* is still higher in female chickens 33/67 (49.25%) than in male ones 61/203 (30.05%). Transmission of *Candida* among poultry flocks is mainly by fecal contamination of feed or water (Dhama et al 2013). This is why poor hygiene could readily predispose chickens to *Candidia* infection. In the study area, the female chickens were mostly kept in cages. Meanwhile, the cockerel and roosters – especially those for cockfighting – were kept in sheds in an open area. This difference in management might have a significant role in the difference of infection rate between male and female chickens. However, the ways of keeping chickens, as a variable, was considered and forced in the final model but did not give a significant result. Despite this statistical insignificance, the authors cannot negate the biological plausibility that poorly sanitized cages, high humidity, and environmental temperature could have effected to increased fungal growth and exposed the female chickens to high concentrations of fungal spores resulting to higher infection rate (Talley et al 2002).

Furthermore, the susceptibility of chickens to fungal infections in relation to sex is still unclear and there are many host factors that need to be considered for further investigation. Taghavi et al (2014) cited that under natural conditions, male compared to female chickens have more ability to transit fungal diseases to other animals and man. It is known that systemic candidiasis in poultry is rare but has been reported in humans and different animals (Ameen 2010).

Table 3. Risk factor associated with the presence of *Candida albicans* by multivariable regression analysis

Term	Odds ratio	95% CI		Coefficient	Standard Error	Z-Statistic	*p*-value
		Lower	Upper				
Male (Yes/No)	0.4426	0.2	0.7	-0.8151	0..2884	-2.8267	0.0047
Constant	*	*	*	-0.0299	-0.2444	-0.1222	0.9028

Score test: p=0.0042, statistic=8.1865
Likehood ratio test: p=0.0048, statistic=7.9511

With the result of this study, raisers are encouraged to employ effective hygiene and sanitation management practices to the whole flock. This is to prevent predisposition of any chicken type to infection. Flock owners must also observe the proper disposal of chicken manure. *Candida albicans* is an important zoonotic agent causing various types of mycoses (El-Hariri et al 2013). *Candida spp.* are known to be associated cause of skin lesions in poultry workers, commonly known as "chicken poison disease" (Mayer and Daclam 2012). Ideally, to prevent exposure to fungal spores poultry raisers should avoid situations where material that might

be contaminated can become aerosolized and subsequently inhaled. Therefore, work practices and dust control measures that eliminate or reduce dust generation during the removal of chicken manure from the cage will also reduce risks of infection and subsequent development of disease. To the best of the author's knowledge, this is the first isolation of *C. albicans* from domestic chicken droppings that has been done in the Philippines.

CONCLUSION AND RECOMMENDATIONS

A total of 94 isolates from 270 fecal samples examined were positive for *C. albicans* with prevalence of 34.81%±5.68%. Eight factors were unconditionally associated with *Candida albicans* infection in chickens but only one factor (sex of chicken) remained in the final logistic regression model (male, OR=0.44, p=0.0047). With the zoonotic potential of *C.* albicans, it is highly recommended that efficient health and sanitary practices must be implemented in smallhold chicken flock production systems.

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