Univ. of Min. Intl. Mult. Disc. Res. Jour. Vol. 3, Issue 1, Dec. 2018
http://www.umindanao.edu.ph/journal

Ectoparasites in domesticated goats and associated bacterial species from Davao City, Philippines

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Date received: September 4, 2018
Date accepted: December 18, 2018
Date published: December 20, 2018

ABSTRACT

This study focused on identification of ectoparasites associated with goats from Davao City, Southern Mindanao, Philippines and molecularly detected the bacterial species associated with these ectoparasites. Ectoparasites were collected from three goats in three different sites within Davao City, of varying breed and gender in three different locations in Davao City. Ectoparasites were combed from the head, body, and tail, pooled and were subjected to molecular procedures. Eighty-nine ectoparasites were collected, composed of: *Linognathus* spp, *Ornithodoros* spp and *Boophilus* spp. Molecular data revealed four bacterial species associated with the ectoparasites but none were pathogenic. The presence of bacterial species allied with the goat ectoparasites may suggest the capacity of these ectoparasites to transmit bacterial species that could either be pathogenic or not. Moreover, future studies may be explored to establish the relationship of the detected bacterial species from the ectoparasites.

***Keywords**: Animal Raising, Disease, Domestication, Parasitism*

INTRODUCTION

Goats (*Capra hircus* L.) are one of the most common livestock raised in the Philippines due to their market value (Orden et al., 2005). Goat raising is an appealing venture because of its low shelter and feeding requirements and potential for increased production in a relatively short period (Orden et al., 2016). Just like any other domesticated animal, goats are not spared from parasite infestation. Black Bengal goats form Bangladesh are reported to be infested with several species of lice, ticks, and fleas (Rony, Mondal, Islam, and Begum, 2014). Domesticated ruminants in Lebanon including goats were also reported to harbor tick species known to be invovled in pathogen transmission (Dabaja et al., 2017). Portugaliza and Bagot (2015) reported that in Leyte, Philippines, goats serve as host for the following species of lice: *Damalinia caprae* and *Linognathus* spp.

Ectoparasites can facilitate disease transmission from animals like goats to other susceptible hosts. Ectoparasites like ticks and lice are potential vectors of bacteria, which can be transmitted to other hosts via blood meal from an infected source host (Fournie, Pfeiffer and Bendrey, 2017). In Giza, Egypt, mobile and household goats were reported to be infected with the same strain of *Brucella*, with potential increase in Brucellosis onset (Nour et al., 2017). Fournie, Pfeiffer and Bendrey (2017) also reported that even at low levels of transmission in goats, *Brucella melitensis* can be sustained in the population of domesticated goats and that suitable conditions may have promoted the exposure of humans to this pathogen.

Given the popularity of goats being raised for economic purpose and the looming potential of ectoparasite-mediated zoonosis of microorganisms from goats, this study is directed at determining the ectoparasites associated with domesticated goats and the bacterial species the ectoparasites from these goat hosts harbor.

MATERIALS AND METHODS

Collection and identification of ectoparasites

Ectoparasites were collected from domesticated goats raised in three sites in Davao City, Southern Mindanao, Philippines. Three goats per site were inspected. The ectoparasites were collected from the goats' furs following the protocol of Adang et al. (2015) and modified version of the protocol of Israel et al. (2015). A technical expert from the Department of Agriculture Davao del Norte Provincial Office identified the breeds of the goats: Alpine, Anglo Nubian, Saanen, Boer, Anglo Saanen, and Ntive. Ectoparasites were identified following morphological descriptions in Wall and Shearer (2001), and Johnson and Triplehorn (2005).

Molecular detection of bacterial species

The ectoparasites were first surfaced cleaned with 85% ethanol and pooled together following the modified protocol of Aktas et al. (2010). The nucleic acid from the ectoparasites were extracted using phenol-chloroform method following the protocol used by Zumbo (2015) and Halos et al. (2004). The extracted genome was subjected to PCR amplification using the 16srRNA primer sequence developed by Barghouti (2011): QUGP-F3 5'-GATACCCTGGTAGTCCA-3', QUGP-R3 5'-TGGACTACCAGGGTATC-3'. The PCR kit used for amplification was TaqMaster Mix (Vivantis). Mixture proportion of the PCR components was followed according to the manufacturer's recommendations. DNA bands detected after gel electrophoresis were sent to Korea (Macrogen Inc)

for sequencing. Sequence identity was determined by finding a match from the available database from NCBI using BLAST (BLASTN) Analysis (Donkor et al. 2014).

RESULTS AND DISCUSSION

Percent occurrence of ectoparasites based on goat's breed, gender and body parts

Eighty nine ectoparasites were collected from nine goat individuals from three different locations within Davao City (Table 1). All the goats were infested with *Linognathus* spp., a chewing lice, while soft ticks, *Ornithodoros* spp. (soft tick), were collected from Anglo-nubian and Saanen breeds. Samples of hard ticks (*Boophilus* spp.) were solely observed from the Anglo-nubian breed. The Anglo-Saanen breed harbored the highest number of ectoparasites (n=32) but it was in the Anglo Nubian breed where diverse groups of ectoparasites were collected.

Since the collection of ectoparasites was conducted during the months of October to December 2017, the occurrence of *Lignognathus* spp. in all goat breeds during these months appears to be consistent with previous reports. Yeasmin, Khanum and Zaman (2014) documented that some ectoparasites like *Linognathus* sp. show higher infestation during the rainy months of the year which is also attributed to the sensitivity of the reproductive system of the female lice as it can only produce greater number of eggs on colder months (Brown et al. 2005).

The *Linognathus* spp. spend their lifetime attached to a host and may only be transferred to others through direct contact thus making it common among herding animals (Adang et al. 2015). The soft ticks belonging to the *Ornithodoros* spp. were also documented in the study.

Table 1. Percent occurrence of ectoparasites according to goat breed. (N = 89)

TYPE OF ECTOPARASITE/ BREED	LICE 	SOFT TICKS 	HARD TICKS 
Alpine	16%		
Anglo Nubian	1%	6%	1%
Saanen	7%	4%	
Boer	10%		
Anglo Saanen	36%		
Native	19%		

According to Smith and Sherman (2009), there are only two genera of soft ticks found to be common and significant in goats: *Otobius* spp. in America and some parts of India and *Ornithodoros* spp. found in most Asian countries with warm tropical weather (Roman et al. 2012). In the study of Nabian and Rahbari (2008), hard tick (*Boophilus* spp.) was reported to be present in almost all ruminants located within the same area including goats. Although the goats sampled in this study were free to roam, they stayed in the same cage albeit separated according to gender at the end of the day. Goats that live together are infested with the same ectoparasites and their degree of occurrence could be affected by their living conditions (Adang et al. 2015). This could be the possible reason why ticks were observed in both the Anglo-Nubian and the Saanen breed since were housed in the same cage by the end of each. High infestation rates may be caused by a few factors including climate, malnutrition, poor husbandry practices, poor awareness of farmers regarding ectoparasites, and inadequate animal health services (Kumsa et al. 2012).

Six out of the nine goats sampled were female and three were male. A total of 53 ectoparasites were collected from the male individuals while only 36 ectoparasites were obtained from the female goats. These data parallels the results reported by Seyoum et al. (2015), wherein they attribute the high ectoparasite prevalence on male goats to social behavior. Male ruminants usually do not do as much self-grooming as females and have more activities that require interaction with other ruminants, thus increasing the potential to be infected (Mooring et al. 2006). This is the reason why ectoparasites can transfer from one animal to another as they cling onto shrubs and grasses and unsuspectingly latch on to whatever host that passes by (Walker et al. 2003).

The body of the goat was observed to have the highest infestation rate of ectoparasites while the head was observed to be infested with various parasites (Table 2). The ventral section of the goat's body harboured the most number of ectoparasites (*Linognathus* spp). As the warmest part of the goat, the lice aggregate in this area (Brown et al. 2005). Ohaeri and Ugwu (2013) noted that lice are found on the neck and head while ticks are found on the head, abdomen and tail part of the goat. Other sites for attachments are the upper part of the neck and head, especially the back of the ears (Desta et al. 2010). Both the *Ornithodoros* spp. and *Boophilus* spp. were only observed on the head. The lumpy body of the tick makes it easy for the host to remove the ectoparasite through self-grooming. Part of the adaptation of ticks is to reside on the head to avoid being chewed on or licked off by the host while grooming (Yacob et al. 2007).

Table 2. Percent occurrence of ectoparasites according to goat body parts.

TYPE OF ECTOPARASITE/ BODY PARTS	LICE 	SOFT TICKS 	HARD TICKS 
Head	21%	1%	10%
Body	42%		
Tail	26%		

Bacterial species associated with goat ectoparasites

A total of 11 pooled ectoparasite samples were subjected to molecular detection of bacterial species. The pooling was based on the sample location, breed of goat, and ectoparasite species identified. Only two out of the 11 pooled samples were negative for any bacterial species (Sample ID 1ST and 1SU). Six of the nine samples had 16srRNA gene sequence most similar to *Lactobacillus paracasei* while others were most similar to *Bifidobacterium longum*, Bacterium C08, and uncultured actinobacterium clone (Table 3).

Table 3. Sequence analysis results of the amplified PCR products based on NCBI BlastN database.

Sequence Code	Query Cover (%)	% Identity	Sequence Id	Accession Number
2NL	93	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1
2ASL	51	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1
1SL	24	94	Bacterium C08 16S ribosomal RNA gene	DQ329326.1
1BL	73	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1
1ASL	23	85	*Bifidobacterium longum* partial 16S rRNA gene	AM990172.1
1ANL	15	78	Uncultured actinobacterium clone YC5 16S ribosomal RNA gene, partial sequence	DQ4114825.1
1NL	41	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1
2ANT	74	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1
1AL	33	91	*Lactobacillus paracasei* 16S rRNA gene	AJ508362.1

All the bacterial species appear to be non-pathogenic to humans. In fact, *L. paracasei* and *B. longum* are commonly used as components of probiotics (Eutamene et al. 2007; Kitaoka et al. 2005). The *Lactobacillus* genus is one of the many bacterial taxa that persist as a commensal of the human gastrointestinal tract and may have a role in pathogen protection, immune system development, and improved host nutrition (Heilig et al. 2002; Walter et al. 2001). The study of Furrie et al. (2005) further highlighted the commensalistic nature of *B. longum* and it being a component of the human gastrointestinal tract. Results of the study also revealed that the synergy of this bacterium with other microflora of the gut can be utilized to address gastrointestinal diseases such as ulcerative colitis. Although *B. longum* is a commensal of human intestine, finding it in the associated ectoparasites of the goats may imply that the goats have eaten food items contaminated with human fecal matter. This is also significant since the species of *Bifidobacterium* found in animal and human guts are entirely different (Gavini et al. 2006). Hence, it is also possible that the ectoparasites acquired the bacteria through biting other species and transferred the bacteria through blood meals, though this was not observed during the collection of ectoparasites. More studies however may clarify this assumption. The Bacterium CO8 16s rRNA and the uncultured actinobacterium clone 16s rRNA that are most similar to two different nucleotide sequence samples do not have a clear pathogenic role but appear to be significant components of the environment. Bacterium CO8 was reported by Niu et al. (2006) as a

constituent of the microbial load of the wastewater while the uncultured actinobacterium clone appears to be a methanogenic bacterium found in the soil (Zhou et al. 2008).

RECOMMENDATIONS

We recommend that future researchers who will work in similar studies must increase the number of goats to be inspected for ectoparasites to establish if what was collected are the only ectoparasites infesting goats in Davao City. It will also be more productive if several primers targetting other genes for bacterial identification be considered.

Future research endeavors may also focus on metagenomics as some bacterial species which could be pathogenic occurred in levels below detection.

ACKNOWLEDGEMENT

We are indebted to the generosity of the goat owners who allowed us to collect samples from their raised animals. We are also grateful to the logistical support of the Arts and Sciences Department of San Pedro College through its dean, Dr. Ana Julia P. Enero. Sincerest thanks to Ms. Helen Ancla, head of laboratories of San Pedro College, for generously providing the materials and lending the equipment needed for the experimentation of the study. The Department of Agriculture Davao del Norte Provincial Office generously provided technical assistance in the identification of the breeds of the goats.

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