Vector Biology Journal

Research Article

A SCITECHNOL JOURNAL

Rattus norvegicus (Berkenhout 1769) as Potential Reservoir Host of Zoonotic Parasites from Selected Backyard Pig Farms in Basag, Butuan City, Agusan Del Norte, Philippines

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Abstract

Rodents play a significant role in public health as a biological reservoir of various parasitic zoonoses. Preliminary detection of zoonotic parasites among wild rats, *Rattus norvegicus* in selected backyard livestock farms in Basag was carried out to assess their vectored zoonotic parasites. The results of the study showed that 100% of the collected rats were infected with zoonotic helminths and these parasites were identified as *Hymenolepis diminuta*, *Hymenolepis nana*, *Cysticercus fasciolaris*, *Taenia spp*., hookworms, *Angiostrongylus cantonensis*, *Capillaria spp*. and *Echinostoma spp*. with prevalence at 66.7%, 33.3%, 33.3%, 6.70%, 100%, 6.70%, 20% and 6.70%, respectively. Moreover, infection rate has significant association to the farming practices (average farming experience rs=-0.402, p =0.038; distance of pig pen to the neighboring household rs=0.402, p =0.038), and to some morphological features of rats such as the head-body size (rs=0.534, p =0.040), length of the tail (rs=0.534, p =0.040) and hind-foot measurement (rs=0.570, p =0.026). In addition, possible cross-infection of hookworm between rats and backyard raised pigs was also recorded. The results indicate that there was significant relationship between the intensity of zoonotic parasites and the rat's behavior and morphology; the presence of some susceptible host (pigs, stray dogs/cats); and the hygiene practices and knowledge of farmers pertaining to backyard-livestock faming. Consequently, it was recommended that rodents must be eliminated in livestock farms and the conceptualization of a community-based intervention programs for animal/ public health aspects must be address in backyard farming to prevent rodent- borne disease outbreak in the future.

Keywords

Rattus norvegicus; Reservoir host; Zoonotic parasites; Helminths

Introduction

Rodents can play various roles in distributing parasitic diseases: they can be the accidental host, significant reservoirs, vectors, or a final host, reliant on the illness and location. Consequently, rodents can be part of the transmission of diseases to humans without direct contact with them and even more being far away from the place of infection [1]. The wild brown rats *Rattus norvegicus* is the most dominant rodent species in the Philippines that have been recognized as destruction to foodstuffs, pests of agronomic crops resulting to some significant economic losses and apart from that, they have been responsible for spreading some diseases far beyond their original boundaries (Leptospirosis, Angiostrongyliasis, Echinostomiasis, etc.) Preliminary detection of zoonotic parasites among wild rats *R. norvegicus* in selected backyard pig farms in Barangay Basag, Agusan del Norte was carried out to assess their potential as reservoir host for zoonotic parasites [2]. Provision of the data that was generated in this research is of great importance in promoting public health awareness and formulation of rodent control policy in the area to prevent the people from becoming infected by these rodent-borne parasitic diseases [3].

Materials and Methods

Study area

Barangay Basag is a small community and is one of the 86 barangays of Butuan City within the province of Agusan del Norte located in Caraga Region, Mindanao, Philippines. The barangay is situated at 8.92° North latitude, 125.62° East longitude and 99 meters above the sea level. The community of Barangay Basag has total population of 3,573. There are two classifications of Land in Basag: The residential land, and agricultural land (e.g. rice land, corn land, etc.). The study was conducted in both residential and agricultural areas of Barangay Basag. Within the residential area, three households of swine raisers were selected as sampling sites [4]. The selected households were randomly selected based on the swine they owned that are proved positive for helminth infection and soil contamination (based on the unpublished study). The accessibility, peace and order situation, and the willingness of the community to participate in the research study were also considered (Figure 1) [5].

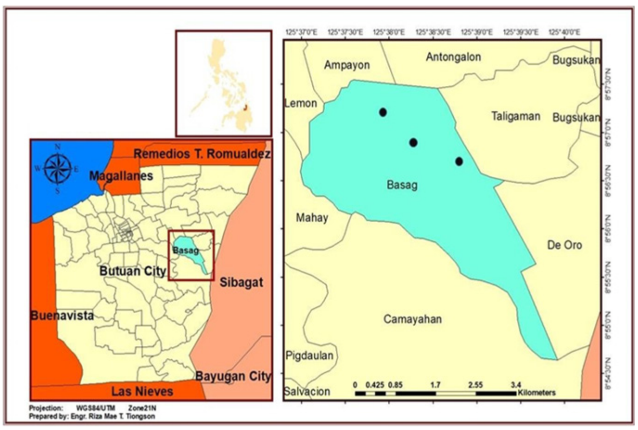

Figure 1: Map showing the study area, Barangay Basag, Butuan City, Agusan del Norte, Philip- pines.

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Received: 25 May, 2021, Manuscript No. VBJ-21-32231; **Editor assigned:** 28 May, 2021, PreQC No. VBJ-21-32231 (PQ); **Reviewed:** 11 June, 2021, QC No. VBJ-21-32231; **Revised:** 05 September, 2022, QI No. JNRD-19-5514, Manuscript No. VBJ-21-32231; **Published:** 03 October, 2022

Collection of rats: The study was done last January to February 2018. Trapping of rodents was done in three weeks during 17:00 pm to 6:00 am in the three sampling sites with permission and help of some residents in Basag. A minimum of five rodents were collected in each sampling site using a custom-made steel wire traps measuring 29 X 22 X 50 cm with dried fish as baits [6]. Eight traps were set every day and placed in different locations: Inside the swine raisers house; near the backyard pig farm; neighboring household near the swine raisers pig farm; and the agricultural areas near the swine raiser's residency (e.g. rice field, corn field, etc.). Trapped rodents undergo fasting for 24-48 hours and were killed by placing the rodents into a plastic box containing cotton wool soaked with chloroform [7]. The biometrics of rodent was employed such as sex, diagnostics features and morphometric measurements (head- body, hind foot, tail, and weight) for identification purposes of the collected rodents and was recorded [8].

Isolation, examination and identification of parasites: Ectoparasites collected rodents was isolated from the external parts of the rodent's body such as fur, anal area, armpits, behind ears, between fingers and toes. The collected Ectoparasites were observed under the dissecting microscope for the proper visualization of its morphology and identification [9]. The collected rodents were dissected after anaesthetizing to expose the gastrointestinal tract and the other internal organs; the body cavity was examined for the presence of any macroparasite visible to the naked eye [10]. The gut region (stomach; small and large intestine; and caecum) was weighed and cut opened. Parasite worms in the gut region that are visible to the naked eye were collected and placed in the vial containing 10% ethyl alcohol for preservation. Small and large intestines were placed in the different petri plates containing distilled water prior to examination of parasite worms and eggs that are not visible to the naked eye. Intestinal tissues soaked in distilled water were placed in the glass slide and observed in the light microscope under 40X (Scanner); 100X (LPO); and 400X (HPO) magnification [11]. The observed parasites were documented using a motic camera. Parasite eggs from the fecal pellet of the gut contents were observed using a simple salt floatation method. Liver was examined for the presence of cysts and migratory tracts of larvae, and refrigerated in the laboratory of the Biology Department before preservation for further analysis. Parasites collected were identified using available parasitology books and journals, published data, and consultation of experts [12]. The prevalence and intensity of the collected parasites were calculated using the formula [13].

Prevalence (%)=(Number of positive sample/Total number of samples) X 100

Mean Intensity=(Total Count of Parasite/Total number of Positive Samples)

Survey on household information, knowledge about parasitic infection and farming practices of locals

The administered, modified and well-structured survey questionnaire based on the known associated risk factors of parasitic infections was developed [14]. The survey questionnaire was addressed to the farming practices of the swine raisers; presence of latrine and its utilization; source and treatment methods for drinking water and other domestic uses; hand washing facilities; zone of residence (across the river, upland, middle and lowland plateau zones); presence of animals in the participant's residency (e.g. pigs, dogs, cats, chickens, carabao and other animals that may serve as vector of parasitic infection); and other risk factors was carried out by the researcher through survey analysis on the surrounding environment that would support the data for the possible risk factors for parasitic infection [15].

Data analysis: Statistical Package for the Social Sciences (SPSS) software version 16 was used for the statistical analysis. Spearman's Correlation coefficient was used in examining the association between the prevalence and mean intensity of parasitic infections among rats *R. norvegicus*, morphometric measurements, gender, and farming practices [16]. No statistical analysis that was used between the secondary data based on parasitic infection in swine (unpublished data) and rats since both samples are not taken at the same time. Kruskal-Wallis test was used in the comparison of parasite infection in rats relative to sex and between farms [17]. The differences were statistically significant if the p-value is at 0.01 and 0.05 level [18].

Results

Prevalence and mean intensity of endoparasites

A total of fifteen rats, 11 (73.3%) males and 4 (26.7%) females of which one was pregnant were caught within three weeks from January to February 2018 [19]. All the captured rat in this Brown rats *R. norvegicus* is the most dominant rodent species in the Philippines 15 [20]. The result of this study demonstrated that all examined *R. norvegicus* has an overall 100% infection rate of Helminth Endoparasites [21]. These findings are similar with other prevalence rate studies: 95.3% in Kandy district, Sri Lanka 49, 90.9% in Grenada 10, and 100% in Akure, Nigeria where in H. was identified as *Rattus norvegicus* (verified by Angelica Tujan from University of the Philippines, Los Baños). An observation that conform to the findings in the prevalence study in the Philippines, in which it was confirmed that the ubiquity of this rat species is the most common wild rats 15 [22]. All the captured rats were active and some exhibited aggressive behaviors in captivity prior to dissection [23]. Some external appearances indicate symptoms of any diseases such as skin rashes and loss of fur of the 15 rats examined, 100% were infected with helminth endoparasites. One species of trematodes (flatworms), four species of cestodes (tapeworms) and three species of nematodes (roundworms) has been isolated from the examined rats *R. norvegicus*. Among nematodes, hookworm infections were the most prevalent type of infections (100%) followed by cestodes (Rat tapeworms), *H. Diminuta* (66.7%), *H. nana* (33.3%) and *Cysticercus fasciolaris* (33.3%) [24]. Three individuals (20%) were infected with nematode parasite *Capillaria spp*. and one individual (6.7%) was infected with nematode parasite *A. cantonensis*, cestode *taenia spp*. and Trematode *echinostoma spp*. Though Hookworms has the highest prevalence rate, *A. cantonensis* has the highest mean intensity (1507) followed by *H. nana* (989.2), *Echinostoma spp*. (222), Hookworms (204.6), *H. diminuta* (155.7), and *C. fasciolaris* (4) and *Capillaria spp*. (2.33) [25]. While *taenia spp*. has the lowest mean intensity of one. Diminuta, and *H. nana* and *Taenia spp*. are the most prevalent intestinal helminth parasites that were recovered from examined rats [26]. However, the prevalence rates were relatively lower in Sudan (70%) 11, and in Ile-Ife, Nigeria (58%)72, five species of intestinal helminth parasite were recovered in this study. In Saudi Arabia, there is 23.7% prevalence rate of Helminth infection among rats wherein two species of helminth were recovered, *H. Diminuta* and *H. nana* 18 [27]. The findings of this study show that the rat tapeworms, *H. diminuta*, *H. nana* and *Taenia spp*. were the most common Endoparasites found in *R. norvegicus* rats in Basag, Butuan City, Agusan del Norte [28]. This finding agrees with that of the prevalence study in Taiwan in which *H. Diminuta* and *H. nana* has the highest prevalence rate of infection in rats [29]. Moreover, recent study of Endoparasites in rats found in wet markets of Luzon, Philippines, *H. diminuta* and *Taenia spp*. were the most common helminth endoparasites in rats. Though, most of the research study mentioned that rat tapeworms (*H. diminuta*, *H. nana*, *Taenia spp*.) are the most prevalent, the results in this research study shows that hookworm infection are the most prevalent (100%). Hookworms is a type of unsegmented intestinal helminth parasites that naturally infects rats [30]. Hookworms are mainly spread because of poor hygiene practices and when rats encounter feces of infected rats and other animals. However, *A. cantonensis* has the highest mean intensity of 1507 with a least prevalence rate of 6.7%. The results show that there is inverse relationship between the mean intensity and prevalence rate of *A. cantonensis* infection. *A. cantonensis* is a rat lungworm that naturally infects wild rodents especially brown rat *R. norvegicus* and black rat Rattus rattus [31]. This parasite species has been found to naturally infect several rodent species in the Philippines [32]. The results show low prevalence of *A. cantonensis*, however, the prevalence rate (46%) of *A. cantonensis* infection in *R. norvegicus* is relatively higher in Muñoz, Nueva Ecija, Philippines [33]. This indicates that the intensity and prevalence of *A. cantonensis* infecting the rats in the area might be due to a seasonal infection *A. cantonensis*. It is observed that most life stages of the recovered parasites were eggs. Female nematodes (Hookworms, *A. cantonensis*, and *Capillaria spp*) [34]. Can lay thousands of eggs per day 58,79,80, cestodes (Rat tapeworms: *H. diminuta*, *H. nana*, *C. fasciolaris* and *Taenia spp*.) produces an average 1,000 proglottids and each worm may produce 50,000 to 250,000 eggs per day 42-45, and Trematode *Echinostoma spp*. which is similar to Hermaphroditic cestodes that has both male and female reproductive organs that is capable for self-fertilization, and can produce thousands of eggs per day. Of the 15 rats examined, 100% are infected with two or more parasites [35]. No single infection has been recorded in this research study. Ten (80%) rats have double infections, two (13.33%) rats have triple infections and three (6.67%) of rats have multiple infection [36]. These findings revealed that 100% of the examined wild rats *R. norvegicus* has double to multiple infections, the same as the findings in the prevalence study in India wherein all the wild rodent species found are naturally infected with one or more species of helminthes [37]. However, it contradicts with the research study about experimental rats in Iraq in which it was found out that single infection (52%) is much higher than double (16%) to multiple (8%) infections. Hence, the result of determining the prevalence rate of parasitic infection in experimental rats is in contrast with prevalence rate of parasitic infection in wild rats [38]. Observation in this study reveals the small intestine as the preference site of the Endoparasites might be due to the helminth worm characteristics that generally don't have digestive system and thereby depend mainly on the high abundance of absorbable and digested food materials in the small intestine of their host and thus absorb the nourishment into their body (Table 1) [39].

Table 1: Double, triple and multiple helminth parasite infection of the 15 examined *R. norvegi- cus* collected from selected backyard pig farms of *Basag, Butuan City, Agusan del Norte*.

Types of infection	No. of infected rats	Percentage (%)
Double infection		
Hookworm+*H. diminuta*	5	33.33
Hookworm+*H. nana*	5	33.33
Total Double	10	66.67
Triple infection		
Hookworm+H. diminuta+C. fasciolaris	2	13.33
Total Triple	2	13.33
Multiple Infection		
Hookworm+H. diminuta+Capillaria spp.+C. fasciolaris	2	13.33
Hookworm+H. diminuta+Taenia spp.+A. cantonensis +Capillaria spp+Echinostoma spp+ C fasciolaris	1	6.67
Total multiple	3	20
Total	15	100

Prevalence and mean intensity of parasites relative to gender

The prevalence and mean intensity of the parasites relative to gender of the examined rats are presented in since almost all the examined rats are males (73.3%) than females (26.7%), it is observed that there is higher prevalence and mean intensity rate of parasites in males compared to females [40]. This observation agrees with the prevalence study in Abeokuta, and Akure Nigeria 50 in which prevalence and mean intensity of parasitic infection is relatively higher in males compared to female rats. It has been suggested that higher exposure to parasitic infection could be pronounced in male rats due to their due to their active foraging habit [41]. However, the prevalence (p value=0.16) and mean intensity (p value=0.33) of parasitic infection between sex has been compared through Kruskal-Wallis Test and shows no significant association [42]. This indicates that parasite infection in collected rats in the study area does not vary between genders. This result was supported by the research study in southwest Nigeria, in which it was stated that there are no certain explanations that could attribute to the fact that the males carry the higher rate of parasitic infection than females since both sexes are exposed to the same environmental conditions [43]. Hence, feeding habit of rats have high predisposition for getting parasite infestation due to their Euryphagic food habit (Figure 2) [44].

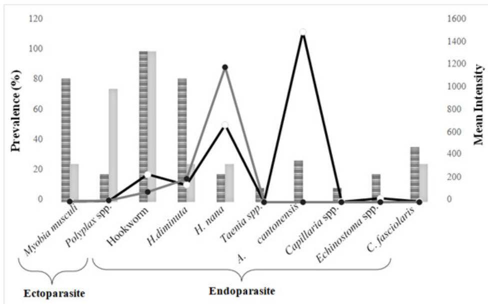

Figure 2: Prevalence and Mean Intensity of parasites by sex of the 15 examined R. norvegicus col- lected from selected pig farms of Basag, Butuan City, Agusan del Norte Note: Prevalence in male (n=1), Mean intensity in male, Prevalence in female (n=4), Male intensity in female.

Parasites in the rat liver: The fifteen *R. norvegicus* examined, five were infected with multiple hepatic cyst (*Cysticercus fasciolaris*) and Capillaria hepatica. *C. fasciolaris* is a larval form of *T. taeniaeformis* that is commonly encountered by rats. Cysts appeared creamy white color, oval/round shaped that are embedded within the liver [45]. Each cyst contained a single larva that measuring 14-31 cm, with a scolex containing two rows of hooks, and four suckers followed by a very long neck. Gross examination of the liver hepatica infection with firm yellowish white nodulesor appeared in patches/streaks, that are randomly scattered in the serosal surface (Figure 3).

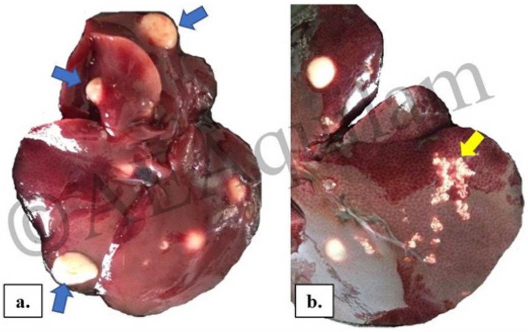

Figure 3: Liver of the rats R. norvegicus collected from selected pig farms of Basag, Butuan City, Agusan del Norte showing the coinfection with Capillaria hepatica and Cysticercus fasciolaris. a) Cysticercus fasciolaris are embedded in the liver (Blue arrows) containing the larva; b) Capillaria hepatica appears as yellowish white patches/tracts or streaks on the liver surface (yellow arrow).

In this research, there are three collected helminth groups such as nematodes (Roundworms), cestodes (Tapeworms), and trematodes (Flukes). Nematodes such as hookworms, *Capillaria spp*., and *A. cantonensis* varies in their egg morphology. Collected hookworm eggs are oval or elliptical in shape measuring 60 µm by 40 µm and has thin transparent hyaline membrane. Hookworm eggs are released by adult worms in the intestine, the egg contains an unsegmented ovum and later develops into segmented ovum when passed in feces which usually has 4 to eight blastomeres. Hookworm eggs are undistinguishable to identify the genus and species because they have the same morphological features in eggs. They must be cultured in the lab to allow larvae to hatch out and so eggs may no longer be evident. In such a case, it is important to distinguish hookworm species, as infection with latter has more serious implications that requires different management. *Capillaria spp*. egg is a peanut or barrel shaped measures 26-45 µm long and 21 µm wide and have striated cell wall, with prominent polar plugs. *A. cantonensis* is another type of nematode that cause human eosinophilic meningitis. *A. cantonensis* eggs is oval shaped measuring 45-60 µm long and 15-25 µm wide (Figure 4) [46].

Figure 4: The identified parasite eggs (400X magnification) collected from 15 examined rast from selected backyard pig farms in Basag, Butuan City, Agusan del Norte: a) Hookworm; b) H. diminuta; c) H. nana; d) Capillaria spp. with its (blue arrows) prominent polar plugs, and (black arrow) striated cell wall; e) A. cantonensis; f) Echinostoma spp. with its (red arrow) uperculum.

Cestodes such as *H. diminuta* and *H. nana* is another type of helminth that is commonly known as rat tapeworms. *H. diminuta* and *H. nana* varies in both egg and worm morphology. *H. diminuta* egg is slightly oval to round in shape, 70-86 µm by 60-80 µm with striated outer membrane and a thin inner membrane. The space between the membranes is smooth or slightly granular, and six hooks in the oncosphere. *H. diminuta* egg is typically larger than *H. nana* egg, lacks polar filaments, and has yellow to transparent cover. *H. nana* egg is oval to spherical in shape, size ranging from 40-60 µm long by 30-50 µm. There are two poles in the inner membrane, from four to eight polar filaments spread out between two membranes and six hooks in the Oncosphere. *H. nana* egg in shows the extra detail of the two polar thickenings on the membrane of the oncosphere with filaments extending into the space around the hexacanth embryo. *Echinostoma spp*. is the only trematode collected from examined *Rattus norvegicus*. *Echinostoma spp*. Egg has wide size range depending on the species, the egg size ranged from 80-135 µm long and 55- 80 µm wide. They have an inconspicuous Operculum and the Abopercular end that is often thickened [47,48].

Hookworms: Hookworms is a type of soil-transmitted Helminth that is the second most common human helminthic infection (after Ascariasis). Hookworms are in worldwide distribution, mostly in areas with moist, and warm climate. Hookworms usually has direct life cycles and doesn't need an Intermediate host for development but infect directly their definitive/final host, where they inhabits in the small intestine and complete the development to adults. Inside the final host, pregnant female hookworms produce thousands of eggs that are usually defecated with the feces of the host and contaminates the environment for example meadows, wetlands, soils and etc. There are other instances in which feces of the infected animals are used as fertilizer, eggs are deposited in the soil and mature. Mature eggs can also be spread if the rodents and other animals travels through fecally contaminated soil and then contaminates other surfaces, that would possibly be the reason that hookworms eggs/larvae may come into contact with humans. L1 larvae hatch out of the eggs in a few hours under favorable climatic conditions. The hatched L1 larvae lives in the vegetation or in the aquatic environment and feed on other microorganisms. The L1 larvae later on molt into L2 larvae and then molt to L3 larvae, the infective stage. The L3 larvae migrate to the intestines where they complete their development to adults and reproduce [49].

There are some known zoonotic hookworms that live in animal that can be transmitted to humans. Dogs, cats, pigs and some rodents can become infected with several hookworm species, including *Ancyclostoma brazilense*, *Ancyclostoma ceylanicum*, *Ancyclostoma caninum* and *Uncinaria stenocephala*. Zoonotic hookworm infection is primarily acquired by walking barefoot on contaminated soil wherein larvae penetrate unprotected skin and cause inflammation. This hookworm disease is called Cutaneous Larva Migrans (CLM) (Figure 5) [50].

Figure 5: Hookworms collected from 15 examined rats from selected backyard pig farms in Ba- sag, Butuan City, Agusan del Norte: a) Larval stage (400X); b) Rhabdi tiform larva (100X) ; c) Filariform larva (L3) (100X); d.) Mature female worm with egg (Red arrow) (100X).

***Angiostrongylus cantonensis*:** *A. cantonensis* are roundworms with a thin cylindrical body which causes eosinophilic meningitis and is spreading rapidly too many parts of the world. Measurement of first stage larvae (L1) is 0.3 mm long by 0.015 mm wide. Second stage larvae (L2) range from 0.45 mm by 0.3 mm and is similar to the size of the third stage larvae (L3), fourth stage larvae measures in about 1.0 mm by 0.4 mm. Newly molted sub-adults measures in about 2 mm long by 0.06 mm wide. Female worms grow to about 12 mm and 11 mm for males before leaving the rats brain and migrate to the pulmonary arteries, where they mature reaching the size up to about 35 long by 0.6 mm wide for females and 25 mm by 0.4 mm for males [51]. The morphological features of both sexes doesn't only vary in size but also in their structures, males can be distinguished based on the appearance of caudal copulatory bursa and long spicules, the apparatus that is used to clasp the female during mating. Female worms can also be distinguished from male through the presence of "Barber pole" that is observed in their internal structure (Figure 6) [52].

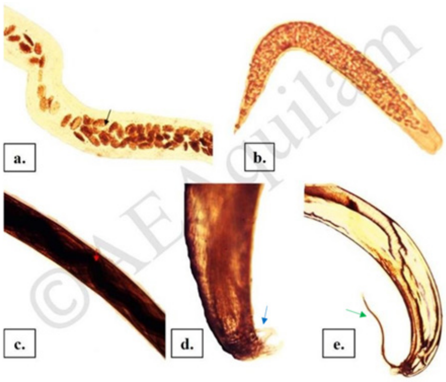

Figure 6: A. cantonesis recovered from the lungs of 15 examined rats from selected backyard pig farms in Basag, Butuan City, Agusan del Norte: a) Thousands of eggs inside a female A. cantonesis (100X); b) Infective larvae (L3) (400X); c) Female adult worm with a "Barber pole" spiral (Red arrow) characteristic (100X); d) Tail of adult male worm showing the copulatory bursa (Blue arrow); e) Long spicules (Green arrow) (100X).

Cestodes: Cestodes or tapeworms possess many basic structural characteristics but also show striking differences shows the general features of the collected tapeworms. The morphological structure of cestodes *H. diminuta*, *H. nana*, worm are divided into scolex or head that bears the organs for attachment, a neck that is the region of segment proliferation and a chain of proglottid called strobila which elongates as new proglottid form in the neck region. Immature segments are found nearest to the neck region (undeveloped sex organs) and those segments near the posterior region are mature. The gravid segments in the terminal region, has the most prominent feature of egg-filled uterus. The scolex contains the cephalic ganglion that serves as the brain of the tapeworm's nervous system. Superficially, the scolex is characterized by holdfast organs. Depending on the species, these organs consist of a Rostellum, the backward facing hooks that helps anchor the worm in the small intestine of the animal host, the bothria are long, narrow, weakly muscular grooves, and suckers called acetabula (Figure 7) [53].

The definitive classification of cestodes or tapeworms not only varies on their egg morphology, but also in both larva, and adult stages [54]. Adult *H. diminuta* measures from 20 to 60 centimeters in length. This tapeworm has a long cylindrical body with 4 suckers and an apical organ at its scolex with no rostellar hooks. Like other cestodes, *H. diminuta* lacks any trace of a digestive tract and absorbs all essential substances through its outward covering [55]. The proglottid of *H. diminuta* contains both male and female sex organs. This tapeworm is distributed worldwide, and approximately 200-300 human cases have been recorded in India, Japan, Italy, and some areas in southern United States. *H. nana* is commonly known as dwarf tapeworm and is the most encountered tapeworm that infects humans and is distributed worldwide [56]. Adult *H. nana* are usually smaller than all other tapeworms measuring 25 to 40 millimeters in length, and 1 mm in width. This tapeworm is transparent and has a long slender neck with segments. They have unilateral genital pores on the side of the segment, wherein each segment contains a single proglottid with a single set of reproductive organs. The scolex of this tapeworm are retractable with 20-30 and has four suckers. *Taenia spp*. worms are usually large that can grow to several meters long and up to 6 centimeters wide, depending on the species. They are whitish in color and like other tapeworms, their scolex or head is armed with hooks and has four suckers for attaching the intestinal wall [57]. Their strobila has hundreds of proglottids which contains both male and female reproductive organ. Numerous *Taenia spp*. are found in both carnivores and herbivores worldwide. Various Taenia species appear to have scientific names because the larval stages in herbivores are frequently named as Cysticercus, Strobilocercus or Coenurus spp. These tapeworms are distributed throughout South and Central America, Eastern Europe, South Africa and some countries in Asia including Indonesia, China and Philippines. In this research study, the species name of the Taenia worm that was collected from the examined *R. norvegicus* was unidentified because eggs of this tapeworm were not observe [58].

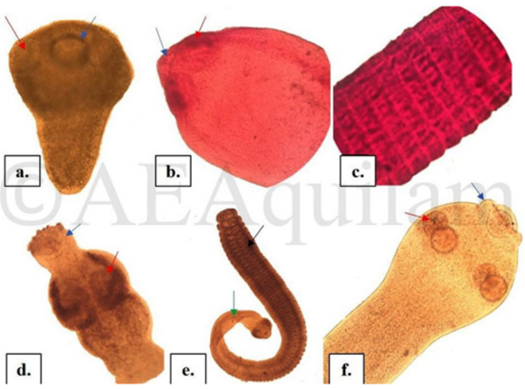

Figure 7: Cestodes showing the common morphological characteristics of their body such as the (blue arrows) rostellum and (red arrows) four suckers, these groups of parasites were recovered from the intestines of 15 examined rats from selected backyard pig farms in Basag, Butuan City, Agusan del **Note:** a) Immature; b) Mature scolex of H. diminuta (100X); c) Proglottid of H. diminuta (100X); d) Immature; e) Mature scolex of H. nana (100X), showing the (green arrow) neck region and (white arrow) proglottid (100X); f) Taenia spp. (100X).

***Trematode (Echinostoma spp.)*:** *Echinostoma spp*. is a type intestinal fluke that has three-host life cycle with their intermediate hosts such as snails and other aquatic organisms various animals including humans may serve as their final host. These parasites are type of Digenean trematode which infects the intestine and bile duct of their hosts. The measurement of adult Echinostoma varies between species but tend to be 2-10 mm long and 1-2 mm wide. They have two suckers: the oral and ventral sucker. Moreover, they are Hermaphroditic parasites that has both male and female reproductive organs. The testes are found in the posterior part of the flukes' body and the ovary that is also found near the testes. Echinostoma has global distribution that is particularly common in South East Asia most especially in the Philippines, Japan, Indonesia and South Korea (Figure 8) [59].

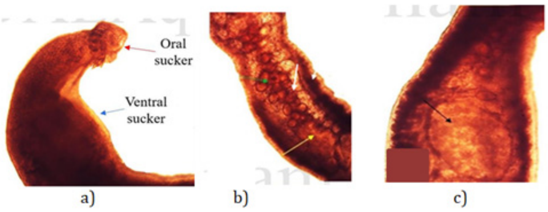

Figure 8: Echinostoma spp. recovered from the small intestine of 15 examined rats from selected backyard pig farms in Basag, Butuan City, Agusan del Norte showing the morphological characteristics of their body a) with two suckers (100X); b) uterus with eggs (Green arrow), ovary (Yellow arrow) and vitellaria (White arrow) (100X); c) Testes (Black arrow).

Echinostoma ilocanum is a widespread Trematode parasite in the Philippines that causes Echinostomiasis. This parasitic disease has been recorded in the Northern Luzon, particularly in Ilocos. Rattus spp. were reported as the most important animal hosts. Positive experimental infection has been attained by feeding metacercariae to a variety of laboratory animal species, but rats, mice, and arid hamsters are the utmost susceptible target [60].

Collected ectoparasites: Direct contact with infected rodents it is theoretically possible that other animals could become infected through direct contact with shed fur containing the eggs or through mobile adults *M. musculi* in the environment. There are no known cases that this type of rat ectoparasite infest human such that, this mite is generally known to spend its entire life cycle on the fur of rodents. The life cycle of this mite is completed in approximately 23 days. *M. musculi* eggs are usually attached to the proximal part of the among the collected ectoparasites, *M. musculi* (66.70%) has the highest prevalence than *Polyplax spp*. (33.30%). The mean intensity of *Polyplax spp* is much higher than *M. musculi* (5.5). Myobia musculi is a mite species which naturally infects rats. It has eight legs. Its first two legs are extremely adapted to grasp hair, so the mite appears to have six legs and a pair of pincers. The body of *M. musculi* has bulges between the legs, and each of the walking legs has a single empodial claw. The male (285-320 µm) is much smaller than female (400-500 µm). M musculi is transmitted through hair shaft. The survival duration of *M. musculi* eggs in the environment is unknown. When laid on animal, eggs hatch in 7-8 days. *Polyplax spp*. is a sucking louse which was distributed worldwide and normally infects brown rat *R. norvegicus*, black rat R. Rattus and other rodent species. *Polyplax spp*. lives as an obligate parasite such that they are all specialized that they can feed and develop as parasites. They are flattened dorso-ventrally and appears pale yellow to dark brown color. *Polyplax spp*. is transmitted through direct close contact with other rodent species. No known cases that this type of ectoparasite infects humans (Figure 9).

Household information: Farmers in each swine farms were interviewed, 66.7% were females and 33.3% were males. Formal education has been attained by the farmers. The level of education for the majority 66.7% of the farmers attained elementary school and 33.3% of the farmers have attained secondary school education. All the farmers 100% obtained their income in both swine farming and other businesses. Among three farms, two farms were in the lowland plateau near the rice farm while the one farm was in the middle plateau. All the farmers (100%) has animals owned that is greater than ten (including dogs, cats, chickens, etc.), has running water at home, has bathroom present at home, and has never been dewormed in the past.

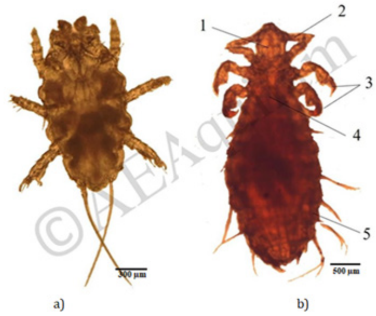

Figure 9: Ectoparasites recovered from the external body parts of 15 examined rats from selected backyard pig farms in Basag, Butuan City, Agusan del Norte: a) Myobia musculi (100X); b) Polyplax spp. (100X) showing the morphological characteristics of their body 1) Head; 2) Antenna; 3) Tibial spurs; 4) Elongated abdomen; 5) Prominent abdominal paratergal plates.

Pig production and management practices: All the farmers have the same farming practices: They owned the same breeds of pigs, the same commercial hog feeds given to pigs, maintains the cleanliness of the pig pens, uses deep well as the source of water given to pigs, has no source of information in pig farming, never dewormed there pigs regularly, and knows the clinical signs of worm infection. Majority of the farmers (66.7%) have greater than ten years of average farming experience, drained the swine manure in the septic tank, sold 5 to 10 pigs in a year and a 5 to 10 meters distance of pig pens from the neighboring household, while 33.3% has 5 to 10 years average farming experience, drained the swine manure in the creek, sold more than 10 pigs in a year and 20 meters distance of pig pens from the neighboring household respectively.

Relationship between farming practices and parasite infection in examined rats: Farming practices such as average farming experience (r_s=-0.402, p-value=0.038) and distance of pig pen and the neighboring household (r_s= 0.402, p-value=0.038) shows significant association to the parasite infection in rats with p-value of 0.038. Farming experiences with no good sanitation will seldom eliminate rodents. Pig farms that have poor sanitations can sure attract rodents and permit them to thrive and reproduce in greater abundance (Table 2).

Table 2: Relationship between the farming practices and parasitic infection of the 15 examined rat from selected backyard pig farms of Basag, Butuan City, Agusan del Norte.

Farming practices	Mean Intensity of Parasitic Infection in Rats		Remarks
	Correlation coefficient (rs)	p-value	
Average farming experience	-0.402	0.038	Significant
Distance between pig pen and the neighboring household	0.402	0.038	Significant
Swine manure management	0.152	0.449	Not Significant
No. of pigs sold in a year	-0.25	0.208	Not Significant

On pig farms, where feed grains are handled and stored, or other farms where livestock are housed and fed, it is generally impossible to ignore rodents from all available food. In such situations, neighboring household near swine, poultry and other farms can be invaded by rodents that thrive in farms. The closer, the household to the farms, the greater the chance that rats could invade and use their houses as shelter where they thrive and reproduce rapidly. However, swine manure management (rs=0.152, p-value =0.449) is not associated with the intensity of parasitic infection of the examined rats because although swine manures can cause poor sanitation and can participate to a more serious rodent problem, rodent infestation (particularly, brown rats *Rattus norvegicus* and house mice mus musculus) does not necessarily mean that sanitation is inadequate. Such that, there are some other techniques not only good sanitation practice could integrate with the cost-effective control of rodents. Rodents have remarkable capacities for reproduction, which makes it needed to control them as early and diligently, before their population levels increase and may cause significant damages. It is very impossible to eliminate rodents through sanitation alone, particularly in farms, because they can survive in some very small areas with limited amounts of food, water and shelter. Rodent infestation needs population reduction techniques which includes trapping, fumigation and toxic baits. Additionally, number pigs sold in a year (rs=-0.250, p-value =0.208) is not significantly correlated with the intensity of parasitic infection in rats. Mortality of pigs that may result in low production of pigs is not just because of the parasitic zoonosis that was being harbored by rats. There are some other factors of faming that could lead the mortality of pigs such as: limited experience of livestock husbandry skills, culling practices, rapid expansion, parity and others.

Relationship between the intensity load of parasites and the morphometric measurements of the examined rats: There is significant association between the intensity load of parasites, and the rats morphometric such as the head-body size (rs=0.534, p-value =0.040), tail-length (rs=0.534, p-value=0.040) and the hind-foot measurement (rs=0.570, p-value =0.026). This indicates that intensity load of parasites could influence some morphometric measurements (head-body, tail, and hind- foot) of the parasitically infected rodents. However, weight, and head measurement, has no significant association with the parasite load in rats. This result was supported by the research study in Nigeria, in which it was stated that the number of parasites harbored by rats will not always influence the morpho metric measurements of highly parasitized rats. There are some other factors that would influence the morphometric measurements of the parasitically infected rats like sex, age (immature, adult, juvenile), food availability, environmental conditions in tropical settings that has the climate and vegetation that is all year round beneficial to the habitation of many various lives of organisms including parasites, rodents, humans and other animals. Good and adequate environmental sanitations can either be breached or compromised the frequency of deliberate or non-deliberate parasite-to-rodents-to-humans interaction (Table 3).

Table 3: Relationship between the intensity load of parasites and the morphometrics of the 15 examined rats from selected backyard pig farm of Basag, Butuan City, Agusan del Norte.

Morphometric measurements	Intensity load of parasites	
	Correlation coefficient (rs)	p-value
Weight	0.425	0.114
Head Measurement	0.505	0.055
Head-Body Measurement	0.534	0.04
Tail Measurement	0.534	0.04
Hind-Foot Measurement	0.570	0.026

Relationship between the intensity load of parasites and the weight of the digestive tract of the examined rats: The results in this study shows no significant association between the intensity load of parasites and the weight of the digestive tract (rs=0.379, p-value=0.163) of the rats. This indicates that the intensity load of parasites could not influence the weight of the digestive tract of the rat host. But since the experimental sampling size in this research study is small, it cannot fully establish an evident explanation about the correlation of the parasite load and the weight of the digestive tract of the rat host. Thus, this research would support the parasite diversity study in Bangalore, India, in which it was stated that a large host has higher intake of food and water and a larger home range, thus apparently harbors a higher parasite load. Since transmission of parasite is directly proportional to the host population density, it is expected that parasite load is positively correlated to the host body morphology including the digestive tract which is the preference site for most helminth endoparasites.

Possible cross-infection of hookworms between rats and pigs: Hookworms are the only found parasite that infects both examined rats and feces of backyard raised pigs in Basag. Hookworms observed in pigs were examined in the month of August-November 2017, while the hookworms observed in rats were examined on January-February 2018. Though both samples are not taken and examined at the same time, there is still a possibility of hookworm cross-infection between the backyards may infect rats and continue their life cycles and housed in their preference site (mostly intestines) wherein they can mature and reproduce. Moreover, hookworms have a wide host-range in which it can infect many susceptible hosts, mostly mammals. It would also be possible that the transmission of hookworm infection in rats was also facilitated by some susceptible hosts observed in the study area such as stray dogs, and cats 80-82. Since there are many types of hookworm species that is capable of infecting more than one species of closely related hosts, they most likely have greater parasite diversity. The mean observed prevalence of hookworms is relatively higher in Visayas (18%) and Mindanao (11.3%), compared to Luzon which is 4.5%. This indicates that hookworms are one of the most known soil- transmitted helminths in the Philippines. Based on the results, there is possible cross- infection hookworms between rats and backyard-raised pigs (Figure 10).

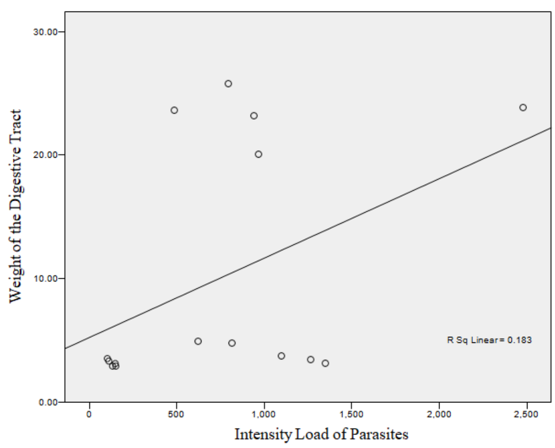

Figure 10: Relationship between the intensity load of parasites and the weight of the digestive tract of the 15 examined rats from the selected pig farms of Basag, Butuan City, Agusan del Norte.

Implication to public health: Rodents play an important role as hosts of various parasites that are transmissible to humans. By maintaining the parasite cycle in nature, rats serve as a reservoir for zoonotic parasites. Route of infection of the collected parasite eggs. Zoonotic parasite infection occurs in two ways: Direct route is by seeding the environment with the infective stage of parasite and human become infected through ingestion of contaminated food and water. Direct route of infection Hookworm, *H. nana*, *Capillaria spp.* and *A. cantonensis* infection possessed direct route of transmission. Farmers and other residents in the community of Basag may have a higher risk in becoming infected with this type of parasites such that, children in the area walks barefoot and play near the pig pens, they might accidentally ingest fecally contaminated soil. Most of the pig pens in the area are located near the rice farms, and pig excrements that are sometimes used as fertilizers in the area could have a great impact for soil contamination. When rats travel to parasitically infected feces of pigs, contaminated soil and water, there is a great possibility that rats may harbor infective stages of parasites and distribute or spread the parasites several feet away including the nearby household and agricultural farms (rice farms) in the area where rats thrive and reproduce. Rats thereby contaminate food, soil, water and other surfaces. The swine manures disposed in the creek which is connected to the rice farm may have a great impact for the possible parasite infection to the rice farmers. Indirect route of infection Hookworm, *Capillaria spp*. *A. cantonensis*, *Echinostoma spp*., *H. nana*, and *H. diminuta* infection. Rats and other rodent species contaminate the environment with the parasite larva, and then infects the intermediate hosts and multiplies in them. Humans become infected by eating and accidental ingestion of infected intermediate hosts. The impacts of rodents are therefore twofold especially in rice and swine farming communities 12. Wild rats *R. norvegicus* causes a great problem in the farms and the parasites that they carry can have a major impact on the health of the residents in Basag. For example, in rice farming, the situation can become worse in times of scarcity of grain foods because of the destruction caused by rats. To a greater chance, farmers may be enforced to look for an alternative food, like intermediate hosts of these parasites (mainly snails, clams, frogs, etc.), which are eatable and readily accessible in the area, and thus upsurge the possibility of people in the area to become infected by these parasites that were harbored by rats. The people in Basag share territories with rats, domesticated animals like pets (e.g. cats, and dogs) and livestock (e.g. carabaos, cows, pigs, chickens, etc.) which would contribute a major impact for the transmission of parasitic diseases. Such that, these animals have been a human companion, transportation, a great help for work and a main source of food. Moreover, wild animals are also important in maintaining the balance in nature and preservation of ecosystems. Zoonotic parasites in rodents are tremendously harmful to animals and humans that may cause severe economic losses, which would result in not just mortality but also cause a decrease in meat, milk, egg-laying and rice production1. Rodent-borne parasitic diseases cause major impact to public health since most of the parasite they harbor has zoonotic potentials (Figure 11).

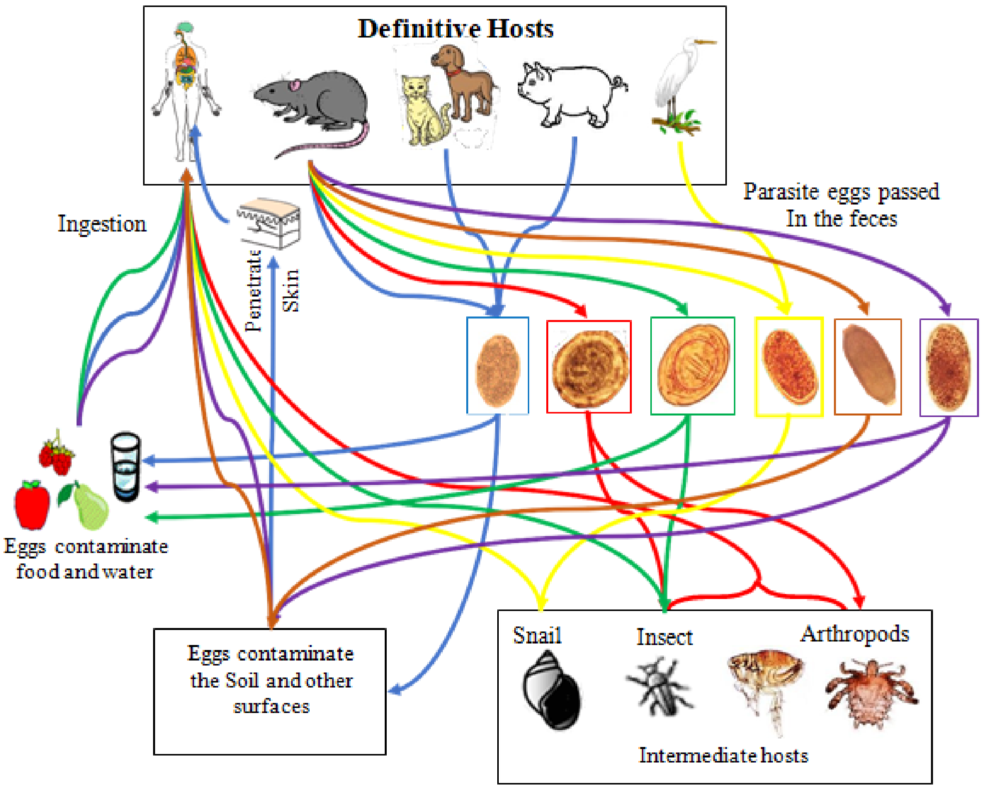

Figure 11: Inferred route of transmission of the collected parasite eggs that may infect humans from the examined rats from selected backyard pig farms in Basag, Butuan City, Agusan del Norte. **Note:** (■) Hookworm Infection, (■) H. diminuta infection, (■) H. nana infection, (■) Echinostoma spp. infection, (■) Capillaria spp, infection, (■) A. Cantonens is infection

Discussion

Rodents play various significant roles as host and reservoir of various zoonotic parasites. Brown rats *Rattus norvegicus* is the most widespread rodent species in the Philippines that have been recognized as destruction to foodstuffs, pests of agronomic crops resulting to some significant economic losses and apart from that, this rat species has spread some diseases far beyond their original boundaries (leptospirosis, angiostrongyliasis, echinostomiasis, etc.). The purpose of this research study was to investigate the ecto and Endoparasites among rodents in Basag, Butuan City, Agusan del Norte to assess their potential as biological reservoirs for zoonotic parasites and its implication to public health. A total of fifteen norvegicus were caught alive using custom-made steel wire rat traps. Eight rat traps were set within three weeks (during 17:00 to 06:00 hrs). from January to February 2018. Of the fifteen examined rats, 100% were infected with zoonotic Helminth Endoparasites (%=prevalence rate; mean intensity) namely, four cestodes: *H. diminuta* (66.7%; 155.7), *H. nana* (33.3%; 989.2), *C. fasciolaris* (33.3%; 4), *Taenia spp*. (6.70%; 1); three nematodes: Hookworms (100%; 204.6), *A. cantonensis* (6.70%; 1507), *Capillaria spp*. (20%; 2.33); and one trematode: *Echinostoma spp*. (6.70%; 222) were isolated [61]. Infection rate has significant association to the farming practices (average farming experience rs=-0.402, p value=0.038; distance of pig pen and the neighboring household rs=0.402, p value =0.038), and rats body morphology such as the head-body size (rs=0.534, p value =0.040), length of the tail (rs=0.534, p value=0.040), and hind-foot measurement (rs=0.570, p value =0.026). There is a possible cross-infection of hookworm between rats and backyard raised pigs. The result of the study indicates that there is a significant relationship between the zoonotic parasites level of infection to the rat's behavior and morphology, presence of some susceptible host (pigs, stray dogs and cats) or vectors, and hygiene practices and the knowledge of the farmers pertaining to parasite transmission dynamics [62]. The findings in this research study indicates that rats *R. norvegicus* play significant implications to public health in the study area, being a biological reservoir host of many zoonotic parasites. Most of the zoonotic parasites they harbor are tremendously harmful not just in humans but also with other animals (livestock: pigs, cows, chickens). Proximity to the rodent populations may possess health threats to humans [63].

Conclusion

It is noted that the sample size is small in this research study. A larger sample size is recommended to better improve the ascertain prevalence and intensity of zoonotic parasites in rodents and to test the relationship between infection rate with rodent's sex, age, morphometric measurements as well as the farming practices, attitudes, and knowledge of the residents in the study area about parasite infection that can be transmitted from rodents. Since parasites that both infects rats and backyard raised pigs were not statistically correlated, it is recommended to take the experimental samples at the same time to better investigate and establish the possible cross-infection of parasites. In view of this, rodent control eradication measures must be put in place to prevent rodent-borne parasitic disease outbreaks in Barangay Basag, Butuan City, Agusan del Norte. Educating the farmers on the risk of parasitic transmission and prevention can play major role in reducing parasitic infection. However, control of zoonotic parasites in rodents requires attention of concerned government agencies, and with the cooperation of veterinarians, physicians, biologists, and public health workers to become successful in this campaign.

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