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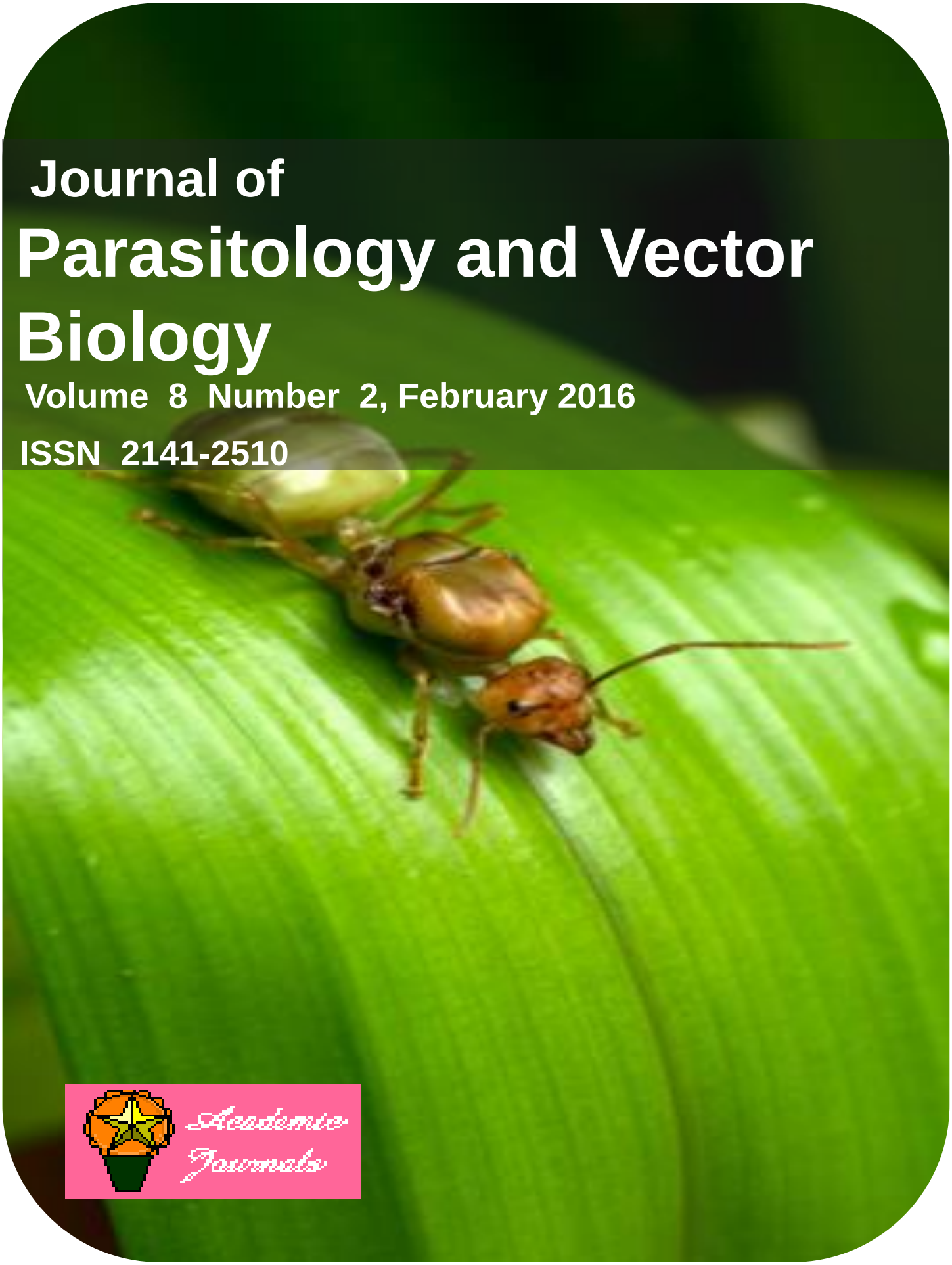

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Full Length Research Paper

Selection of *Plasmodium falciparum Pfmdr-1* N86Y alleles by Amodiaquine-Artesunate and Artemether-Lumefantrine in Nanoro, Burkina Faso

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***Plasmodium falciparum Pfmdr1*-86 gene polymorphisms were investigated in blood samples of patients > 6 months of age treated with Amodiaquine-Artesunate (ASAQ) and Artemether-Lumefantrine (AL) in Nanoro, Burkina Faso. Treatments outcome was determined with a 28-day follow-up. The prevalence of *Pfmdr-1* N86Y alleles was determined before and after treatment. The PCR-adjusted Adequate Clinical and Parasitological Response (ACPR) was higher in the ASAQ arm (100%) than in the AL arm (87.5%) [Risk difference = -12.50; 95% CI: -20.13; - 4.86 (p=0.001)]. The prevalence of *Pfmdr-1* Y86 mutation in the ASAQ arm was significantly higher among patients who had a recurrent parasitaemia (54.54%) than those classified as ACPR (12.70%) (p = 0.007). Similarly, the prevalence of the mutant allele *Pfmdr-1* Y86 before treatment (20.00%) was significantly lower than that found in post-treatment (55.56%) in the ASAQ arm (p = 0.01). However, we did not see such difference in the AL arm either for post-treatment samples versus pre-treatment samples (p = 0.88), nor for patients with recurrent parasiteamia compared to those classified as ACPR (p = 0.65). In conclusion, our study showed that ASAQ is selected for parasites carrying the *Pfmdr-1* Y86 mutation; however we were not able to demonstrate the reverse relationship between *Pfmdr-1* 86N and AL treatment as previously reported in Africa.**

Key words: Malaria, *Plasmodium falciparum*, Amodiaquine-Artesunate, Artemether-Lumefantrine, Efficacy, *Pfmdr-1*, Burkina Faso.

INTRODUCTION

Worldwide use of Artemisinin-based combination therapies (ACT) combined with other control measures (vector control, seasonal malaria chemoprophylaxis, intermittent preventive treatment in pregnant women, etc) has led to significant decreases in malaria transmission and thus subsequent malaria-related morbidity and

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mortality over the past decade in many endemic countries (Maude et al., 2009; WHO, 2006). Nevertheless, malaria still kills approximately 584,000 people a year worldwide and causes illness in hundreds of millions more, most of them children living in sub-Saharan Africa (World Malaria report 2013).

ACTs, mostly artemether–lumefantrine (AL) and artesunate–amodiaquine (ASAQ), are deployed worldwide, including Burkina Faso where a new treatment policy was adopted in 2005 recommending the use of ASAQ or alternatively AL for the treatment of uncomplicated falciparum malaria (Gansané et al., 2009; Zwang et al., 2009). However, following the reports on the decreasing susceptibility of *P. falciparum* to artemisinin derivatives along the Thailand and Myanmar border (Dondorp et al., 2009; Lim et al., 2009; Noedl et al., 2010; Rogers et al., 2009), several studies have reported a decline of adequate parasitological response rate to treatment with ACTs in African patients, possibly due to the emergence of parasites with reduced drug sensitivity (Beshir et al., 2013; Borrmann et al., 2011). In such context, the regular monitoring of *P. falciparum* sensitivity against artemisinin derivatives and its partner drugs in Africa is needed. Several studies have identified the polymorphisms of *Pfmdr-1* N86Y gene as one of the main molecular markers involved in the development of tolerance / resistance to Lumefantrine and Amodiaquine; the two partner drug of AL and ASAQ respectively (Baliraine and Rosenthal, 2011; Baraka et al., 2015; Dokomajilar et al., 2006; Eyase et al., 2013; Holmgren et al., 2006, 2007; Sisowath et al., 2005, 2007; Somé et al., 2010). Here we report the results of a pilot study investigating the relationship between the polymorphism of the *Pfmdr-1* N86Y alleles and the treatment outcome of ASAQ and AL in Burkina Faso.

MATERIAL AND METHODS

The study was carried out between October and December 2012 in Nanoro, Burkina Faso where malaria is hyper-endemic with a seasonal transmission from July to December. *Plasmodium falciparum* is the predominant malaria parasite and the commonest vectors are Anopheles gambiae ss, An. funestus and An. Arabiensis (Tinto et al., 2002). This was part of a pilot study that investigated the therapeutic efficacy of AL and ASAQ in patients ≥ 6 months of age with a molecular markers study nested into it. The study methodology has been described in detail elsewhere (ClinicalTrials.gov Identifier: NCT01697787). Briefly, patients with fever (axillary temperature of 37.5°C) or history of fever with a suspicion of malaria were screened after informed consent. Then, patients meeting the inclusion criteria were treated and followed up according to the WHO 28-day *in vivo* test (WHO, 2003). Outcomes were defined according to the WHO guidelines for monitoring antimalarial drug resistance (WHO, 2003).

Blood samples for the molecular analysis were collected on filter paper (Whatman 3, Maidstone, England) at day 0 before treatment and at the time of recurrent parasitaemia. DNA was extracted from dried blood spots using QIamp DNA miniKit (Qiagen, Germany) following the manufacturers procedures. Detection of *Pfmdr1* N86Y polymorphisms was performed using nested PCR method followed by a restriction fragment length polymorphism (RFLP) (Dokomajilar et al., 2006): Briefly, the first round was done by using primers MDR1 5'-ATGGGTAAAGAGCAGAAAGA-3' and MDR2 5'-AACGCAAGTAATACATAAAGTCA-3' and then nested PCR was done by using primers MDR3 5'-TGGTAACCTCAGTATCAAAGAA-3' and MDR4 5'-A TAAACCTAAAAAGGAACTGG-3'. The second round (nested PCR) product was subjected to enzyme digestion with *AflIII* (New England Biolabs), which cuts only the mutant gene into 226 bp and 295 bp fragments. For each series of samples, water was used as a negative control, 3D7-clone DNA was used as the wild-type control and Dd2- DNA was used as the mutant control. Nested PCR was performed as well for the analysis of *Msp*-1 and *Msp*-2 to distinguish between recrudescence and new infection (Ranford-Cartwright et al., 1997). Data were double entered in an Excel database. Statistical analysis was performed using STATA (IC), version 8.0 software. *Pfmdr-1* N86Y genotype was determined by the presence or absence of wild/mutant alleles. Differences between groups were assessed using the Chi-square test for proportions and a P-value $\leq$ 0.05 was considered as statistically significant.

RESULTS

Out of 246 patients screened, 78.45% (193 patients) had a microscopically confirmed malaria infection. Out of them, 150 were randomized to receive either ASAQ (n=75) or AL (n=75). The two treatments' outcomes are summarized in Table 1. At day 28, 74 patients completed their follow-up in the ASAQ arm against 72 patients in the AL arm. Unadjusted Adequate Clinical and Parasitological Response (ACPR) at day 28 was significantly higher in the ASAQ (85.13%) than in the AL arm (61.11%) [Risk difference = -24.13; 95% CI: -38.00; - 10.25 (p=0.001)]. Similarly, The PCR-adjusted ACPR was significantly higher in the ASAQ (100%) than in the AL arm (87.5%) [Risk difference = -12.50; 95% CI: -20.13; - 4.86 (p=0.001)]. The *Pfmdr-1* N86Y gene was successfully genotyped in the blood samples of the 150 patients randomized at day 0. The overall prevalence of the mutant allele *Pfmdr-1* Y86 was 18.67% [CI 95% (12.78 to 25.84)].

The prevalence of the two alleles before treatment in relation with treatments outcomes is summarized in Table 2. The prevalence of *Pfmdr-1* Y86 mutation in the ASAQ arm was significantly higher among patients who had a recurrent parasitaemia (54.54%) than those classified as ACPR (12.70%) (p = 0.007). However, we did not see such difference in the AL arm (p = 0.65). Similarly, the prevalence of the mutant allele *Pfmdr-1* Y86 before treatment (20.00%) was significantly lower than that found in post-treatment (55.56%) in the ASAQ arm (p = 0.01). However, we did not see such difference in the AL arm (p = 0.88).

DISCUSSION

Good efficacy of an ACT is reported when the partner drug is also efficacious (The Four Artemisinine-Based Combinations (4ABC) Study Group, 2011). The high rate of 100% ACPR reported in the ASAQ arm is an indication

Table 1. Efficacy rates of ASAQ and AL at day 28

Outcome	AL (n=72)	ASAQ (n=74)	Difference [95%CI]	P-value
PCR unadjusted				
ACPR	44 (61.11)	63 (85.13)		
ETF	0 (0.0)	0 (0.0)		
LCF	15 (20.83)	4 (5.41)	-24.13 [-38.00 ; -10.25]	0.001
LPF	13 (18.06)	7 (9.46)		
TTF	28 (38.89)	11 (14.87)		
PCR adjusted*				
ACPR	63 (87.5)	74 (100)		
ETF	0 (0.0)	0 (0.0)		
LCF	0 (0.0)	0 (0.0)	-12.5 [-20.13 ; -4.86]	0.001
LPF	9 (12.5)	0 (0.0)		
TTF	9 (12.5)	0 (0.0)		

AL: Artemether-Lumefantrine; ASAQ: Artesunate-Amodiaquine; ACPR : Adequate Clinical and Parasitological Response ; ETF : Early Treatment Failure ; LCF : Late Clinical Failure ; LPF : Late Parasitological Failure ; TTF : Total treatment Failure.

Table 2. Selection of *Pfmdr-1* N86Y alleles by ASAQ and AL.

Treatment outcome	ASAQ		AL	
	Wild(N86)	Mutant(Y86)	Wild(N86)	Mutant(Y86)
TTF % (n/N)	0(0/0)	0(0/0)	66.66 (6/9)	33.34 (3/9)
New infection % (n/N)	45.46 (5/11)	54.54(6/11)	94.73 (18/19)	5.27 (1/19)
ACPR% (n/N)	87.30 (55/63)	12.7 (8/63)	81.81 (36/44)	18.18 (8/44)

AL: Artemether-Lumefantrine; ASAQ: Artesunate-Amodiaquine; TTF: Total Treatment Failures; ACPR: Adequate Clinical and Parasitological Response.

of a good efficacy of Amodiaquine and confirm the results of previous studies conducted in Burkina Faso and comparing the two ACTs (Siribié, Diarra, Tiono, Soulama, & Sirima, 2012; Sirima et al., 2009; The Four Artemisinine-Based Combinations (4ABC) Study Group, 2011; Tinto et al., 2008, 2014; Zongo et al., 2007). The overall prevalence of the mutant allele *Pfmdr-1* Y86 regardless of the treatment arm was surprisingly low when compared with that reported by previous studies; indicating a decrease of this mutation in Burkina Faso (Baraka et al., 2015; Somé et al., 2010; Tinto et al., 2003, 2008).

The *Pfmdr-1* Y86 and *Pfcrt* T76 mutations have been identified as the main determinants for 4-aminoquinolines resistance including Amodiaquine (Tinto et al. 2003; Djimdé et al. 2015; Sondo et al. 2015). The decrease of the *Pfmdr-1* Y86 mutation observed in our study may follow the same trend observed with *Pfcrt* T76 mutation after the malaria treatment policy change in endemic countries. Indeed, a decrease of the prevalence of *Pfcrt* T76 mutation was reported following the withdrawal of chloroquine from the treatment policy in many Africa endemic countries (Kublin et al., 2003; Laufer et al., 2010; Mwai et al., 2009; Sondo et al., 2015). These findings suggest that Amodiaquine resistance may be decreasing following the implementation of the new anti-malarial drug policy based on ACT in Burkina Faso.

An association between the polymorphism in *Pfmdr-1* gene and the parasite response to arylaminoalcohols including lumefantrine has been reported with a significant increase of *Pfmdr-1* 86N wild type allele after exposure to the drug; suggesting that Lumefantrine exerts the opposite effect of amodiaquine on this locus (Duraisingh and Cowman, 2005; Sisowath et al., 2005). In addition, *Pfmdr1* 86N–carrying parasites have been associated with decreased sensitivity to lumefantrine *in vitro*, suggesting this allele as a potential marker of lumefantrine resistance (Baliraine and Rosenthal, 2011). We noticed in our study an overall high prevalence of *Pfmdr-1* 86N allele. However we did not observed an increase in the prevalence of the *Pfmdr-1* 86N in patients who had a recurrent parasitaemia samples than those classified as ACPR. Similarly, we did not observe an increase of the prevalence in the post-treatment samples than in pre-treatment samples. Therefore there was no significant selection of the *Pfmdr1* 86N allele after AL

treatment in our study as reported previously in Africa (Baliraine and Rosenthal, 2011; Sisowath et al., 2005, 2007).

Overall, we did not see any linear association between the *Pfmdr1*-N86Y alleles and both AL and ASAQ treatments outcome. This lack of relationship confirms the difficulty to predict the individual treatment outcome by looking at molecular markers alleles in pre-treatment samples (Holmgren et al., 2006). This can be explained by the effect of the host immunity which could modify the relation between molecular markers and resistance; a phenomenon similar to what has been reported previously for CQ resistance (Djimdé et al., 2015; Tinto et al., 2003, 2008). Indeed, in our study, patients of all age groups including adults were enrolled when in most of studies where this relationship was established, study participants were mostly children (Baliraine and Rosenthal, 2011; Sisowath et al., 2005, 2007). However, further studies should be carried out exclusively in children to confirm this assumption in our study area.

In conclusion, our study showed a decrease of the *Pfmdr-1* Y86 mutation in parasites strains circulating in Burkina Faso. ASAQ selected for parasites carrying the *Pfmdr-1* Y86 mutation, however we were note able to demonstrate the reverse relationship between *Pfmdr-1* 86N allele and AL treatment as reported previously.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Prevalence of *Schistosoma Japonicum* infections among field rats (*Rattus rattus norvegicus)* in schistosoma infested areas of Northern Samar, Philippines

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Forty-nine field rats collected from six municipalities of Northern Samar through the use of rat traps were necropsied, examined for schistosome infection, assessed for macroscopic lesion characteristics, and evaluated for parasite burden. Evaluation showed that out of the 49 field rats clinically examined, a prevalence rate of 42.86% was obtained. From this infection rate, 24.49% were males and 18.57% were females parasitized. Rats collected from Catarman had the highest prevalence rate (14.28%) of schistosoma infection. Schistosome burden was low with one adult schistosome on the average per infected field rat. All schistosomes recovered were retrieved from the mesenteric veins. Majority (42.86%) of the schistosome infected field rats demonstrated granulomatous liver surfaces with edematous intestines. About 23.81% infected rats showed multiple lesions with fibrosis and nodular mass (granulomas) of the liver and edematous intestine as prominent lesions. These findings attest to the fact that about 43% of the field rat population in Northern Samar is infected with *S. japonicum*. It can therefore be concluded that field rats in the province may play as one of the multiplier hosts of schistosomiasis among humans and animals.

Key words: *Schistosoma Japonicum*, fibrosis, infected rats, edematous intestines, granulomatous.

INTRDUCTION

Schistosomiasis is endemic in the countryside and remains a major health problem for the Filipino farmers and their family members. At present there are twenty eight (28) out of eighty one (81) provinces nationwide considered to be endemic for the said dreadful zoonotic disease. Record shows that about 12 million Filipinos

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are exposed to Schistosomiasis (Leonardo et al., 2012). In Northern Samar for instance a notable prevalence rate of infection was noted among family members living in the rural areas based on the case reports of the Schistosoma Control Unit of the Department of Health, Northern Samar from 2002-2007. Despite the massive treatment programs for 20 years of human cases by the Department of Health (DOH), the prevalence rate of infection remains alarming and even increasing in other areas of the country. One of the problems encountered in the total eradication of schistosomiasis is the fact that aside from human beings, various domestic animals and wildlife particularly field rats serve as reservoir hosts and they play a major role in the disease transmission.

Schistosoma japonicum is the specific causative agent for schistosomiasis found in the Philippines. Both humans and animals are infected upon exposure in the field by skin penetration or by drinking water containing the infective larval form of the parasite called cercaria.

The transmission of schistosomiasis from wild animals such as the field rats to man could be a great possibility why the disease remains prevalent in a certain locality. Reports showed that in China, cattle and water buffaloes are considered to be important reservoir hosts of the schistosomes whereas in the Philippines those considered are pigs, dogs, and field rats (Willingham, 2002).

It is on this context that this study was conceived so as to assess *Schistosoma japonicum* prevalence rate among field rats and thus confirming further their role as reservoir hosts even in the province of Northern Samar where schistosomiasis continues to cause death among the Samarenos. Hopefully, the findings of this study if found as envisioned could serve as baseline data in research and in the formulation of effective control measures by the concerned agencies under Northern Samar conditions.

Objectives of the study

This study in general was aimed to determine the prevalence rate of *Schistosoma japonicum* infections among field rats in Northern Samar. Specifically, the study also aimed to determine the prevalence rate of schistosome infection among male and female field rats, among field rats of either sex from selected representative municipalities representing the province and further determined the parasite load of infected field rats and assessed the state of macroscopic pathology of vital visceral organs of schistosome infected field rats.

Time and place of the study

The study was conducted for four months, from July 2014 to October 2014. Based on the records of the Department of Health (DOH) Schistosoma Control Unit, Northern Samar Office on the prevalence of schistosome infection among humans in the province, the sampled rat populations used in the study were collected from the first six top ranking infested municipalities of the province, three municipalities from each of the two districts of the province; namely, Catarman, San Jose and Lavezares for the 1st Disrict and Las-Navas, Catubig and Palapag for the 2nd District.

The collection and identification of adult schistosomes, parasite load determination and assessment on the degree of pathological condition of visceral organs of positively infected rats with schistosomes were done at the Parasitology Laboratory, Department of Paraclinical Sciences, College of Veterinary Medicine, University of Eastern Philippines, University Town, Northern Samar.

MATERIALS AND METHODS

General study approach

Selection of sampling areas from the two congressional districts of the province was based on the report of schistosomiasis incidence among Samarenos in the province as per report of the Schistosoma Control Unit of the Department of Health, Northern Samar (2013). Thus the municipalities of Catarman, San Jose, and Lavezares showing highest incidence rate in human schistosomiasis within the First District and the municipalities of Las Navas, Catubig and Palapag likewise showing highest incidence of human schistosomiasis within the Second District were purposively chosen to represent the entire province. From each of the municipalities in both districts, two barangays were likewise chosen based again on highest incidence rate of human schistosomiasis (Table 1).

From each barangay, a rice field area of about a hectare was identified and ten (10) rat traps numbered accordingly were strategically positioned in equal distances. Each trap was provided with baits. Rats caught for a period of three days were considered samples from the area. This procedure was applied to all selected barangays in the municipalities included in the study. Caught live rats were brought to the Parasitology Laboratory College of Veterinary Medicine, University of Eastern Philippines, University Town, Northern Samar where they were examined thoroughly by necropsy. Data on source of sampled rats, date of collection, weight and sex of rats were properly recorded. The rats were humanely euthanized and necropsied for the recovery of adult schistosome parasites (Figures 1 and 2).

Search for the adult schistosomes was made in all vital visceral organs of the body through dissections. Macroscopic pathological characteristics of organ parasitized were described and properly noted and recorded. Presence of species of parasites other than the schistosomes in affected organs was also recorded for reason that they might have some kind of influence on the characteristic pathology of the parasitized organs (Figures 3 and 4).

Collection of adult *Schistosoma japonicum*

A systematic examination of the specific body parts of each sampled rat was conducted right after euthanasia. The abdominal cavity was opened with a sharp scalpel blade and the gastrointestinal and thoracic viscera exposed. Parasites found outside and on surfaces of organs immediately after visceral

Table 1. Municipalities purposively chosen to represent the province of Northern Samar based on top three ranking schistosome infested towns (Schistsoma Control Unit, DOH, Northern Samar, 2013).

Municipalities	Barangays	Incidence rate (%)
1st District:		
Catarman	Cal-igang	37.50
	New Rizal	24.80
San Jose	Bonglas	28.10
	San Lorenzo	25.70
Lavezares	San Miguel	12.19
	Libas	4.25
2nd District:		
Las-Navas	Bulao	16.60
	San Francisco	15.50
Catubig	Bonifacio	10.61
	Magtuad	9.66
Palapag	Campedico	10.70
	Pangpang	8.30

exposure were collected and properly placed in appropriate containers. Each of the organs was then properly and carefully separated using sharp surgical scissors and placed on containers pre-prepared for the purpose. Adult schistosomes were searched from each organ in their respective containers under a dissecting stereoscope. The small and large intestines with their mesenteries intact were examined for easy recovery of the adult schistosome in a well-lighted container filled with warm water to easily observe movement of the parasites in the mesenteric veins. Recovered schistosomes were separated from their organ attachment and placed in Petri dishes or clear flat glass and allowed to relax so as to come up with well stretched out specimens. The liver, lungs and other solid internal organs were thoroughly dissected for the recovery of schistosomes and other parasites if any. All parasites collected were identified and properly fixed and preserved in their respective containers containing 10% formalin.

Identification of Adult Schistosomes

All specimens collected from each sampled rat were identified morphologically following the keys of Soulsby (1982) and Bowman and Lynn (1999) which anatomy of The Schistosomes and other parasites has not been revised up to the present (Appendix A).

Documentation

Permanent mounts of adult male and female schistosomes were made and micro-photographs taken. Pictures were presented in appropriate portions of the manuscript.

Statistical tools

Non-parametric statistical tools (percentage, average) were used in the data analysis. Data were presented in figures and in tabular forms. The prevalence rate was computed based on the following formula:

$$\text{Prevalence Rate (\%)} = \frac{\text{Total Number of infected}}{\text{Total Number of Sample}} \times 100$$

RESULTS AND DISCUSSION

Prevalence rate

The prevalence rate of *Schistosoma japonicum* infection among field rats in Northern Samar are presented in Table 2 by municipality as representative sampling sites for field rat collection in the province. As shown in the table for the course of the study, 49 field rats were sampled and examined for the presence of *Schistosoma japonicum* infection. Out of this sampled field rats, 36.73% (18/49) were males and 63.27% (31/49) were females. Further, from this sampled field rats 21 were diagnosed during necropsy examination to be infected with *Schistosoma japonicum,* giving rise to a prevalence rate of 42.86% for the province (Figure 5). Prevalence rate

Figure 1. Sampled rats caught in rat traps.

Figure 2. Visceral organs of necropsied rats examined thoroughly for the presence of adult schistosomes.

between sexes likewise showed to be 24.49% (12/49) among the males and 18.37% (9/49) among the females. Among representative municipalities for the province, prevalence rate of schistosome infection among sampled field rats ranked from highest to lowest were 14.28% (7/49) for Catarman, 12.24% (6/49) for San Jose, 8.16%(4/49) for Las Navas, 4.08% (2/49) for Lavezares and 4.08% (2/49) for Palapag and 0% (0/49) for Catubig. These results are further plotted in Table 2.

As shown in the result, none (0%) from among the sampled rats from Catubig was found upon microscopic examination to be infected with *Schistosoma japonicum*. This particular finding contradicts the findings of Olegario (2009) who reported that 9.76% of the sampled rats from the municipality were infected with the blood fluke, schistosome. Further, cases of human schistosomiasis have been recorded from this municipality being the top third in human incidence as reported by the Provincial Schsitosoma Office, Department of Health (2013). The present work however collected samples from different barangays of Catubig other than those included in the work of Olegario.

The work of Cabrera (1976) in Leyte demonstrated that male rats apparently have higher schistosome infection rate than female rats. The present study revealed parallel result in that males (24.49%) had higher infection rate than the females (18.37%). Cabrera (1976) and the present worker could not find any reason why males had higher infection rate than females. Nevertheless, considering that males do not look after the young cubs' nutritional needs, they have more time to scavenge for food with a wider area to search for, thus, exposing them to schistosome infection more than the females. Moreover, a similar result is shown in the work of

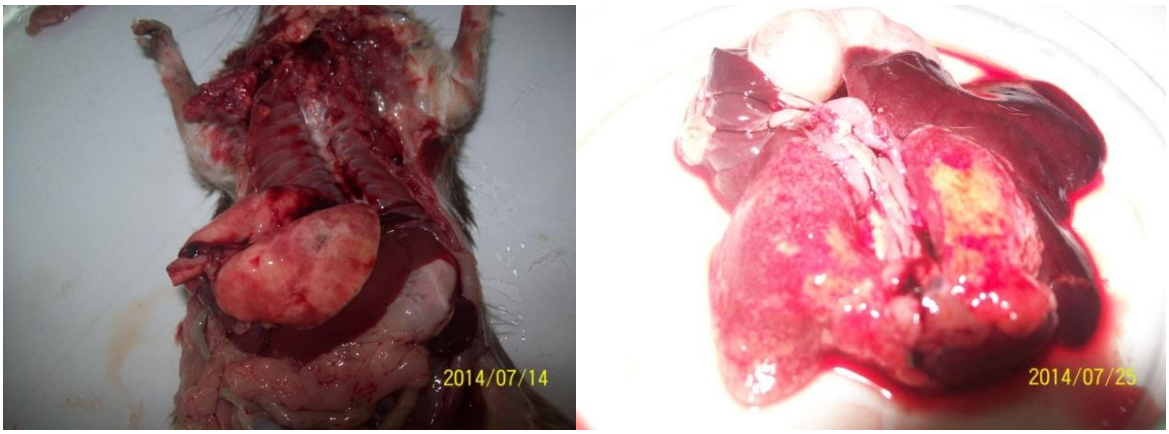

Figure 3. Visceral organs exposed in search for macroscopic lesions.

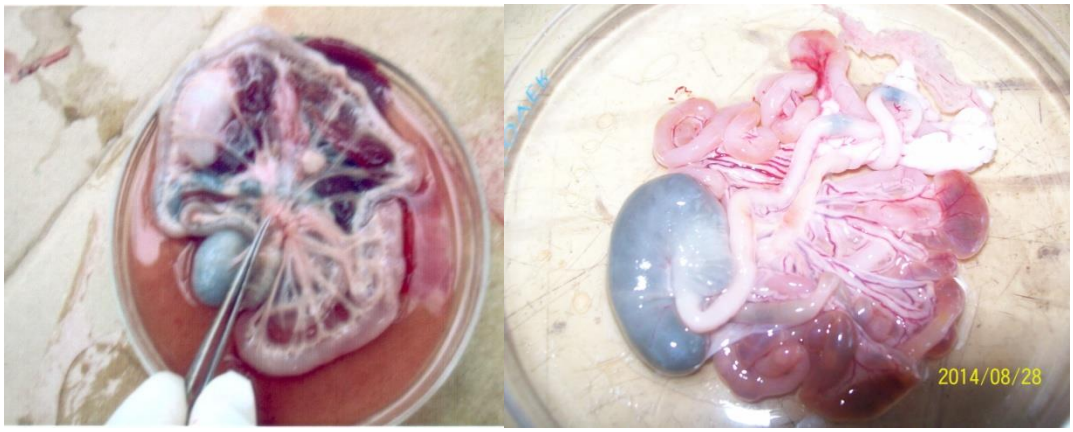

Figure 4. Rat viscera displayed in search for the adult schistosomes.

Fernandez et al. (2007) on schistosomiasis in the Philippines examining a number of field rats caught through rat traps together with 35 cats, dogs, pigs, and water buffalo randomly sampled from 50 villages in the province of Samar (Northern Samar not included) and Leyte. Among rats from different villages prevalence rate ranging from 0.7 to 95.4% was recorded; while in this present study the recorded prevalence rate ranges from 0 (Catubig) to 14.28% (Catarman).

Furthermore, the present findings contribute and attest to the affirmation that schistosomiasis particularly that which is caused by *S. japonicum* is not limited to man as the disease also occurs in wild life (field rats and monkeys) as it commonly occurs in domestic animals such as pigs, dogs, goats, cats, carabaos, cows (Blas et al., 1990; He et al., 2001; Gray et al. 2011). It is accepted by the cited authorities that cross infection of the parasite from man, cow, dog, pig, and rat have proven as such. McGarvey et al. (2006) likewise stated that *Schistosoma japonicum* is a true zoonosis, infecting mammals, including humans, all of which are definitive hosts necessary for transmitting the infection. In the Dongting Lake region of China, for instance, the average prevalence rate in the *Rattus* species was 14.2% (17/120), revealing that *S. japonicum* infection within field rodents was widespread (Guo et al., 2013). They concluded that wild rodents can be important reservoir hosts for *S. japonicum* transmission in the region and are of a public health concern. Thus, with this finding it is now clearly established that in the province of Northern Samar transmission of schistosomiasis from human to domestic and wild animals could be attributed to all these sources and that field rats in particular seemingly appear to be an effective multiplier host in the wild that control measures to protect the public health need more serious considerations. Gonzales (2014) added that among animals serving as reservoir hosts of *Schistosoma japonicum,* rats play a crucial role in transmission because they

Table 2. Prevalence rate of *Schistosoma japonicum* infection among field rats in Northern Samar.

Municipality	Sampled field rats		MALE				Female				Total(N=49)	
			Infected		Not Infected		Infected		Not Infected		No.	%
	No.	%	No	%	No.	%	No.	%	No.	%		
San Jose	9	18.37	4	8.16	0	0.00	2	4.08	3	6.17	6	12.24
Lavezares	6	12.24	2	4.08	0	0.00	0	0.00	4	8.16	2	4.08
Catarman	10	20.41	4	8.16	**1**	2.04	3	6.12	2	4.08	7	14.28
Catubig	8	16.33	0	0.00	2	4.08	0	0.00	6	12.24	0	0.00
Las Navas	10	20.14	2	4.08	2	4.08	2	4.08	4	8.16	4	8.16
Palapag	6	12.24	0	0.00	1	2.04	2	4.08	3	6.12	2	4.08
Total	49	100.0	12	24.49	6	12.24	9	18.37	22	44.90	21	42.86

Table 3. Organ locations of adult schistosomes in the body of infected field rats.

Organ-location	Male		Female		Total (N= 49)	
	No.	%	No.	%	No.	%
Portal Veins	0	0.00	0	0.00	0	0.00
Mesenteric Veins	11	22.45	9	18.37	20	40.82
Both Portal and Mesenteric veins	1	2.04	0	0.00	1	0.04

remain in the wild and therefore their movements are beyond control.

Organ location of adult schistosomes

As noted earlier out of the 49 field rats examined, there were 24.49% (12/49) male rats and 18.37% (9/49) female rats (Table 2) found to have been infected with *Schistosoma japonicum*. At necropsy the adult schistosomes were recovered from the mesenteric veins of the 11 (22.45%) infected male rats and 9 (18.37%)female infected rats. From the 2.04% (1/49) infected male rat schistosomes were recovered from both portal and mesenteric veins. On the other hand, not a single schistosome was recovered from the portal veins among the female infected rats (Table 3). Majority of the worms recovered were females (Figures 7 to 14).

This result conforms to the findings of most authorities (Soulsby, 1982; Urquhart, et al., 1987; Bowman and Lynn, 1999; McManus et al., 2010; Leonardo et al., 2012) that adult schistosomes especially the females inhabit the mesenteric veins which location is ideal for their laying movement activity near the intestinal capillaries of the venous endothelial lining. In the Philippines, according to Conlan et al. (2011) a prevalence of 85% for *Rattus norvegicus* and 56.5 – 95.5 % *Rattus rattus* have been recorded in natural

Population but in most cases the adult worms were found trapped in the lungs and few produced viable eggs. Kamiya et al*.* (1990) did a similar study in Dagami Leyte, Philippines with a prevalence of 81.9% but the adult parasites were recovered from the portal mesenteric veins (85.7%) and from the lungs of (44.2%) infected rats. However, in this study, adult schistosomes were not recovered from organs other than the portal and mesenteric veins in spite of the thorough search done.

Parasite burden

There appeared to be very low level of parasitism that was noted among the schistosome infected rats that were examined through necropsy and microscopy. Virtually, one adult schistosome was recovered from each of the infected field rats (Figures 7 to 13) except with 2 female rats with adult schistosomes recovered in a state of copula (Figure 6a and b). This observation strongly suggests that all the infected field rats were suffering from chronic schistosomiasis. Such observation can be noted from the reports of authorities (Soulsby, 1982; Urquhart et al*.,* 1987; Bowman and Lynn, 1999; McManus et al., 2010; Leonardo et al., 2012) that schistosomiasis is basically a chronic parasitic disease. The pathology therefore is also of a chronic nature that are debilitating in effect. Though parasite burden is low their effect is however cumulative and enduringly destructive. Richard (2013) explained that in about five to 15 percent of cases, especially in those who get the infection repeatedly, the eggs of a schistosome in hundreds which are deposited

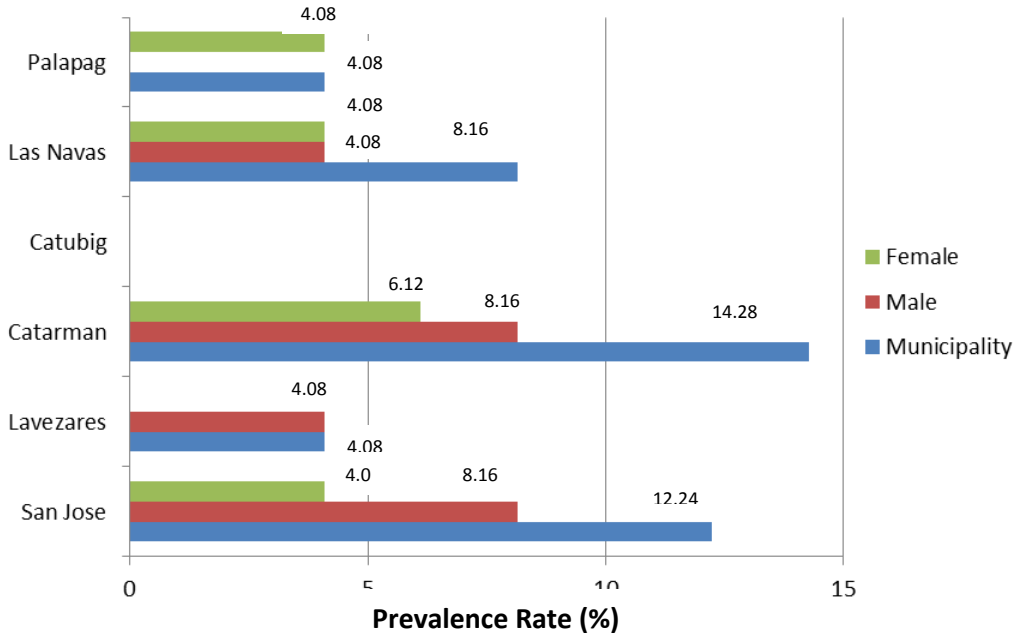

Figure 5. Prevalence rate of *Schistosoma japoniucm* infection in field rats in Northern.

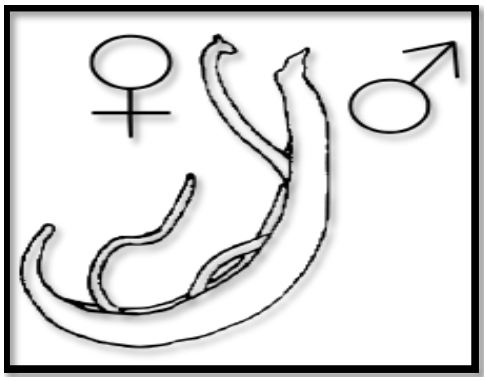

Figure 6a. Schematic drawing of adult male and female schistosomes in copula.

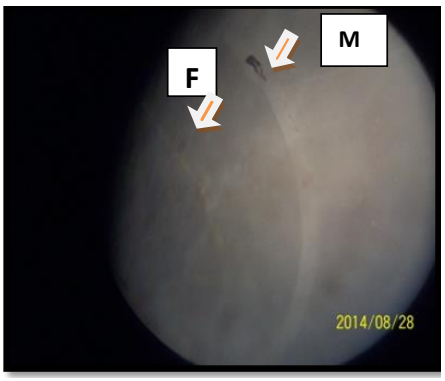

Figure 7. A microphotograph (LP10X) of dead adult male and female schistosome.

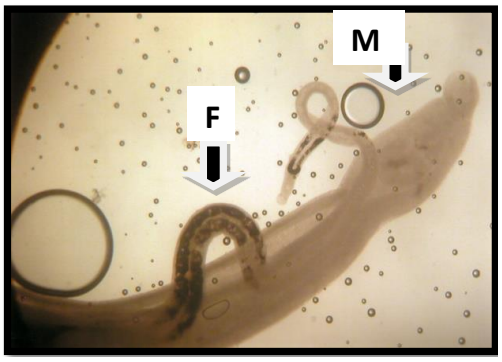

Figure 6b. A microphotograph (LP10X) of adult live male and female schistosomes in copula.

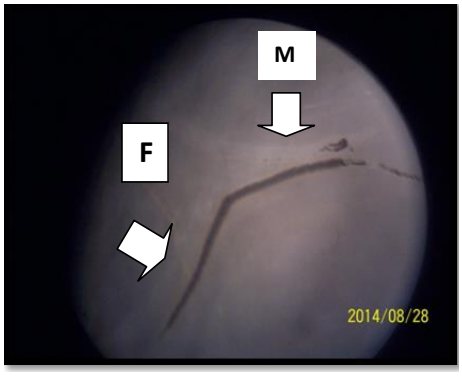

Figure 8. A microphotograph (LP10X) of dead adult male and female schistosome.

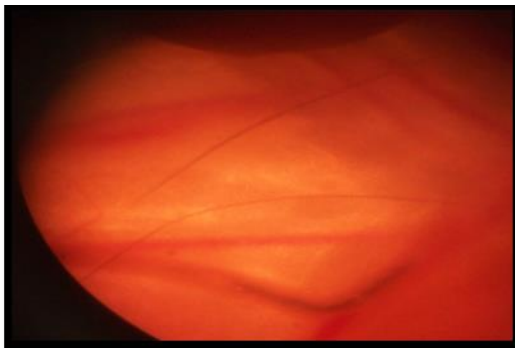

Figure 9. A live female schistosome (LP10X) inside the mesenteric vein.

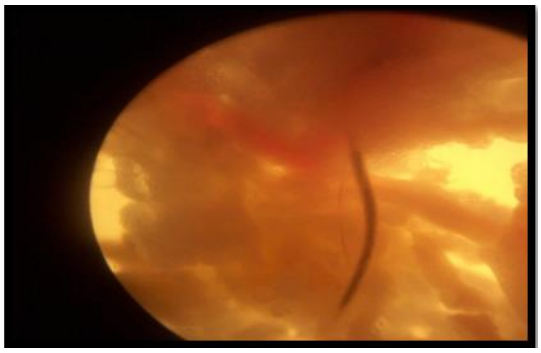

Figure 10. A live female schistosome (LP10X) outside the mesenteric vein.

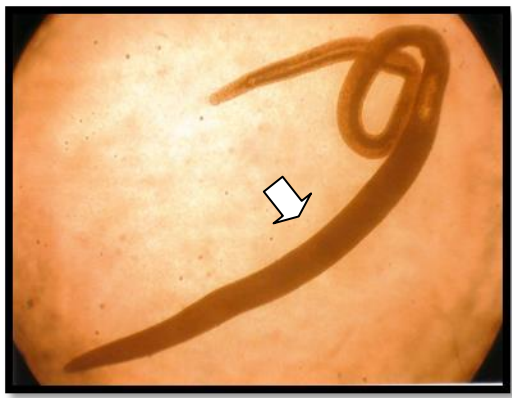

Figure 11. Mounted adult female *S. japonicum* (LP10X).

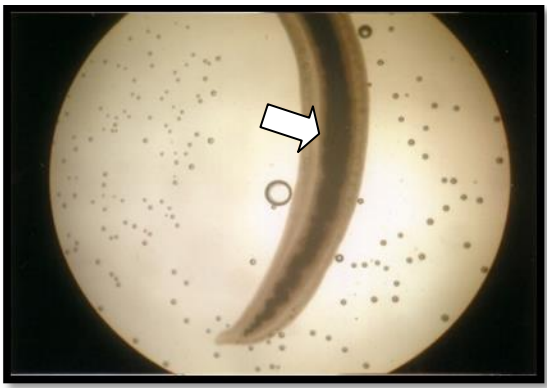

Figure 12. Posterior end of adult female schistosome (LP10X) showing the yolk or vitelline glands that occupy the posterior half of the body.

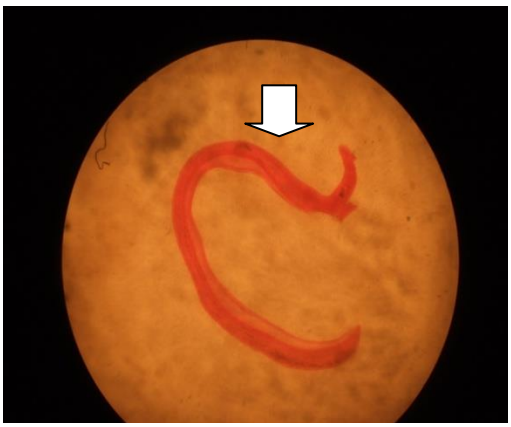

Figure 13. Mounted specimen of a male *S. japonicum (*LP10X).

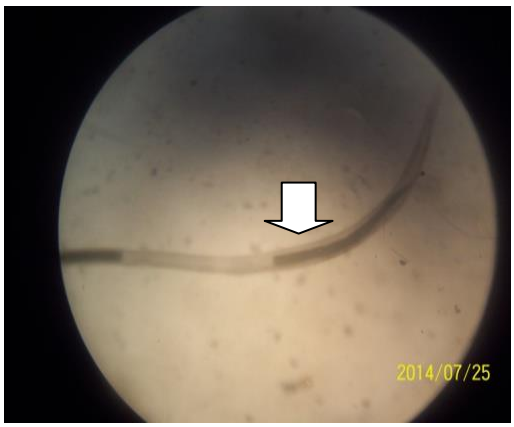

Figure 14. *Nippostrongylus sp.* (LP10×).

in various tissues and organs of the body induce inflammation that over time(five to 15 years or longer) results in a variety of complications. Some of these complications such as portal hypertension and liver cirrhosis can be life-threatening. Such pathology is more likely to occur in field rats as shown also by the lesions

Table 4. Macroscopic lesions clinically examined on some visceral organs of schistosome infected field rats.

Macroscopic lesions	Infected rats (N = 21)				Total	
	Males		Females			
	No.	%	No.	%	No.	%
Single lesion:						
Nodules in liver	4	19.05	3	14.28	7	33.33
Edematous intestine	1	4.76	2	9.52	3	14.28
Multiple lesions:						
Fibrosis, nodules and edematous intestines	2	9.52	3	14.28	5	23.81
Nodular mass (granulomas)	5	23.81	4	19.05	9	42.86
Nodules and edematous intestines	1	4.76	2	9.52	3	14.28

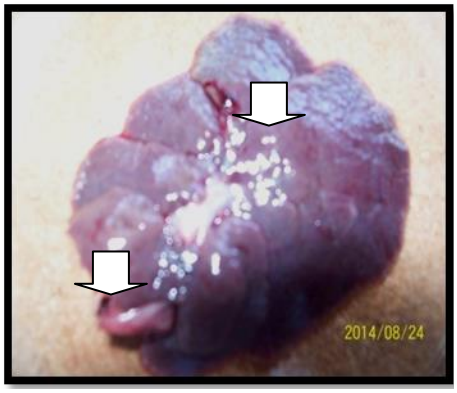

Figure 15. Liver of a field rat with hepatic fibrosis and nodular mass.

diagnosed and presented in this study.

Further, there were five rats parasitized with other species of worms, two were already schistosome infected and were also infected with strongyle worms while another three rats all from the municipality of Catubig were parasitized with strongyles (Figure 14) and tapeworms but were not infected with schistosomes. Parasite load as to the stronglye and tapeworm species were very minimal ranging from 1 to 3 per affected rat. With these worm populations other than the schistosome, the lesions observed to have been established in the viscera of the infected rats could not be attributed to their presence and that their population is beyond capability to producing pathological lesions.

Lesions in some visceral organs

The visceral organs of schistosome infected rats were thoroughly examined during necropsy for the presence of macroscopic lesions (Table 4). Clinical observations on different visceral organs both abdominal and thoracic were noted and that lesions were found only on the liver and intestines. However, occurrence of lesions on organs was found either singly in some rats or on multiple conditions in others. These lesions are herein presented in Table 4. Thus, as shown in the table, aside from being already parasitized by the blood fluke, schistosomes, 33.33% (7/21) infected rats of which 19.05% (4/21) were males and 14.28% (3/21) were females had single lesion in form of granulomas on the surface of the liver; 14.28% (3/21) infected rats of which 4.76% (1/21) were males and 9.52% (2/21) were female showed also to have single lesion of having edematous intestines. On the other hand, there were schistosome infected rats that showed multiple lesions affecting two or more organs. Thus, 42.86% (9/21) infected rats of which 23.81% (5/21) were males and 19.05% (4/21) were female's demonstrated nodules on the liver. Further, 23.81% (5/21) showed fibrosis and granulomas on the liver coupled with edematous intestine. Multiple occurrences of lesions in visceral organs was further seen in 14.28% (3/21) schistosome infected rats of which 4.76% (1/21) were males and 9.52% (2/21) were females. These rats showed liver with nodules, edematous intestine and lungs that were very pale. Figures 15 to 21 show these kinds of lesions.

With these observations, it is indicated that nodules on the liver are attributed to *Schistosoma japonicum* infection considering that majority of the schistosome infected rats either male or female and either having single or multiple affection of lesions appeared to be very common. Pathologists may discern this condition to be an effect on the chronicity on the migratory phase of the adult schistosome in the liver tissues before proceeding to the mesenteric veins or spread of the eggs within the liver from adult schistosome that copulate and lay early fertilized eggs in the portal vessels or eggs that are swept back to the liver laid by females from the intestinal mucosal lining. According to Soulsby (1982) and Urquhart et al. (1987) eggs of schsitosomes contain allergenic

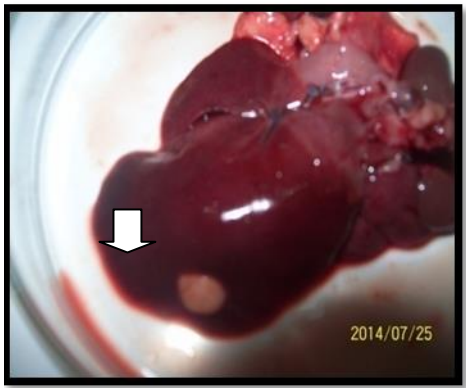

Figure 16. Liver of a field rat with nodule (granuloma).

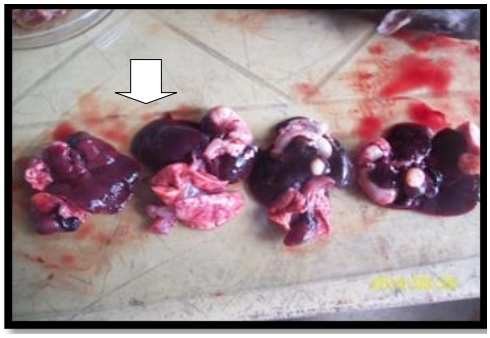

Figure 17a and b. Liver with nodular mass granuloma.

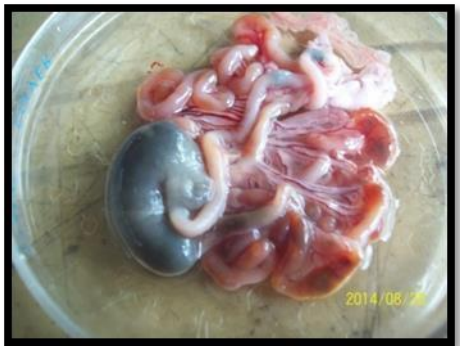

Figure 18. Distended intestines.

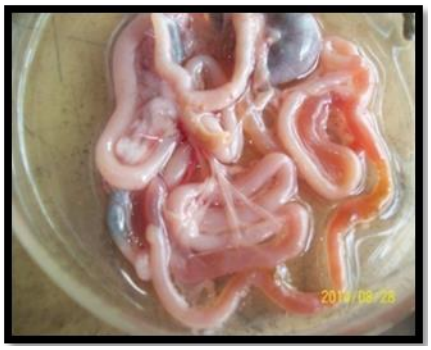

Figure 19. Distended intestines with Sloughing of mesenteric tissues.

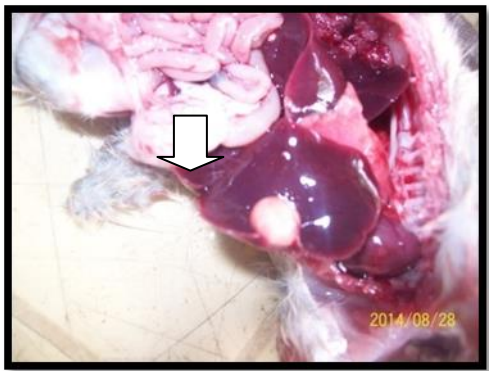

Figure 20. Liver with nodular mass intestine edematous.

properties that trigger the formation of pathological lesions in the liver. In addition, movement of the adult parasites within the organ parasitized causes chronic irritation most often resulting to fibrosis of the tissues affected. Further, Conlan et al. (2011) reported that in their study on prevalence of *Schistosoma japonicum* among field rats, in most cases the adult worms are trapped in the lungs and produced few viable eggs. This condition would therefore trigger into granuloma lesion formations in the lungs, the lungs being very soft parenchymatous tissues.

SUMMARY AND CONCLUSION

This study was conducted for a period of four months

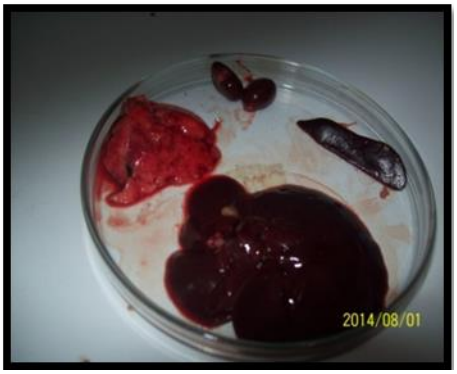

Figure 21. Liver with nodular mass granuloma.

with the aim of determining the prevalence rate of *Schistosoma japonicum* infection among field rats in Northern Samar. Forty-nine field rats collected from six municipalities through the use of rat traps were necropsied, examined for the presence of schistosome adults, assessed for macroscopic lesion characteristics, and evaluated for parasite burden.

Results of the evaluation showed that out of the 49 field rats clinically examined, a prevalence rate of 42.86% was obtained. From this infection rate, 24.49% were males and 18.57% were females parasitized. Rats of either sex collected from the municipality of Catarman had the highest prevalence rate to schistosoma infection of 14.28%.

Schistosome burden appeared to be low with one adult schistosome on the average per infected field rat. Almost all of the schistosomes recovered were retrieved from the mesenteric veins of all infected field rats of both sexes. Of the macroscopic lesions characterized, majority (42.86%) of the schistosome infected field rats demonstrated granulomatous liver surfaces with edematous intestines. About 23.81% infected rats showed multiple lesions with fibrosis and nodular mass (granuloma) of the liver and edematous intestine as prominent lesions.

These findings conform to various reports of the existence of schistosomiasis among field rats in many areas of the country and attest to the fact that about 43% of the field rat's populations in Northern Samar are infected with *S. japonicum*. It can therefore be concluded that field rats continue to serve as multiplier hosts of schistosomiasis among humans and animals in the province.

RECOMMENDATIONS

Based on the results of the study the researcher would like to recommend the following:

1. Agencies concerned should come up with measures such as province-wide use of rodenticides (synthetic or organic), any kind of rat poison, campaign on search and destroy rats as farm pests or giving some kind of monetary incentives to anybody who could catch rats to a specified number per day to control or possibly annihilate wild field rats.
2. Research should be conducted on this line of interest searching for endogenous materials that can be used as rodenticides against field rats as well as molluscicides that can destroy *Oncomelania quadrasi*, the snail host of *S. japonicum* responsible for the asexual multiplication of the infective form (cercariae) of schistsomes.
3. Agencies concerned should conduct public awareness campaign of the role of field rats in the transmission of schistosomiasis.
4. Since schistosomiasis is endemic in the province, the disease together with other notifiable zoonotic diseases should be made part of the school science courses in the elementary, secondary and tertiary levels within the province.
5. Sustain the presently existing program of the government on the control of Schistosomiasis throughout the country

Conflict of interests

The authors have not declared any conflict of interests.

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