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Study on the prevalence of *Schistosoma japonicum* among water buffalo (*Bubalus bubalis*) in selected Barangays of Gonzaga, Cagayan

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Key words: *Schistosoma japonicum,* Infection, Water buffalo, Infection, Severity

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Abstract

The study aimed to evaluate the presence and distribution of *Schistosoma japonicum* in water buffalo in terms of the number of eggs present per sample, number of animals affected with the, parasite, level of infection of animals per area and a GIS mapping of the area where the parasite exists. It also determined possible association of infection with the age and sex of the animals. The study tested 480 heads of water buffalo from fourteen (14) barangays: San Jose, Baua, Sta. Maria, Cabiraoan, Sta. Cruz, Amunitan, Ipil, Magrafil, Tapel, Calayan, Sta. Clara, Pateng, Isca and Rebecca. Water buffaloes listed in recent health records of the Municipal Agriculture Office were selected and included in the study. The sex and age of the animals were recorded, and they were tested for infection prevalence of *Fasciola* and *Schistosoma japonicum* in terms of total count and number of eggs per gram. On the prevalence by number and percentage of animals affected, out of 480 heads, 400 were infected with *Fasciola* which comprises 83.68% of the total number of animals tested, while 109 heads were infected with *S. japonicum* which is 22.60% of the animals tested. There were animals infected with *Fasciola* alone, while others were infected with *S. japonicum* alone, while still others were infected by both parasites. All of the fourteen (14) barangays involved in the study were infected with *Fasciola*, while only ten (10) barangays were infected with *S. japonicum*. The age of the animal is positively related to the number of eggs per gram of *S. japonicum* but not with *Fasciola*, which means that the older the animal, the more severe the infection with *S. japonicum*. Infection prevalence of *S. japonicum* has nothing to do with *Fasciola* prevalence.

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Introduction

The continued increase in human population accounts for an increase in the demand for food, shelter, clothing, and other basic human needs. With regards to the country's performance, the Philippine Statistics Authority (2020) recorded to have a drop in the production of cattle (-12.1%), swine (-6.7%), carabao (-14.4%), chicken (-6.1%), and even dairy products (-12.8%). It should be noted that the country's performance in food production is still below the production line thus effort must be made to meet the demand.

Many factors that affect the production of animals include the incorrect bull-to-cow ratio, low calving percentage, and high mortalities and losses caused by drought, flooding, and diseases (Hangara *et al.,* 2011). In addition, the Food and Agriculture Organization (2021) claimed that scarcity and fluctuating quantity and quality of year-round feed supply had also greatly affected animal performance specifically in developing countries. Furthermore, the health or the overall well-being of the animals plays a vital role in the overall animal performance.

Bacterial and viral diseases can be successfully controlled through conventional vaccination and quarantine procedures. However, for parasitic diseases, these approaches are either not yet possible or impractical. Chemotherapy coupled with grazing management is the only methods of control currently available. The report shows that in developing countries like the Philippines, the losses induced by clinical and subclinical parasite infections have been estimated to equal the value of the present output of animal industries. Parasites can cause weight loss, low weight gain, poor breeding efficiency, low work capacity, and high mortality, which all spell heavy losses. Although there are government interventions in the possible increase in the animal population, it was only focused on genetic quality enhancement thus productivity index cannot still be met due to losses caused by unchecked parasitic conditions.

Schistosoma japonicum is an internal parasite of water buffalo caused by trematode worms and was reported to have an outbreak in the municipality of Gonzaga. The infection can be transmitted to the snail as its intermediate host and can even cause harm to humans. It should be made clear that the activity of the farmers such as their direct exposure to land preparation and management of the rice production area and the wallowing behavior of the water buffalo to dissipate heat during daytime, especially during summer are the prime reasons for the transmission of the parasite *Schistosoma japonicum*. Considering the threat of the said parasite that could induce the livestock industry, the researcher thought of assessing its transmission and its cycle, particularly in water buffaloes. This research will also serve as baseline data to know the level of infection of *Schistosoma japonicum* in water buffalo and the distribution of the parasite in Gonzaga, It will also provide recommendations to concerned individuals to lessen its transmission of the parasite, hence this paper.

Generally, the study aimed to determine the prevalence rate of schistosomiasis in water buffaloes and to determine the significance of water buffaloes in the transmission of *S. japonicum* to the environment.

Specifically, it aimed to evaluate the presence and distribution of *Schistosoma japonicum* in water buffalo in terms of the following parameters:

1. Number of eggs present per sample.
2. Number of animals affected by the parasite.
3. Level of infection of animals per area.
4. GIS mapping of the area where with the parasite exist.

Materials and methods

Hypothesis

Ho1: There is no significant difference in schistosomiasis infection levels between male and female animals

Ha2: There is a significant difference in schistosomiasis infection levels between male and female animals

Ho1: There is no significant relationship between the age of animals and their infection level with schistosomiasis

Ha2: There is a significant relationship between the age of animals and their infection level with schistosomiasis

Ho1: There is no significant relationship between *Fasciola* and *Schistosoma* infection levels

Ha2: There is a significant relationship between *Fasciola* and *Schistosoma* infection levels

Scope and delimitation of the study

This study was centered on the prevalence of schistosomiasis in different barangays of Gonzaga Cagayan. It also included *Fasciola* a parasite that is closely associated with *S. japonicum*; being in the same family. The seashore barangays that are predominantly fishing communities were not included in the study. Fourteen (14) inland barangays that are farming communities and with water buffalo populations were included only in the study, the selection of selected barangays is based on the Geographic information system which shows the river basin and its tributaries.

Locale of the study

A cross-sectional study was conducted in fourteen (14) barangays based on river tributaries: San Jose, Baua, and Sta. Maria, Cabiraoan, Sta. Cruz, Amunitan, Ipil, Magrafil, Tapel, Calayan, Sta. Clara, Pateng, Isca, and Rebecca.

Materials

The following materials were used in the experiment: rectal palpation gloves, rubberized gloves, plastic cups, marking pen, styrofoam, refrigerator, beaker, graduated cylinder, mesh, gauze, formalin, ethyl acetate, potassium hydroxide, stirrer, digital weighing scale, centrifuge, test tube, McMaster glass slide, aspirator, spatula, microscope, and thermometer.

Experimental design

A descriptive design with correlation analysis was used in the experiment. The T-test was also used to assess the significance of the study.

Collection and examination of samples

The collection of stool samples was done by direct insertion of a hand with moistened gloves into the rectum of the test animals. One (1) fecal sample was collected and placed in a clean disposable cup with a lid**.** Samples were labeled accordingly to avoid interchanging specimens. All samples were brought and examined at the College of Agriculture, Cagayan State University-Gonzaga Campus in Flourishing, Gonzaga, Cagayan.

Parasitological examination technique

The study used the novel copro-parasitological technique for the detection of *S. japonicum* eggs in ruminant fecal samples, the FEA-SD technique with few modifications. The whole volume of the sample was determined and recorded using a McMaster 4 chamber worm egg counting slide 4×0.5 ml (Whitlock Sfelo©). All of the samples were examined only once. Full details of the procedure on FEA-SD technique were as follows:

1. First, bovine stool samples were collected rectally from the animals (approximately 500 g each) by veterinarians or trained personnel.
2. Each stool sample is homogenized with an applicator stick. From there, fisty (50) grams of the stool mixture is taken and mixed into a slurry in a beaker with 300 ml of water.
3. The slurry is then sieved, by pouring the material into a 60-copper mesh (Tyler scale with a pore opening size of 250 µm). Water is also used to flush the smaller sediment into a 260-copper mesh (61 µm), held below the 40–60 mesh. Particles caught on the 260 mesh are washed with water into a conical flask and allowed to sediment naturally for 30 minutes.
4. The excess water is removed leaving only the sediment which later was poured into a 50 ml tube. Approximately 50 ml of water is added to the conical flask and natural sediment for 30 minutes. After this, excess water was removed and pouring sediment into the same 50 ml tube.
5. Ten (10) % formalin (v/v) is added to the tube and well mixed by whirling the substance. To fix the eggs, let the substance sit at room temperature for 30 minutes. The Falcon tube is then vortexed, and 10 ml of the suspension, or 10 grams of feces, is transferred using a pasteur pipette into two 15 ml tubes, each of which is labeled A and B.

6. The volume of tubes A and B is increased to 8 ml using 10% (v/v) formalin solution, and the tubes are well mixed by vortexing. Next, 4 ml of 100% (v/v) ethyl acetate is added using a glass pipette, and the tubes are violently vortexed once more for 30 seconds. The tubes are centrifuged at 500 g for 10 minutes with the cap slightly loosened, producing a four-layer separation. It is important for this step that the tubes are spun at 500 *g* for stable and efficient debris removal.

7. The ethyl acetate is removed from both tubes by gently rimming the bulk debris layer with a thin applicator stick and decanting the top three layers which are then discarded. Ethyl acetate and 10% (v/v) formalin can be added and spun again if necessary to remove further bulk debris. If the middle layer of bulk debris is very thin the tube should be shaken vigorously and re-spun for better efficiency.

8. Water is added to the remaining pellet to take the volume up to 5 ml and an equal volume of 10% (w/v) potassium hydroxide (KOH) is added to each tube. The tubes are mixed gently by vortexing to resuspend the pellet and the sample is digested overnight at 37°C.After digestion, the sample is vortexed vigorously and centrifuged at 900 *g* for 10 minutes.

9. The pellet is washed once with 10–15 ml of water to remove any residual KOH by centrifuging the solution for 10 minutes at 900 *g*, the supernatant is removed and the final pellet is resuspended in 4–6 ml of water (as the samples are now fixed) and stored at 4°C. The sample is now ready for counting the *S. japonicum* eggs in a microscope.

10. Before counting, the suspension is mixed gently with a pipette and the total volume of tubes A and B are counted for each sample, pipetting 200–300 µl onto each slide for microscopy. It should be noted that the sensitivity of the procedure increases with the amount of suspension examined. The microscopy was performed blindly by two independent microscopists. Infection intensity (eggs per gram of feces, EPG) was calculated based on the total egg number in 10 g of feces (i.e., the contents of tube A plus tube B).

Statistical analysis

The prevalence of *S. japonicum* infection among water buffaloes was determined based on the presence of schistosome eggs in fecal samples. Egg counts in 5 g of feces were noted. The age of the animals was also considered to assess its significance with the prevalence of *S. japonicum* infection. This was determined using Pearson Product-Moment Correlation analysis (Pearson rho) and the corresponding t-test of the significance of the correlation. The relationship between sex and prevalence was determined through a t-test of the difference in prevalence between male and female animals with the use of a t-test for two independent samples. The statistical significance was determined at a 0.05 level.

Specifically, infection intensity among water buffaloes within each endemic setting was determined based on the total egg count in 10 g of fecal samples. In addition, the intensity of infection was determined by egg counts in 10 g of feces and was categorized as light (1-400 eggs), moderate (101-400 eggs), or heavy (>400 eggs), as described by Ardina (2014). The bovine contamination index (BCI) per animal was measured.

Prevalence rate

The PR of schistosomiasis within the water buffalo population was determined using the following formula:

PR= (No. of cases in the population at one time)/(No. of animals in the population at the same time)

Bovine contamination index

The number of *S. japonicum* eggs excreted by each bovine, on average, was calculated through the formula, as used by Gordon et al. (2012) and Tenorio and Molina (2020).

BCI= (Arithmetic mean of eggs per gram × (EPG) × Number of infected)/ (Bovine × 25,000 g) (average fecal weight)

Results

Prevalence of infection

Number of eggs per sample

Table 1 shows that out of 478 water buffalo tested *Fasciola* had a total of 16,920 eggs with a mean of 35.4, while the *S. japonicum* had a total of 2,265 eggs with a mean of 4.74.

Table 1. Number and percentage of eggs per sample

Variables	Total	Mean
Total Number of Treatments	478	
Total Eggs per gram (Fasciola)	16920	35.4
Total Eggs per gram (*S. japonicum*)	2265	4.74

Table 2. Number of animals affected by the parasite

Parasite	Number of animals affected	Percentage
Number of animals with *Fasciola*	400	83.68
Number of animals with *S. japonicum*	109	22.80
Total	509	106.49

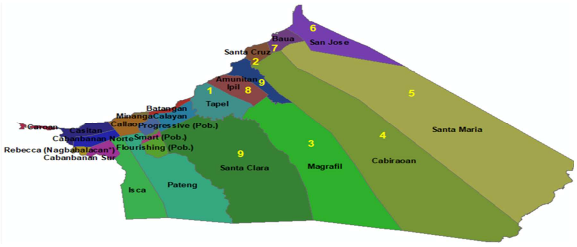

Fig. 1. Ranking of affected Barangay with *Schistosoma japonicum*

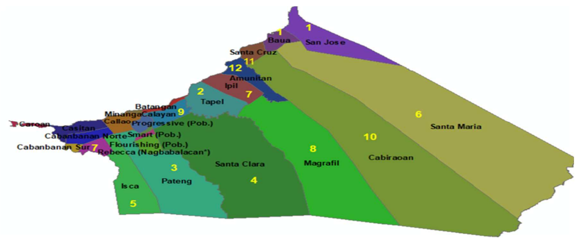

Fig. 2. Ranking of affected Barangay with *Fasciola*

Prevalence by number and percentage of animals affected

Out of this number of 478 samples, 400 of them were infected with Fasciola which comprises 83.68% (Table 2). There were 109 heads infected with *S. japonicum* which is 22.60% of the animals tested. Of the total animal infected, there were those who were infected with Fasciola alone, others were infected with *S. japonicum* alone, and still, others were infected by both parasites.

Prevalence of schistosoma in different barangays

Table 3 shows the number of animals tested per barangay, their mean ages, and the prevalence of parasite infection in terms of the number of eggs per gram. Of the fourteen (14) barangays involved in the study, all of them were affected by Fasciola, while only ten (10) barangays were infected with *S. japonicum* (Fig. 1).

In *Fasciola* infection, Barangay San Jose and Baua had the highest level of infection while Barangay Amunitan had the lowest. In *S. japonicum* infection, of the ten (10) barangays infected, Barangay Tapel had the highest while Barangay Amunitan and Barangay Sta. Clara had the lowest (Fig. 2).

It is good to note that animals from four barangays; Calayan, Pateng, Isca, and Rebecca had no infection in Schistosomiasis.

Bovine contamination index

The Table 4 shows how many eggs are produced per day per Barangay, it shows that Magrafil has the Highest BCI and Barangay Baua has the lowest.

Parasite infection level and sex of the animals

In the study, there were more female than male animals involved, with 286 females comprising 60% and 192 males comprising 40%.

The t-test of difference revealed that the numerical differences between the male and female animals were not statistically significant (Table 5) at a 0.05 level of significance. This result shows that the parasite infection level is not related to the sex of the animal. It further means that whether the animal is male or female, it has nothing to do with the level or severity of parasite infection. This is true with both Fasciola and *S. japonicum* parasites.

Relation between Parasite Prevalence and Age of Animals

In terms of the relation between the prevalence of infection and the age of the animals (Table 6), it was found that age and the numbers of eggs per gram of Fasciola have no significant relationship. The Pearson Product-Moment Analysis of Correlation yielded a low positive correlation coefficient of r = .091 (Table 7), but the t-test of significance revealed that the correlation is not significant at the 0.05 level.

Table 3. Prevalence by Barangay

Barangay	Number of heads surveyed	Mean age	Prevalence rate					
			Fasciola +	Mean	Rank	Schisto +	Mean	Rank
San Jose	33	4.03	33	100	1	4	12	6
Baua	13	4.77	13	100	1	1	8	7
Sta. Maria	38	4.74	32	84	6	11	29	5
Cabiraoan	67	4.48	52	78	10	29	43	4
Sta. Cruz	11	4.91	7	64	11	6	55	2
Amunitan	34	5.71	20	59	12	2	6	9
Ipil	27	5.11	22	81	7	2	7	8
Magrafil	59	6.2	47	80	8	28	47	3
Tapel	36	5.28	35	97	2	22	61	1
Calayan	14	5.71	11	79	9			
Sta. Clara	69	4.77	62	90	4	4	6	9
Pateng	23	3.83	21	91	3			
Isca	27	3.96	23	85	5			
Rebecca	27	4.74	22	81	7			
Total	478	68.24	400	84		109	23	
Grand mean		4.87	28.57			10.9		

Table 4. Bovine contamination index

Barangay	# Of animal infected with *schistosoma*	Bovine contamination index		
		EPG	Mean	BCI
San Jose	4	40	1.21	4,848,485
Baua	1	10	0.77	192,308
Sta. Maria	11	180	4.74	234,473,684
Cabiraoan	29	475	7.09	2,441,464,552
Sta. Cruz	6	140	12.73	267,272,727
Amunitan	2	35	1.03	1,801,471
Ipil	2	50	1.85	4,629,630
Magrafil	28	750	12.71	6,673,728,814
Tapel	22	530	14.72	4,291,527,778
Calayan				-
Sta. Clara	4	55	0.80	4,384,058
Pateng				-
Isca				-
Rebecca				-

BCI= (Arithmetic mean of eggs per gram × (EPG) × Number of infected)/ (Bovine × 25,000 g) (average fecal weight)

Table 5. Analysis of parasite infection prevalence by sex

	Sample size	Percentage	Prevalence(actual count)				Mean eggs per gram	
Sex			*Fasciola*	Mean	*Schistosoma*	Mean	*Fasciola*	*Schistosoma*
Male	192	40	160	8.42	44	4.00	4243.8	2130.8
Female	286	60	240	8.49	65	4.26	4209.4	2000.0
Total	478	100						

This means that the age of an animal has nothing to do with the level of infection or prevalence of Fasciola in terms of the number of eggs per gram.

This result for *Fasciola* is in contrast with *S. japonicum*, because the positive correlation coefficient r = .205 (Table 7) has a t-test computed value of 2.168 that is higher than the tabular value of 1.960, therefore the null hypothesis (Ho) is rejected and the alternative hypothesis (Ha) is accepted. This means that the age of the animal is positively related to the number of eggs per gram of *S. japonicum*. It means further that the older the animal, the more eggs per gram of *S. japonicum* in it. In other words, the older the animal, the more severe the infection with *S. japonicum*.

Table 6. T-test of difference in prevalence between male and female animals

Parasite	T-test probability of rejecting Ho	Decision at .05	Result
Fasciola	0.843	Accept Ho	No difference between male and female animals
S. japonicum	0.806	Accept Ho	No difference between male and female animals

Table 7. Pearson product-moment analysis of correlation between parasite prevalence and age of animals

Variables	Pearson rho coefficient	T-test of significance		Decision at .05	Result
		Computed value	Tabular value		
Age and *Fasciola* eggs/gram	0.091	1.816	1.960	Accept Ho	No significant relationship
Age and *S. japonicum* (eggs/gram	0.205	2.168	1.960	Reject Ho	Significantly related

Table 8. Pearson product-moment analysis of correlation between prevalence of *Fasciola* and *Schistosoma* (in eggs per gram)

Variables	Pearson rho coefficient	T-test of significance		Decision at .05	Result
		Computed value	Tabular value		
Fasciola and *S. japonicum* prevalence (eggs/gram)	0.37	8.68	1.960	Reject Ho	There is a significant relationship between *Fasciola* and *S. japonicum*

Relation between schistosomiasis *and Fasciola prevalence*

On the relation between prevalence of *Fasciola* and *S. japonicum* (Table 8), the Pearson rho correlation analysis yielded a positive correlation coefficient of r = .37, but this relationship is significant at .05 level in the t-test of significance. Therefore, the result means that there is a positive relationship between the two variables. It means that if the water buffaloes is infected with fasciola the animal is more susceptible to *Schistosoma japonicum*.

Discussion

The study tested a total of 478 heads of water buffalos (carabaos) in 14 barangays of Gonzaga, Cagayan. Stool samples were collected from the test animals and stored in a refrigerator for about 3 to 7 days with a temperature regime of 8°C. The gathered stool sample came from the 14 identified barangay of the municipality of Gonzaga. Sample materials were processed and analyzed at the laboratory center of Cagayan State University Gonzaga campus. The descriptive correlation design was used in the experiment. The T-test was used to compare the significance of result generated in the study.

Based on the result of the study, animals were tested for infection of *Fasciola* and *Schistosoma japonicum* in terms of total count and number of eggs per gram. On the prevalence by number and percentage of animals affected with the parasite, out of 478 heads of water buffalos, 400 (83.68%) of them were infected with *Fasciola* while 109 or 22.60% were infected with *S. japonicum*. Of the total 509 infections, there were those who were infected with Fasciola alone, others were infected with *S. japonicum* alone, and still, others were infected by both parasites.

Fourteen (14) barangays tested positive for *Fasciola* while only ten (10) barangays were infected with *S. japonicum*. In *Fasciola* infection, Barangay San Jose and Baua had the highest level of infection while Barangay Amunitan recorded the lowest infection rate. In *S. japonicum* infection, of the ten (10) barangays infected, Barangay Tapel had the highest infection rate while Barangay Amunitan and Barangay Sta. Clara had the lowest infection rate. No incidence of infection was recorded in the following barangay: Calayan, Pateng, Isca, and Rebecca in terms of schistosomiasis.

In terms of sex, female (286 or 60%) animals outrank the male population (192 or) 40%. However, male animals had a numerically higher number of eggs per gram, with a mean of 4243.8 in Fasciola and 2130.9 in *S. japonicum*, while the female animals had a mean of 4209.4 in *Fasciola* and 2000 in *S. japonicum*. Statistically no significant relationship was observed in terms of parasite infection level in relation to the sex of the animal.

The number of eggs per gram of *Fasciola* is not associated with the age of the animals. But in the case of *S. japonicum*, the age of the animal is positively related to the number of eggs per gram of *S. japonicum*. It means that the older the animal, the more eggs per gram of *S. japonicum* in it. In other words, the older the animal, the more severe the infection with *S. japonicum*.

There is a significant relationship between the *Fasciola* and *Schistosoma*. If the water buffaloes is infected with *Fasciola* the immunity of the animal is weaker which is more susceptible to acquire *Schistosoma*.

Conclusion

Based on the findings of the study, the following conclusions are drawn:

1. On the prevalence by number and percentage of animals infected with *S. japonicum*, the area covered by the study has 22.60% prevalence. This is based on the number of animals tested and the number of animals infected.
2. Although all of the barangays involved in the study had cases of *Fasciola* infections, not all of them had cases of *S. japonicum* infection.
3. The prevalence and infection level is not related to the sex of the animal. Whether the animal is male or female, it has nothing to do with the level or severity of parasite infection. This is true with both *S. japonicum* and *Fasciola* infections.
4. The infection with *S. japonicum* is positively related to the age of the animals. It means that the older the animal, the more eggs per gram of *S. japonicum* in it. In other words, the older the animal, the more prone to *S. japonicum* infection.
5. The infection prevalence of *S. japonicum* has something to do with *Fasciola* prevalence.

Recommendations

Based on the findings of the study, the following recommendations were made:

1. Buffalo owners and raisers in the municipality of Gonzaga, Cagayan should be more proactive in the care and monitoring of the health of their animals and in asking for assistance from the expertise of the Municipal Agriculture Office.
2. Extension workers of the Cagayan State University and other agencies should include animal care in their technology transfer activities. Animal raisers and owners need to be more empowered with knowledge and skills about animal health.
3. Conduct of researches on prevalence of *Schistosoma japonicum* on other animals should be conducted in the locality.

A follow-up study should also be conducted involving Municipal Health Office and Municipal Agriculture Office on the occurrence of *Schistosoma* in the Locality

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