Zoonotic Transmission and Infection from Bovine Feces in Selected Ricefields of Lake Mainit, Philippines

Joycelyn C. Jumawan*, Maria Karla M. Balamad, Leonardo A. Estaño

Department of Biology, Caraga State University, Ampayon, Butuan City, PHILIPPINES.

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ABSTRACT

Ricefields are suitable nidus of active parasite transmission via bovines due to often unprotected exposure by humans and livestock that utilize the habitat for farming and foraging. This study was conducted to assess infection of protozoans, trematodes and nematodes from bovine feces from ricefields of Brgy Magpayang, San Isidro and Matin-ao surrounding Lake Mainit, Philippines. Bovine fecal samples collected from these ricefields were processed using the Formalin Ethyl Acetate Sedimentation (FEA-SD) technique. A total of five species from three major parasite groups (protozoans, trematodes and nematodes) were observed. Bovines from ricefields of Brgy San Isidro harbored the highest number of fecal parasites. The liver fluke *Fasciola* sp. was the most predominant species observed with the highest prevalence (69%) across study areas. In contrast, multiple parasite burden of *Fasciola* sp., hookworms, coccidian oocysts and *Ascaris* sp. eggs were observed from bovines from Brgy San Isidro and Matin-ao. This study is the first report of parasites from bovine feces in selected ricefields of Lake Mainit. The high prevalence of *Fasciola* eggs in bovines calls for an extensive assessment of possible widespread bovine fascioliasis in the area.

Key words: Bovines, Lake Mainit, Zoonosis Fascioliasis, Parasites.

Correspondence:
Joycelyn C. Jumawan, Ph.D.
Department of Biology, Caraga State University, Ampayon-8600, Butuan City, PHILIPPINES.
Phone no: +63 (085) 341-3249

Email: joycejumawan1981@gmail.com

INTRODUCTION

Livestock animals, such as bovines, are essential partners of farmers as on-farm power in ricefields and contributes to the sustainability of agricultural systems.[1,2] These livestock animals feed mostly only on grasses and other non-food biomass, which, in return, supply manure to act as fertilizer in agricultural lands such as ricefields.[3] These animals, however, serve as crucial hosts to the transmission of zoonotic diseases that affects food production, reduced animal productivity and cost of interventions.[4]

The use of bovines for agricultural rice cropping is a common feature of farmland preparation in ricefields surrounding Lake Mainit, Philippines. Studies in key habitats surrounding the lake have indicated probable link of snail and bovine-associated diseases, particularly the active transmission of schistosomiasis and other zoonotic diseases, respectively.[5,6] Moreover, studies on bovines as a critical host remains limited with some, even unreported.[4] This study aimed to report bovine infection of trematodes and nematodes using bovine fecal samples from selected ricefields in Lake Mainit following the Formalin Ethyl Acetate Sedimentation Concentration Technique (FEA-SD). This study aimed to determine the prevalence of bovine infection, their medical and veterinary importance that may help in understanding the complex life cycle to manage parasite infection in bovines in ricefields effectively.

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MATERIALS AND METHODS

Study Sites

Ricefields of three barangays, namely San Isidro, Magpayang and Matin-ao surrounding Lake Mainit were utilized for the collection of bovine fecal samples (Figure 1). The selection of ricefields was based on an existing research study on ricefields surrounding Lake Mainit, where snails harbored trematodes of agricultural and medical importance.[5]

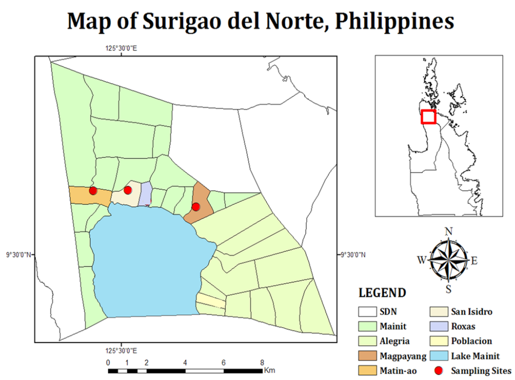

Figure 1: Map showing the three ricefield study sites of Lake Mainit, Philippines.

Collection of Bovine Fecal Samples

A total of 16 domestic bovines were purposively sampled in the study sites in February 2020. The selection considered the following criterion: First, carabaos must be exposed to rice fields at the time of collection; and second, the freshness of fecal samples must not be over 24 hr before collecting. Consent from the Local Government Units (LGUs) was secured before the collection of samples. The collection of fecal samples was done by collecting samples from freshly-dropped bovine feces that were scooped out of about 3-5 g using a fecal scooper and stored in fecal containers with 2 ml of 10% formaldehyde for fecal preservation.[7] The fecal samples were then immediately transported to the laboratory no later than 1 hr after collection.

Formalin Ethyl-Acetate Sedimentation Concentration Technique (FEA-SD)

The formalin–ethyl acetate sedimentation-digestion (FEA-SD) technique used for the processing of bovine fecal samples was adapted with modifications.[8,9] Briefly, about 1-2 g of the formalin homogenized feces was strained using a 3-layered surgical gauze (4x4 in.) into a 15 ml centrifuge tube. Ten percent (10%) formalin (v/v) was added unto the suspension to attain a total volume of 7 ml and mixed thoroughly, following the addition of 3 ml of 100% (v/v) ethyl acetate to come up with the 10 ml total suspension volume. Each lid of the centrifuge tube was covered with insulating tape and vigorously shaken for 10 s. Each centrifuge tube was spun at 1500 rpm for 5 min to create four separate layers of ethyl acetate, bulk debris, formalin and sediment to be used for microscopy, respectively. Three layers: ethyl acetate, bulk debris and formalin; were carefully decanted into a waste bottle. The remaining layer of resulting sediment for egg count was pipetted and carefully transferred to a 1.5ml microcentrifuge tube for storing and microscopic examination. Laboratory examination of processed bovine fecal samples was performed by four trained personnel. The presence/ absence of parasite eggs was recorded as "positive (+)" and "negative(-)."[10] The identification of isolated parasite eggs was based on morphological features from key literature.[9,11,12]

Analysis

The intensity of parasitic fecal eggs per species was counted manually per bovines and referred to as eggs per count (epc). Prevalence (%) for each bovine infection was computed using the formula by dividing the number of positive bovine fecal samples to the total number of bovine fecal samples examined from the three barangays of Lake Mainit.[13]

RESULTS

Prevalence (%) and Intensity of Parasitic load

Three major parasite groups (protozoans, trematodes, nematodes) were recovered and examined comprising of coccidian oocysts and eggs of *Fasciola* sp., hookworm, decorticated *Ascaris* sp. and *Strongyloides* sp. (Table 1; Figure 2). An overall prevalence rate of fecal parasite infection of 67% was observed (Table 1). Among these

Table 1: Prevalence of bovine feces parasites from rice fields in Lake Mainit, Philippines.

Parasite	Total number of infected Bovine (n=16)	Total Egg Count	Prevalence (%)	Participation in the Zoonotic Transmission
Fasciola sp.	14	57	69	Definitive Host [4,14-16]
Hookworm	3	15	18	Potential Host[17,18]
Coccidian oocysts	3	5	0.05	Reservoir Host[19,20]
Ascaris sp. (Decorticated)	2	3	0.025	Potential Host[17]
Strongyloides sp.	1	1	0.012	Potential Host[19,21]

barangays, San Isidro had the highest infection rate (100%; Table 1); however, with fecal samples varying in the parasitic load. No fecal infection from bovines was observed from the ricefields of Magpayang. Fourteen out of sixteen fecal samples processed were positive for infections of some protozoans, trematodes and nematodes.

Laboratory examination shows that eggs of the liver fluke *Fasciola* sp. had the highest number of egg counts among other parasites recorded from bovine fecal samples (Table 2). Other parasites were also observed, particularly coccidian oocysts and eggs of hookworm, *Ascaris* sp. and *Strongyloides* sp.. *Fasciola* sp. and hookworm eggs garnered the top two highest prevalence rates of infection with 69% and 18%, respectively (Table 1). Other parasites were observed but with low prevalence. The high prevalence of *Fasciola* eggs from the fecal samples indicate that bovine fascioliasis exists in most bovines in the ricefields studied surrounding Lake Mainit. High incidence of dual parasite burden mostly comprising *Fasciola* sp. and hookworm in bovine fecal samples was observed from ricefields of San Isidro and Matin-ao (Table 2, 3). *Oncomelania quadrasi* from San Isidro ricefields was reported to harbor *Schistosoma japonicum*[6] however; the current study was not able to observe *Schistosoma* eggs from the bovine fecal samples from the area. Nonetheless, multiple infections of hookworm, coccidian oocysts and *Ascaris* sp. were also noted but of low occurrence in Brgy San Isidro and Matina-ao (Table 3).

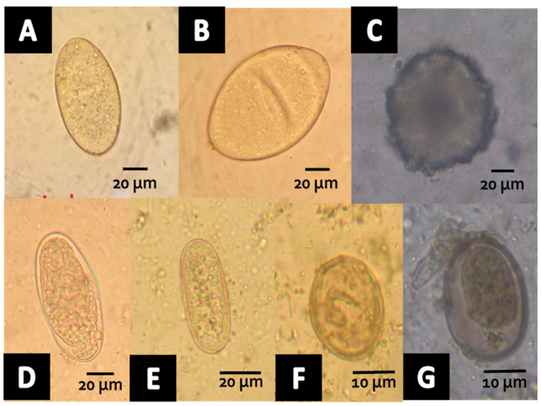

Figure 2: Zoonotic parasites isolated from bovine fecal samples in selected ricefields in Lake Mainit, Philippines. A-B. *Fasciola* sp.; C. Decorticated *Ascaris* sp.; D. Hookworm; E. *Strongyloides* sp.; and F-G. Coccidian oocysts. (400x).

DISCUSSION

Trematode infection such as fascioliasis is high in areas where there is exposure to existing vegetation and the presence of such intermediate snail hosts.[22] Fascioliasis or liver fluke infection has been considered a significant parasitic disease that affects ruminants, such as bovines.[4,23] Several studies reported that among other parasitic animals, *Fasciola* species had been the most prevalent and leading parasite harbored by bovines. Studies indicate that bovines have acquired resistance to anti-helminthic treatment hence the need for a more holistic approach in controlling fascioliasis.[24]

Table 2: Distribution of isolated bovine fecal parasites from ricefields of Lake Mainit, Philippines.

Parasite	Eggs per count (epc)		
	Magpayang	San Isidro	Matin-ao
Fasciola sp.	0	47	10
Hookworm	0	15	2
Coccidian oocysts	0	2	3
Ascaris sp. (Decorticated)	0	3	0
Strongyloides sp.	0	1	0

Table 3: Incidence of multiple parasitic infection of bovines from ricefields in Lake Mainit, Philippines.

Recorded Multiple Infection	Magpayang	San Isidro	Matin-ao	Over-all
No. of fecal sample examined	2	6	8	16
Fasciola sp. + hookworm	0 (0.0)	4 (67%)	6 (75%)	10 (62.5%)
Hookworm + Coccidian oocyst + *Ascaris* sp.	0 (0.0)	1 (33.3%)	2 (25%)	3 (18.8%)
Fasciola sp. + hookworm + coccidian oocyst + *Strongyloides* sp.	0 (0.0)	1 (16.6%)	0 (0.0)	1 (6.25%)

Bovines are the known definitive host of *Fasciola,* which uses lymnaeid snails as its snail intermediate host for the asexual development of the infective stage. Eggs of *Fasciola* are passed out in the feces of its relative host where it hatches and uses snails for full asexual development from miracidium, sporocyst, rediae and full-grown cercariae. Cercariae of *Fasciola* encyst in aquatic vegetation where the definitive host ingests it.[14] The adult *Fasciola* becomes a common parasite found in hepatic biliary ducts, liver, lungs and gall bladder of domesticated and herbivorous animals.[25] Lymnaeid snails, such as *Radix* are abundant in the ricefields of Lake Mainit.[5] Infection to *Fasciola* is reportedly dependent on the presence of intermediate snail host and bovine exposure to water-bodies. In some cases, a low prevalence may be reported due to bovines being stall-feed; hence less exposure to aquatic vegetation and possible transmission could occur.[19] Reports on cases of human fascioliasis in the area may be scarce because the animals responsible for the transmission are not usually studied.[25] The common symptoms of animals with fascioliasis are associated with prolonged intake of low to moderate numbers of herbage that results in an immense loss of bodily function and condition. Anemia is often severely diagnosed among undernourished infected bovines. Inflammation of the infected organ such as hepatic biliary ducts, gall bladder and liver (fibrosis) were heavily observed in animals exposed and highly infected with fascioliasis.[14] Incidence of multiple infections, such as *Fasciola* sp., hookworm, coccidia and *Strongyloides* sp., in the feces of bovines, were also reported in India.[19] Cases of coccidian infection were mostly of avian hosts.[20] Lake Mainit is home to many migratory birds, which might explain the pathway of transmission from the excretion patterns of birds[21] to some wetlands where bovines are highly exposed.[6]

Hookworms are blood-feeding nematodes that inhabit the alimentary system of mammals. Their transmission and infection in both humans and domestic animals are well-documented, making them a significant neglected tropical disease-causing agent not only to primates but also to ruminants,[26] and has implications on animal and agricultural productivity.

Incidence of *Ascaris* sp. eggs in bovine fecal samples was noted to be of low prevalence rate. Existing studies on helminthiasis on stool samples among school children from Lake Mainit indicate ascariasis, particularly of *A. lumbricoides*, which was observed in 24.9% of pupils examined. Hookworm infections in school children were also observed, but low at only 1.6% of pupils examined.[27] The current findings could serve as a baseline for the possible link of bovines to the transmission of this nematode in ricefields. Ascariasis is a common intestinal infection of nematodes in pigs. However, while *Ascaris* uses pigs as its host and infects humans typically, other animals that are in contact with pigs can be a potential tool for the diagnosis of ascariasis.[28] Atypical and natural infection of *A. suum*[29,30] and *A. lumbricoides*[31] in calves and adult cattle have been reported elsewhere.

CONCLUSION AND RECOMMENDATION

Zoonosis, particularly of bovine fascioliasis, was found to be prevalent in two ricefield barangays surrounding Lake Mainit. *Fasciola*, a genus of liver flukes, is one of the most popular disease-causing parasites of bovines. Other parasites such as hookworms, *Strongyloides* sp., coccidian oocysts and *Ascaris* sp. were also noted to be infecting bovines and must be therefore given attention for control measures of the transmission to animals and humans. In the ricefields assessed, eggs of *S. japonicum* were not observed in the fecal samples and would require a more extensive coverage of ricefields for the survey. Molecular analysis to further delve into the species-level identification of parasites is recommended to understand better the zoonotic transmission with implication to livelihood and public health. Proper hygiene and the wearing of protective clothing for farmers exposed to these ricefields reported are also recommended.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

EPC: eggs per count; **FEA-SD:** formalin ethyl acetate sedimentation-digestion; **LGU:** Local Government Unit.

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