Detection of Juvenile Fasciola Using Cathepsin BReverse Transcription-Loop-Mediated Isothermal Amplification (Rt-Lamp) Test Kit in Snails

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Abstract

The study was conducted to detect the presence of fasciola spp. infection using the Reverse Transcription – Loop Mediated Isothermal Amplification (RT- LAMP) test kit in ten (10) randomly selected snails from Cevallos farm and UEPCVM Goat project in Catarman, Northern Samar. Collected snails were brought and processed at the Diagnostic Laboratory of the Veterinary Teaching Hospital (VTH), University of Eastern Philippines. Results of this study revealed a 30% (3/10) fasciola infection in snails. These findings provide an evidence that RT-LAMP test kit can amplify the target gene Cathepsin B in the snails. RT –LAMP test kit is a quick, early and reliable diagnostic method for the detection of fasciolosis and can contribute in providing an advance management and control of the disease. Based on the result of the study, it can be inferred that fasciolosis is present at Cevallos farm and at the UEPCVM Goat project. The researcher recommends a larger number of samples to be tested for fasciola spp. infection and should be conducted in the different Municipalities of the Province.

Keywords Fasciolosis, fasciola species, snails, RT-LAMP test kit

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INTRODUCTION

Fasciola species are helminth parasites that cause fluke pestilence in cattle, buffaloes, goats and sheep worldwide and became a critical pathogen of humans. Fasciolosis is a zoonotic disease with various reports on the incidence of human infections. It is brought about by two trematode species, *Fasciola hepatica* and *Fasciola gigantica*. *F. hepatica* is a concern in Europe and America but the distribution of these species overlaps in Africa and Asia while *F. gigantica* is the most common species infesting ruminants in the tropical parts of Asia and Africa which includes the Philippines. Many of fluke species contribute to a great loss in economic and medical aspects, with losses of economic productivity and lives of livestock particularly in ruminants and illnesses in humans (Domingo, et al, 2018).

Piedrafita, et al (2010) stated that the geographic distribution of *Fasciola* species

is dependent on the distribution of suitable species of snails. Lymnaea species play a significant role in the parasites life cycle, proliferation and transmission of the disease. The snails carry the larval stage of fasciola which carries the gene that expresses the protein cathepsin B, responsible for liquefying the liver when the juvenile fluke enters the body of susceptible animal.

According to Cancela, et al (2008), cathepsin L and B are the significantly expressed cysteine proteases in many stages of Fasciola species which are also the virulence gene targets and proposed vaccine antigen candidates. Cathepsins obtained from newly excysted juvenile (NEJ) and immature juvenile flukes were shown distinctive from the enzymes of adult parasites. In a report, mature cathepsin B3was present only in Fasciola spp. NEJs but none in adult parasites and its ability to digest fibronectin, a native component of host's connective tissue, suggest that the possible function of secreted cathepsin B3may be in digesting the host's tissues to facilitate the migration of the excysted parasite.

RT - LAMP exhibits high specificity and selectivity because of the use of 4 primers recognizing 6 distinct regions on the target base sequence, and can be completed in a short time (1 hr as standard) due to the high amplification efficiency under isothermal conditions. It was utilized in the detection of Fasciola species during the prepatent period or the period between infection and the demonstration of the parasite targeting cathepsin B3 gene in snails. This can be a new platform for early diagnosis of fasciolosis and can contribute a novel approach for the improvement of the disease management and control (Domingo et al, 2018).

Lack of sensitive and convenient tests for diagnosis of Fasciola spp is one of the main problems in the control of Fasciolosis. This disease will contribute a negative impact on animal health and welfare which can cause a compromised food security which will incur economic losses to the agricultural sector.

This research aimed to detect fasciolosis in snails using RT-LAMP test kit.

MATERIALS AND METHODS

This study was conducted at the rice paddies of Cevallos farm, UEP, College Goat project and at the Veterinary Teaching Hospital-Diagnostic Laboratory, University of Eastern Philippines, University Town, Catarman Northern Samar from May 1 - 31, 2021.

A 10 snails at the rice paddies of Cevallos farm and at the College Goat project, UEP, University town, Catarman, Northern Samar. After collection, the samples were transported using aseptic packaging to the laboratory of the CVM Veterinary Teaching Hospital for the test detection of Fasciola pathogen using RT-LAMP test kit. Random sampling technique was used in collecting the snails while frequency counts and percentages were used to interpret the results.

The first procedure was crushing the sample, preferably about 500-700 grams, placed in a 1.5 mL microcentrifuge tube using a sterile mini grinder until homogenized. After which, one ml syringe was used to aspirate the contents of reagent 1, then added 3 drops into the tube with pulverized snail and was ground until it became fully homogenized. Another one ml syringe was used to aspirate all the contents of reagent 2 then 3 drops were poured into the pulverized snail. The researcher used a yellow stick and was dipped into the corresponding tube with homogenized snail. The wet end of the stick was dipped once again into the smaller PCR tube containing the LAMP premixes and the tube was tapped to settle the liquid at the bottom. The PCR tube was then incubated at 65°C for 90 minutes into the water bath.

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After completion of the reaction, a dye, which was also included in the kit, was added into the LAMP tube using the white stick and change in color reaction was observed. Positive reaction displayed a visible green color whereas negative reaction displayed an orange color or remained colorless.

RESULTS AND DISCUSSION

Ten (10) snail samples were collected and pulverized individually. The samples were tested using the LAMP test kit. In this study, three (3) were found positive to fasciola pathogen and seven (7) tested negative. The result of this study revealed that RT-LAMP test kit can detect fasciola infection in snails and the presence of Fasciolosis at Cevallos farm and at the College of Veterinary Medicine Goat project, University of Eastern Philippines, Univeristy town, Catarman, Northern Samar. A few studies on Fasciola spp. prevalence are available from Palapag, Northern Samar (Gordon et al., 2015), Baybay, Leyte (Portugaliza et al, 2019) and Nueva Ecija (Domingo, 2014).

Table 1. ***RT LAMP test results among snails at Cevallos farm and CVM Goat project, UEP, Catarman, Northern Samar***

Number of snails	*Positive*	*Negative*	*Percent (%) positive*
1		-	
2	*+*		
3		-	
4		-	
5		-	
6	*+*		
7		-	
8	*+*		
9		-	
10		-	
Total 10	***3***	***7***	***30%***

Table 1 shows that out of 10 snails, 30% (3/10) were found infected with fasciolosis as it displayed a green color in the LAMP tube whereas sample number 1, 3, 4, 5, 7, 9 & 10 displayed an orange color or colorless which means negative for fasciolosis. The results of the current study are in disagreement with those of other studies such as the study of Gordon et al., 2015 and Domingo, 2014 in terms of high intensity of infection, however, despite the low prevalence, this study revealed that Fascilosis is present at the Cevallos farm and at the UEPCVM Goat project, Catarman, Northern Samar and the potential transmission of this parasite in majority of the ruminants like carabaos, cows and goats cannot be discounted. In the study conducted by Gordon, et al (2015), there was a high prevalence and infection intensity of *F. gigantica* in one of the municipalities of Northern Samar specifically in Palapag. According to Martin & Cabrera, 2018, lymnaeid snails that inhabit rice fields and man-made water ducts are potential carriers of zoonotic parasites that have both medical and veterinary public health significance. The presence of cercariae in the lymnaeid snails reflects the diversity of parasites and possibly of reservoir hosts, may significantly pose potential health hazards to animals and humans. Moreover, this study proved that RT-LAMP test kit can amplify the target gene Cathepsin B in the snails, therefore, LAMP tests are newer, simple, quick, highly sensitive and reliable technique used in the detection of pathogens which can produce results within 90 minutes and test result can be seen visually without requiring a machine to read the results.

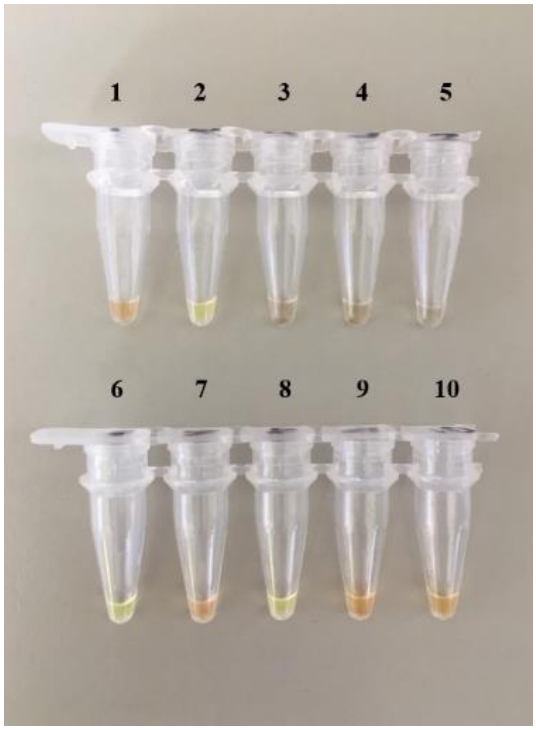

Figure 1.The LAMP tube premixes showing the test results wherein positive reaction displayed a visible green color which are the snail numbers 2, 6 and 8 whereas negative reaction displayed an orange color or remain colorless which are the snail numbers 1, 3, 4, 5, 7, 9 and 10.

CONCLUSION

Based on the results of the study, Fasciolosis is present in snails collected in the rice paddies at Cevallos farm and at the College of Veterinary Medicine Goat project, University of Eastern Philippines, University Town, Catarman, Northern Samar and that the RT- LAMP test kit is a highly specific and sensitive test that can be carried within 90 min under field conditions for the detection of fasciola pathogen, therefore, this can be a new platform for controlling fasciolosis by detecting Fasciola infected snails in the area.

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