# Geographical Mapping of Liver Fluke (Fasciola hepatica) Prevalence through Snail Sampling in Mayantoc, Tarlac

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Abstract— The livestock industry continues to face challenges that hinder growth and productivity, with fasciolosis remaining a significant parasitic disease of concern. Fasciolosis, caused by Fasciola hepatica and Fasciola gigantica, impairs the health, productivity, and reproductive performance of ruminant animals such as cattle, goats, and sheep. This study was conducted to detect Fasciola DNA in snail intermediate hosts and to map potential infestations in goats within selected barangays of Mayantoc, Tarlac. Laboratory techniques employed included DNA extraction, loop-mediated isothermal amplification (LAMP) using Cathepsin B primers, and GIS-based interpolation mapping. Snail samples from selected sites were pulverized, homogenized with extraction reagents, and incubated in LAMP tubes, with positive results indicated by a distinct color change. Surprisingly, no detectable Fasciola DNA was identified in snail samples collected from different barangays. This result may be attributed to effective deworming programs, both oral and injectable, initiated by the Department of Agriculture (DA) and Local Government Unit (LGU) of Mayantoc. The study further explored the demographic profile, knowledge, and practices of 35 smallhold ruminant farmers in the municipality. Results revealed reliance on tethering, limited awareness of fasciolosis, and minimal preventive strategies despite ongoing animal health campaigns. GIS interpolation maps highlighted variations in barangay elevations, providing useful insights into the role of topography in parasite transmission. Collectively, findings confirm that Mayantoc remains liver fluke-free, reinforcing its suitability for ruminant production while underscoring the importance of sustained farmer education and surveillance.

Keywords— Cathepsin B-RT Lamp Test, infestation, liver fluke, mapping, snail.

# I. INTRODUCTION

Goats play an important role in the rural economy of the Philippines. They are essential for development because they can convert fodder, crops, and household waste into meat, fiber, hides, and milk, contributing significantly to the nutrition of the rural poor. As tangible financial assets, goat product consumption and sales enhance households' economic stability during crop failures. However, despite its potential, the growth and development of this industry have remained low in the past decades.

Fasciolosis is one of the most widespread and most dangerous parasitic diseases in ruminant farms. These are caused by parasitic flatworms (liver fluke), which pose a serious threat to ruminant animals like goats, cattle, and sheep. The life cycle of liver flukes involves two main hosts: snails and ruminants. The parasites start by infecting snails, where they multiply and develop. The infected snails then release larvae into the water, eventually attaching to plants or floating free. When ruminants consume these contaminated plants or water, the larvae migrate to their liver and mature into adult flukes. This stage of the fluke's life cycle is particularly harmful to ruminants, as the parasites cause severe liver damage, leading to symptoms such as fever, abdominal pain, reduced growth rates, and lower birth rates. Infected animals also suffer from inefficient feed conversion and compromised quality of milk, meat, and fiber, which can even lead to death. The presence of liver flukes significantly affects the overall health and productivity of ruminant animals, posing a significant challenge for farmers and the livestock industry.

In this study, the researchers gathered and explored epidemiologic data that suggest high endemicity of *Fasciola* infection in goats and the risk factors independently associated with the parasite prevalence in Mayantoc, Tarlac. The study aimed to create a GIS map to easily visualize the geographic pattern of the intermediate host and *Fasciola* infection. The goal was to provide valuable information for developing future intervention strategies in Mayantoc, Tarlac, and its neighboring localities.

### II. MATERIAL AND METHODS

# 2.1 Description of the Study Site:

Mayantoc is known as the agricultural haven of Tarlac, with a total land area of 31,142 hectares. Currently, it is classified as a third-class municipality in Tarlac, comprising 24 barangays with a total land area of 311,420,000 square meters, of which 68,960,000 square meters is agricultural land and 242,460,000 square meters is residential.

A total of 35 snail (*Lymnaea* ssp.) samples were randomly collected in the waterways near the pasture area of goat farms in selected barangays of Mayantoc, Tarlac. These samples were subjected to a Cathepsin B RT-Lamp test kit at the Animal Diagnostic Laboratory at Tarlac Agricultural University. Before the study was conducted, researchers coordinated with the Local Government Unit and the Department of Agriculture of Mayantoc, Tarlac, for guidance on receiving assistance in the area. A semi-structured questionnaire was used to collect information regarding the goat production system from the raiser area.

### 2.2 Data Gathered:

A questionnaire was used to collect information regarding the ruminant production system from the raisers in the area.

### Farm Survey Form:

- 1. Farm Name This indicates the registered names of farms, which range in size from large to small.
- 2. Farmer Owner(s) this determines who owns the farm, if any.
- 3. Barangay Details about the farm's location in the area.
- 4. Farm Class
- 5. Number of Animals The inventory predominantly consists of ruminants.
- 6. Breed This refers to a distinct group of domestic animals that can be identified by consistent appearance, behavior, or other characteristics.
- 7. Sex Respondents were identified as either male or female.
- 8. Marital Status Respondents were categorized as single, married, widowed, widower, divorced, or separated.
- 9. Age The Age of the farm owner.
- 10. Educational Attainment The education level of the interviewed farmers was classified as None, Primary School, Secondary School, College, or Post-Graduate.
- 11. Farming Experience The duration of ruminant raising experience was categorized into three periods: one to five years, five to ten years, and thirty years.
- 12. Management System The management systems of farmers are classified into four categories: Tethering, Intensive, Extensive, and Semi-Intensive.
- 13. GPS Coordinates The exact location of each farm was marked using latitude and longitude from the Geographic Information System (GIS) mapping.
- 14. Elevation The farm's elevation was measured in meters (m) or feet (ft).
- 15. Remark Samples that tested positive or negative that is carrier of liver fluke were noted as (+) or (-).

TABLE 1
FARM CLASSIFICATION

Farm Class	Classification (Goats)	Type of Farm	Classification (Cattle)	Type of Farm
A – Small scale	5 -30 heads of animals	Backyard farms with limited livestock	1 – 20 heads of cattle	Backyard farms or small family-operated farms
B – Medium Scale	165 heads of animals	Small to medium-sized commercial farms	21 – 100 heads of cattle	Small to medium-sized commercial cattle operations
C – Large Scale	166 heads and above	Large commercial farms	101 heads and above	Large commercial cattle ranches and feedlots

## 2.3 DNA Extraction and Analysis:

Samples taken from several sampling locations were transferred to the laboratory. Snails were thoroughly cleaned and prepared for DNA extraction by placing each snail in a 1.5ml microcentrifuge tube and crushing it with a tiny pestle until pulverized.

Loop-mediated isothermal amplification (LAMP) test kits were used to determine the presence of *Fasciola* DNA in the snails. There were two reagents used; Reagent 1 and Reagent 2. The crushed snails were homogenized using Reagent 1. Three drops of Reagent were added to the tube with the powdered snails before crushing with a tiny pestle until entirely homogenized. Reagent 2 was then introduced by infusing three drops into the homogenized snail tube.

The yellow tips wet ends were dipped into a smaller PCR tube containing the LAMP premixes. The tubes were tapped to allow the liquid to settle. The dry bath was prepared at 60°C before the LAMP tube samples, as well as the positive and negative controls, were put inside and incubated for 1 hour and 30 minutes. The tubes were withdrawn from the dry bath after incubation. The white plastic tips were dipped into the dry tubes, followed by the LAMP tubes. A shift in the color of the LAMP tube to green indicated a favorable result, this may be seen with the human eye or under the blue light of a counterfeit detector. A green color shift in the LAMP tube show a positive result which may be seen with the naked eye or with a counterfeit bill detectors blue light. The positive snails were utilized to map the *Fasciola* infestation in goats in Mayantoc, Tarlac.

# 2.4 Process of Detecting Fasciola:

Snails were put inside the 1.5 ml microcentrifuge tube. The frozen snails were crushed with a mini pestle and ground until pulverized. All contents of Reagent 1 were aspirated using a syringe, and three drops were added into a tube with pulverized snail. The pulverized snail in the tubes was ground continuously using a mini pestle until fully homogenized. Using a separate syringe all contents of Reagent 2 were aspirated, and three drops were added into another tube with pulverized snail. The ends of each of the yellow plastic tips were dipped into corresponding tubes with homogenized snail. The wet ends of the yellow tips were then placed into corresponding smaller PCR tubes containing the LAMP premix. The tubes were then tapped to allow the mixture to settle. The dry bath was preheated at 60°C. The LAMP tube samples were placed together with the positive and negative controls in a dry bath and allowed to incubate for 1 hour and 30 minutes. The tubes were then removed from the dry bath. The ends of the white plastic tips were then dipped into the Dye tube, after which these were dipped into the LAMP tube. Similar to the procedure for DNA extraction, a shift in the color of the LAMP tube to green indicated a favorable result, this may be seen with the human eye or under the blue light of a counterfeit detector.

### 2.5 Geographical Information System Mapping:

Inverse distance weighted (IDW) used in the interpolation with formula was:

$$F(x,y) = \sum_{i=2}^{n} w_i f_i \tag{1}$$

where n< is the number of scatter points in the set, fi are the prescribed function values at the scatter points (e.g. the dataset values), and wi are the weight functions assigned to each scatter point. The classical form of the weight function is:

where p is an arbitrary positive real number called the power parameter (typically,p=2) and hi is the distance from the scatter point to the interpolation point or:

$$w_i = \frac{h_i^{-p}}{\sum_{j=i}^n h_j^{-p}}$$
 (2)

where (x,y) are the coordinates of the interpolation point and (xi,yi) are the coordinates of each scatter point. The weight function varies from a value of unity at the scatter point to a value approaching zero as the distance from the scatter point increases. The weight functions are normalized so that the weights sum to unity.

$$h_i = \sqrt{(x - x_i)^2 + (y - y_i)^2} \tag{3}$$

#### 2.6 Biosecurity:

Learning the sources of diseases is one of the first stages in developing a biosecurity program and avoiding illness. Pathogens that cause diseases and bad health include: bacteria that produce caseous lymphadenitis, viruses that cause caprine arthritis encephalitis (CAE), and parasites such as coccide. Many variables contribute to the transmission of a disease, it is determined by the features of the host (health, immunological function, etc.), the surrounding environment (temperature, stocking rate, pasture condition, etc.), and the disease agent itself.

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#### 2.7 **Statistical Analysis:**

The data that collected were presented in frequency and percentage formats to provide summaries about the samples and the observations. This were obtained through a research questionnaire to the respondents, covering socio-demographic profiles, liver fluke awareness, and interventions in farming experiences.

The formula for percentage used was:

$$\frac{n}{N} * 100 \tag{4}$$

Where:

n= Frequency of the given score in a set

N= Total number of the participant

#### III. RESULT AND DISCUSSION

#### 3.1 GIS Map of the Municipality of Mayantoc, Tarlac:

The mapping distribution of selected barangays in Mayantoc, Tarlac is depicted in Figure 1. The limits are depicted in the image, and the study sites' location is indicated on the map. The map outlines the geographic boundaries and specific locations pertinent to the study sites. The mapping offers a clear depiction of the barangays' borders, highlighting their closeness to significant environmental features and land use patterns that could have affected the prevalence of Fasciola hepatica infections.

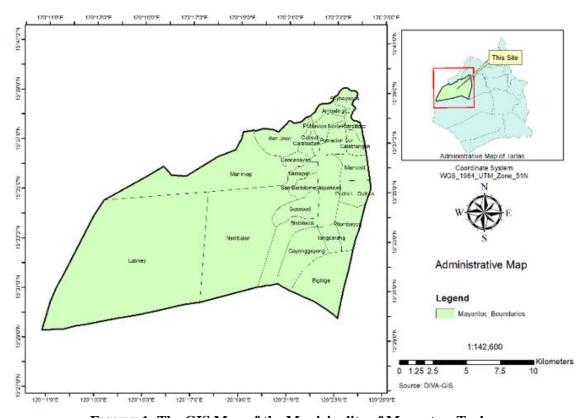


FIGURE 1: The GIS Map of the Municipality of Mayantoc, Tarlac

#### 3.2 GIS Map of the Distribution of Quadrant of Selected Barangays in Mayantoc, Tarlac:

Figure 2 shows the map of the municipality of Mayantoc, Tarlac, displaying the quadrant distribution with orange representing Quadrant 1, yellow for Quadrant 2, green for Quadrant 3, and blue for the final quadrant (4). Quadrant 1 includes the barangay of Maniniog, Quadrant 2 encompasses the barangays of Baybayaoas, Ambalinguit, Cubcub, San Jose, Carabaoan, Poblacion Sur, Mamonit, Mapandan and San Bartolome, Quadrant 3 contains the barangays of Gossood, Binbinaca, Pitombayog and Gayong-Gayong, and the blue quadrant represents the barangays of Labney and Nambalan.

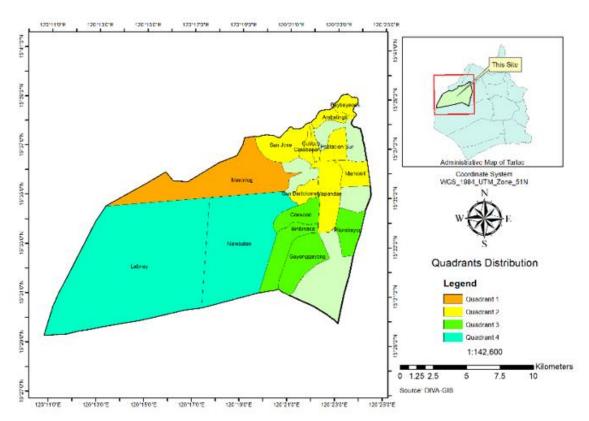


FIGURE 2: GIS Map of the Distribution of Quadrant of Selected Barangays in Mayantoc, Tarlac

# 3.3 GIS Map of the Elevation in Selected Barangays in Mayantoc, Tarlac:

A color gradation (shown in Figure 3) indicates height, with red denoting the highest elevations, which are 1400 meters. Yellow denotes the second highest elevations with 690 meters; and green denotes the last highest elevation with 20 meters.

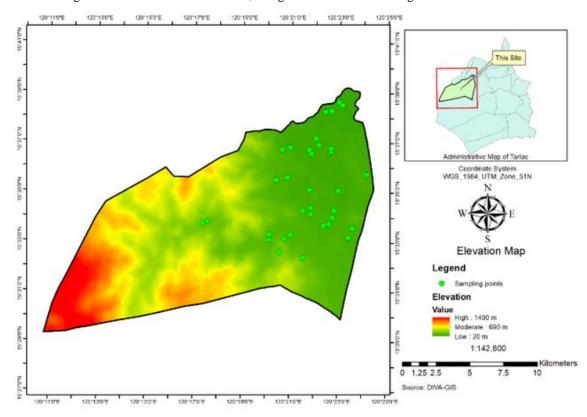


FIGURE 3. GIS Map of the Elevation in Selected Barangays in Mayantoc, Tarlac

# 3.4 Inventory and Breed of Ruminant Population Surveyed Farms in Mayantoc, Tarlac:

The survey conducted in Mayantoc, Tarlac, revealed a total of 266 small ruminants (goats) across the surveyed farms (Table 4). This included 50 bucks, 129 does, and 87 kids. Mapandan reported the highest population with 34 goats, including 6 bucks, 13 does, and 15 kids. In contrast, Mamonit reported the lowest count with only 2 goats, consisting of 1 buck and 1 doe. Additionally, all goats surveyed were classified as native breeds. This distribution aligns with existing literature that emphasizes the predominance of smallholder farming systems within the Philippine livestock sector. According to the Philippine Statistics Authority (2023), smallholder farms account for approximately 99.3% of the country's goat population manage around farms, underscoring the critical role that these agricultural operations play in local economies and food security.

TABLE 4
INVENTORY AND BREED OF RUMINANT POPULATION SURVEYED FARMS IN MAYANTOC, TARLAC

D	Small Ruminant (Goat)			
Barangay	Buck	Doe	Kid	TOTAL
Maniniog	1	15	6	22
Baybayaoas	2	4	1	7
Mapandan	6	13	15	34
San Jose	1	3	1	5
Mamonit		1	1	2
San Bartolome	1	4	6	11
Carabaon	2	2	1	5
Ambalinguit	3	16	1	20
Pob. Sur	1	3	4	8
Cubcub	2	4	2	8
Gayong-Gayong	4	15	8	27
Gossood	2	9	10	21
Pitombayog	4	15	14	33
Binbinaca	7	10	12	29
Labney	4	7	1	12
Nambalan	10	8	4	22
TOTAL	50	129	87	266
BREED	NATIVE			266

# 3.5 Sex of Respondents:

Table 5 shows the gender of the 35 respondents across various barangays, with a 19 male respondents and 16 female respondents. The male participants were from Maniniog, Baybayaoas, Mapandan, San Bartolome, Carabaoan, Ambaliguit, Pob. Sur, Cubcub, Gayong-Gayong, Gossood, Pitombayog, Binbinca, and Labney. Meanwhile, barangays Nambalan, San Jose, Mamonit, Maniniog, Baybayaoas, Mapandan, San Bartolome, Carabaoan, Ambaliguit, Pob. Sur, Cubcub, Gayong-Gayong, Gossood, Pitombayog, and Binbinca had female respondents.

TABLE 5
SEX OF RESPONDENTS

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Sex	Frequency	Percentage
Male	19	54.29
Women	16	45.71
Total	35	100

## 3.6 Marital Status of Respondents:

Table 6 shows the marital status of respondents across various barangays. This included 74.29% married individuals, 22.86% single individuals and 2.86% that were widow/er. Respondents who were married were from the following barangays: Maniniog, Baybayaoas, Mapandan, San Jose, Mamonit, San Bartolome, Carabaon, Pob. Sur, Cubcub, Gayong Gayong, Gossood, Binbinaca, Pitombayog, and Labney. Respondents that were single were from: Maniniog, Mapandan, San Jose, Ambaliguit, Gosood and Nambalan. Meanwhile, one respondent from Nambalan was a widow. According to the Philippine Statistics Authority (PSA) in 2022, a total of 50,633 marriages were recorded in Central Luzon.

TABLE 6
MARITAL STATUS OF RESPONDENTS

Marital Status	Frequency	Percentage
Single	8	22.86
Married	26	74.29
Widow/Widower	1	2.86
Total	35	100

# 3.7 Respondents:

Table 7 shows that the age distribution of respondents across selected barangays in Mayantoc, Tarlac, demonstrated extensive variability, with ages ranging from as young as 24 years old (from Ambalinguit) to 69 years old (from Mapandan). Notable concentrations of older individuals were found in Barangays Mapandan, San Bartolome, Mamonit and Gayong-Gayong suggesting a substantial elderly population that may have required targeted community services and healthcare resources. Conversely, younger respondents were concentrated in Ambalinguit, with additional younger individuals in Cubcub (26 years). The age profile across other barangays, including Pob. Sur (30 years), Pitombayog (40 years), Maniniog (54 years), and Labney (56 years old) indicated a balanced demographic distribution.

TABLE 7
AGE OF RESPONDENTS

Age Group	Frequency	Percentage
21-30	7	20
31-40	7	20
41-50	7	20
51-60	10	28.57
61-70	4	11.43
Total	35	100

### 3.8 Educational Attainment of Respondents:

Table 8 details the educational attainment levels across various age groups. Survey results show that the primary school level had the highest representation, accounting for 48.57% of the respondents. This category covers all age groups. Respondents who attained secondary school level made up 34.28%, while college-level category included 14.29% of the population. Lastly, 2.86% (one respondent) reported to have undergone no formal education.

TABLE 8
EDUCATIONAL ATTAINMENT OF RESPONDENTS

Educational Attainment	Frequency	Percentage
Educationally unaccomplished	1	2.86
Primary Certificate	17	48.57
Secondary Certificate	12	34.28
College Diploma	5	14.29
Post-Graduate Studies	0	0
Training/Seminars	0	0
Total	35	100

## 3.9 Farming Experience of Respondents:

Table 9 shows the respondents' number of years in farming. The most common farming experience was 1-5 years, representing 65.71% of the population. This was followed by 5-10 years of farming experience, with 25.71%, followed by 10-20 years of farming experience with 5.71%. Lastly, the farming experience category of 20-10 years had 2.86% (one respondent).

Respondents in selected barangays of Mayantoc, Tarlac exhibited a diverse range of farming experiences. The majority of respondents, including those from Mapandan, Binbinica, Gossood, Baybayaoas, San Jose, Ambalinguit, and Pitombayog fell within the 1-5 years of experience category, indicating a rather recent entry into agricultural practices. Nambalan stood out with a respondent possessing over 30 years of experience, reflecting extensive expertise in livestock management. Additionally, some respondents from Mapandan, Mamonit, San Bartolome, Gayong-Gayong and Labney reported 5-10 years of experience, while Maniniog had a respondent with 10-20 years of farming experience. This variability in farming experience suggests differing levels of knowledge and practice, which could influence management decisions and overall agricultural productivity within the community.

TABLE 9
FARMING EXPERIENCE OF RESPONDENTS

Years in Farming	Frequency	Percentage
1-5 years	23	65.71
6-10 years	9	25.71
11-20 years	2	5.71
21-30 years	1	2.86
31-40 years	0	0
Total	35	100

# 3.10 Management Systems of Respondents for their Livestock:

In selected barangays of Mayantoc, Tarlac, respondents primarily utilized tethering and extensive grazing management systems (Table 10). Tethering was the dominant practice among respondents in Maniniog, Baybayaoas, San Jose, Mamonit, San Bartolome, Carabaon, Pob. Sur, Cubcub, Gayong Gayong, Gossood, Binbinaca, Pitombayog, Labney, and Nambalan, which facilitated controlled grazing and livestock movement. Only two respondents in Mapandan opted for extensive grazing systems, providing their livestock with broader grazing areas but making health monitoring more challenging, balancing resource availability with livestock productivity.

TABLE 10
MANAGEMENT SYSTEM OF RESPONDENTS FOR THEIR LIVESTOCK

Management System	Frequency	Percentage
Tethering	33	94.29
Intensive	0	0
Extensive	2	5.71
Semi-Intensive	0	0
Total	35	100

# 3.11 Purposive and Stratified Sampling of Snail Samples in Mayantoc, Tarlac:

Table 16 provides a purposive and stratified sample of snail samples collected from waterways adjacent to pasture areas in selected goat farms in Mayantoc, Tarlac. A total of 35 snail samples were collected using a combination of purposive and stratified sampling, with the criteria that the samples come from areas with waterways. Specifically, the barangays of Maniniog, Baybayaoas, Mapandan, San Jose, Mamonit, San Bartolome, Carabaon, Ambaliguit, Pob. Sur, Cubcub, Gayong-Gayong, Gossood, Binbinaca, Pitombayog, Labney, and Nambalan were included. The consistency in sampling efforts across these barangays ensured equal representation in the study. The total sample size of 35 provided comprehensive data to analyze potential snail carriers responsible for spreading liver fluke infections. This approach aligns with the findings of Perez-Saez *et al.* (2019), who demonstrated the effectiveness of purposive and stratified sampling in monitoring snail populations.

TABLE 16
PURPOSIVE AND STRATIFIED SAMPLING OF SNAIL SAMPLES IN MAYANTOC, TARLAC

Barangay	No. of Snail Sample Collected	Percentage
Maniniog	2	5.71
Baybayaoas	2	5.71
Mapandan	6	17.14
San Jose	2	5.71
Mamonit	1	2.86
San Bartolome	1	2.86
Carabaon	2	5.71
Ambaliguit	2	5.71
Pob. Sur	2	5.71
Cubcub	2	5.71
Gayong-Gayong	1	2.86
Gossood	3	8.57
Binbinaca	3	8.57
Pitombayog	2	5.71
Labney	2	5.71
Nambalan	2	5.71
Total	35	100

# 3.12 Result of the Cathepsin B RT-LAMP Test Kit Analysis of Snail sample Collected from Selected Barangays in Mayantoc, Tarlac:

Table 17 details the results from a Cathepsin B RT-LAMP test kit analysis conducted on snail samples collected from various barangays in Mayantoc, Tarlac. All samples from the different Barangays tested negative for liver fluke, indicating no detectable levels of the parasite. This outcome may be attributed to the deworming programs (both oral and injectable) implemented by DA-LGU Mayantoc.

TABLE 17
RESULT OF THE CATHEPSIN B RT-LAMP TEST KIT ANALYSIS OF SNAIL SAMPLE COLLECTED IN SELECTED BARANGAYS OF MAYANTOC, TARLAC

Barangay	Results
Maniniog	Negative
Baybayaoas	Negative
Mapandan	Negative
San Jose	Negative
Mamonit	Negative
San Bartolome	Negative
Carabaon	Negative
Ambaliguit	Negative
Pob. Sur	Negative
Cubcub	Negative
Gayong-Gayong	Negative
Gossood	Negative
Binbinaca	Negative
Pitombayog	Negative
Labney	Negative
Nambalan	Negative

# IV. CONCLUSION

- 1. The study included 35 backyard farmers (19 men and 16 women), with 74.29% married, 22.86% single, and 2.86% widowed. Respondents ranged from 24 to 69 years old. The range of educational levels shows a clear trend: the majority, 48.57%, have attained primary education. Following this, secondary education accounts for 34.28%, while 14.29% have pursued college education. Only a small group, 2.86%, have no formal education. This distribution highlights the significant emphasis on primary education within the population. Farming experience showed 65.71% had 1-5 years, 25.71% had 5-10 years, 5.71% had 10-20 years, and 2.86% had over 30 years. All the respondents reported a lack of awareness about liver fluke and its implications, indicating a critical need for targeted educational initiatives. The assessment highlighted a complete lack of knowledge among respondents regarding snail sampling, liver fluke-related activities, and seminars, with all 35 respondents indicating unawareness. Additionally, all respondents were unprepared regarding the transmission vectors of liver fluke, particularly the role of snails and environmental factors.
- 2. The assessment of management practices among ruminant smallhold farmers in Mayantoc, Tarlac, revealed that tethering is the predominant method used. This practice was prevalent in barangays such as Maniniog, Baybayaoas, San Jose, Mamonit, San Bartolome, Carabaon, Pob. Sur, Cubcub, Gayong Gayong, Gossood, Binbinaca, Pitombayog, Labney, and Nambalan. Tethering facilitates controlled grazing and movement of livestock, helping to manage grazing areas efficiently and prevent overgrazing. Additionally, specific areas within these barangays have been identified as suitable for different management systems based on the practices of the respondents. While tethering emerged as the most common method, practiced by 94.29% of respondents, only 5.71% from Mapandan utilized an extensive grazing system, which allows livestock to roam freely over larger areas.
- 3. Results on the Cathepsin B RT-LAMP test showed no presence of liver fluke in Mayantoc, Tarlac. Therefore, it can be said that Mayantoc is a liver fluke free town in Tarlac and safe to raise ruminants.

#### **CONFLICT OF INTEREST**

Authors declare no Conflict of Interest.

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