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Prevalence and risk factors associated with *Fasciola gigantica* and *Paramphistomum cervi* infection through actual retrieval in Philippine Carabaos in Ormoc City Abattoir, Philippines

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ABSTRACT

This study determined the prevalence and risk factors associated with *Fasciola* and *Paramphistopmum* and the co-infection of both parasites in 246 Philippine carabaos slaughtered in Ormoc City abattoir. Upon postmortem examination, adult parasites were collected from the organ of localization and identified based on morphological characteristics. Intrinsic factors [sex, age, weight, Body Condition Score (BCS)] and origin of the carabaos were recorded. The parasites identified were *Fasciola gigantica* and *Paramphistomum cervi.* The study showed a prevalence of 50.81% for *F. gigantica,* 4.31% for *P. cervi* and 32.11% for co-infection. *Fasciola* was more prevalent in female carabaos (57.80%), carabaos more than three years of age (58.41%), >325kg bodyweight (60.36%) and higher than three body condition score (59.09%). *Paramphistomum* was common in female carabaos (51.37%), carabaos less than or equal to three years of age (46.82%), ≤325kg bodyweight (52.59%) and less than or equal to three (≤3) body condition score (49.64%). Carabaos originating from Leyte have a higher infection rate to *Fasciola* (53.30%) and *Paramphistomum* (46.70%), compared to carabaos supplied by other provinces. Logistic regression modeling indicates that male carabaos are a protective factor (OR 0.59) against *Paramphistomum* infection while carabaos within Leyte have a greater likelihood of being infected with both parasites (OR 4.27 to 4.57). Pearson's Correlation Coefficient (r-value) showed a strong and positive result (r=0.997**) that the likelihood of *Fasciola* and *Paramphistomum* co-existing (co-infection) together is high. A high prevalence rate of fluke infection has been recorded in carabaos admitted for slaughter, implying that most of the backyard carabao raisers are not practicing sound deworming, pasture and grazing management and proper deworming protocol. To prevent and control flukes and improve production and health of carabaos, the above-mentioned factors must first be considered.

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INTRODUCTION

As of January 1, 2016, the total carabao inventory in the country was 2.9 million, about 0.78 percent higher than the previous year (Ocampo 2016). The inventory reveals that carabao raising is predominantly (99.6%) a backyard endeavor where food supplementation and health maintenance input is lacking. The animal is fed with grass and other lush herbage for food and nutrition. The said practice increases the susceptibility of the animals to parasitic infection. Because parasites compete with the nutritional absorption of the animal, production loss is not uncommon.

Trematodes are one of the major problems affecting ruminant production around the world, with *Fasciola* as the most common (Vercruysse & Claerebout 2001). Fascioliasis is a serious endoparasitic disease of domestic ruminants and humans (Haridy et al 2002). It tops all the zoonotic helminths around the world and is often responsible for condemnation of meat and marketable organs during postmortem inspection (Jemal et al 2016).

In the Philippines, the occurrence of the parasite *Fasciola* has been reported in many studies. Lameta and Manuel (1981) studied the prevalence of *Fasciola gigantica* among 420 carabaos slaughtered in Metro Manila abattoirs through the demonstration of eggs from the collected feces, with the use of flotation technique and the retrieval of adult flukes from the liver. The study reported a 33% prevalence among sampled carabaos. Molina et al (2005) reported a 64.9% prevalence of *Fasciola gigantica* in 32 carabaos slaughtered in Cotabato City based on egg detection using sedimentation technique.

In addition to *Fasciola, Paramphistomum* is also one of the common parasites of goats, sheep, cattle, and water buffaloes, which resides in the animal's rumen and reticulum. The severity of infection depends on the number of parasites present. A small number of the parasite does not cause serious damage and can go unnoticed, but in massive infection, it may cause acute parasitic gastroenteritis. High morbidity and mortality have also been recorded, especially in the young ones (Castro-Trejo et al 1990). Paña (2001) reported a prevalence of 36% of *Paramphistomum* infection among carabaos raised in different barangays (villages) in Baybay, Leyte.

Moreover, several studies have shown that *Paramphistomum* may co-exist with *Fasciola* in the digestive system of ruminants. This is due to the fact that the snail intermediate hosts of the parasite, such as *Bulinus, Planorbids, Lymnaea, Fossaria, and Pygmanisas* for *Paramphistomum* and *Lymnaea* for *Fasciola*, may be present in the same river or swamp and can serve as an intermediate host for both parasites (Paña 2001). This is documented in the study of Abrous et al (1996) which revealed that *Lymnaea (Galba) truncatula* is an intermediate host for both *Paramphistomum daubneyi* and *Fasciola hepatica* in France.

In Leyte, limited information is available on *Fasciola, Paramphistomum,* and co-infection of the parasites; available studies are focused on carabaos that are sampled in the field. Actual retrieval is the method of diagnosis employed in the study for a higher sensitivity rate of detection in comparison with the conventional sedimentation technique. The results of the study will provide baseline data that could be used as basis for more in-depth epidemiological study on individual and co-infection of parasites and also in improving health management of the backyard ruminant farming in the locality and in the country as a whole.

MATERIALS AND METHODS

Sample Size Determination

To determine the sample size, the formula of Cannon and Roe was used (1982) with a 20% prevalence by Bantugan (2004) and 95% level of confidence. Hence the total number of carabaos examined was 246. A systematic random sampling was employed by assigning respective numbers to carabaos and those with even numbers were sampled for the study.

Body Condition Scoring and Intrinsic Factor

The body condition score and other intrinsic factors (age, sex, weight, & origin) were recorded during antemortem inspection. Only the sample were all Philippine carabao breed. These data were associated with trematode infection in carabaos. A score of 1 was given to carabaos that were emaciated, 2 if thin, 3 for average body condition (neither thin nor fat), 4 if fat, and 5 if obese. An increment of 0.5 was used as an adjustment point in the body condition scoring for the animals, since the carabaos admitted in the abattoir were transported from different locations that might decrease body fat reserves during the process and influence the BCS reading (Anitha et al 2011).

Carabaos slaughtered in Ormoc City abattoir came from three provinces: Bohol, Cebu, and Leyte. Naturally, Leyte provided most of the supply of carabaos to the city and other neighboring municipalities. The sampled carabaos were grouped into two, based on the origin, with carabaos raised in Leyte are termed (Within Leyte) and those coming from other provinces (Outside Leyte).

Postmortem Detection of Adult Parasites

After the removal of the whole internal organs of the carabaos, the liver and the whole forestomach were separated for visual examination, palpation and incision in specific areas.

For detection of *Fasciola*, the common bile duct and the gall bladder were opened using a scalpel blade. The presence of *Fasciola* was recorded as “Positive” and “Negative” if absent.

For detection of *Paramphistomum*, the forestomach was opened using a sharp knife starting from the rumen, then to the reticulum, omasum, and abomasum. The stomach contents were removed to aid visualization of the parasite. The whole rumen, reticulum, omasum and abomasum were checked for the presence of *Paramphistomum* and recorded as “Positive” and “Negative”.

Parasite Identification and Collection

Representative parasites were gathered randomly from ten collections. With the use of serrated forceps, the parasites were harvested from the organ and stored in alcohol-formalin-acetic acid (AFA) solution. The parasites were mounted and the shape and the size were measured using a ruler and recorded.

Fasciola

The species of *Fasciola* were identified based on morphological characteristics as described by Soulsby (1982). The most common *Fasciola* are *Fasciola gigantica* and *Fasciola hepatica* which can be differentiated primarily by their size and shape. *F. hepatica* may reach a size of 30mm in length and 13mm in breadth. The shape of the parasite conforms with a leaf and has an anterior cone-shaped projection which is followed by a pair of “broad shoulders.” It is greyish-brown in color, changing to grey when preserved. *F. gigantica*, though resembling *F. hepatica*, is readily recognizable by its larger size that is 25-75mm in length and up to 12mm in breadth. Also, *F. hepatica* has a smaller anterior cone, lesser prominent shoulders and a more transparent body. *F. gigantica* is endemic in warm countries including Africa and Asia (Soulsby 1982).

Paramphistomum

Similarly, morphological description by Soulsby (1982) was used in identifying *Paramphistomum*. The most common paramphistome present all over the world is *Paramphistomum cervi.* The color of the live adult specimen is light red; it is pear-shaped, slightly concave ventrally and converse dorsally, with a large posterior subterminal sucker. The worm measures about 5-13mm in length and 2-5mm in breadth.

Statistical Analyses

Microsoft Excel was used to record the gathered data. To determine the animal-level prevalence and risk factors associated with the parasite infection, descriptive statistics and regression analyses were employed with the use of Epi Info version 7 program by the Centers for Disease Control and Prevention, USA. For continuous variables (BCS, age, & weight), t-test was performed for the analysis. A univariate and multivariate logistic regression was used for the assessment of the association between the dependent variable (presence of fluke) and the independent variable (risk factors). Risk factor variables that showed $p<0.20$ during the univariate analyses, were selected and further analyzed in a multivariate logistic regression model. To derive the significant variables, a backward stepwise elimination process was done with $p<0.05$ as the limit, while the least significant variables were removed until the p-value of the whole model was <0.05.

RESULTS AND DISCUSSION

Overall Prevalence of Trematodes

Of the 246 Philippine carabaos examined in Ormoc City abattoir, the prevalence of *Fasciola* was 50.81% (125 carabaos), *Paramphistomum* was 44.31% (109 carabaos), while co-infection was found in 32.11% (79 carabaos) as shown in Table 1.

Table 1. Prevalence of *Fasciola gigantica* and *Paramphistomum cervi* and co-infection by actual

Species Detected	Prevalence	
	N	95% CI
Fasciola	125	50.81±0.09
Paramphistomum	44.31	44.31±0.09
Co-infection	32.11	32.11±0.10

Prevalence of Trematode Infection in Association with the Animals Intrinsic Factors

The study examined 137 carabulls and 109 caracows. Actual detection showed that *Fasciola* and *Paramphistomum* were more frequent in females at a prevalence rate of 57.80% and 51.37%, than in males at 45.25% and 38.68%, respectively (Table 2a). Co-infection was also greater (38.53%) in females than in males (27.00%). These data showed that parasitism by trematodes was present in carabaos regardless of sex, due to the fact that both sexes were exposed to similar field conditions and grazed in the same area (Cabuenas 2009). Gordon et al (2015) reported a similar finding where *Fasciola* infection in female carabaos from Mabaras, Northern Samar were significantly (p=0.0001) higher (68.8%) in comparison to males (26.5%). Susceptibility by female carabaos could be attributed to stress brought about by physiological influences, such as estrous, lactation and calving, that decreases resistance of carabaos to infection (Duval 1996, Molina et al 2010).

Table 2a. Univariate analysis for factors associated with *Fasciola*, *Paramphistomum,* and co-infection

Variable	N	Code	Stat	*Fasciola* +		*Fasciola* -		ODDS Ratio (95% CI)	*p*-value
				n	% Prev	n	% Prev		
Sex			X^2						
Male	137	1		62	45.25	75	54.74	0.6063	0.0677
Female	109	0		63	57.80	46	42.20	[0.3633-1.0028]	
Origin			X^2						0.0138
Within Leyte	227	1		121	53.30	106	46.70	4.2807	
Outside Leyte	19	0		4	21.05	15	78.95	[1.3782-13.2960]	

Table 2a continued

Variable	N	Code	Stat	*Paramphistomum* +		*Paramphistomum* -		ODDS Ratio (95% CI)	*p*-value
				n	% Prev	n	% Prev		
Sex			X^2						
Male	137	1		53	38.67	84	61.31	0.5972	0.0627
Female	109	0		56	51.38	53	48.62	[0.3589-0.9937]	
Origin			X^2						0.0180
Within Leyte	227	1		106	46.70	121	53.30	4.6722	
Outside Leyte	19	0		3	15.79	16	84.21	[1.3247-16.4781]	
				Co-infection +		Co-infection -			
				n	% Prev	n	% Prev		
Sex			X^2						
Male	137	1		37	27	100	72.99	0.6036	0.0544
Female	109	0		42	38.53	67	61.47	[0.3633-1.0049]	
Origin			X^2						0.1832
Within Leyte	227	1		76	33.48	151	66.52	2.6843	
Outside Leyte	19	0		3	15.79	16	84.21	[0.7587 -9.4973]	

Variables with a $p<0.20$ in univariate analyses are further analyzed in multivariate analyses.

Majority (131/246) of the carabaos sampled were between the age of 1-3 years, but the average age was 4.81±0.22 years and the age range was 1-20 years. "Within Leyte" carabaos older than 3 years were highly infected with *Fasciola,* with a prevalence rate of 58.41% compared to *Paramphistomum,* which was prevalent in carabaos three years or younger (46.82%). Co-infection was prevalent at 34.65% in carabaos older than three years. Carabaos coming from other provinces aged greater than three years have a higher prevalence of *Fasciola*, *Paramphistomum* and co-infection (40%, 59%, 41% respectively). Several studies established that younger carabaos (1-3 years old) are more susceptible to gastrointestinal parasitism (Raza et al 2007, Mamun et al 2011), although helminth species may vary between young and adult animals (Raza et al 2007). One explanation points to the practice of backyard farmers of early weaning of the caracalf which interferes with the complete colostral transfer. With lower immunity transferred, the calf cannot mount a good immune response against infection (Tongson 2004). In addition, in their first year of pasture, young animals are susceptible to parasites since they do not have a great deal of immunity against it (Duval 1996). The present study contrastingly recorded a higher prevalence rate of *Fasciola* and *Paramphistomum* in older animals (>3 years) which is similar to other findings (Iqbal et al 2013, Molina et al 2010). The utilization of carabaos for draft purposes, lactation and pregnancy in female carabaos are stress factors that increase susceptibility of the animal to infection. Older animals are left to dwell longer in the fields and gained access to different areas where the metacercariae are present. In general, carabaos of any age that graze on pasture are vulnerable because of access to contaminated grass and water (Bantugan 2004).

Most carabaos slaughtered at the Ormoc City abattoir during the period of the study had a bodyweight of ≤325kg and the average weight was 323±5.91kg. The weight range of carabaos slaughtered in Ormoc City abattoir was 120-685kg. "Within Leyte" carabaos weighing >325kg had a recorded *Fasciola* prevalence rate of 60.36%, while *Paramphistomum* (52.59%) and co-infection (31.90%) are prevalent in carabaos weighing ≤325kg. For carabaos coming from other provinces, *Fasciola* (33.33%), *Paramphistomum* (16.77%) and co-infection (40%) are all high in carabaos weighing ≤325kg. These could be attributed to the pasture and grazing process wherein carabaos are moved from one grazing area to another and, it is natural for soil and grasses to be contaminated by internal parasites (Duval 1996). According to Sothouen (2007), severe parasitism affects nutrient absorption resulting in weight loss of about 20-40kg.

Based on body condition scoring (BCS), almost 50% (122/246) of the carabaos slaughtered in Ormoc City abattoir had a BCS of three (3±0.04), which means that the animals had an average body score, that is, they were neither thin nor fat. For carabaos coming from Leyte, *Fasciola* was prevalent at 59.09%, especially those with BCS greater than three (>3) (upon palpation, bones are rounded with fats and bone margins such as the ribs and pelvis, are non-discernible) compared to *Paramphistomum* (49.64%) and co-infection (35.97%) which were prevalent in carabaos with less than or equal to a BCS of three (≤3) (bone margins are clear with less fats felt upon palpation and the ribs and pelvis are discernible). Prevalence of *Fasciola*, *Paramphistomum* and co-infection were all high in carabaos coming from other provinces with a BCS of less than or equal to three (≤3) (25%, 16.67% & 16.67%, respectively). According to Raza (2009), parasitic infection has a higher prevalence in animals with poor body condition (76.3%), followed by animal with medium (23.9%) and good body condition (6.9%). Based on the results, trematode infection was present in carabaos regardless of body condition score.

Trematode infection rate was highest in carabaos coming from Leyte, with *Fasciola* having a prevalence rate of 53.30%, *Paramphistomum* at 46.70% and co-infection at 33.48%. On the other hand, carabaos from other provinces or outside Leyte has a prevalence rate of 21.05% for *Fasciola*, 15.79% for *Paramphistomum* and 15.79% for co-infection. The prevalence of *Fasciola* and *Paramphistomum* infection can be attributed to two factors: proper management of carabaos and presence of lowland areas in the localities. Proper management like deworming practice and care of carabaos are not well-practiced by smallholder farmers, who have fewer resources and knowledge in raising carabaos (Bardillon 2012). Carabaos that are usually kept tied in a stick in large natural pastures can get the infective stage of the parasites, the metacercaria (Faylon 1992). Also, deworming of carabaos is not practiced by smallholder farmers due to financial incapacity.

This study reveals the encompassing character of trematode infection, where infection was present regardless of intrinsic and extrinsic factors.

Risk Factors for Fluke Infection

Tables 2a and 2b show the independent variables/risk factors associated with *Fasciola* and *Paramphistomum* infection and co-infection. In univariate analyses (p<0.20), origin and sex of the carabaos become significant in both parasite infection and in co-infection. Multivariate logistic regression model was performed on those variables that are significant (p<0.05), using a backward elimination process to identify the most significant risk factor. The age of the carabaos were also included as forced variable in the multivariate analyses to determine if it is associated with the prevalence of parasite, since it is an important intrinsic factor. Results (Table 3) showed that carabao origin (p=0.019) was a significant risk factor associated with *Fasciola* infection, while both carabao origin (p=0.016) and sex (p=0.047) were significant for *Paramphistomum* infection.

Table 2b. Univariate analysis for factors (continuous variables) associated with *Fasciola*, *Paramphistomum,* and co-infection

Statistical Test: t-test		
		p-value
Age Range:1-20 yrs	Mean 4.81 Median 3	0.8702
Weight Range:120-685	Mean 323 Median 325	0.0876
BCS Range:1-5	Mean 3.31 Median 3	0.2681

Table 3. Multivariate analysis for factors associated with *Fasciola*, *Paramphistomum* and co-infection based on actual detection of adult parasite

Variable	Odds ratio	95% C.I. Lower Limit	95% C.I. Upper Limit	Coefficient	S.E.	Z-Statistics	*p*-value
Fasciola							
Sex	0.6036	0.3633	1.0028	-0.5048	0.2590	-1.9493	0.0513
Origin	4.2781	1.3777	13.2843	1.4535	0.5781	2.5142	0.0119
Forced variable							
Age	0.8457	0.5122	1.3964	-0.1676	0.2559	-0.6551	0.5125
Paramphistomum							
Sex	0.5972	0.3589	0.9936	-0.5156	0.2598	-1.9845	0.0472
Origin	4.6712	1.3246	16.4729	1.5414	0.6430	2.3972	0.0165
Forced variable							
Age	1.1381	0.6871	1.8853	0.1294	0.2575	0.5025	0.6153

Table 3 continued

Variable	Odds ratio	95% C.I. Lower Limit	95% C.I. Upper Limit	Coefficient	S.E.	Z-Statistics	*p*-value
Co-infection							
Sex	0.5902	0.3441	1.0123	-0.5272	0.2752	-1.9155	0.0554
Origin	2.6842	0.7587	9.4966	0.9874	0.6447	1.5316	0.1256
Forced variable							
Age	0.9948	0.5818	1.7010	-0.0052	0.2737	-0.0189	0.9849
Weight	0.6763	0.4093	1.1177	-0.3911	0.2563	-1.5259	0.1270

Variables with $p<0.05$ in multivariate analyses are considered significant and are associated with parasite infection.

Due to constant exposure to the metacercariae in the environment, male carabaos develop a certain level of immunity that their body weight is barely affected, unless they live in a poor condition area (Duval 1996). Female carabaos differ from male carabaos in terms of physiological responses (Ferrer 1981). Working under the heat of the sun, the female carabao's body parameters become unstable with an increase in its body temperature, pulse rate, and respiration. Sharing the same workloads with male carabaos and even utilizing them for draft purposes up to 2/3 of their gestation period, female carabaos become predisposed to infection (PCARRD 1992).

On one hand, origin appeared to be an important factor for both *Fasciola* (p=0.091) and *Paramphistomum* (p=0.016) infection. Data showed that carabaos coming from Leyte had a higher likelihood [4.27 to 4.57 times (odds ratio)] of getting infected with *Fasciola* and *Paramphistomum*.

As cited by Raza et al (2007), regional variation of helminths may be attributed to different geographical distributions, host factors, and climatic conditions required for the development of free-living stages of the helminths. Various studies had conveyed the prevalence of parasites in different regions of Leyte. Leyte province has a wide area of marshy and swampy grounds that are used for farming in which intermediate hosts and metacercariae are present. According to Vencilao (1980) and Cabarrubias (2002), carabaos raised in lowlands are more at risk to fasciolosis and paramphistomosis because of proximity to stagnant wetlands that favor the existence of snail intermediate host in endemic areas.

Correlation Analysis for Fasciola and Paramphistomum Infection in Philippine Carabaos

Pearson correlation coefficient (r) of 0.997** implies a strong and positive correlation between *Fasciola* and *Paramphistomum* infection (Table 4). The r-value (0.997**) implies that when *Fasciola* is present, the likelihood that *Paramphistomum* is also present is very high, and vice versa. This might be because both parasites can infect a snail intermediate host at the same time. Snails of the genus of *Lymnea* and *Galba* are the most common intermediate host of the parasite (Soulsby 1982). *Galba* snails are distributed worldwide with a higher density in Europe (Mas-Coma et al 2005). The most common snail species that are used by the parasites is the *Lymnaea philippinensis,* a related species to *Galba* since they belong to the same family of *Lymnaeidae* (Jesus 1935).

Table 4. Pearson correlation to determine the association of *Fasciola* with *Paramphistomum* infection

		r-value	
		Fasciola	*Paramphistomum*
Fasciola	Pearson Correlation	1	.997**
	Sig. (2-tailed)		.000
	N	246	246
Paramphistomum	Pearson Correlation	.997**	1
	Sig. (2-tailed)	.000	
	N	246	246

**. Correlation is significant at the 0.01 level (2 tailed): strong and positive correlation.

According to Abrous et al (1996), snails of the genus *Lymnaea (Galba) truncatula* can serve as an intermediate host for the two parasites and that they could co-exist together in one snail and give rise to the development of the metacercariae. Carabaos harbor the infection if they gain access to contaminated water and blades of grass with either or both of the parasites. Moreover, pasture larval counts and helminths fecundity play a role in the epidemiology of helminths. As a single factor, pasture contamination directly influences the population dynamics of helminths (Raza et al 2007).

Table 5. Measurement of body length and width of *Fasciola*

Sample No.	Body Length (mm)	95% CI	Body Width (mm)	95% CI
1	70	4.21-90.99	10	3.71-16.09
2	72	2.97-92.23	14	1.22-18.58
3	56	13.44-81.76	12	2.46-17.34
4	41	22.19-13.01	11	3.08-16.72
5	41	22.19-13.01	9	4.32-15.48
6	39	23.43-71.77	9	4.32-15.48
7	42	21.3-73.9	10	3.71-16.09
8	31	28.39-66.81	9	4.32-15.48
9	43	20.95-74.25	8	4.94-14.86
10	41	22.19-73.01	7	5.56-14.24
Range	31-72		7-14	

Endoparasite Identification

Representative parasites gathered randomly from ten collections were measured for identification. The body length of *Fasciola* ranges from 31-72mm (47.6mm average), while the width ranges from 7-14mm (9.9mm average). The shoulders were not broad and the shape is not leaf-like. Based on these features, the flukes were likely to be *Fasciola gigantica.*

Meanwhile, the body length of isolated *Paramphistomum* measurement was between 9-12mm (11.25mm average) in length and between 3-5mm (3.75mm average) in width. The shape of the parasites conforms to a cone and the color of the live specimen was light red. Based on these characteristics, the amphistome is most likely to be *Paramphistomum cervi* which has a body length of 5-13mm and width of 2-5mm.

Table 6. Measurement of body length and width of *Paramphistomum*

Sample No.	Body Length (mm)	95% CI	Body Width/Subterminal sucker (mm)	95% CI
1	12	3.86-18.74	5	0.71-6.89
2	11	4.48-18.12	5	0.71-6.89
3	9	5.72-16.88	3	1.94-5.66
4	9	5.72-16.88	3	1.94-5.66
5	11	4.48-18.12	3	1.94-5.66
6	12	3.86-18.74	3	1.94-5.66
7	14	2.62-19.98	4	1.32-6.28
8	12	3.86-18.74	4	1.32-6.28
9	11	4.48-18.12	5	0.71-6.89
10	12	3.86-18.74	3	1.94-5.66
Range	9-12		3-5	

Parasite identification was based on its organ of localization and its morphological characteristics. But for a more sensitive and accurate identification of the specific species of the parasite, a molecular approach can be done since helminth dynamics are rapidly changing (Mas-Coma et al 1997).

In a study of Valero (2001), morphological features, such as body length and width, have been traditionally used to differentiate the two species of Fasciola. However, variations in size, discrepancy of morphological features and the presence of intermediate forms, makes this approach unsuitable for differentiation of these two species. Spithil (1999) added that the presence of *F. hepatica* and *F. gigantica* in the same animal creates an opportunity for cross-fertilization and hybrid formation, and the most reliable method of differentiating the two is through molecular methods by sequencing of different genes (Mas-Coma et al 2007).

CONCLUSION

A high prevalence rate of fluke infection has been recorded in carabaos admitted for slaughter, implying that most of the backyard carabao raisers are not practicing sound deworming, pasture and grazing management and proper deworming protocol. To prevent and control flukes and improve the production and health of carabaos, the above-mentioned factors must first be considered. The present study recorded co-infection by helminths of similar genus for survival. This baseline information can be taken into consideration especially in deworming schedule, techniques, and diagnosis.

IMPLICATION

The results of the present study showed that trematode infection appears to be endemic in Leyte and neighboring provinces. This information can be a basis to enhance education in smallholder farming regarding the economic importance of the infection and also to develop effective control strategies such as selective deworming, rotational grazing management and conventional or advance method for early parasite infection in carabaos.

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