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Suitability of Danish Bilharziasis Laboratory Technique (DBL) as Detection Test for Trematode Infection in Buffaloes

John Philip Lou M. Lumain[1] and Lotis M. Balala[1]

[1]College of Veterinary Medicine, Visayas State University, Visca, Baybay City, Leyte, Philippines
Email for correspondence: johnphiliploum.lumain@gmail.com

ABSTRACT

Trematodes are one of the devastating parasites in ruminants with significant public health and economic importance. Continued evaluation and development of novel detection methods for accurate diagnosis are highly warranted. This study aimed to assess the suitability of the Danish Bilharziasis Laboratory (DBL) technique as a detection test for *Fasciola* spp. and *Paramphistomum* spp. infection in buffaloes. Sensitivity and specificity were compared to *post-mortem* recovery of parasites from specific organs. A total of 246 buffaloes were examined in this study, of which 125 (50.81%) were infected with *Fasciola* spp. and 109 (44.31%) with *Paramphistomum* spp. by *post-mortem* detection. In contrast, 107 (43.50%) were positive for *Fasciola* spp. and 92 (37.40%) for *Paramphistomum* spp. by the DBL method. Sensitivity of DBL was high in detecting *Fasciola* spp. (85.6%) and *Paramphistomum* spp. (84.4%). The specificity was 100% for both parasites. Kappa coefficients (κ) indicated an almost perfect agreement of the two tests in detecting *Fasciola* spp. (0.8539) and *Paramphistomum* spp. (0.8577). Based on these findings, DBL is a suitable technique for the detection of trematodes in buffaloes.
Keywords: DBL, *Fasciola*, *Paramphistomum,* sensitivity, specificity

INTRODUCTION

Foodborne trematodiasis is one of the neglected tropical diseases affecting primarily the poor and marginalized people in rural settings of developing countries (Mas-coma et al., 2009). This disease is caused by trematodes which include infectious flukes such as *Fasciola* spp. and P*aramphistomum* spp. that are endemic worldwide. The flukes are two of the most common trematodes affecting ruminants resulting to vast economic loss in meat and milk production. These parasites also exhibit broad zoonotic reservoir with cattle and buffaloes being the most important natural end hosts.

Fasciola spp. which induces fascioliasis is responsible for the inflammation of the bile duct, formation of gallstone, and fibrosis of the liver in infected animal. *Paramphistomum* spp., on the other hand, produces severe economic loss to milk production as the fluke saps nutrients from their hosts, resulting to weight loss and decrease in milk production (Haridy et al., 2002). Because of their economic impact, development of a highly sensitive and specific diagnostic tool is warranted to achieve successful prevention and control of parasitic infection. Direct coprological diagnostics, immunodiagnostics, molecular diagnostics, and imaging techniques are the main ante-mortem tools to

diagnose trematode infections (Keiser et al., 2006). Among these, coprological exam has advantage of being cost-effective, relatively easy to perform, and is most practical in resource-constrained settings (Pullan and Brooker et al., 2008). Several coprodiagnostic methods have been used to recover fluke eggs including flotation, Kato- Katz thick smear, and the formalin-ether/ethyl acetate concentration techniques (WHO, 2009). However, none of these techniques are satisfactory (Sindberg et al., 2014). The Danish Bilharziasis Laboratory technique (DBL), developed at DBL- Centre for Health Research and Development, is a novel fecalysis technique combining filtration and centrifugation (Willingham et al., 1998). The DBL method is gaining popularity in detecting eggs of small trematodes in domestic animals. It has been found suitable in quantifying eggs of fish-borne zoonotic trematodes in dogs, cats, and pigs (Anh et al., 2008; Sindberg et al., 2014). However, the use of DBL in detecting trematodes in buffaloes has not been explored. We therefore investigated the suitability of the DBL technique in detecting the eggs of *Fasciola* spp. and *Paramphistomum* spp. in buffaloes and compared sensitivity and specificity to the *post-mortem* detection of flukes in specific organs being the gold standard.

MATERIALS AND METHODS

Fecal collection in buffaloes. The study involved 246 randomly selected buffaloes at the Ormoc City Abattoir, Ormoc City, Leyte, Philippines. The sample size was determined using the formula of Cannon and Roe (1982) based on the 20% prevalence reported by Bantugan (2004, unpublished). Prior to slaughter, 100 grams of feces were collected directly from the rectum using a lubricated hand glove. The glove later served as temporary container of the fecal material upon removal from the hand. Fecal samples were properly labeled and stored at 4°C until use.

Fecal examination for eggs of trematodes. The DBL technique described by Willingham et al. (1998) was used in screening fecal samples for the presence of trematode eggs. Briefly, 35 grams of fecal sample were homogenized in a mortar and pestle to allow even distribution of the eggs in the feces. After homogenization, five grams of subsamples were washed in saline solution (1.2%) and shaken mechanically for 10 minutes in a shaker machine. The fecal mixture was then poured into a series of sieve stacks measuring 400 µm, 100 µm, and 45 µm from top to bottom. The residual sediments were washed with saline and allowed to settle for 10 minutes. Washing with saline solution was repeated with in between discharge of the supernatant. Retained sediments were centrifuged at 1500 rpm for 3 minutes and resuspended in saline to 2.25 mL volume. A drop of methylene blue was then added in the preparation to provide a good contrast for the visualization of eggs (Hansen & Perry, 1994). About 150 µl of the fecal suspension was mixed with 850 µl of saline solution in a DBL slide for microscopic examination. The presence of the eggs of *Fasciola* spp. and *Paramphistomum* spp. in the feces was recorded "positive" and "negative" if eggs were not detected.

Trematode egg identification. The eggs were identified based on the description provided by Soulsby (1982). Eggs of *Fasciola* spp. were operculated, ovoid in shape, light yellow to greyish in color with length ranging from 130 to 150 µm and width from 60-90 µm. The eggs of *Paramphistomum* spp. were operculated, ovoid in shape, brown to greenish in color with a length ranging from 114-176 µm and width from 73-100 µm. The eggs were further distinguished through the diffusion of methylene blue in the eggs of *Paramphistomum* (Figure 1) 35–40 minutes after staining due to thinner egg capsule (Hansen & Perry, 1994). The eggs were viewed at 400x magnification and measured by ocular micrometry with 26 µm per ocular division (Zajac, 2012).

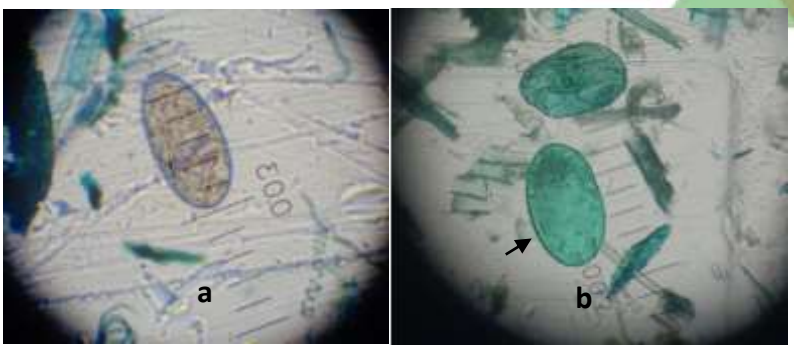

Figure 1. Egg of *Fasciola* spp. (156 µm) unstained in methylene blue (a); egg (arrow) of *Paramphistomum* spp. (156 µm) absorbing the methylene blue stain (b) at 400x magnification.

Post-mortem detection of parasites. *Post- mortem* recovery of trematodes in their predilection sites was the gold standard in concluding positive infection in the animals. After slaughter, the liver and forestomach of each of the sampled animal were removed for visual inspection. To detect *Fasciola* spp., the common bile duct and the gall bladder were palpated and cut-open using a scalpel blade. *Paramphistomum* spp. was traced from the rumen, reticulum, omasum, and abomasum. The stomach contents were flushed with running water to visualize parasites adhering to the stomach wall. The recovery of trematodes was regarded as "positive" and "negative" if undetected.

Adult parasite identification. Species of *Fasciola* and *Paramphistomum* were identified based on morphological characteristics described by Soulsby (1982). Briefly, *Fasciola hepatica* is leaf-shaped with broad shoulders and may reach a size of 30 mm in length and 13 mm in breadth. *Fasciola gigantica,* though resembling *Fasciola hepatica,* is recognized by its larger size that is 25–75 mm in length and up to 12 mm in breadth, shoulders are not prominent, and the body is more transparent. *Paramphistomum* is pear-shaped, slightly concave ventrally and converse dorsally, with a large posterior subterminal sucker. It measures 5–13 mm in length, and 2–5 mm in breadth. It is light red in color when alive.

Statistical analysis. Data were recorded in Microsoft Excel ® and analyzed in Epi Info® version 7. Prevalence was computed based on the formula:

$$Prevalence\ Proportion = \frac{Number\ of\ infected\ animal}{Total\ number\ of\ animal\ examined}\ x\ 100$$

A Chi-square test was used to compare the frequency of parasitic infections and considered significant at $p < 0.05$. Diagnostic sensitivity was calculated as the probability that a true positive animal was classified as positive while diagnostic specificity was calculated as the probability that a true negative animal was classified negative (Anh et al., 2008). Kappa coefficients (κ) was interpreted using the guidelines outlined by Landis and Koch (1977), where strength of the kappa coefficients is slight (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and almost perfect (0.81-1.00).

RESULTS AND DISCUSSION

In this study, 256 buffaloes were examined using DBL technique and *post-mortem* recovery of parasites from their predilection sites. The latter was used as gold standard to evaluate diagnostic sensitivity and diagnostic specificity of DBL. Originally, DBL was developed to detect *Schistosoma japonicum* in animals by the Danish Centre for Health Research and Development (Willingham et al., 1998). It was later used to screen buffaloes for *S. japonicum* in Western Samar and Sorsogon Provinces in the Philippines (Carabin et al., 2005). Table 1 outlines the prevalence of trematodes in buffaloes using the two methods. The prevalences of *Fasciola* spp. and *Paramphistomum* spp. were higher by *post-mortem* detection of parasites ($p < 0.05$). There was generally a higher prevalence of *Fasciola* spp. compared to *Paramphistomum* spp. Co-infection was significantly higher by post-mortem detection compared to DBL ($p < 0.05$). The occurrence of co-infection is parallel in many studies where gastrointestinal nematodes are also present aside from the trematodes. *Fasciola* spp. and *Paramphistomum* spp. are among the most prevalent gastrointestinal helminths in ruminants (Raza, et al., 2007; Mamun, et al., 2011). Most of the buffaloes examined in this study were owned by smallhold farmers who have limited resources to maintain good management and improve the health status of the animals. More often, deworming was not applied due to financial constraint.

Table 1. Prevalence of *Fasciola* spp. and *Paramphistomum* spp. in buffaloes

	***Post-mortem* detection**		**DBL technique**	
	Positive (n=256)	**Prevalence (95% CI)**	**Positive (n=256)**	**Prevalence (95% CI)**
Fasciola spp.	125	50.81±0.09	107	43.50±0.09
Paramphistomum spp.	109	44.31±0.09	92	37.40±0.10
Co-infection	79	32.11±0.10	66	26.83±0.11

The presence of helminths in ruminants can be predisposed by some factors such as host age, sex and breeding status, and grazing habits. Additional factors are the level of education and economic capacity of farmers and their standard of management (Asanji &Williams, 1987; Gulland & Fox, 1992). In addition, geoclimatic conditions and poor awareness of livestock farmers contribute towards a conducive environment for the development and growth of parasites (Raza et al., 2007). The prevalence of *Fasciola gigantica* in this study was similar to the report of Molina et al. (2010), while prevalence of *Paramphistomum cervi* is higher than in the study of Iqbal et al. (2013). Dorny et al. (2011) reported prevalence estimates of 5–20% and 45–95% for *Fasciola* and *Paramphistomum*, respectively, using coprological examination. Lower prevalence (*Fasciola hepatica* - 32% and *Paramphistomum cervi* - 11%) was reported by Kobak and Pilarczyk (2012) in water buffaloes raised in the Notecka Forest Region in Poland. This regional variation may be attributed to different geographical distributions, host factors, and climatic conditions required for the development of free-living stages of the trematodes (Raza et al., 2007). Throughout South East Asia, infections with gastrointestinal trematodes are very common in ruminants because of appropriate climatic conditions year-round that favor the development and transmission of the infective stages of trematodes from grasslands (Iqbal et al., 2013; Dorny et al., 2011). Buffaloes that are usually kept tied in a stick in large natural pasture may acquire the infective metacercaria (Faylon, 1992). In addition, the propensity of buffaloes to seek rivers, pools, or swamps for wallowing raises the risk of infection to snail-borne helminthes (Cockrill, 1974).

Table 2. Specificity and sensitivity of DBL as detection test for trematodes

	***Post-mortem* detection**	**DBL**	**Sensitivity (%)**	**Specificity (%)**	**Kappa coefficients**
Fasciola spp.	125	107	85.6	100	0.8539
Paramphistomum spp.	109	92	84.4	100	0.8577
Co-infection	79	66	83.5	100	0.8733

Table 2 shows that the sensitivity of DBL to detect *Fasciola* spp. and *Paramphistomum* spp. was 85.6% and 84.4%, respectively. Specificity was 100% for both parasites. So far, no data is available to compare sensitivity and specificity of DBL in detecting trematodes in buffaloes. However, Sindberg et al. (2013) recorded an 85% sensitivity of DBL in detecting small trematode eggs in dog feces. The same level of sensitivity (80-92%) was also reported by Anh et al. (2008) for small trematodes, mostly Heterophyidae and Echinostomatidae belonging to the genera *Haplorchis* and *Echinochasmus*, in dogs, cats, and pigs. Specificity was 100% in all animal species. In this study, the specificity of DBL was high, however, a 100% specificity may be unreal if the possibility of false positives data is to be considered (Tarafder et al., 2010). It should also be noted that test sensitivities are strongly influenced by intensity of infection and this variation needs to be taken into account for the choice of a diagnostic test in a specific setting (Nikolay et al., 2014). Furthermore, the kappa coefficients (κ) indicated an almost perfect agreement between DBL and *post-mortem* recovery in detecting *Fasciola* spp. (0.8539) and *Paramphistomum* spp. (0.8577) with 92.68% and 93.08% degree of agreement, respectively. This confirms the suitability of DBL as detection test for trematodes in buffaloes.

Results of this study show that DBL can be a satisfactory detection test in the field or any setting where slaughter or necropsy of the buffaloes is not necessitated. As a test, DBL offers several advantages over other detection methods being simple, quantitative, and does not require special reagents or equipment to perform the examination (Ahn et al., 2008). This technique can easily be performed by even the simplest veterinary laboratory to produce data that are particularly important for epidemiological analyses.

CONCLUSION

Occurrence of trematodes continues to affect development in the buffalo industry. The deleterious effect of these parasites led to production losses and condemnation of affected organs after slaughter. A highly sensitive and specific diagnostic tool is therefore appropriate to design effective control strategies. To the best of our knowledge, the present study is the first to publish data on the sensitivity and specificity of DBL in detecting trematodes in buffaloes. The results highly suggest considering DBL as a tool for routine diagnosis. Moreover, the use of serological and molecular tools may be considered to address concerns in sample processing, low infection rate, and overlapping morphological features.

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