

Original papers

A comparison of FLOTAC and CFF techniques in detecting gastrointestinal parasites in water buffaloes (*Bubalus bubalis*)

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ABSTRACT. The objective of the study was to compare the usefulness of FLOTAC and centrifugal fecal flotation (CFF) techniques. More specifically, the taxonomic classes (Nematoda and Cestoda) of endoparasites present in fecal samples of buffaloes are identified, the sensitivity and specificity of FLOTAC relative to CFF are calculated, and the agreement of both techniques is evaluated using Kappa statistics. Fresh fecal samples from 220 buffaloes in 10 municipalities were collected. Sheather's sugar was used as a flotation solution for both the FLOTAC and CFF techniques. Of the 220 animals, 109 samples were nematode positive and 111 samples were nematode negative according to the FLOTAC technique, while 74 were found to be positive and 146 negative according to the CFF technique. No cestodes were detected by either technique. The calculated sensitivity for FLOTAC is 89.19% and its specificity is 70.55%. Kappa statistics revealed moderate agreement (k=0.535) between the two techniques in detecting nematodes. The prevalence observed based on FLOTAC and CFF test were 49.54% (109/220; 95% CI: 47.75–56.34) and 33.64% (72/220; 95% CI: 27.42–40.3), respectively.

Key words: prevalence, nematodes, FLOTAC, CFF, Philippines, water buffaloes

Introduction

Parasitism is one of the most important health problems facing domestic animals worldwide [1]. Gastrointestinal parasitism in buffalo has been identified as one of the major factors disrupting the development of the industry in Asia and causing substantial economic loss to poor subsistence farmers in developing countries [2]. Numerous helminth species parasitize the body of animals, and some of them cause a remarkable disease burden to the animal and heavy losses to the livestock industry [3]. Internal parasites adversely affect the health and productivity of animals and also decrease the resistance of animals to various diseases, which may ultimately lead to higher mortality [4].

Helminthosis is a disease caused by a helminthic infection which may take in a number of forms. The term "helminths" designates wormlike animals of several phyla applied only to the parasitic and non-parasitic species belonging to the Phyla Platyhelminthes (flukes, tapeworms and other flatworms) and Nemathelminthes (roundworms and their relatives) [5].

The effects of parasitism on production are well documented. It has been said that the economic loss in livestock production from gastrointestinal parasites is greater than any other disease [6]. In the Philippines, the most important parasites among ruminants include roundworms in the genera *Haemonchus* and *Trichostrongylus*, and a smaller proportion of *Strongyloides* species. According to

Montes et al. [7], the exact contribution of roundworm parasitism to annual mortality losses is large, but its economic value has rarely been estimated. The livestock industry assumed that 15% of adult ruminant mortality, or 1% of adult cattle, arises from roundworm-related diseases [7,8]. Selected studies of productivity in parasitized ruminants have been undertaken in Southern Luzon [9]. Sani and Gray [10] note that, while smallholder farmers might be well aware that parasites lead to sickness and death, they might not know of the fertility losses they cause.

In the humid tropics, forage pasture land is often affected by complexities brought about by the parasitic development of trichostrongylate nematodes, namely *Trichostrongylus* spp., *Haemonchus* spp., *Cooperia* spp., and *Oesophagostomum* spp. [11]. These are common nematodes, and with *Trichostrongylus* spp. was found to be the dominant nematode by a study conducted in Southern Mindanao, Philippines [12].

As polyparasitism is so common and in response to the recent trend toward integrated control of multiple parasitic diseases, there is a need for sensitive diagnostic tools that is simple to apply [13]. Though standard tests are already available, continuous efforts should be made to find new techniques.

Processing fecal samples by centrifugal flotation has emerged as the new gold standard due to its improved sensitivity over traditional, gravity, flotation. Not only has centrifugation become the method of choice among veterinary parasitologists, but in addition, there are many published papers indicating that this methodology has a significantly higher yield of parasites than the current floatation method [14].

"The FLOTAC technique is a recently-developed mulitivalent, copromicroscopic technique based on the same principle as the flotation technique but with a host of different potential applications" [15]. It was developed by Professor Giuseppe Cringoli (University of Naples Frederico II, Italy) to detect and quantify the number of eggs, oocysts, cysts and parasite larvae found in animals and humans. "This technique uses the FLOTAC apparatus (Fig. 1) and is based on the centrifugal flotation of a fecal sample suspension and subsequent translation of the apical portion of the floating suspension" [15].

No existing data regarding the sensitivity and specificity of this technique currently exist in the Philippines. However, the results of the present study may serve as a basis for its use in the parasitic evaluation of large ruminant feces. Should the findings be found favorable, this technique can be used as an additional approach to test animal feces in the Philippines.

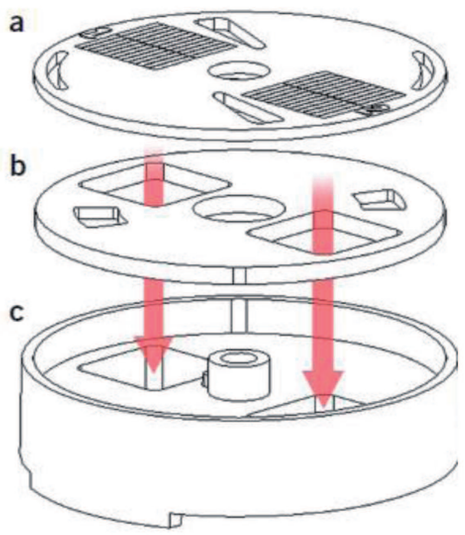

Fig. 1. Physical components of the FLOTAC apparatus. The core device is composed of reading disk (a), translation disk (b) and base (c). Arrows indicate flotation chambers modified by Cringoli et al. [15].

The study primarily compares the effectiveness of the FLOTAC and centrifugal fecal flotation (CFF) methods as techniques for gastrointestinal parasite diagnosis, more specifically, their potential to identify the taxonomic classes of roundworms; calculate the sensitivity and specificity of the two techniques; evaluate the tests agreement by Kappa (k) statistics; calculate the prevalence of roundworm infection in water buffaloes in the province of Nueva Ecija; and present the prevalence of parasitism in the covered municipalities using maps.

Materials and Methods

Sampling method and sample size

A total of 220 fecal samples were collected from water buffaloes in different dairy cooperatives in 10 municipalities of Nueva Ecija province. The number of samples collected per municipality was 22. Subject animals were randomly selected by raffling the identification numbers of the animals present in every collection site.

Sample collection and processing

Fecal samples were collected directly from the rectum by manual extraction. Samples were packed

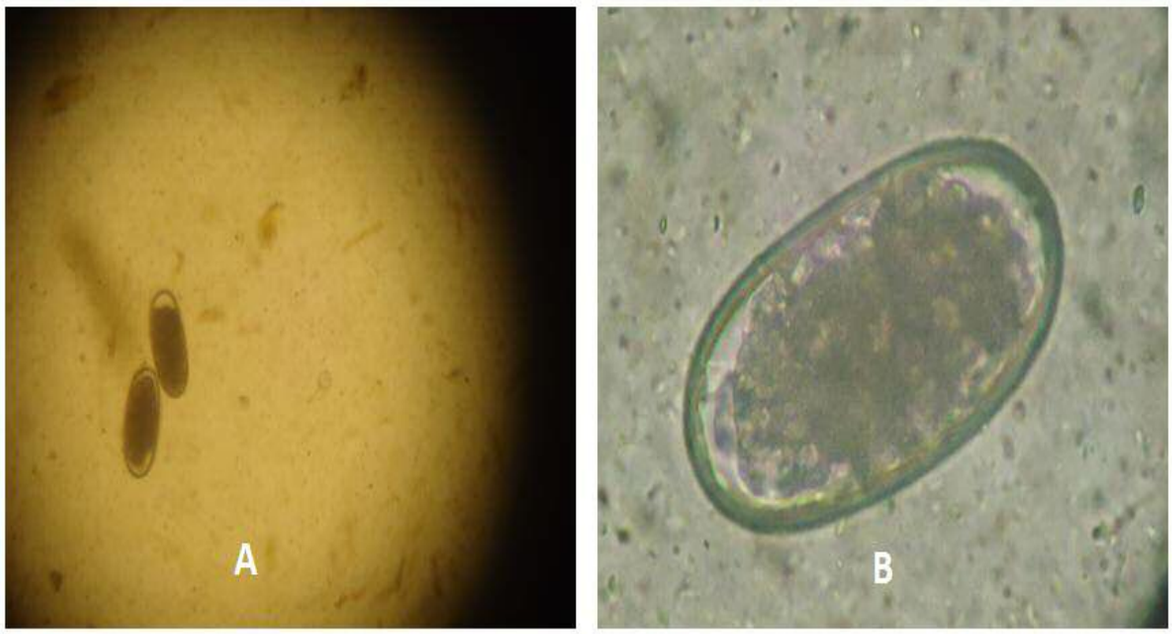

Fig. 2. Strongyle-type eggs from infected water buffalo after FLOTAC (A) and CFF (B) assays

in a 12”×14” plastic bag and then properly labeled with the animal identification number, date, and name of the cooperative. The collected feces were stored in an ice box and transported to the laboratory for processing.

The fecal samples were processed immediately upon collection. The taxonomic classes (Nematoda and Cestoda) identified were recorded for each technique used.

Procedure for FLOTAC technique [15]

Ten grams of feces were taken and added to 90 ml of tap water. The suspension was thoroughly homogenized using a glass rod. The suspension was filtered through a wire mesh and 11 ml of the filtered suspension was placed into a tunic tube. The two flotation chambers of the FLOTAC required 6 ml each to fill the two flotation chambers. The suspension was centrifuged for 3 min at 1500 rpm. The supernatant was discarded, leaving only the sediment as a pellet in the tube. The tube with Sheather’s sugar solution was filled to the previous 11 ml level. The homogenized suspension was used to fill the two flotation chambers of the FLOTAC. The FLOTAC was closed and centrifuged for 5 min at 1000 rpm. After centrifugation, the top parts of the flotation chambers were transferred, read under microscope and examined under LPO.

Procedure for CFF [16]

Between three and five grams, about one teaspoonful, of feces with 10 ml of Sheather’s sugar solution were mixed in a paper or plastic cup. The mixture was filtered using a double layer of cheesecloth or gauze. A tea strainer was used to pour the mixture into a 15 ml centrifuge tube. The mixture was centrifuged for about 5 min at approximately 500–650 x g. The supernatant was discarded and the sediment was resuspended with flotation solution. Following centrifugation, the surface layer of fluid containing parasite eggs was harvested. The tube was spun without the coverslip and the tube was removed from the centrifuge after spinning and placed in a test tube rack. Then it was added with Sheather’s sugar solution to form a reverse meniscus. Coverslip was placed on the tube and allowed to sit for an additional 5–10 min. Then the solution was placed on a slide [16].

Case definition

A diseased animal is an animal that tested positive by the CFF technique, while a non-diseased animal is one that tested negative. The identification of cestode eggs and nematode eggs in a slide was interpreted as cestode positive or nematode positive, respectively. The same criterion was used to interpret the results from the results of the FLOTAC technique.

Results and Discussion

Using FLOTAC and CFF techniques, only eggs from Class Nematoda were detected. The nematode eggs identified in both FLOTAC and CFF techniques were strongyle-type eggs (Fig. 2),

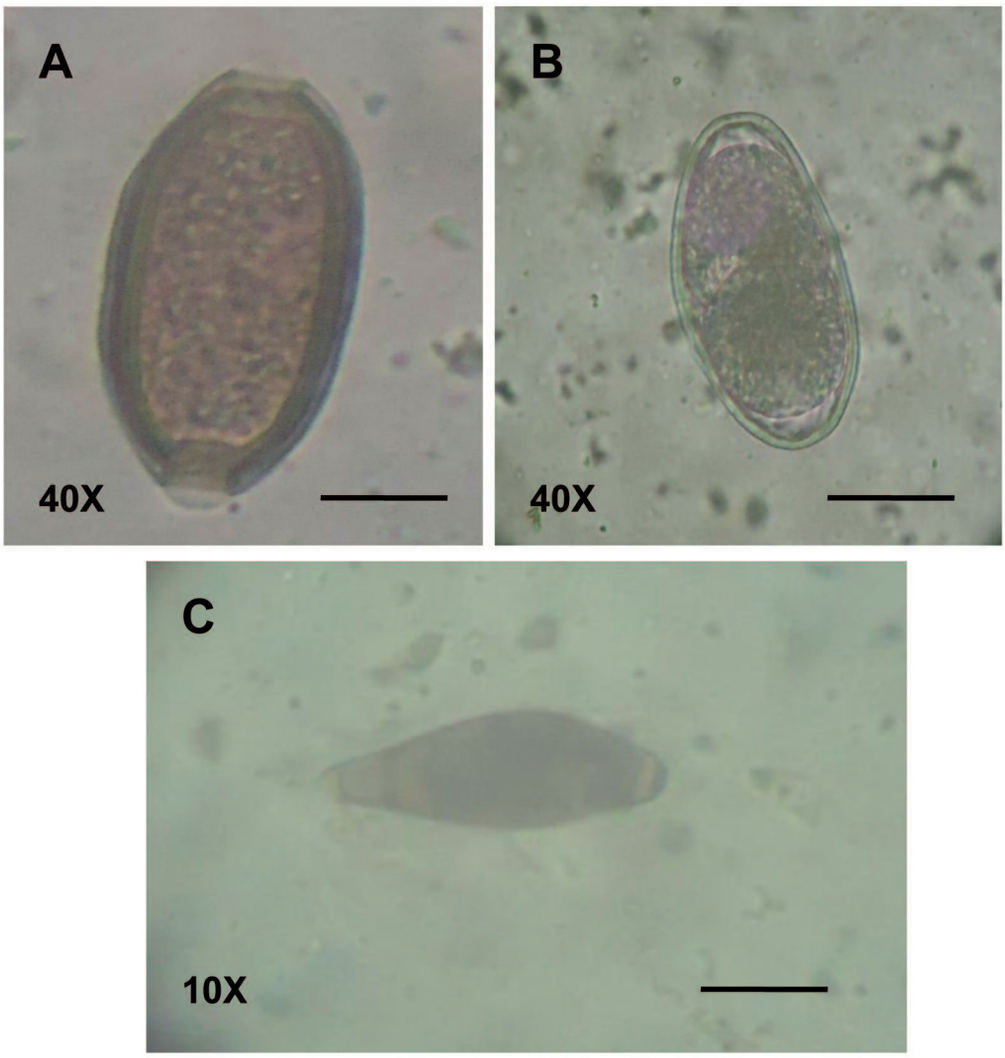

Fig. 3. Eggs of *Capillaria* spp. (A), *Strongyloides* spp. (B), *Trichuris* spp. (C) from infected water buffalo after CFF assay

Capillaria spp., *Strogyloides* spp. and *Trichuris* spp., (Fig. 3). These eggs are commonly found in ruminants such as buffaloes [5,17].

Strongyloides spp. and *Trichuris* spp. eggs can be more clearly seen using CFF than FLOTAC because samples for FLOTAC can be seen only under LPO (100×) while samples for CFF can be seen using the HPO (400×). However, the samples in FLOTAC were centrifuged twice while CFF centrifuged only once. Less debris can be seen if the samples are centrifuged more than once and this can ease parasite detection. The higher prevalence observed in the FLOTAC results could be associated to the clarity of the field during microscopic evaluation.

No cestodes were detected using both techniques considering the life cycle of *Moniezia benedini,* a cestode of ruminants which is chiefly found in cattle [17]. Ruminants are infected by the ingestion of infected oribatid mites, which serve as the intermediate host of the tapeworm [5]. Although the disease has a high prevalence in summer, which corresponds to the active period of oribatid mites during this season [18], the collection was performed from November to December when there is low prevalence of the cestode. In addition, eggs are seldom seen during routine fecalysis, as they are more likely to be found either in the soil or in the oribatid mites [17].

Flotation solutions play a fundamental role in determining the analytic sensitivity of any copromicroscopic technique, either qualitative or quantitative, based on flotation, including the FLOTAC technique [15,19]. Most gastrointestinal strongyles floated in a sucrose-based flotation

Table 1. A two-by-two table used in determining the sensitivity and specificity of FLOTAC and CFF techniques

	CFF		
FLOTAC	**Positive**	**Negative**	**Total**
Positive	66	43	109
Negative	8	103	111
Total	74	146	220

solution at specific gravity between 1.200 and 1.350, but few *Moniezia* spp. did so despite the gold standard flotation solution for FLOTAC in detecting *Moniezia* spp. in fresh feces is Zinc sulfate [15].

The results of the CFF and FLOTAC techniques used on positive and negative animals are shown in a 2×2 table (Table 1). Based on the calculated data, the sensitivity of FLOTAC relative to CFF is 89.19%. This sensitivity may result in 10% probability of not detecting other sick animals, particularly those with low parasite load. On the other hand, FLOTAC specificity (70.55%) is comparable with that of the CFF since they are both direct microscopic techniques.

The agreement of the two techniques was evaluated using Kappa statistics (k=0.535) with a 95% Confidence Interval (0.429–0.641). Statistical analysis revealed moderate agreement between the two techniques.

Therefore, FLOTAC is a good screening test to identify infected stock. However, its test properties may change if a more sensitive gold standard test is used. The sensitivity of a test is always relative to the gold standard, and the CFF technique was set as the gold standard in the study since it is the most commonly used method in laboratories.

Table 2 summarizes the prevalence of nematode infection in the selected areas using CFF and FLOTAC techniques. Out of 220 samples, the estimated prevalence is 33.64% (74/220; 95% CI: 27.42-40.3) when using CFF and 49.54% (109/220; 95% CI: 47.75-56.34) for FLOTAC. The Science City of Muńoz recorded the highest prevalence, with 72.73% in CFF and 90.91% in FLOTAC technique. This is followed by Llanera 68.18% and 63.64%, Sto. Domingo 57.14% and 72.73%, San Jose 54.55% and 72.73%, Guimba 26.09% and 47.83%, Rizal 18.18% and 22.73%, Talavera 13.64% and 40.91%, General Tinio 13.64% and 45.45%, General Natividad 13.64% and 22.73, Aliaga 0% and 13.64%, for CFF and FLOTAC, respectively.

The same areas recorded the highest nematode burden among buffaloes using the CFF technique. The findings correspond to those of a summary of dead animals recorded previously, which notes that these areas also have the highest mortality in terms of gastrointestinal parasitism.

Accordingly, same areas have the highest buffalo population in the province for the year 2012. The high prevalence of nematodes in these areas can be attributed to the difficulties in carrying out deworming programs caused by the high animal population. Higher populations will require higher maintenance in terms of preventive practices such as deworming.

On the other hand, the five municipalities found to have the lowest prevalence of nematodes by CFF and FLOTAC were Rizal, Talavera, General Tinio, General Natividad and Aliaga. These areas have lower mortalities due to gastrointestinal parasitism among buffaloes in Nueva Ecija.

Aliaga has the lowest prevalence of nematodes in buffaloes among the top ten municipalities, with 0% and 13.64% prevalence according to CFF and FLOTAC techniques, respectively. This can be attributed to the small buffalo population in the area, which results in most of the animals being

Table 2. Prevalence of nematode infection in the selected areas of the Nueva Ecija province (N=220)

Municipality/City	**CFF (%)**	**FLOTAC (%)**
Science City of Muńoz	72.73	90.91
Llanera	68.18	63.64
Sto. Domingo	57.14	72.73
San Jose City	54.55	72.73
Guimba	26.09	47.83
Rizal	18.18	22.73
Talavera	13.64	40.91
General Tinio	13.64	45.45
General Natividad	13.64	22.73
Aliaga	-	13.64
Total	33.64	49.54

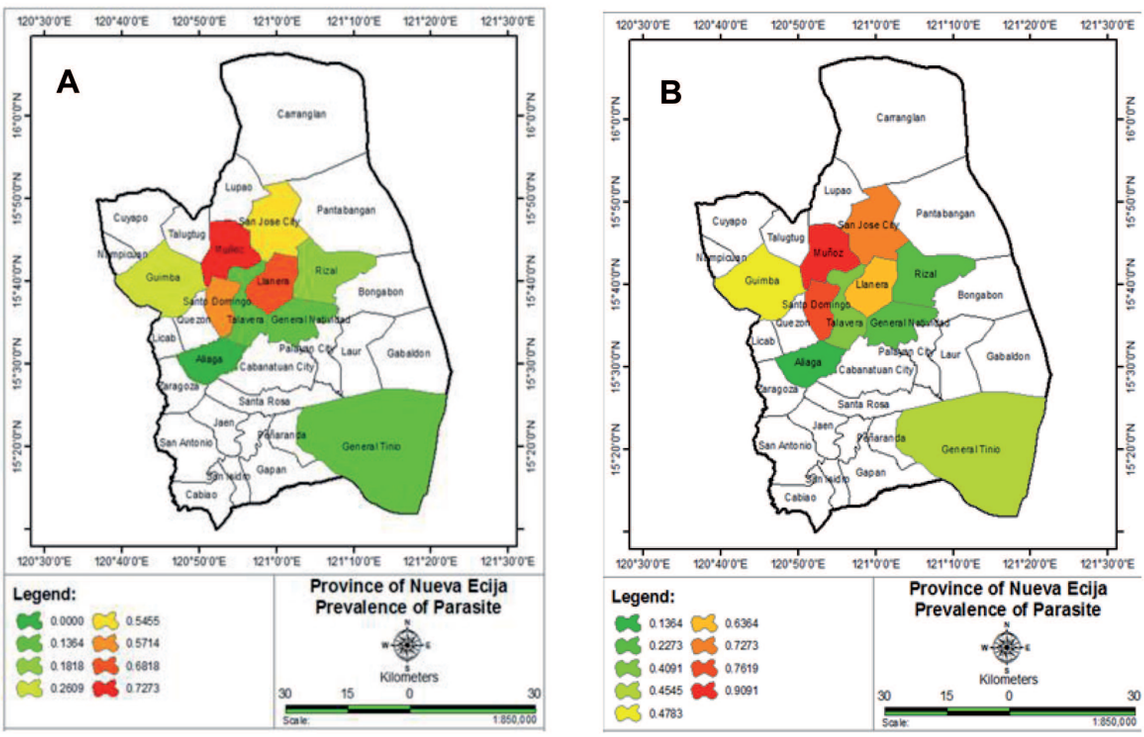

Fig. 4. Maps showing the prevalence of nematode infections using CFF (A) and FLOTAC (B) techniques

dewormed regularly. Based on the deworming records, the animals in Aliaga were already dewormed before the collection of the samples, while the rest of the areas covered were collected before deworming.

The prevalence of the nematodes detected using CFF by municipality is shown in Fig. 4A, while the distribution revealed by FLOTAC is shown in Fig. 4B. Maps were drawn using ArcGIS.

All the samples were negative for cestodes in both techniques. No cestodes were detected by fecalysis because, *Moniezia benedini,* a cestode of ruminants, is chiefly found in cattle [17,20]. Based on the tapeworm's life cycle, ruminants are infected by the ingestion of infected oribatid mites, which serves as intermediate host of the tapeworm [5]. In addition, the collection was performed between November and December when the prevalence of *Moniezia benedini* is low, because the active period of the oribatid mite is during the summer months [18,21]. Moreover, eggs are seldom seen during routine fecalysis because the eggs are mostly seen either in the soil or in the oribatid mites.

Conclusions

Out of 220 fecal samples, only nematode eggs were seen and cestode eggs were not detected. In total, 109 animals were found to be nematode positive and 111 nematode negative according to the FLOTAC technique, while 74 positives and 146 negatives were identified by the CFF technique. Based on the results, the prevalence according to FLOTAC (49.55%) is higher than that of CFF technique (33.63%). The FLOTAC technique has 60.55% sensitivity and 92.79% specificity for nematodes. Kappa statistics demonstrate a moderate agreement (k=0.535) between the results of the two tests in detecting nematodes.

Since *Moniezia benedini* is mostly seen in the summer season, it is recommended that another study be peformed during this period to confirm the results. The use of other flotation solutions, such as zinc sulfate, is highly recommended to establish the sensitivity and specificity of FLOTAC to cestodes.

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