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Short communication

# Evaluation of four coproscopic techniques for detection of small trematode eggs in dog faeces

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ABSTRACT

The study was conducted to evaluate copro-diagnostic techniques for detection of small trematode eggs in dogs. FLOTAC, a novel flotation technique, and DBL, a sieving and sedimentation technique developed at the former Danish Bilharziasis Laboratory (DBL), were compared using 53 subsamples from four copro-positive dogs. Moreover, a modified version of the DBL technique and the Kato-Katz (KK) thick smear were later compared using faecal samples from 21 dogs. The four techniques were pair-wise compared regarding sensitivity, infection intensity and practical applicability. For the former two techniques, egg recovery subsequent to storage and reproducibility were also compared. The DBL technique detected all 53 subsamples positive for small trematode eggs. Based on 17 subsamples, mean infection intensity of $47 \pm 49$ eggs per gram of faeces (EPG) was detected by the technique. Due to large amount of sediment, examination of a single subsample required an average of 3 hours. The FLOTAC technique was found less sensitive (82%) than the DBL technique and recovered significantly less eggs ($4 \pm 6$ EPG). Both sensitivity and intensity were further reduced following storage. As the FLOTAC technique requires specialised equipment, safety disposal and personal protective equipment, it was found less suited than the DBL technique for a basic laboratory. Additionally, poor reproducibilities were found for both the DBL and FLOTAC techniques ($30 \pm 15$% and $38 \pm 33$%, respectively). Based on the 21 faecal samples, the modified version of the DBL technique was found more sensitive (85%) than the KK technique (68%), whereas egg counts were significantly higher for the latter ($23 \pm 26$ EPG vs. $482 \pm 909$ EPG). By modifying the DBL technique, it was possible to diminish the retained sediment and examination time to a maximum of an hour, which was also the time required by the KK technique, although the latter was faster and more easily processed. Based on the results obtained in this study, none of the techniques evaluated were found applicable in their current form for detection of small trematode eggs in faeces from dogs in Vietnam.



## 1. Introduction

Fishborne zoonotic trematodes (FZT) are emerging and are currently considered a significant health problem in Vietnam and other parts of Southeast Asia (Chai et al., 2005; WHO, 2004). Attention is primarily directed towards the small liver flukes, *Clonorchis sinensis* and *Opisthorchis viverrini* (WHO, 2010), although high prevalences of a mix of several small intestinal flukes, especially *Haplorchis* spp., were recently found in both fish intermediate hosts and domestic animals in the Nam Dinh Province, a highly FZT endemic area in Northern Vietnam (Anh et al., 2009a; Anh

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et al., 2009b; Hop et al., 2007; Phan et al., 2010). The role of domestic animals as reservoir hosts maintaining the transmission of the FZT by contributing to the environmental contamination with eggs remains to be elucidated (Anh et al., 2009b; De et al., 2003). However, the eggs of the small liver and intestinal flukes are indistinguishable by light microscopy as they are small (< 50 μm) and morphologically similar (Ditrich et al., 1992). Several techniques are being used to recover fluke eggs; especially the Kato-Katz (KK) thick smear and the formalin-ether/ethyl acetate concentration techniques are used routinely for human diagnosis (WHO, 2009). However, none of the two techniques are ideal (WHO, 2009) and there is a need for a sensitive, simple, fast and safe method. A technique combining sieving and sedimentation, developed for detection of *Schistosoma japonicum* eggs (Willingham et al., 1998) at the former Danish Bilharziasis Laboratory (DBL) – today part of the Section for Parasitology and Aquatic Diseases at the Department of Veterinary Disease Biology, University of Copenhagen, was recently found efficient for qualitative and quantitative detection of the small trematode eggs in domestic animals (Anh et al., 2008). Since 2007, a modified version of the McMaster flotation method, the FLOTAC technique has been promoted for detection of foodborne trematodes in both human and veterinary parasitology (Cringoli et al., 2010; WHO, 2009), but the technique remains to be validated for small trematode eggs. The KK technique was evaluated for veterinary use by Anh et al. (2008). Although the technique was not found adequate, it has the advantage of being fast, safe and simple to perform in the field.

The objective of this work was to evaluate different coproscopic techniques for detection of small trematode eggs in dog faeces. The DBL and FLOTAC techniques were tested regarding sensitivity, infection intensity, reproducibility and practical applicability. To reduce the amount of sediment retrieved by the DBL technique, a modified version of the DBL technique was later compared to KK thick smear with regards to sensitivity, infection intensity and practical applicability.

## 2. Materials and methods

The study was conducted in May–July 2010 and July 2011 at the National Institute of Veterinary Research (NIVR) in Hanoi, Vietnam. Faecal samples were either collected from four copro-positive dogs purchased from farmers in Nghia Lac commune in the Nam Dinh Province and housed at NIVR (53 subsamples) or taken rectally from household kept dogs in the same geographical area (21 samples). The dogs kept at NIVR were housed in a group according to EU-guidelines and walked twice daily, during which faecal material was collected. Samples were directly stored at 5 °C without preservation.

From the four dogs housed at NIVR, 17 faecal samples were randomly collected and individually homogenised. Subsequently, 1–7 subsamples were isolated from each of the 17 faecal samples in the following way (Fig. 1): one subsample was isolated from 17/17 faecal samples for sensitivity and intensity analysis (producing 17 subsamples); two subsamples (day 0/day 7) were isolated from 8/17 faecal samples for egg recovery following storage analysis (producing 16 subsamples); and finally, four subsamples were isolated from 5/17 faecal samples for reproducibility analysis (producing 20 subsamples). In total, 53 subsamples were isolated and processed by each of the two techniques: 1) The DBL technique as described by Willingham et al. (1998) and 2) the FLOTAC technique according to Cringoli et al. (2010). In brief, the DBL technique was performed as follows: Subsamples of 5 g were washed with saline (0.9%) through a series of three sieves, measuring 400, 100 and 45 μm. The residual was allowed to sediment for 20 and 15 minutes, with discharge of the supernatant in-between. Retained sediment was centrifuged for one minute at 100 g and re-suspended with saline to 2.25 ml. For each subsample, three slides, each representing ⅓ eggs per gram of faeces (EPG), were examined to obtain the result directly in EPG. Hence, 3 × 150 μl of suspension were examined using a 1 ml Sedgewick Rafter counting chamber slide and adding 0.85 ml saline/slide. Floating particles on the surface were removed by cotton buds. For the FLOTAC technique, faecal subsamples of 5 g were homogenised with tap water (dilution ratio: 1:10), filtered through a sieve (500 μm mesh size) and from this, 11 ml was centrifuged for three minutes at 170 g. Retained sediment was suspended in $ZnSO_4{\cdot}7H_2O$ (CAS no. 7446-20-0; Shantou Xilong Chemical Co. Ltd.) to a total volume of 11 ml, corresponding to 1 g faeces, and filled into the two chambers of the FLOTAC apparatus, each holding 5 ml. As no hydrometer was available, the specific gravity of the flotation fluid (sp. gr.: 1.35) was approximated by comparing to the weight of tap water (1 g/ml). A hand centrifuge was used to spin the FLOTAC apparatus prior to examination of both chambers to obtain the EPG.

Analysis of the 53 subsamples were comparatively by the two techniques regarding the 17 and 16 subsamples isolated for sensitivity/intensity and egg recovery, respectively, while the additionally 20 subsamples for reproducibility were isolated from each five different faecal samples and thus non-comparative. Egg recovery was assessed on the day of collection and following storage at 5 °C seven days after collection. Reproducibility was assessed from inter and intra-sample variations. Inter-sample variation was measured for both techniques, as the variation in EPG between 4 replicate egg counts of 5 subsamples. Intra-sample variation was measured as the variation between the 3 consecutive egg counts conducted to obtain EPG for the DBL technique. This was done for each of the 4 replicate egg counts of 5 subsamples (3 × 20 samples). As a measure of reproducibility, the coefficient of variation (CV = standard deviation / mean) was estimated for the 4 × EPG values (inter-samples) or the 3 × 1/3 EPG values (DBL-Intra-samples), when all replica counts were found positive. Furthermore, practical applicability was assessed from the overall performance of all techniques and included simplicity, rapidity, safety and equipment required. Rapidity was measured by estimating the time required for preparation and examination of all samples conducted by each technique.

The 21 faecal samples collected rectally from 21 dogs were processed by two techniques: 1) A modified version of the DBL technique and 2) by KK thick smear. The