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Short Communication

Need for Improved Diagnostics to Screen for Soil-transmitted Helminths

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Soil-transmitted helminths (STHs), namely *Ascaris* spp. (roundworm), *Trichuris* spp. (whipworm) and *Ancylostoma* spp. (hookworm) are parasitic worms that infect both animals and humans. Conventionally, the identification of STH species in animals have relied on coprological or post-mortem examination of the adult worms to identify morphological differences across various species. STH species have also been identified based on egg size (*Trichuris* spp. [1], Hookworm [2]). Misdiagnosis, on the other hand, might arise due to a mismatch in egg size across species. In recent years, the focus of diagnostics on the veterinary side has shifted from merely detecting the presence/absence of infection, to detecting its influence on productivity., in terms of reduced growth, cost of control (anthelminthics), increased risk of co-infection and potential interference with vaccination. An early sensitive and accurate diagnosis can therefore aid in targeted and accurate treatment, reducing economic losses.

The transmission of both roundworm and whipworm occurs through the oral uptake of their infectious eggs. Hookworms, on the other hand, are transmitted through the cutaneous penetration of L_3 larvae. An infected animal sheds eggs through their feces, and the number of eggs shed, defines the intensity of infection [3]. Microscopic demonstration and quantification of eggs in stool are the most popular methods of diagnosing STH infections. Over the last few decades, several microscopic techniques have been used, each with its own set of pros and cons in terms of -- principle of demonstrating eggs, sensitivity, ability to provide accurate egg counts (quantitative vs. qualitative techniques), user-friendliness, and equipment requirements.

Microscopic techniques are broadly classified into qualitative and quantitative. Although qualitative approaches can be used to assess the presence or absence of parasites, they yield imprecise estimates of egg excretion, or fecal egg counts (FECs; expressed as eggs per gram of stool (EPG)). This is in contrast with quantitative techniques that provide accurate FECs. The latter techniques being important to determine the intensity of infections. In addition, they are also essential in determining the efficacy of anthelminthic drugs by means of reduction in egg excretion after treatment [4]. Some popular qualitative techniques are the wet mount smear and the formol-ether concentration methods. On the other hand, widely used quantitative techniques are Kato-Katz thick smear, McMaster, FLOTAC and Mini-FLOTAC. However, the sensitivity of microscopic techniques has always been a cause of concern. A meta-analysis study done by Nikolay et al. to compare the sensitivity and quantitative performance of the commonly used coprological-microscopic diagnostic methods for STH, found varying levels of sensitivity depending on the type of STH being screened for each method namely direct microscopy (43-63%), formol-ether concentration (53 - 81%), Kato-Katz (60 - 83%), McMaster (59 - 82%), FLOTAC (80 - 91%) and Mini-FLOTAC (76 - 79%) [5]. This study employed the Bayesian latent class analysis to estimate the true, unobserved sensitivity of compared diagnostic tests for each of the different STH species.

To date, the Kato-Katz smear is the benchmark diagnostic of STH for the WHO. One of the main reasons for this recommendation is the simplicity of this technique and its ability to screen both STH and *Schistosoma* spp. (*S. mansoni* and *S. japonicum*), other important Neglected Tropical Diseases (NTDs). A major disadvantage of the Kato-Katz thick smear is the restriction of its application to fresh specimens or to those refrigerated for a relatively short period of time [6]. In addition, the amount of stool examined is volumetrically measured, as a consequence of which the multiplication

factor may not always be equal to 24 as the density of stool varies across both between and within subjects [7].

In recent years, there has been a surge in the interest in molecular approaches, despite the obvious benefits of screening stool samples with limited infrastructure and human capability [8-12]. The main rationale being the apparent lack of sensitivity of the microscopic techniques (none of the microscopic techniques shows a sensitivity of more than 93%) [5]. Other reasons put forward are the ability to (i) screen a wide spectrum of pathogens in stool, including but not limited to parasites (ii) speciate the different STH species [10,13], and (iii) detect early development of anthelminthic resistance in STH [14]. The majority of the molecular techniques target the conserved Internal Transcribed Spacer (ITS) 1, 2 and 18s region of the worms [15-17]. Other targets are the mitochondrially encoded cytochrome c oxidase I [18], 16s and 18s rRNA [19]. Indeed, the majority of the recent studies confirm the superior sensitivity of molecular techniques (e.g. qPCR; [20,21] and show clear correlation between FECs obtained through microscopic examination and qPCR outputs (Ct or amount of DNA) [22].

Despite this, studies also indicate some important challenges when employing molecular techniques in the detection of the helminth genome. Beyond the obvious need for better equipped and staffed laboratories, an increasing number of studies are highlighting that molecular techniques do not always detect DNA in microscopic positive samples. For example, a study by Traub et al. failed to pick two percent microscopic stool samples by PCR [23]. This is because, complete lysis of the STH eggshell is crucial for successful extraction of DNA, and subsequently for accurate and precise test results downstream. In the past, a variety of DNA extraction methods have been used to extract DNA from STH eggs in the stool, including heat-shock

steps, chemical treatment with detergents (e.g. SDS) and enzymes (e.g. proteinase K), and bead beating [12,24]. Although these methods have been used to diagnose STH eggs using microscopy, the recovery rate has been low, especially for *Ascaris* and *Trichuris* species [25]. A recent study showed a combination of bead beating and proteinase K treatment (Qiagen kit) helped improve the overall sensitivity of multiplex real-time qPCR [26]. Despite convincing evidence of molecular approaches' enhanced sensitivity, speed, and scalability, there have been only few large-scale investigations using molecular techniques to screen for these parasites. Molecular detection of STH continues to be restricted to the research settings. Nevertheless, having gained crucial technical lessons on molecular techniques' accuracy and cost-effectiveness, it is recommended to adopt this method for routine diagnosis. More importantly, it is crucial to integrate technologies that would aid in successful large-scale, cost-effective screening for various STH infections. For example, use of next-generation sequencing based methods have been shown to be field friendly [27], and allowing to pool various samples, making it cost-efficient.

As most parasitic disease research relies on public-private partnerships, this would require a significant amount of political will and/or philanthropic investment. Given that STH infection adversely affect animal health and threaten profitable animal production, there is a growing need to appropriately diagnose and manage the spread of this infection to reduce the overall economic impact. There is a need to develop and validate newer, rapid, sensitive, specific, cost-effective tests for the detection of STHs, even more so because many STH species are known to crossover and infect human.

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