Laboratory Diagnosis of Selected Neglected Parasitic Diseases in the Philippines: Can we do better?

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ABSTRACT

Background. Several Philippine studies on selected neglected parasitic diseases cite major challenges in laboratory diagnosis that require review of standards, policies and practices.

Objectives. This review aims to: (1) describe the epidemiology of selected neglected parasitic diseases in the Philippines; (2) describe the current status of laboratory diagnosis of these diseases in terms of standards, policies and practices; and (3) identify challenges and opportunities for improvement of laboratory diagnosis.

Methods. Epidemiologic data were collected from published scientific papers and monographs. International standards were gathered from journal articles and the World Health Organization (WHO). Philippine policies were obtained from interviews with the Department of Health (DOH) and other agencies. Recommendations for policy and practice were formulated based on evidence and feasibility.

Results. High prevalence of neglected parasitic diseases in the country, coupled with lack of national policies on diagnosis and quality assurance guidelines specific to parasitology, pose significant challenges to accurate diagnosis.

Conclusion. Results of this review merit the development of a comprehensive Philippine policy on a quality assurance scheme for parasitology laboratories and stronger licensing standards. This policy should be supported through a network of reference centers that coordinate parasitology training, staff certification, and laboratory accreditation.

Key Words: parasitic diseases, laboratories, quality assurance, quality control

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Introduction

Neglected tropical diseases (NTDs) are infectious diseases of poverty that affect over one billion people worldwide, mostly in Africa and the Western Pacific Region. A subset of these diseases is of parasitic etiology, which includes soil-transmitted helminth (STH) infections, schistosomiasis, and foodborne trematode (FBT) infections. Intestinal protozoan infections may also be considered neglected, as they are also seen in areas with poor environmental sanitation and contaminated water supplies.[1]

Several of these selected neglected parasitic diseases have readily available tools for control and elimination, one of which is intensified case management that relies on prompt and accurate diagnosis.[2] In the Philippines, however, major challenges such as lack of training, inappropriate procedures, and inadequate techniques compel a review of current laboratory standards, policies and practices.[3-5] A review of the epidemiology of selected neglected parasitic diseases in the country will also describe the magnitude and distribution of these diseases and more importantly justify the need for more effective disease control through improved diagnosis.

Method

Published scientific papers and monographs containing epidemiology of selected neglected parasitic diseases in the Philippines were retrieved and reviewed. International standards on diagnosis and quality assurance were gathered from journal articles and documents from the World Health Organization (WHO). Philippine policies and practices on diagnosis and quality assurance were obtained from the Department of Health (DOH), as well as key-informant interviews with the DOH Bureau of Health Facilities and Services (BHFS), the Research Institute for Tropical Medicine (RITM), and the University of the Philippines Manila–College of Public Health (UPM-CPH) and College of Medicine (UPM-CM). Information on diagnosis were limited to fecal-based parasitologic techniques. Recommendations for policies and practices were based on available evidence and applicability to the local setting.

Results

Epidemiology of Neglected Parasitic Diseases in the Philippines

Biomedical surveys from the 1960s to the early 1980s have been conducted for selected neglected parasitic diseases in the Philippines. These surveys have reported prevalence rates for *Trichuris trichiura* (65%), *Ascaris lumbricoides* (44%), hookworm (35%), *Schistosoma japonicum* (3%), *Echinostoma ilocanum* (11%), and less than 1% for heterophyids, opisthorchids and *Paragonimus* spp. Intestinal protozoan infections were also reported for non-pathogenic *Entamoeba coli* (21%), *Endolimax nana* (9%) and *Chilomastix mesnili* (1%) infections, as well as pathogenic *Entamoeba histolytica* (5%) and *Giardia lamblia* (6%) infections.[6]

Soil-transmitted Helminth Infections

Soil-transmitted helminth infections remain a persisting public health problem in the Philippines. These infections are highly prevalent in preschool (66%) and school-age children (SAC) (54-66.9%), with heavy intensity prevalence rates as high as 22.1% observed in the latter group.[7-10] The general population are also affected, with prevalence rates ranging from 2 to 43.1% at the provincial level.[3,11,12] Reinfection appears to be the primary mechanism that contributes to persistently high prevalence; a follow-up assessment in a school-based study revealed that STH prevalence rates remained above 40% despite mass drug administration (MDA) strategies.[13]

Schistosomiasis

Schistosomiasis has been reported to have a decreasing prevalence rate from the 1980s to the 1990s. As of 2010, however, schistosomiasis remains endemic in 190 municipalities in 28 provinces of 12 regions in the country.[14] A survey in Visayas and Mindanao noted prevalence ranging from 0 to 3.95% according to province.[12] In contrast, prevalence in northeastern Leyte and Samar in Visayas were as high as 47% in selected villages.[15,16] A large proportion of SAC (31.8%) in Agusan del Sur in Mindanao were also found to have schistosomiasis; 19.3% of which were of moderate to heavy intensity infections.[17] These rates are much higher than the reported 2.5% national prevalence (Lydia R. Leonardo, University of the Philippines Manila, personal communication).[12] In addition, schistosomiasis has been confirmed in newly described areas such as Cagayan Valley.[3]

Foodborne Trematode Infections

Recent studies have revealed FBT prevalence that contrast with initial findings. *Echinostoma* spp. infection has been confirmed in Siargao Island (11.4%) and Davao del Norte (0.2%).[18,19] Heterophyid infection rates ranging from 0 to 27.5% have been observed in Visayas and Mindanao, with a reported prevalence of 5.6% in a recent school-based survey.[12,18] Surveys in Compostela Valley reported much higher heterophyid prevalence (36%, 32.4%), with a substantial proportion (44.7%) of moderate to heavy intensity infections found in one of the surveys.[20,21] Paragonimiasis is another FBT infection that is often misdiagnosed due to symptoms that resemble those of pulmonary tuberculosis (PTB). A study in the province of Sorsogon described paragonimiasis infection rates ranging from 16 to 25% in patients suspected to have PTB.[22] Prevalence of paragonimiasis has also been noted in Davao del Norte (18.8%) and Zamboanga del Norte (14.8%).[23,24]

Intestinal Protozoan Infections

Intestinal protozoan infections also persist, though non-pathogenic infections are predominant. A study in Cagayan Valley found higher prevalence of non-pathogenic species such as *Blastocystis hominis* (8.3%), *E. nana* (6.3%), and *E. coli* (21%); compared with the pathogenic *G. lamblia* (4.9%) and *E. histolytica* (0.8%).[3] Non-pathogenic species also comprise the majority of protozoans found in food handlers and military personnel.[25,26] High *B. hominis* prevalence (40.7%) has been noted in both children and food handlers.[27,28] Overall *E. histolytica* prevalence is low, with infection rates ranging from 0 to 1.9%.[29] It is important to note, however, that these rates may be overestimates; a study in Northern Luzon using PCR-assisted detection discovered that more participants were diagnosed with *Entamoeba dispar* (7.318%), a non-pathogenic species morphologically indistinguishable from *E. histolytica*, compared with the latter (0.961%).[30]

International Standards on Selected Neglected Parasitic Diseases

Laboratory Diagnosis

The laboratory diagnosis of selected neglected parasitic diseases utilizes different stool processing techniques, depending on the parasitic organisms being recovered and the purpose of the diagnosis (e.g. clinical diagnosis, surveillance). These techniques include the direct fecal smear (DFS), Kato Thick, Kato-Katz, and ether-based methods.

Direct fecal smear, also known as direct wet mount, requires mixing 2 mg of stool with 0.85% normal saline solution (NSS) or iodine solution in a glass slide and then examining under a cover slip.[31] NSS mounts detect motile protozoan trophozoites and helminth eggs and larvae, while iodine mounts detect only protozoan cysts. DFS is useful for clinical diagnosis but lacks the sensitivity and quantitative assessment ability required for field surveys. This technique demonstrated varying efficiency in terms of recovery of unfertilized *Ascaris* eggs (69.8%), fertilized *Ascaris* eggs (84.4%), *Trichuris* eggs (67.9%), hookworm eggs (28%), and protozoan cysts (30%).[32] A comparison study found that

more participants were correctly identified with protozoan trophozoites processed via trichrome stain (58.5%) versus DFS (4.8%).[33] Light-intensity infections are also difficult to detect due to the small amount of stool used.[34]

Another technique is the Kato Thick method which requires 50 to 60 mg of stool to be placed over a glass slide and covered with cellophane paper soaked in glycerine-malachite green solution.[35] This method is effective for detection of thick-shelled eggs (e.g. *Ascaris* and *Trichuris*) but not thin-shelled ones (e.g. hookworm); it also cannot detect protozoan cysts or trophozoites. Like DFS, this technique is practical for clinical diagnosis but is limited in field surveys due to its inability to quantitatively assess infection intensity. This method was found to have a high efficiency rate for recovering fertilized (94.4%) versus unfertilized (78.4%) *Ascaris* eggs and *Trichuris* eggs (94.1%), but it performed poorly with hookworm eggs (37.8%).[32] Compared with formalin-ether concentration technique (FECT), this method was reported to have a higher detection rate for *Ascaris* (92.7 versus 90.0%) and *Trichuris* infections (62.3% and 51.1%) and a lower detection rate for hookworm infection (5.8 versus 14.9%).[35]

The Kato-Katz technique differs from the other methods in that it allows for quantitative diagnosis that can describe infection intensity. To perform this technique, a small amount of stool is sieved through a wire screen. A template is used to measure 41.7 mg of stool, which is then covered with cellophane paper soaked in glycerine-malachite green solution. Preparations should be examined within 10 to 20 minutes to avoid clearing of hookworm eggs.[31] This technique is recommended for field surveys of STH and schistosome infections due to ease of use and ability to quantitatively assess infection intensity.[36] However, it is not common in clinical laboratories since diagnosis does not require assessment of infection intensity. In the case of STH infections, Kato-Katz demonstrates sensitivity rates that rarely exceed 70-80%; it also has low sensitivity to light-intensity STH and schistosome infections.[5,37-39] For FBT infections, it may be adequate for identifying infected people for treatment and epidemiologic studies. Exceptions include *Paragonimus* spp., which is better diagnosed by sputum examination, and *Fasciola* spp., which deposits eggs in the bilary tract.[20,40]

Ether-based concentration methods such as FECT recover parasites through sedimentation. These methods involves preserving 1 g of stool in formalin or sodium acetate-acetic acid. After the addition of ether, the sample is centrifuged and the resulting sediment examined for parasite elements. Use of ethyl acetate is a safe and effective alternative for ether, which is highly flammable. These methods can recover STH and schistosome eggs as well as intestinal protozoan cysts, but not trophozoites, as these are destroyed during centrifugation. These methods also facilitate detection of light-intensity infections, which makes them useful for verification of negative DFS results. Limitations include resource requirements and the inability to quantify infection intensity.[34,41] Compared with Kato-Katz, it is more sensitive to *Ascaris* and *Trichuris* infections but less sensitive to hookworm infection.[41,42] FECT demonstrated high efficiency in the recovery of fertilized (93.3%) *Ascaris* eggs and *Trichuris* eggs (95.1%); efficiency was much lower for unfertilized *Ascaris* eggs (38.8%) hookworm eggs (58.6%).[32] For intestinal protozoan cysts, FECT was reported to have a high rate of recovery (98.6%) as well as superior recovery of *E. histolytica* and *G. lamblia* cysts compared with DFS.[32,43] A relatively new ether-based method, FLOTAC, can quantitatively assess helminth and protozoan infections and outperform Kato-Katz in the diagnosis of STH infections.[41,44,45] However, time and resource-intensive preparation limits applicability in the field.[46]

Quality Assurance (QA) of Parasitology Laboratories

Quality assurance (QA) is the set of processes that ensures correct and relevant diagnosis. It involves the maintenance of a quality management system as well as standards of laboratory personnel and equipment.[47] A quality management system encompasses documentation, Standard Operating Procedures (SOPs), Quality Control (QC), and an External Quality Assessment Scheme (EQAS).[48]

QC is an internal monitoring of working practices and technical procedures.[49] It ensures collection of satisfactory specimens, proper preparation and maintenance of reagents, correct processing techniques and stool examination, and routine recording and reporting of results.[34] For the surveillance of STH and schistosome infections, it is recommended that additional QC measures be practiced, such as validation of slides through comparison of the readings of the microscopists with those of the reference microscopist.[36]

EQAS is also known as proficiency testing. It evaluates the entire process of diagnosis, from receiving and testing of samples to reporting of results.[48] In the United States (U.S.), the Clinical Laboratory Improvement Amendments (CLIA) requires all parasitology laboratories to undergo proficiency testing.[50] In the United Kingdom (UK), there is a national EQAS (NEQAS) for parasitology that tests identification of parasites in fecal smears, formalinized fecal samples, and urine and cyst suspensions. Vital to the UK NEQAS are its training programs that help identify reoccurring problems in parasite identification, which are subsequently addressed by providing teaching materials.[51]

In addition to a quality management system, QA for parasitology laboratories also requires maintenance of equipment and supplies such as protecting microscopes from dust, vibrations and moisture, as well as routine cleaning of lenses and alignment of condensers. Centrifuges should be cleaned regularly, with speed checks every six months to ensure proper function of brushes and bearings.[52]

Proper storage conditions should be followed for all reagents and solutions. Although the ideal frequency of replacement varies among solutions, labeling with the date of preparation will facilitate timely replacement. For example, while formalin and isotonic saline solutions can last indefinitely if properly stored, iodine solution must be replaced every 14 days. Extra precautions should also be taken for flammable reagents such as ether, which should be stored in stoppered containers on a cool and open shelf.[34]

Philippines Policies on Selected Neglected Parasitic Diseases

Laboratory Diagnosis

Existing guidelines include those for schistosomiasis, which specifies Kato-Katz; and paragonimiasis, which specifies sputum processing and FECT.[14,53,54] There is also an existing policy on the diagnosis of intestinal protozoa, but it is limited to FECT examination of food handlers and is not properly implemented.[55,56] Only DFS is required in clinical laboratories, though this is not explicitly mentioned in License to Operate (LTO) standards (Cynthia Rosuman, Department of Health, personal communication).[57] However, several neglected parasitic diseases in the Philippines lack policies on laboratory diagnosis. There is currently no existing national policy on the diagnosis of STH infections, as well as non-*Salmonella* foodborne diseases, which include other FBT infections aside from paragonimiasis.

Quality Assurance (QA) of Parasitology Laboratories

There is currently no Philippine policy on QA for laboratories diagnosing neglected parasitic diseases. There are, however, general standards that clinical laboratories must satisfy to renew their LTO, including practice of Internal QA and documentation of Internal QC. These standards require employment of qualified staff with documented training and experience. Facilities should be well-lighted, clean, and safe, with adequate space and ventilation. Equipment and reagents should be in good working order. There should also be routine maintenance of equipment and monitoring of facilities, as well as recording and reporting of results. None of the aforementioned standards, however, explicitly mention requirements specific to parasitology. There is also no mention of reference or accreditation schemes for parasitology laboratories.[57,58]

All laboratories are required to participate in a NEQAS administered by designated National Reference Laboratories (NRLs). The LTO may be revoked, suspended or modified for laboratories that refuse to participate; in the case of parasitology, there are no penalties for poor performance.[59] The Research Institute for Tropical Medicine (RITM) is the NRL in charge of the NEQAS for parasitology. For the NEQAS, formalin-preserved fecal samples with helminth eggs (*A. lumbricoides*, *T. trichiura*, hookworm) and cryovials containing intestinal protozoan cysts (*G. lamblia*, *E. coli*) are given to participating laboratories for diagnosis.[60] Feedback is limited to regional meetings attended by personnel from the DOH and participating laboratories; there is no distribution of individual feedback to each laboratory (Donato G. Esperar, RITM, personal communication). Furthermore, the NEQAS in 2009 found that only 60.8% of the 288 participating laboratories scored higher than the cut-off score of 75.0%. Although high identification rates of *A. lumbricoides* (98.6%), *T. trichiura* (97.1%), hookworm (99.1%), *E. coli* (92.3%) and *B. hominis* (100%) were noted; 126 laboratories (43.8%) made errors of over-diagnosis, 43 (14.9%) made incorrect identifications, and 68 (23.6%) overlooked parasites.[60]

The Philippine Council for Quality Assurance in Clinical Laboratories (PCQACL) has carried out over 20 NEQAS for hematology, clinical chemistry, coagulation and blood banking.[61] Although plans of a NEQAS for parasitology were mentioned in 2009, there has been no implementation (Ariel M. Vergel de Dios, University of the Philippines Manila, personal communication).

Practices and Challenges of Laboratory Diagnosis in the Philippines

Parasitology laboratories in the Philippines lack adequate QC measures, facilities, and resources. For instance, out of 55 parasitology laboratories in Iloilo, only five (9.1%) had updated SOP manuals. The majority (76.4%) of laboratories did not have an exhaust fan; only four (7.3%) possessed safety hoods. Almost a third (32.7%) of laboratories had insufficient working space. Over half (58.2%) had necessary reagents and supplies but no QC; 20 (36.8%) lacked both. Only two (3.7%) laboratories used non-DFS techniques. Most (61.8%) had high quality microscopes but did not carry either spare bulbs or automatic voltage regulators.[4] Microscope maintenance issues such as fungi on the eyepiece and unclear objectives have also been observed (Esperar, personal communication).

Several reports indicate low levels of parasite recognition among laboratory staff. Only 2.2% of Iloilo laboratory staff passed a parasite identification test.[4] Validation conducted in an STH study revealed a sensitivity rate of 64.3%.[18] In a schistosomiasis survey, only 58.5% of positive slides were read correctly.[17] FBT infections are also underreported, as evidenced by survey findings of high heterophyid prevalence and misdiagnosis of paragonimiasis. Low sensitivity to *E. histolytica* detection was noted for both specially trained (56.8%) and regular (16.2%) laboratory staff.[31]

In addition, existing programs for parasitology training in the Philippines provide limited opportunity. Medical technology programs require one semester of parasitology, as well as a clinical laboratory internship that includes a

period of two weeks in the parasitology section. Continuing training programs offered by the UPM-CPH and the RITM cover stool processing techniques, parasite identification, and QA measures. However, both programs can only accommodate a limited number of participants.[62] Trainings on the diagnosis of STH infections, schistosomiasis, and FBT infections are supported by the DOH but on a non-regular basis. A current WHO-RITM training initiative aims to build sub-national capacity for detection of infectious disease outbreaks (Esperar, personal communication).

Discussion

The high prevalence of neglected parasitic diseases despite current control efforts underscores the need for prompt and accurate diagnosis. Schistosomiasis surveys suggest that control efforts have not achieved the 2010 target of less than 1% national prevalence.[63] The persistence of these infections mirrors the situation in China, where prevalence remains high in several areas despite existing control efforts.[64,65] Reported FBT infection rates also indicate a need for control in selected areas; such areas may need to be identified through nationwide surveys. These surveys should also include intestinal protozoan infections, since current data on these infections are limited. Accurate diagnosis is essential to the success of these surveys, as the resulting data will form the basis of treatment, control and surveillance efforts.[17,66]

Progress towards accurate diagnosis is hampered by the lack of national policies and guidelines in the country. There is no explicit LTO standard for stool examination. Most laboratories in the country use DFS, which may overlook parasites. Other techniques have their own limitations; Kato-Katz sensitivity decreases for light-intensity infections, while ether-based methods can be resource-intensive.[39,34] As such, Philippine policy on diagnosis should require multiple examinations and techniques as necessary to improve sensitivity (Table 1).[38] Moreover, the combination of techniques used for epidemiologic studies should depend on the parasitic organisms being recovered.

Table 1. Proposed Stool Processing Techniques For Specific Situations.

Situation	Appropriate technique(s)
Routine clinic or hospital stool examination	DFS + Kato Thick (if initial diagnosis is negative)
STH and schistosomiasis surveillance	Kato-Katz
Health certification of food handlers and overseas Filipino workers	FECT, DFS + Kato Thick (alternative)
Epidemiologic investigation	DFS, Kato Thick, Kato-Katz, FECT

Accurate diagnosis is also hindered by the absence of a comprehensive QA policy for parasitology laboratories. Existing LTO standards do not mention training requirements or internal QC measures specific to parasitology. Standards regarding facilities, equipment, and supplies fail to cite minimum requirements for parasitology. The exclusion of emerging parasites as well as the lack of penalties for poor performance and individual feedback for laboratories limits the effectiveness of NEQAS. No reference scheme is in place to assist with difficult diagnoses, and the absence of an accreditation scheme leaves no incentive for improvement. These policy gaps are evident in observed laboratory deficiencies. This policy situation is not unique to the Philippines; with the exception of Thailand, several countries in the Western Pacific region either lack QA policy or are still in the early stages of policy development and implementation.[67,68] These countries are potentially missing out on opportunities for capacity building, as various studies have shown that proper implementation of QA can improve proficiency in parasitology work.[69,70]

Thus, it is recommended that comprehensive QA policy for parasitology laboratories be developed. SOPs should be updated under the guidance of recognized parasitology experts and subsequently properly implemented. LTO standards should require laboratory staff to be certified in parasitology, through completion of recognized training programs, in addition to parasitology QC measures such as validation of slides. Standards regarding facilities, equipment, and reagents should include a list of minimum requirements for parasitology. The NEQAS should have a licensing cut-off score and include emerging parasites. Results of NEQAS should be communicated in a timely manner, through individual feedback, to all participating laboratories. The establishment of a reference scheme will provide invaluable technical support to the laboratory staff. An accreditation program should also be created to reward parasitology laboratories fulfilling local and international standards.

An integral aspect to timely identification of endemic parasitic diseases is proper training of laboratory staff.[11] Low proficiency in the diagnosis of neglected parasitic diseases has been noted by several studies, with sensitivity rates as low as 16.2%. Low diagnostic proficiency has also been noted for other parasitic diseases; one malaria study revealed a sensitivity rate of 55% among laboratory staff in Agusan del Sur.[71] In order to improve training, the current medical technology curriculum should be revised to emphasize NTDs of global importance. There should also be capability building through creation of an institutionalized network of parasitology reference centers, which offers training for basic diagnostic parasitology, trainer and expert certification, as well as a periodic refresher course for technology updates and its applications to delivery of health services in the laboratory setting. The government or other funding agencies may need to allocate funds for these centers, as laboratory diagnosis falls under the services prioritized in the Magna Carta for public health workers and the current Philippine President's Health Agenda.[72,73]

Conclusion

Results of this review merit the development of a comprehensive Philippine policy on laboratory diagnosis of neglected parasitic diseases. Epidemiological data show persistence of these diseases, while gaps in laboratory diagnosis and processes pose significant challenges for accurate laboratory diagnosis. Therefore, a QA scheme for parasitology laboratories in terms of procedures, facilities, supplies, training, and support should be implemented, along with strengthening of LTO standards. Mandatory parasitology training should also be offered through an institutionalized network of reference centers. A national policy for quality assurance in parasitologic laboratories will improve control and prevention efforts of neglected parasitic diseases in the country. More importantly, this national policy will indicate that challenges are being addressed head-on and that indeed, the country can do better.

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