Diagnosis of *Opisthorchis viverrini* Infection with Handheld Microscopy in Lao People's Democratic Republic

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Abstract. Opisthorchiasis is a neglected tropical disease, yet it is of considerable public health importance in Southeast Asia given the predilection for chronically infected persons to develop cholangiocarcinoma. We evaluated a handheld microscope for the diagnosis of *Opisthorchis viverrini* in a community-based setting in Lao People's Democratic Republic in comparison with conventional light microscopy. In stool samples collected from 104 individuals, handheld microscopy revealed a sensitivity of 70.6% and a specificity of 89.5% for *O. viverrini* infection. Pearson's correlation for quantitative fecal egg counts between the two devices was 0.98 (95% confidence interval: 0.98–0.99). With small adjustments to further increase diagnostic sensitivity, a handheld microscope may become a helpful tool to screen for *O. viverrini* and other helminth infections in public health settings.

INTRODUCTION

New diagnostic approaches are necessary to meet the needs of individuals living in resource-constrained settings where access to quality diagnostic testing and other interventions are limited.^{1–4} Recently, handheld and mobile phone-based microscopes have been used in public health settings to screen for endemic parasitic diseases, such as malaria, schistosomiasis, and soil-transmitted helminthiasis.^{5–10} Such devices may improve access to laboratory diagnoses for clinical and public health practices in resource-constrained settings given their portability and ease of use, thus facilitating point-of-care diagnosis and subsequent patient management.

Opisthorchis viverrini is a liver fluke, common in Southeast Asia, which is acquired by consumption of infected raw or undercooked fish.¹¹ Over 60 million people are at risk of infection and an estimated 9 million are actively infected with *O. viverrini*, with northern Thailand and central and southern Lao People's Democratic Republic (Lao PDR) heavily affected.^{12,13} Chronic infection with *O. viverrini* poses a major public health threat given the predisposition for individuals to develop cholangiocarcinoma, portending a very poor clinical outcome.¹⁴

Here we compare the diagnostic accuracy of a handheld microscope to traditional light microscopy for the detection and quantification of *O. viverrini* infection in an endemic setting of Lao PDR. We discuss the potential usefulness of such devices for mobile public health teams to screen affected populations and for improved patient management at the point of care.

METHODS

This study was integrated into a pharmacokinetic and dose-finding study of praziquantel, the current drug of choice

against opisthorchiasis. In September 2014, individuals aged 15–65 years who were potentially infected with *O. viverrini* in three rural communities in southern Lao PDR were screened for enrollment. Ethical approval for this study was granted by the National Ethics Committee for Health Research, Ministry of Health, Lao PDR (reference no. 009/NECHR), and the Ethics Committee of Northern and Central Switzerland (reference no. 2014-162). The trial is registered at Randomized Controlled Trials (identifier: ISRCTN77186750). Participants provided written informed consent.

Before praziquantel administration, we collected three stool samples from each participant on different days within a maximum of 5 days. Stool was processed by the Kato-Katz thick smear technique, with 41.7 mg of stool per slide.¹⁵ Slides were examined by senior microscopists via light microscopy using 10×, 20×, and 40× objective lenses on an Olympus CX21 microscope (Olympus, Volketswil, Switzerland). The entire Kato-Katz thick smear was examined for a minimum of 3 min, and the presence or absence of *O. viverrini* eggs was noted for each individual, and eggs were manually quantified if present. Ten percent of all slides were re-examined by a second expert microscopist blinded to the initial reading to ensure quality control.¹⁶

A total of 104 slides from 104 individuals were randomly selected to be re-examined with a Newton Nm1 microscope (Newton Microscopes, Cambridge, United Kingdom) on the same day of stool collection. This is a commercially available, handheld microscope that weighs approximately 480 g and is equipped with modular 10×, 40×, and 100× objective lenses. A senior microscopist, who did not participate in the conventional light microscopy and was blinded to prior results, evaluated slides using this device and recorded the presence or absence of *O. viverrini* eggs, and quantified eggs in positive samples. A reversed lens mobile phone microscope was also initially used to examine slides; however, there were challenges reliably visualizing eggs with this device and it was not used further in this study.

Data were directly entered into an Excel file (Microsoft, Redmond, WA). We measured the sensitivity and specificity of the Newton microscope using conventional light microscopy

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considered as the “gold” standard, calculated exact binomial confidence intervals (CIs), and calculated positive predictive values (PPVs) and negative predictive values (NPVs). We modeled the sensitivity of the Newton Nm1 microscope as a function of eggs per gram of stool (EPG) by conventional microscopy using logistic regression. We compared dichotomous agreement using Cohen’s κ and quantitative agreement using Pearson’s correlation. All analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 104 slides subjected to both diagnostic approaches, conventional microscopy identified *O. viverrini* eggs in 85 slides (81.7%). Eighty-two slides had low egg counts corresponding to light-intensity infections (1–999 EPG), two slides had moderate egg counts (1,000–9,999 EPG), and one slide demonstrated heavy-intensity infection (> 10,000 EPG).¹⁸ The Newton Nm1 microscope was 70.6% (95% CI: 59.6–79.7%) sensitive and 89.5% (95% CI: 65.5–98.2%) specific for *O. viverrini* diagnosis, with a PPV of 96.8% (95% CI: 87.8–99.4%) and an NPV of 40.5% (95% CI: 26.0–56.7%) (Table 1).

As the intensity of infection increased, so did diagnostic sensitivity, with sensitivity exceeding 90% at 75 EPG and reaching 100% at > 120 EPG (Figure 1). Comparing conventional light microscopy to the Newton Nm1 microscope for *O. viverrini* diagnosis, Cohen’s κ was 0.41 (95% CI: 0.41–0.57), indicating moderate agreement. We found a very high Pearson’s correlation of 0.98 (95% CI: 0.98–0.99).

DISCUSSION

New pragmatic strategies are required to diagnose neglected tropical diseases given the lack of readily available tools in most endemic countries. Portable and mobile phone microscopy may be a practical solution to many of the current barriers to quality diagnostic testing in rural, remote, and resource-constrained settings. Herein, we show that the Newton Nm1 field microscope has moderate sensitivity and very good specificity for the diagnosis of *O. viverrini* infection within the frame of a community-based screening approach in a highly endemic area of Lao PDR.

The moderate sensitivity of the Newton Nm1 microscope may be due to a variety of reasons. First, *O. viverrini* eggs are quite small and translucent.¹⁹ Hence, given the high proportion of light-intensity infections in this setting (82 of the 85 *O. viverrini*-positive individuals had fewer than 1,000 EPG,

FIGURE 1. Estimated sensitivity of the Newton Nm1 microscope as a function of infection intensity (EPG) as estimated by conventional microscopy. The shaded bars in the histogram depict the distribution of infection intensity in the study population. At an infection intensity > 120 EPG, the sensitivity was 100%.

which translates to < 42 eggs per slide), microscopists may have simply not visualized eggs while scanning over the Kato-Katz thick smears with this device. Second, as the device is handheld, microscopists may miscount eggs due to motion artifact. We feel that the device is best used with a small tripod attached to ensure image stability and prevent user’s fatigue when examining slides. In addition, the stage of the Newton Nm1 microscope is designed to secure and maneuver a microscope slide in the x and y axes as per conventional microscopes. Because of the Newton Nm1’s compact design, the extent of slide motion is limited compared with conventional light microscopes, and if a Kato-Katz thick smear is spread out over a wide area on a slide, the edges of that thick smear may not be visualized with the Newton Nm1 device. This issue was first noted during a field study evaluating handheld microscopes for the diagnosis of *Schistosoma mansoni* and *Schistosoma haematobium* in Côte d’Ivoire, where, on average, fewer eggs were counted on slides examined with portable microscopes compared with conventional microscopes.⁷ Subsequent quantification and analytic studies demonstrated excellent correlation and concordance between the Newton Nm1 microscope and conventional light microscopy when egg counts were multiplied by a “correction factor.”⁸

Potential solutions to mitigate the Newton Nm1 microscope’s decreased sensitivity include ensuring that the specimen is placed on the center of the slide and that the sample is not spread over too wide an area. This simple adjustment will ensure that the microscopist can visualize the entire sample. Other approaches to increase diagnostic sensitivity include examining multiple slides as the Kato-Katz technique may have false-negative readings while examining slides with low infection intensity.^{20–22} Because of the high prevalence of *O. viverrini* infection and moderate sensitivity of this device, the NPV was somewhat poor, and hence this device was not reliable in ruling out infections. In addition, the prevalence of infection was higher in this study compared with prior field studies.^{23,24} We would expect the PPV of the Newton portable microscope to decline in settings with lower *O. viverrini* prevalence, and the NPV to increase in such settings. However, the device demonstrated excellent sensitivity at higher egg intensities (particularly at 120 EPG, which are still categorized as light-intensity infections) and had good quantitative correlation with conventional microscopy; both of these attributes make it potentially useful for epidemiologic surveys in remote settings. Since only an expert microscopist used the

TABLE 1

Diagnostic operating characteristics of the Newton Nm1 microscope for *Opisthorchis viverrini* infection in a community-based screening in Lao PDR in September 2014

		Newton Nm1 microscope		Total
		Negative	Positive	
Conventional microscopy	Negative	17	2	19
	Positive	25	60	85
	Total	42	62	104
	Sensitivity	70.6% (95% CI: 59.6–79.7%)		
	Specificity	89.5% (95% CI: 65.5–98.2%)		
	PPV	96.8% (95% CI: 87.8–99.4%)		
	NPV	40.5% (95% CI: 26.0–56.7%)		

NPV = negative predictive value; PPV = positive predictive value.

Newton Nm1 microscope, future studies should also evaluate the use of this device by frontline health-care personnel, such as laboratory technicians.

The Newton Nm1 microscope requires three AAA batteries and can provide over 200 hours of continuous use. A differential price structure exists to allow for greater affordability and use in low-income countries. As the Newton Nm1 microscope functions like a miniaturized version of a conventional light microscope, slides must still be prepared as per routine laboratory protocols prior to viewing. The advantages of this device include its light weight (480 g), ease of use, portability, and that sample preparation for many neglected tropical diseases (e.g., intestinal and biliary parasitoses) and malaria can be easily performed in field settings, making this device a potentially helpful diagnostic tool outside conventional laboratories.

In conclusion, handheld light microscopy shows promise as a valuable public health tool for the detection and quantification of neglected tropical infections, such as *O. viverrini* in community-based settings. The role of these handheld devices for improved case management at the point of care warrants follow-up studies.

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