Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* infections in humans in the absence of a ‘gold standard’

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**ABSTRACT**

The accuracy of the Kato-Katz technique in identifying individuals with soil-transmitted helminth (STH) infections is limited by day-to-day variation in helminth egg excretion, confusion with other parasites and the laboratory technicians’ experience. We aimed to estimate the sensitivity and specificity of the Kato-Katz technique to detect infection with *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* using a Bayesian approach in the absence of a ‘gold standard’. Data were obtained from a longitudinal study conducted between January 2004 and December 2005 in Samar Province, the Philippines. Each participant provided between one and three stool samples over consecutive days. Stool samples were examined using the Kato-Katz technique and reported as positive or negative for STHs. In the presence of measurement error, the true status of each individual is considered as latent data. Using a Bayesian method, we calculated marginal posterior densities of sensitivity and specificity parameters from the product of the likelihood function of observed and latent data. A uniform prior distribution was used (beta distribution: \(\alpha = 1\), \(\beta = 1\)). A total of 5624 individuals provided at least one stool sample. One, two and three stool samples were provided by 1582, 1893 and 2149 individuals, respectively. All STHs showed variation in test results from day to day. Sensitivity estimates of the Kato-Katz technique for one stool sample were 96.9% (95% Bayesian Credible Interval [BCI]: 96.1%, 97.6%), 65.2% (60.0%, 69.8%) and 91.4% (90.5%, 92.3%), for *A. lumbricoides*, hookworm and *T. trichiura*, respectively. Specificity estimates for one stool sample were 96.1% (95.5%, 96.7%), 93.8% (92.4%, 95.4%) and 94.4% (93.2%, 95.5%), for *A. lumbricoides*, hookworm and *T. trichiura*, respectively. Our results show that the Kato-Katz technique can perform with reasonable accuracy with one day’s stool collection for *A. lumbricoides* and *T. trichiura*. Low sensitivity of the Kato-Katz for detection of hookworm infection may be related to rapid degeneration of delicate hookworm eggs with time.

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1. Introduction

Soil-transmitted helminthiases include infections with roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm (*Ancylostoma duodenale* and *Necator americanus*), which are the most prevalent and geographically widespread parasitic infections in the world. Soil-transmitted helminth (STH) infections are one of the major causes of cognitive and physical growth impairment, and anemia in children (Ezeamama et al., 2005; Bethony et al., 2006; Lammie et al., 2006). Transmission of these helminthic infections occurs mostly in the tropical and sub-tropical regions and is of major public health importance. Humid soil plays an important role in the development and transmission of these worms (Belo et al., 2005; Bethony et al., 2006). Transmission is also influenced by inadequate sanitation and absence of a clean water supply, and poverty (de Silva et al., 2003; Utzinger et al., 2003; Bethony et al., 2006). The transmission process begins when eggs are passed in human feces and contaminate the soil. Examination of human feces for helminth eggs is the most widely used diagnostic techniques for detection of STH infection.

Among the diagnostic methods that are used to detect the presence of STH ova in stool samples, the Kato-Katz (Peters et al., 1980) is the most commonly utilised technique (Goodman et al., 2007; Knopp et al., 2008; Utzinger et al., 2008). Due to the ease of its use in the field...
and relatively low cost, the Kato-Katz technique is recommended by the World Health Organization (WHO) for surveillance and epidemiological field survey of STH infections (WHO, 1994; Montresor et al., 1998). Reports from previous studies suggest that fecal STH egg output varies from day to day (Hall, 1981; Anderson and Schad, 1985; Booth et al., 2003). The sensitivity of the Kato-Katz technique in determining STH infection from a single stool is limited by this day-to-day variation in egg excretion leading to measurement error in estimating the presence of infection. For this reason, examination of several stool specimens collected over consecutive days is recommended to improve the sensitivity of the test (Utzinger et al., 1999; Booth et al., 2003). However, a collection of several specimens over consecutive days may reduce the specificity of the test. A reduced specificity can occur if STH eggs are confused with eggs of other helminth species. In addition, collection of the desired and equal number of stool samples on consecutive days from all study participants is rarely achieved. Knowing the accuracy of a diagnostic test in detection of infection is important in public health practice as it relates to accurate estimation of infection prevalence and incidence, and to the validity of conclusions drawn from epidemiological association studies.

A ‘gold standard’ test, against which sensitivity and specificity (accuracy) of other tests are estimated, is almost never available in parasitology. A ‘gold standard’ test (with 100% accuracy) does not exist for detection of STH infection. We conducted a literature search to obtain papers that reported accuracy of the Kato-Katz technique in determining STH infection. We found six papers that reported sensitivity and/or specificity of the Kato-Katz technique for detecting STH infection (Booth et al., 2003; Santos et al., 2005; Goodman et al., 2007; Knopp et al., 2008; Steinmann et al., 2008; Utzinger et al., 2008). Four of these papers estimated sensitivity and/or specificity of the Kato-Katz against another test or a combination of tests, none of which is 100% accurate (‘pseudo gold standard’) (Santos et al., 2005; Goodman et al., 2007; Steinmann et al., 2008; Utzinger et al., 2008). Knopp and others (2008) used a mathematical model to calculate sensitivity of Kato-Katz for STH infections assuming perfect specificity for the test (Mullen and Prost, 1983; Marti and Koella, 1993; Knopp et al., 2008). Booth and others (2003) used a Bayesian latent class model to estimate sensitivity of Kato-Katz in detecting hookworm infection (Booth et al., 2003). However, to carry out this estimation procedure the constraint of 100% specificity was imposed.

STHs are prevalent in the Philippines as in most of the developing world. Previous studies, conducted in different provinces and cities of the Philippines, reported a moderate to high prevalence of *A. duodenale*, hookworm and *T. trichiura* (Juco et al., 1973; Carney et al., 1980, 1981; Baldo et al., 2004). According to the national survey on STHs conducted in 2004, 16 out of 17 regions in the country had prevalences greater than 50% (WHO, 2008). Although national surveys are conducted periodically in the region, the prevalence estimates from these surveys likely underestimate the actual prevalence of infection as they often survey only a certain age group (mostly children), are not conducted by highly trained field technicians, and only one stool sample is typically analysed. Moreover, STH prevalence estimates from these surveys and those reported by previous studies conducted in different provinces and cities of the Philippines were not adjusted for misclassification and village level clustering.

A Bayesian technique is available to estimate sensitivity and specificity of a diagnostic test in the absence of a ‘gold standard’ test and without putting any constraint on any of the test parameters (Joseph et al., 1995). The objective of this analysis is to estimate the sensitivity and specificity of the Kato-Katz stool examination technique to detect infection with hookworm, *Ascaris* and *Trichuris*, adapting the Bayesian statistical method described by Joseph and colleagues (1995) to our particular situation.

2. Materials and methods

2.1. Source of data

This study involved secondary analysis of baseline data from a longitudinal study conducted between January 2004 and December 2005 in the province of Samar of the Eastern Visayas, the Philippines. The main purpose of the original study was to assess the effect of water management systems and animal management on the transmission dynamics of *Schistosoma japonicum* infection. The design of that study and sampling approach was, therefore, adopted to answer that particular question (McGarvey et al., 2006; Tarafder et al., 2006).

2.2. Study population

There were 134 villages endemic for *S. japonicum* in the Samar province in 2002 (McGarvey et al., 2006). Of these 134 villages, 75 villages were eligible for participation in the study based on the following criteria: (i) safety of the field team; (ii) relatively accessible (travel time by boat should be no more than 2 h; hike not more than 15 min); (iii) at least 50 households in the village; (iv) not located near seacoast; and (v) not a peri-urban village. All of these villages were rice-farming communities. From these 75 villages, 25 primarily rain-fed villages and 25 villages with some form of man-made irrigation system were selected based on a set of criteria that assessed villages’ irrigation and farming characteristics. The following criteria were used to select the 25 rain-fed villages: (i) 90–100% of rice-farming hectares in the village are rain-fed; (ii) the absence of National Irrigation Administration projects or Department of Agriculture assisted pumps or dams; (iii) the absence of polyvinyl chloride or cemented canals; and (iv) a minimum of 15 hectares committed to rice farming. The 25 irrigated villages were chosen as follows. Fifteen villages were chosen based on (i) the presence of sophisticated irrigation systems and a minimum of at least seven irrigated hectares and (ii) at least 20% of the total hectares were irrigated. Ten additional villages were chosen based on having the largest area of irrigated farm land. The proportion of irrigated farm land in the selected irrigated villages ranged from 22.7% to 100%.

A maximum of 35 eligible households were randomly selected from each eligible village. Only those households that had at least five members were eligible for inclusion. In rain-fed villages, households where at least one member worked full-time on a rain-fed farm were eligible for selection. In irrigated villages, households where at least one member worked most of the time (more than 50% of the time) in an irrigated farm were eligible for sampling. When 35 or fewer households were eligible in a village, they were all invited to participate in the study. The head of each household was asked for his or her consent to participate. When the head of the household refused to participate, the head of the next available and eligible household was invited to participate.

At most six individuals including at least one full-time farmer were selected at random from each household. If there were six members or less in the household, all members were invited to participate. All selected household members were asked for their consent to participate. When an individual refused to participate, they were replaced by another consenting household member.

2.3. Stool collection and parasitological examination

Stool samples collected at baseline were examined for the presence of eggs of several parasites, including the three STHs, namely hookworms (*N. americanus* and *A. duodenale*), *A. lumbricoides* and *T. trichiura*.
trichiura, in addition to S. japonicum. Participants were asked to provide one stool sample (morning or first) per day for three consecutive days. Each participant provided between one and three stool samples. If a participant provided a stool sample on one of the three days but was unable for any reason to provide stool samples on other days, that person was considered as a stool sample provider. Stool envelopes (of wax paper and book paper) with pop-sticks were distributed to participants on the first day of stool sample collection. At least thumb-size stool samples were submitted. Portions from different parts of the stool were taken to fill up the envelope. Although consistency of the stool sample was not recorded, only pasty to formed stool could be accommodated in the stool envelopes. Stool samples were processed 2–3 h after collection. Two slides were prepared from each stool sample. All slides were placed in a styrofoam box with cold packs inside at the end of each collection day. At the end of each collection week, all slides were brought to a designated laboratory and transferred to a refrigerator. The time delay between stool sample processing and microscopic reading associated with day one stool collection (provided by 99.45% of participants) ranged from less than 24 h to as long as 20 days with a median of 4 days (inter-quartile range: 2–6 days). The Kato-Katz technique (Peters et al., 1980) was used to detect the helminth eggs in stool samples. Although eggs of each of the STHs were originally documented qualitatively in five response categories (0, + through ++++), the outcome variable for the present analysis was coded as a dichotomous variable, i.e., presence or absence of helminth eggs in each stool sample (observed infected or uninfected, respectively). Laboratory technicians were blinded to the identity of the provider of the stool sample they were preparing and reading and did not know if two stool samples were from the same participant (two consecutive day’s sample). During microscopic examination, the laboratory technicians did not distinguish between N. americanus and A. duodenale eggs, therefore both were considered together as hookworm eggs, although prior reports from The Philippines found exclusively N. americanus infections (Olds et al., 1999).

2.4. Statistical analyses

We obtained between one and three stool samples on consecutive days from those participants who provided at least one sample. The estimates of sensitivity and specificity of the Kato-Katz technique to detect STH infection based on examination of 1 day stool sample will be different from the estimates of sensitivity and specificity of the technique to detect STH infection based on examination of 2 days or 3 days stool samples due to day-to-day variation in STH egg output (Hall, 1981; Anderson and Schad, 1985; Booth et al., 2003). To estimate the sensitivities and specificities of the Kato-Katz stool examination technique when one, two or three stool samples are available from each participant, we adapted the method of Joseph et al. (1995) to accommodate multiple samples and used previously in the analyses of S. japonicum in animals (Carabin et al., 2005) and in humans (McGarvey et al., 2006; Tarafder et al., 2006) in the Philippines (Joseph et al., 1995).

The probability of any single test being positive is the sum of the probability of a true positive result and the probability of a false positive result. If P is the probability of a positive test, then

\[ P = \text{probability of true positive} + \text{probability of false positive} = [\text{true prevalence} \times \text{sensitivity}] + [(1 - \text{true prevalence}) \times (1 - \text{specificity})]. \]

When there is more than one test per person, probability P is used as the probability parameter of a binomial distribution and the product of binomial distributions obtained from each participant is used (Carabin et al., 2005). True positive and false positive are missing information and are known as ‘latent data’. According to Bayes’ theorem, the joint posterior distribution is proportional to the product of the likelihood function and prior distribution. Using this theorem, we can get marginal posterior densities of sensitivity and specificity parameters from the product of the likelihood function of observed and latent data, and prior distributions of all model parameters. Direct use of this equation is not possible as the latent data are not observed. However, inferences about the marginal posterior distribution of sensitivity and specificity from this equation are possible using a Gibbs sampler algorithm. The main concept of this process is that if values of prevalence and all test parameters are known, then it is possible to derive the posterior distribution of the latent data. Also, if latent data are known, then posterior distributions of prevalence and test parameters can be derived given the prior distributions. A constructed algorithm alternates between these two steps. This algorithm (Gibbs sampler algorithm) can provide random samples from marginal posterior densities of each parameter of interest. Based on these random samples, posterior medians can be constructed and used as point estimates for sensitivity and specificity, reported with 95% Bayesian credible intervals (95% BCI) (Bayesian analogue of confidence intervals) (Joseph et al., 1985).

Three separate Bayesian models were created for each of the STHs based on the statistical method described above. In this analysis, we assumed that prior information has beta distribution. Uniform (uninformative) prior distributions on the range from 0 to 1 (parameters of the beta distribution: \( \alpha = 1, \beta = 1 \)) were used for sensitivity, specificity, and prevalence of all three STH infections. For each of the STH models, informative priors, derived from a combination of information obtained from review of published literature and expert opinion, were also used for sensitivity and specificity to assess how dependent our results were on the choice of priors (Table 1). The estimates of sensitivity and specificity were

![Table 1](image)

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<th>Ascaris</th>
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<td>Informativ(^*) Sensitivity</td>
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<td>Informativ(^*) Specificity</td>
<td>85–100</td>
<td>44.7</td>
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\(^*\) Combination of information obtained from review of published literature and expert opinion.
very similar regardless of the priors used. We therefore present only the results using the uninformative priors.

In our analysis, we assumed conditional independence which means that when more than one sample is available from a person, the test results are independent from each other, conditional on the person's true infection status. This assumption was needed in order to apply the statistical method described.

WinBUGS software (version 1.4.3, MRC Biostatistics Unit, Cambridge, UK) was used to implement the Gibbs sampler algorithm. Posterior medians of random samples derived from marginal posterior densities were used as point estimates for sensitivity and specificity, reported with 95% BCI. Model convergence was assessed using the Gelman–Rubin convergence statistic available in WinBUGS (Spiegelhalter et al., 2002. WinBUGS version 1.4.3 [http://www.mrc-bsu.cam.ac.uk/bugs/]). The programs written in WinBUGS are available by request to the authors.

3. Results

A total of 5624 study participants provided at least one stool sample. Among these individuals one, two and three stool samples were provided by 1582 (28.1%), 1893 (33.7%) and 2149 (38.2%), respectively. The proportions of males (54.5%, 52.8% and 51.4%, respectively) were similar among the three stool sample provider groups. The mean ages (S.D.) of participants who provided one, two, and three stool samples were 20.5 (18.0), 22.7 (18.9) and 25.9 (20.0), respectively, and the age range of the participants in the three groups was almost identical. Proportions of different occupations were also similar among the three groups, with the highest proportion of participants involved in rice farming most of time. Further demographic descriptions of the study participants who provided at least one stool can be found elsewhere (McGarvey et al., 2006).

Table 2 shows the day-to-day variation in Kato-Katz results for the three STHs among those individuals who provided at least two samples. All three STHs showed some degree of variation in test results from day to day. This variation was most prominent for hookworm and least prominent for Ascaris.

Sensitivity and specificity estimates of the Kato-Katz technique for A. lumbricoides, hookworm and T. trichiura are shown in Table 3. For A. lumbricoides and T. trichiura, the sensitivity was very good, even with only one stool sample, while the estimate of sensitivity for hookworm with one stool sample was relatively poor, but improved with two or more samples. The specificity for all three STHs, as expected, decreased as the number of stool samples analysed increased. Nevertheless, the specificity estimates remained at 84% or greater for all three STHs with three stool samples. With one stool sample, the specificity for all STHs was over 94%.

4. Discussion

To our knowledge, this is the first study to report sensitivity and specificity of the Kato-Katz stool examination technique for all three STH infections using a Bayesian statistical method. We demonstrated that such an approach could be used to estimate the sensitivity and specificity of the Kato-Katz technique when not all participants provide stool sample over several consecutive days. These results are robust because of the reasonably large sample sizes in our analyses.

Day-to-day variation in fecal egg output is common among parasites and has been reported for STHs (Hall, 1981; Anderson and Schad, 1985; Marti and Koella, 1993; Booth et al., 2003). Our data also show some degree of day-to-day variation in tests for all three STHs. Many study participants had changes in their test results on consecutive days, going from positive to negative or vice versa. This complicates data analysis since the sensitivity and specificity of the diagnostic test varies according to the number of stool samples provided (de Vlas et al., 1992; Engels et al., 1997; Carabin et al., 2005). Our statistical approach allowed us to adjust for this variability by estimating the most likely infection category (probability) for each participant using all available data.

Several stool examination techniques are available to detect the presence of STH eggs in fecal samples, including direct fecal smear, the formalin-ether concentration technique (Ridley and Hawgood, 1956), the Kato-Katz technique (Peters et al., 1980), McMaster technique (WHO, 2008), and the most recently described FLOTAC technique (Cringoli, 2006). In addition to these techniques, serodiagnostic tools for detection of STHs, although rarely used, are also available. The sero-diagnostic approach has some disadvantages including its invasive nature (collection of blood sample), persistence of antibodies after cure (long half-life of antibodies to...
helminths) which makes it inefficient for detection of current infection status, and cross-reactivity with other helminths (nematodes) (Knopp et al., 2008). Although none of these tests (coprological or serological) is considered as a ‘gold standard’ test, the Kato-Katz technique is recommended by the WHO for field diagnosis of STHs due to its low cost, non-invasive nature and the relatively low level of technical skill required. The McMaster technique, which has the advantage of not needing any apparatus other than the McMaster counting chamber, is the most common technique used in veterinary parasitology (WHO, 2008). Two recent studies claimed higher sensitivity of the FLOTAC technique compared with the Kato-Katz technique, but the authors used a combination of tests as the ‘gold standard’ instead of a true ‘gold standard’ test (Utzinger et al., 2008; Knopp et al., 2009). Also, the feasibility of its use in resource-poor settings is yet to be addressed (Utzinger et al., 2008).

A ‘gold standard’ test is not available for detection of STH infections and the development of such a test may not be feasible (Joseph et al., 1995; Dudukuri et al., 2004). As mentioned earlier, four of the six previously published papers used a combination of two or more tests as the ‘gold standard’ (Santos et al., 2005; Goodman et al., 2007; Steinmann et al., 2008; Utzinger et al., 2008). Moreover, although two of these papers (Goodman et al., 2007; Steinmann et al., 2008) collected stool samples on more than two consecutive days, they did not account for the day-to-day variation in egg output in their estimation of sensitivity and specificity of the test. The other two papers assumed 100% specificity (constrained) of the Kato-Katz technique. This is unrealistic since false positives can arise if STH eggs are confused with eggs of other helminth species, e.g. T. trichiura eggs with Capillaria philippinensis eggs (Walden, 1991) or hookworm eggs with Trichuris suis spp. eggs (Ralph et al., 2006). Hookworm eggs can also be confused with Schistosoma eggs if the stool sample is not processed within 4 h of collection (Olveda, R., unpublished data). Moreover, the value assigned to the constrained parameter is arbitrary since its exact value is almost always unknown. Because of this arbitrary fixed value of the constrained parameter (specificity), it is impossible to account for the uncertainty associated with it when calculating confidence intervals for the unconstrained parameter (sensitivity). Also, with this method we are incapable of estimating the true specificity value (Joseph et al., 1995).

Our results show quite good sensitivity of a single stool examination using the Kato-Katz technique for detection of A. lumbricoides and T. trichiura infections and relatively poor specificity for detection of hookworm infection. Our results are in the range of what had been previously reported although these papers used different methods for estimation of sensitivity and specificity of the Kato-Katz technique. Also, as the sensitivity and specificity estimates were very similar when we used informative priors we can say that our results are robust across a reasonable range of prior information on the sensitivity and specificity of the Kato-Katz test.

Low sensitivity of the Kato-Katz for detection of hookworm infection may be related to rapid degeneration of delicate hookworm eggs with time. It has been reported that detection of hookworm eggs is significantly influenced by the time delay from production of feces to preparation of slides (Dacombe et al., 2007; Knopp et al., 2008) and the time delay from slide preparation to microscopic examination (WHO, 1994; Knopp et al., 2008). Although slides were processed in the field within a reasonably short period of time there had been delays in microscopic reading of the slides at the designated laboratory.

The Kato-Katz stool examination technique is also used to detect infection with different Schistosoma spp. Sensitivity and specificity of the Kato-Katz technique for detection of Schistosoma infection varies by the species, intensity of infection and number of stool samples examined (Ebrahim et al., 1997; Yu et al., 1998; Sayed et al., 2002; Booth et al., 2003; Zhou et al., 2007). Schistosoma japonicum is endemic in the Philippines and frequently co-exists with STH infections (polyparasitism). It has been shown that collecting stool samples over consecutive days improved sensitivity of the Kato-Katz technique for detection of Schistosoma infection (Ebrahim et al., 1997; Utzinger et al., 1999; Booth et al., 2003). Our results show a similar trend for the STH infections.

One limitation of this study is that our model assumes conditional independence of test results within each individual given the latent true infection status which is always uncertain. To assess conditional dependence we first have to build a more complex model assuming that there is at least some dependence. This allows examination of the size of the dependence parameter and whether or not its use is meaningful (Joseph, L., unpublished data). Exploring such a complex model is beyond the scope of this paper. However, several authors have noted that overlooking conditional dependence does not substantially change parameter estimates (Dendukuri and Joseph, 2001; Black and Craig, 2002; Gustafson, 2005).

In the Philippines, the department of health (DOH) initiated an Integrated Helminth Control Program (IHCP) in 2006 (Republic of the Philippines Department of Health, 2006. Strategic and operational framework for establishing integrated helminth control program (IHCP). Administrative Order No. 2006-0028. Available from: http://www.doh.gov.ph/health_policies/admin_order/2006 Accessed: 28 August 2009.). The program involves central, regional and local offices of DOH; hospitals and rural health centers; and concerned institutions and agencies. For children 1–12 years of age, twice yearly mass deworming with anthelmintics (against STH) is recommended. In addition, once a year selective deworming of special population groups (adolescent females, pregnant women, soldiers, food handlers and indigenous people) is recommended. One of the objectives of this program is to reduce the prevalence of STHs below 50% among 1–12 year olds by 2010. In mass treatment campaigns with limited resources, prioritized on the basis of community-level prevalence, high specificity of the diagnostic test might be important due to a desire to conserve resources and repeat sampling to improve sensitivity might be less justified. On the other hand, we may fail to identify some of the communities which need to be included in the mass treatment campaign if we use a test with low sensitivity. In situations like this, a balance between sensitivity and specificity of the diagnostic test is desired.

The Kato-Katz technique is still the method of choice for detection of STH infection in studies conducted in rural environments with poor infrastructure. Our results show that it is capable of diagnosing STH infections with reasonably low proportions of error, particularly with 2 days of stool collection. Correct diagnosis and quantification of STH infections is of considerable importance for successful control of these helminths.

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