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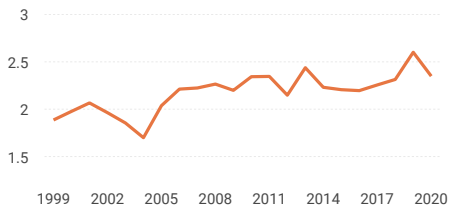

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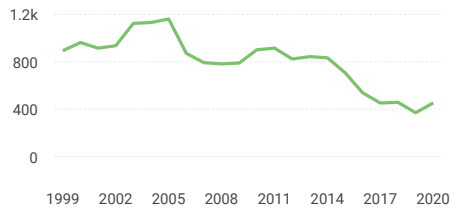

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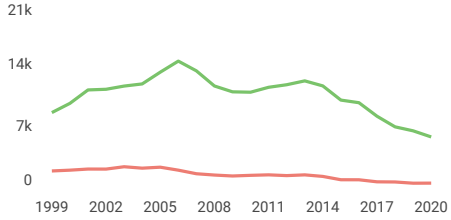

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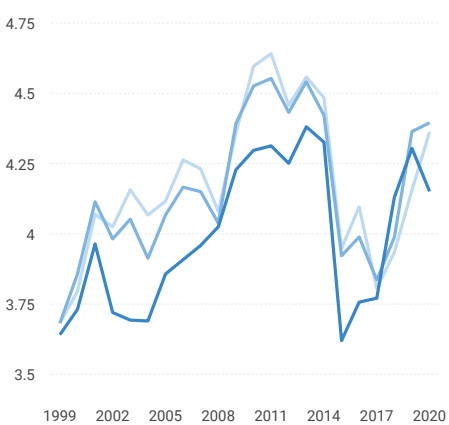

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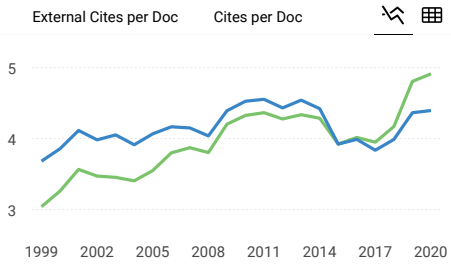

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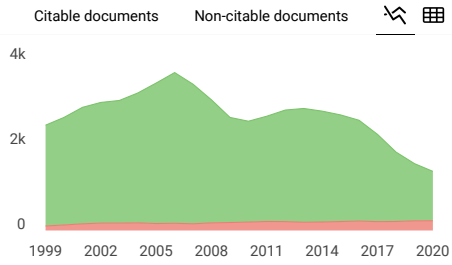

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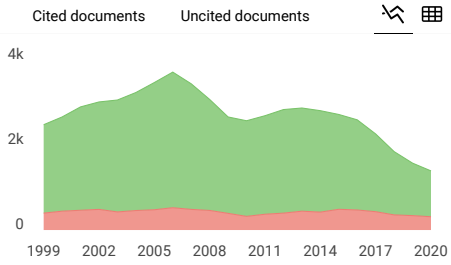

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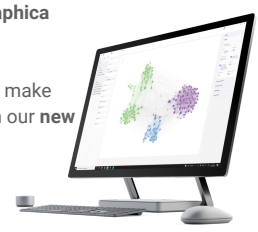

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PARASITOLOGY

Concentration of Urine Samples Improves Sensitivity in Detection of *Strongyloides*-Specific IgG Antibody in Urine for Diagnosis of Strongyloidiasis

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ABSTRACT Detection of IgG in urine is an efficient method comparable to that in serum for diagnosis of strongyloidiasis, but the effects of daily variation in urine dilution on diagnostic accuracy are not clearly known. This study evaluated the effects of urine concentration on the detection of parasite-specific IgG by urine enzyme-linked immunosorbent assay (ELISA), particularly in individuals with borderline results or false-negative diagnosis. Optimal concentration conditions were established by comparing *Strongyloides*-specific IgG antibody levels between unconcentrated and concentrated urine in participants with different infection intensities, namely, healthy control (HC), low-negative (LN), high-negative (HN), and low-positive (LP) groups. The optimal condition was selected and validated in a field trial study. The final urine concentration protocol required centrifugation at 4,000 × *g* at 4°C for 10 mins using the Amicon concentrator tube. This protocol was validated in groups of participants with various diagnoses according to urine ELISA and fecal examination (n = 148). The concentrated-urine ELISA increased the proportion of positive results in the LN group by 68.2% and by 100% in the HN group. Significantly elevated IgG antibody levels were seen in the LP group. In the group that was false negative by urine ELISA but positive by fecal examination (n = 28), concentrated-urine ELISA yielded 100% positive results. Overall, the frequency estimates of *Strongyloides stercoralis* were 23.6% by fecal culture, 27% by standard urine ELISA, and 90.5% by concentrated-urine ELISA. The concentration of urine samples prior to analysis by ELISA improved the sensitivity for diagnosis and is potentially useful in the diagnosis of strongyloidiasis in immunocompromised individuals or in low-prevalence areas.

KEYWORDS *Strongyloides stercoralis*, enzyme-linked immunosorbent assay (ELISA), urine concentration method

Strongyloides stercoralis is an intestinal parasite which causes strongyloidiasis. It is a neglected tropical disease, affecting millions of people worldwide, including Latin America, sub-Saharan Africa, and the southeastern United States. Up to 75% of cases occur in Southeast Asia (1, 2). The infection is acquired by contact with soil contaminated with infective larvae. The life cycle begins when infective filariform larvae penetrate the skin of humans, enter the blood circulation, pass through the lungs, and

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migrate to the small intestine, where they become adult female worms. Eggs released from these adult worms develop to rhabditiform larvae and pass out in feces. In humans, larvae of *S. stercoralis* can cause autoinfection by reinvasion of the intestinal lining (internal autoinfection) or perianal area (external autoinfection), leading to chronic strongyloidiasis (1, 3–5).

Detection of larvae in stool samples can be done using several parasitological techniques, such as Baermann's concentration, the agar culture plate technique (APCT), Harada-Mori filter-paper culture, and the formalin-ethyl acetate concentration technique (FECT). However, these methods have limited sensitivity and require multiple stool samples (5). Molecular and immunological methods are common alternatives (6–10). Enzyme-linked immunosorbent assays (ELISA) are widely used with different types of signaling platforms and types of antigens (11). Various antigenic types, such as *S. stercoralis*, *S. ratti*, *S. venezuelensis*, and recombinant antigen have been reported for strongyloidiasis (12–17). Several protocols for indirect serum ELISA have been reported with various diagnostic accuracies (17–19). Regarding antigen types, a previous study compared the performance of ELISA based on *S. ratti* and *S. stercoralis* antigens for diagnosis of strongyloidiasis and showed that *S. ratti* antigen was an efficient substitute for *S. stercoralis* antigen (17). Another study used filariform larvae of *S. venezuelensis* as an antigen source to screen strongyloidiasis infection in a transplant population. The results suggested using ELISA with *S. venezuelensis* antigen as an additional method for strongyloidiasis detection in transplant candidates (20). However, these serodiagnostic methods require serum to be collected by invasive venipuncture. Recently, detection of antibody in urine has been shown to have high diagnostic accuracy comparable to that of conventional serological assays and be more sensitive than coprological methods (21, 22). Diagnostic accuracy for strongyloidiasis was comparable between urine ELISA and serum ELISA using three types of antigens (NIE recombinant antigen, filariform *S. ratti*, and *S. stercoralis* larvae crude antigens). *Strongyloides*-specific IgG levels in urine were significantly correlated with those in serum (23). The ease and public acceptance of sample acquisition enhance the usefulness of urine ELISAs for population screening in areas of endemicity and clinical diagnosis.

Our study aimed to improve the sensitivity for diagnosis of strongyloidiasis by adding a concentration protocol for urine samples before performing conventional urine ELISA. Specifically, we evaluated how the urine-concentration protocol resolves the problems of false-negative urine ELISAs in parasitologically positive cases as well as in cases with borderline results according to conventional urine ELISA. The results emerging from this study justify inclusion of a urine-concentration protocol in urine ELISA for diagnosis of strongyloidiasis, particularly in low responders, i.e., immunocompromised individuals, in communities where transmission is low and for monitoring responses to drug treatment.

MATERIALS AND METHODS

Participants and clinical specimens. The participants were villagers from Phuwiang District, Khon Kaen Province, and Kamalasai District, Kalasin Province, Northeast Thailand, where strongyloidiasis is endemic. Two separate sets of participants were recruited, one for each part of the project. Part 1 was the optimization study, which enrolled 40 participants from Phuwiang district, Khon Kaen. Part two was the field validation study, which comprised 433 participants from Phuwiang District, Khon Kaen, and Kamalasai district, Kalasin. After acquisition of written informed consent, clinical samples (fecal, urine, and blood samples) were requested from all project participants. Single stool was collected in standard plastic containers and kept at room temperature during transportation to the laboratory, where 4 g of each stool was processed for APCT. Morning urine specimens were collected from the participants after 6 to 8 h without urination in wide-mouthed plastic containers, transferred into 15-mL plastic test tubes, and placed in a chilled insulated box before being processed in the laboratory. The urine specimens were processed by centrifugation at 1,500 rpm for 5 min and stored at 4°C until they were processed for urine ELISA. There were 433 individuals who provided both feces and urine samples. Blood samples were collected by venipuncture and centrifuged at 3,000 for rpm 5 min. Serum was transferred into a separator tube and kept at −20°C until it was processed for serum ELISA. A separate group of healthy people were included in the study to act as negative controls.

Study design. This project was a prospective cross-sectional study, part of a larger longitudinal project on diagnosis and epidemiology of opisthorchiasis and strongyloidiasis. This study used leftover

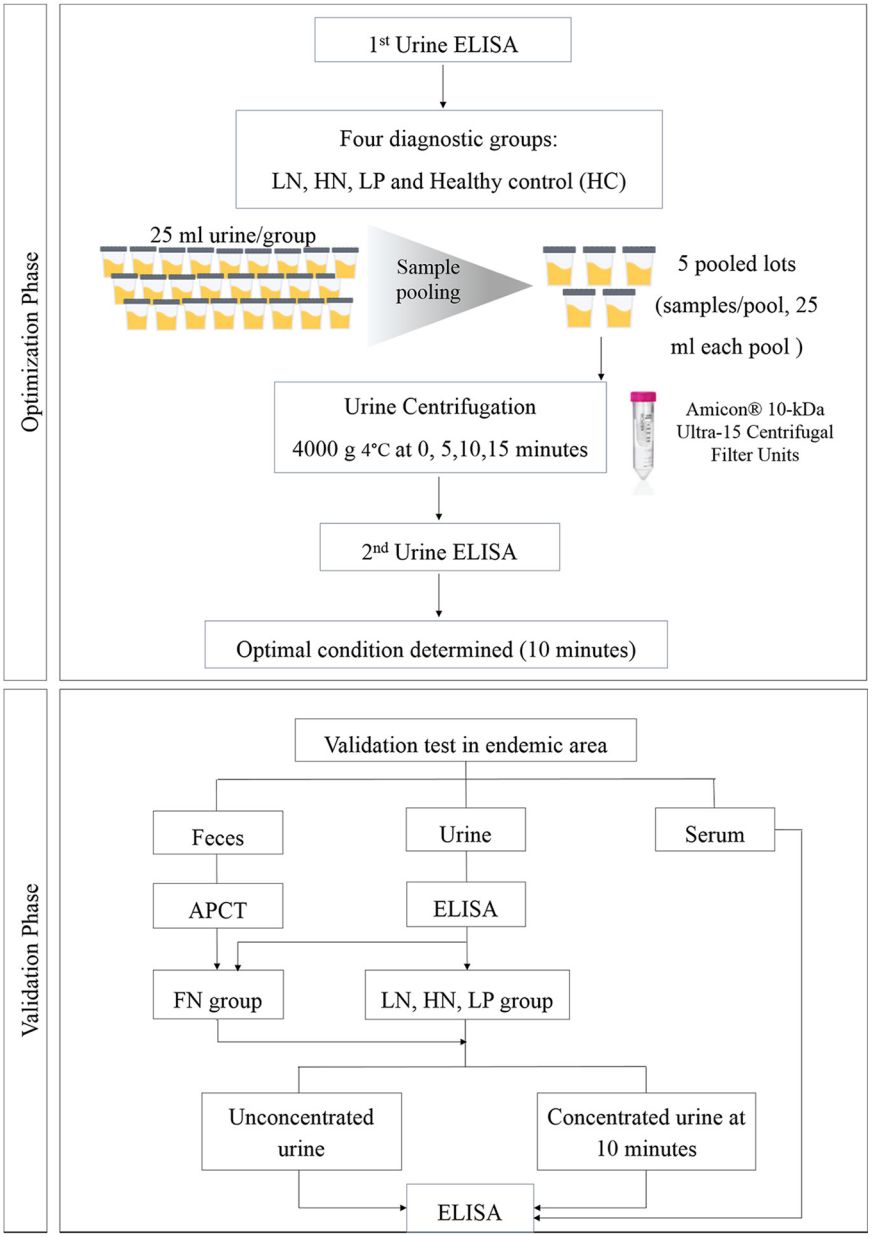

FIG 1 Study design. Phase 1 was an optimization of the urine-concentration protocol for ELISA. Phase 2 was field validation testing of urine samples from areas endemic for strongyloidiasis. LN, low negative; HN, high negative; LP, low positive; HC, healthy control.

specimens from the Cholangiocarcinoma Research Institute under the Cholangiocarcinoma Screening and Care Program, Khon Kaen University, Khon Kaen, Thailand. The use of specimens and the protocols in these studies were approved by the Human Ethics Committee of Khon Kaen University (HE631309).

As shown in Fig. 1, the study design consisted of two parts. Part 1 was the optimization phase in order to establish the urine concentration protocol. The protocol was an outcome of a preliminary experiment using Amicon centrifugal filter units with various durations of centrifugation (4,000 $\times$ *g*, 4°C) for 5, 10, or 15 min. Comparisons between preconcentrated urine samples in the primary or 1st ELISA and concentrated urine samples in the 2nd ELISA were made using pooled lots from 5 individuals in volumes of 25 ml (5 replicates). After the optimal concentration protocol was established, it was subjected to a field trial in project part 2; field-collected urine samples were processed using the concentration protocol (10-min centrifugation) and analyzed by ELISA. The samples were then separated into four groups based on IgG antibody levels. In addition to urine samples, blood and fecal samples were collected from the project participants for reference diagnosis by serological and parasitological methods, respectively.

Fecal examination by the agar plate culture technique (APCT) and formalin-ethyl acetate concentration technique (FECT). At the laboratory, the samples were processed for detection of *S. stercoralis* using the agar plate culture technique (APCT), and this was performed as described previously (19, 24). Briefly, 4 g of fecal sample was placed on a nutrient-agar plate and incubated at room temperature for 4 to 5 days. The presence of *S. stercoralis* larvae was assessed by washing the surface of the plate with 10% formalin, transferring the wash into a test tube, and then centrifuging it. Sediment was examined under a light microscope. To assess other parasitic infection, approximately 2 g of the remaining fecal sample was also processed for a qualitative formalin-ethyl acetate concentration technique (19, 25). The results of fecal examination by APCT and FECT were combined. Individuals positive according to one or both techniques were regarded as infected.

Procedure for urine and serum antibody detection by ELISA. *Strongyloides ratti* was used as the antigen source for urine and serum ELISA. The life cycle of *S. ratti* has been maintained in Wistar rats at the Department of Parasitology, Faculty of Medicine, Khon Kaen University. Feces of infected rats were cultured using a filter-paper culture method to produce third-stage filariform larvae (L3) of *S. ratti*. The larvae were concentrated and washed with normal saline and stored at −20°C for crude soluble antigen extraction, which was done as described previously (21, 26). The L3 larvae were dispersed in phosphate-buffered saline (PBS), pH 7.4, containing protease inhibitor mix (Thermo Fisher Scientific, USA; A32953) and were frozen at −70°C for 30 min and thawed 5 times with boiling and subsequently disrupted by sonication. The homogenate was stored at 4°C overnight and centrifuged at 15,000 × *g* for 30 min at 4°C. The protein concentration of the supernatant was measured by the Bradford protein assay and then stored at –20°C until use.

The ELISA was performed as described by Eamudomkarn and coworkers (21). The 96-well microtiter plates (Thermo Fisher Scientific, China) were coated with 100 μL (5 μg) of *S. ratti* antigen and were kept at 4°C overnight. The plates were washed twice with PBS containing 0.05% Tween 20, and 200 μl of blocking buffer containing 3% skim milk in PBS and 0.5% Tween 20 was added. After incubation at 25°C for 2 h, 100 μl of urine sample or diluted serum (1:8,000) was added to the wells and incubated at 37°C for 1 h. After washing the plates four times with wash buffer (PBS with Tween), 100 μl of horseradish peroxidase conjugate goat anti-human IgG (dilution, 1:4,000) (Abcam, USA; ab6858) was added, and the mixture was incubated at 37°C for 1 h. The plate was washed four times same as previous step and 100 μl of a substrate solution (o-phenylenediamine in citrate phosphate buffer) was added and incubated in a dark box at room temperature for 1 h. Then 50 μl of stop solution (4N sulfuric acid) was added, and the optical density (OD) of each well was measured at 492 nm using an ELISA reader (Sunrise Absorbance reader, Tecan, Switzerland).

Receiver operating characteristic (ROC) curve. For urine and serum antibody-based ELISA, samples from individuals known to be negative or positive for strongyloidiasis (according to FECT and/or APCT) (20 each for urine antibody and 15 each for serum antibody detection) were used to construct ROC curves in MedCal version 11.6.1.0 (MedCal Software, Belgium). The cutoff values for urine and serum ELISA were ≥126 units/mL and ≥177 units/mL, respectively.

Urine concentration optimization procedure. Based on the result of the preliminary ELISA (1st ELISA) of the original (unconcentrated) urine samples, the participants were divided into 4 groups according to the observed levels of urine IgG antibody. The first two groups were urine ELISA negative (IgG antibody, <126 units/mL), which consisted of the low-negative (LN) group, with <60 units/mL IgG, and the high-negative group (HN), with 60 to 125 units/mL IgG. The third group was a low-positive (LP) group, with 126 to 300 units/mL IgG. The fourth group was the healthy control group (HC), who had negative fecal examination and urine ELISA (n = 10). The individual urine samples in each group were pooled to attain a volume of 10 mL with 5 replicates (5 × 10 mL) and were subjected to centrifugation at 4,000 × *g* at 4°C (based on the recommended force for a swinging-bucket rotor) for 5, 10, and 15 min using a commercial concentrator (Amicon Ultra-15 centrifugal filter unit, UFC901024; Merck Millipore, Ireland). The final volumes of concentrated urine obtained at each centrifugation time point at 5, 10, and 15 min were 5, 1, and 0.5 mL, respectively. The levels of *Strongyloides*-specific IgG in the residual supernatant of concentrated urine samples were measured by urine ELISA, and the results were compared with unconcentrated-urine ELISA as shown in Fig. 2.

Field validation test. In order to validate the specimen concentration procedure, urine samples from two communities of endemicity (Phuwiang District, Khon Kaen Province, and Kamalasai District, Kalasin Province) were selected for analysis. After the urine samples had been subjected to the 1st urine ELISA, the samples were divided into 4 groups as for the optimization phase, based on antibody levels, i.e., low negative (LN), high negative (HN), low positive (LP), and false negative, (FN, n = 28). In the FN group the urine samples were negative by urine ELISA but were positive for *S. stercoralis* larvae in feces by fecal examination. These samples were processed using the optimal concentration conditions (10 min of centrifugation at 4°C in an Amicon Ultra-4 centrifugal filter unit [UFC801024; Merck Millipore, Ireland]) and then were reanalyzed by urine ELISA. The results from 1st and 2nd urine ELISAs were compared as shown in Fig. 3.

Statistical analysis. The data obtained were analyzed using SPSS version 19 (IBM, USA) for statistical analysis. McNemar's chi-square tests were used to compare the positive rates of strongyloidiasis determined by ELISA results between unconcentrated and concentrated urine samples. Comparisons between levels of IgG antibody in unconcentrated and concentrated urine from ELISA were done with the Friedman test with multiple comparison. The correlations between IgG antibody levels (log-transformed values of antibody unit/mL) in unconcentrated and concentrated urine and those from serum ELISA were validated by the regression coefficient of the linear regression model.

Diagnostic performances for unconcentrated ELISA in terms of sensitivity, specificity, positive predictive value, and negative predictive value were calculated using fecal analysis by APCT or a composite

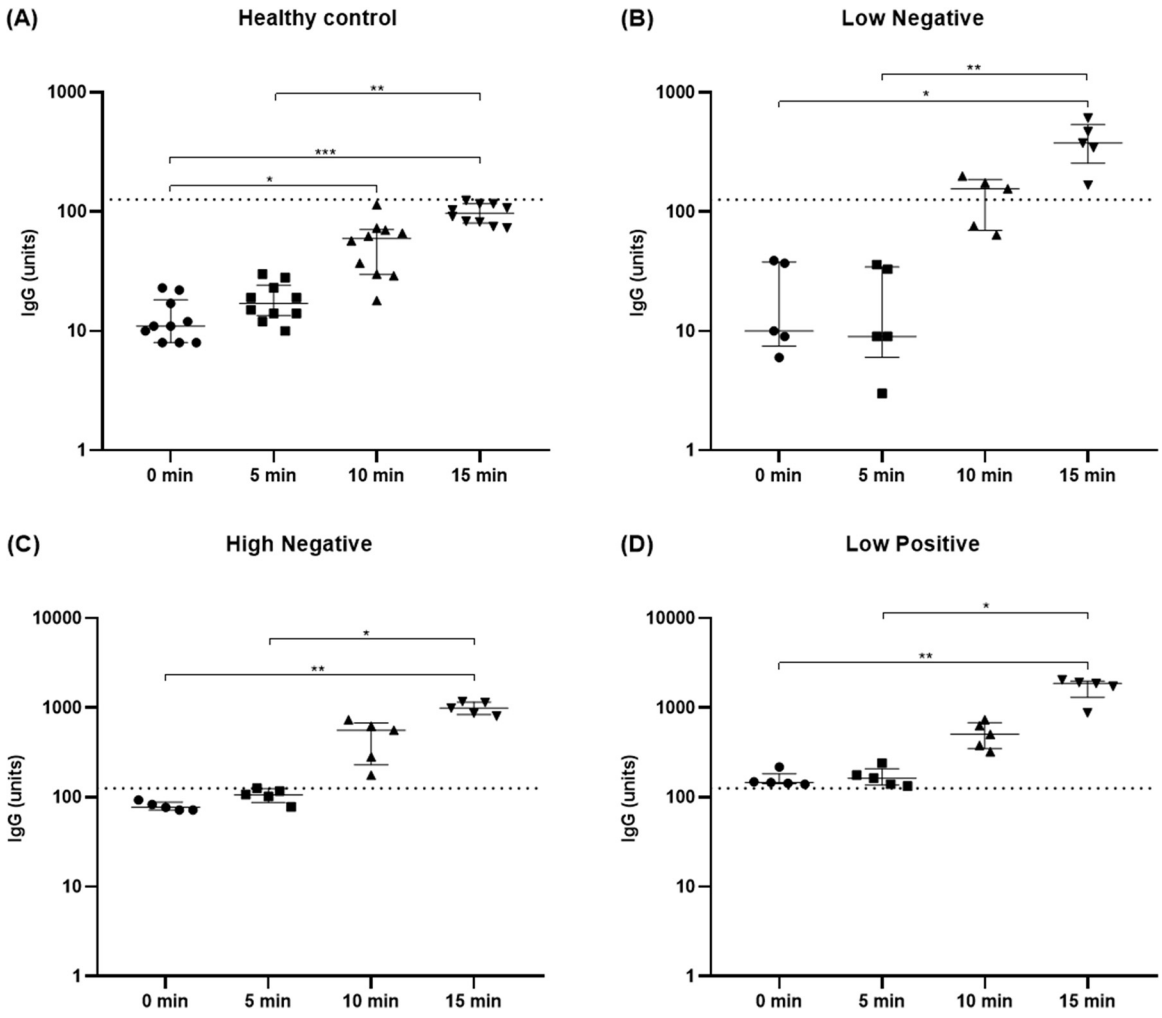

FIG 2 *Strongyloides*-specific IgG antibody levels in urine (unit/mL) in each diagnostic group after the concentration step at 0, 5, 10, and 15 min at 4°C by the concentrator set. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

method combining ELISA and fecal examination as reference methods. All statistically significant levels were set at a *P* value of < 0.05.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Study participants and clinical samples. As shown in Fig. 3, residents from study areas in Khon Kaen and Kalasin provinces were recruited in this study. Two separate sets of participants were recruited, one for each part of the project. Part 1 was the optimization study, which enrolled 40 participants from Phuwiang District, Khon Kaen. Part 2 was the field validation study that included 433 participants from Phuwiang District, Khon Kaen, and Kamalasai District, Kalasin. The clinical samples collected from each participant were processed for screening of *S. stercoralis* infection by urine and serum ELISA. Fecal samples were processed for detection of *S. stercoralis* infection by APCT and FECT.

Optimization of urine concentration protocol. As shown in Fig. 2, no member of the healthy group ($n = 10$) returned a positive result in urine ELISA after any duration of centrifugation. In the HN group, all samples ($n = 5$) exceeded the cutoff value after 10 and 15 min of centrifugation. For the LN group, 3 out of 5 samples (60%) exceeded the cutoff after 10 min, and all 5 (100%) were positive after 15 min of centrifugation. Antibody concentrations in the LP group were always above the cutoff level and

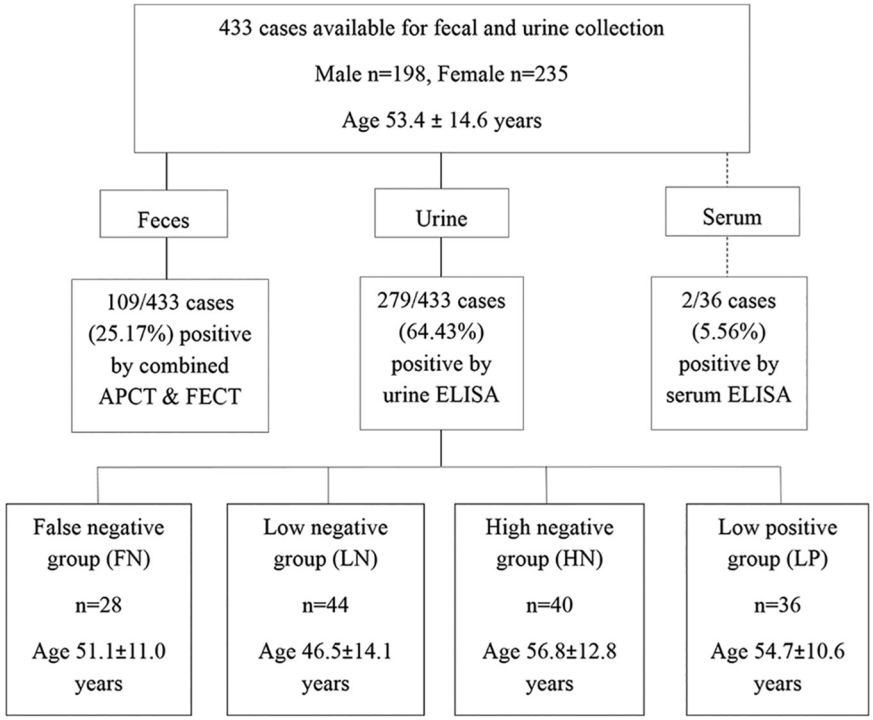

FIG 3 Flow chart of the number of participants, demographic data, and frequency of *S. stercoralis* infection in this study.

increased significantly following centrifugation for 10 and 15 min. Centrifugation for 5, 10, and 15 min reduced the urine sample volumes from the original by 2, 7, and 10-fold, respectively.

Antibody concentrations in all groups increased significantly following centrifugation as shown in Fig. 2. For the HC group (Friedman statistic = 26.64, $P < 0.0001$), the IgG levels after concentration for 10 and 15 min were significantly different from those at 0 min ($P < 0.05$ and $P < 0.001$, respectively), and the IgG levels after concentration for 15 min were significantly different from those at 5 min ($P < 0.01$). For the LN group (Friedman statistic = 14.02, $P < 0.0001$), IgG levels after concentration for 15 min was significantly different from those at 0 and 5 min ($P < 0.05$ and $P < 0.01$, respectively). For the HN group (Friedman statistic = 14.04, $P < 0.0001$), IgG levels after concentration for 15 min was significantly different from those at 0 and 5 min ($P < 0.01$ and $P < 0.05$, respectively). For the LP group (Friedman statistic = 14.02, $P < 0.0001$), IgG levels after concentration for 15 min was significantly different from those at 0 and 5 min ($P < 0.01$ and $P < 0.05$, respectively).

Specific IgG antibody in all diagnostic groups significantly increased following 10- and 15-min centrifugation compared with unconcentrated urine samples. At 5 min of centrifugation, no significant differences between concentrated and unconcentrated samples were seen in any groups. Balancing the length of centrifugation required and the need for an adequate urine volume of sample after concentration for subsequent ELISA (2nd urine ELISA), centrifugation for 10 min at 4°C in an Amicon Ultra-4 centrifugal filter unit was chosen as the optimal protocol for the field validation test.

Validation tests in communities of endemicity. In the study part 2, the field validation, 433 participants (male, 45.73%; female, 54.27%) provided both urine and feces for screening of *S. stercoralis* infection (Fig. 3). Fecal examination by APCT and FECT found that 109 individuals (25.17%) harbored *S. stercoralis* infection, and 279 cases (64.43%) were positive for strongyloidiasis by urine ELISA. A subgroup of participants provided blood samples for diagnostic comparisons, and 2 of 36 participants (5.56%) were positive by serum ELISA.

TABLE 1 Frequency of parasitic infection in the study participants determined by fecal examinations by APCT and FECT ($n = 433$)

Parasite	No. positive (%)
S. stercoralis larvae	109 (25.1)
S. stercoralis	99 (22.9)
S. stercoralis + hookworm	1(0.2)
S. stercoralis + *O. viverrini*	6(1.4)
S. stercoralis + *Taenia* sp.	2 (0.5)
S. stercoralis + *O. viverrini* + MIF	2 (0.5)
Other parasites	26 (6.0)
O. viverrini	19 (4.4)
Minute intestinal fluke	1 (0.2)
Echinostoma sp.	1 (0.2)
Taenia sp.	3 (0.6)
Hookworm	2 (0.4)
Total	135 (31.2)

The frequency of parasite infection determined by fecal examination for *S. stercoralis* was 25.1%, followed by *Opisthorchis viverrini* (4.4%), *Taenia* (0.6%), hookworms (0.4%), minute intestinal flukes (MIF) (0.2%), and *Echinostoma* spp. (0.2%) as detailed in Table 1. Mixed infections of *S. stercoralis* and other parasites were found with *O. viverrini* (1.4%), *Taenia* (0.5%), MIF (0.5%), and hookworms (0.2%).

Using fecal examination as a reference method, diagnostic performance of urine ELISA in terms of sensitivity and negative predictive values (NPV) was 77.1% and 83.8%, respectively (Table 2). With reference to the composite standard by combining fecal examination and urine ELISA, the sensitivity and NPV were 91.8% and 83.8%, respectively. Diagnostic agreement between urine ELISA and fecal examination measured as a kappa value was 0.11.

The participants samples for the second part of the study were divided into four groups according to *Strongyloides*-specific IgG antibody levels (units/mL) in the preliminary urine ELISA (1st urine ELISA). These were the low-negative (LN, $n = 44$), high-negative (HN, $n = 40$), low-positive (LP, $n = 36$) and false-negative (FN, $n = 28$) groups. Their urine samples were concentrated as mentioned above (10-min centrifugation at 4,000 $\times$ *g* at 4°C) before being reanalyzed by 2nd urine ELISAs (Fig. 3). The false-negative (FN) group included the participants who had a negative urine antibody test despite being positive by fecal examination (APCT). The frequency estimates for strongyloidiasis in each diagnostic group determined by APCT and urine ELISA pre- and postconcentration of urine samples are shown in Table 3. The concentration of urine ELISA raised the proportion positive for strongyloidiasis from 0% to 100% in the HN, LP, and FN groups. In the LN group, all the participants ($n = 44$) were negative by unconcentrated-urine ELISA, but levels for 30 individuals (68.2%) rose above the cutoff value with concentrated-urine ELISA. In total, the frequency of strongyloidiasis was 23.6% by APCT, 27% by unconcentrated-urine ELISA, and 90.5% by concentrated-urine ELISA, as shown in Table 3. The distributions of individual IgG antibody levels in unconcentrated and concentrated urine samples by ELISA in each diagnostic group are compared in Fig. 4A and B. The levels of IgG antibody in concentrated urine were significantly higher

TABLE 2 Diagnostic performance of urine ELISA compared with fecal examination as a primary standard and a combined fecal examination and urine ELISA as a composite standard (APCT and FECT) ($n = 433$)

	Diagnostic performance (%)[a]				
Reference method	Sensitivity	Specificity	PPV	NPV	Accuracy
Primary standard	77.1	39.8	30.1	83.8	49.2
Composite standard	91.8	100.0	100.0	83.8	94.2

[a]PPV, positive predictive value; NPV, negative predictive value.

TABLE 3 Frequency of strongyloidiasis determined by fecal examination (APCT) and unconcentrated- and concentrated-urine ELISA in the field-study participants separated into 4 diagnostic groups[a]

Group	Diagnosis	Data [*n* (%)] for:		
		Fecal examination	Unconcentrated-urine ELISA	Concentrated-urine ELISA
LN (*n* = 44)	Positive	0 (0)	0 (0)	30 (68.2)
	Negative	44 (100)	44 (100)	14 (31.8)
HN (*n* = 40)	Positive	0 (0)	0 (0)	40 (100)
	Negative	40 (100)	40 (100)	0 (0)
LP (*n* = 36)	Positive	7 (19.4)	36 (100)	36 (100)
	Negative	29 (80.6)	0 (0)	0 (0)
FN (*n* = 28)	Positive	28 (100)	0 (0)	28 (100)
	Negative	0 (0)	28 (100)	0 (0)
Total (*n* = 148)	Positive	35 (23.6)	40 (27.0)	134 (90.5)
	Negative	113 (76.4)	108 (73.0)	14 (9.5)

[a]LN, low negative; HN, high negative; LP, low positive; FN, false negative.

than those in unconcentrated urine in all diagnostic groups (paired *t* test, $P < 0.001$). When combining the 4 diagnostic groups together, there were positive and significant correlations between IgG antibody levels in unconcentrated and concentrated urine in the LN, HN, LP, and FN groups ($r = 0.89$, $P < 0.0001$; Fig. 5). In addition, the levels of *Strongyloides*-specific IgG antibody in serum were significantly correlated with those in unconcentrated and concentrated urine ($n = 36$) (Fig. 6).

Serum samples were available for 14 of the 28 participants within the FN group. No member of this group had tested positive by unconcentrated-urine ELISA, but all (100%) were positive by concentrated-urine ELISA. Only one of the 14 tested using serum-based ELISA returned a positive (7.14%) result.

The frequency of strongyloidiasis as determined by unconcentrated- versus concentrated-urine ELISA for each category of parasite infection determined by fecal examination is shown in Table 4. Of those 31 individuals infected only with *S. stercoralis*, 7 cases (22.6%) were positive by unconcentrated-urine ELISA, while all 35 individuals (100%) were positive using concentrated-urine ELISA. In 5 cases with *O. viverrini* infection alone, a single positive case became 5 positive cases according to concentrated-urine ELISA. In fecal-negative cases, the positive rate determined by unconcentrated-urine ELISA was 25.9% and became 87% by concentrated-urine ELISA.

DISCUSSION

Although immunological methods are known to be more sensitive than parasitological methods, a considerable proportion of parasite-positive people test negative by

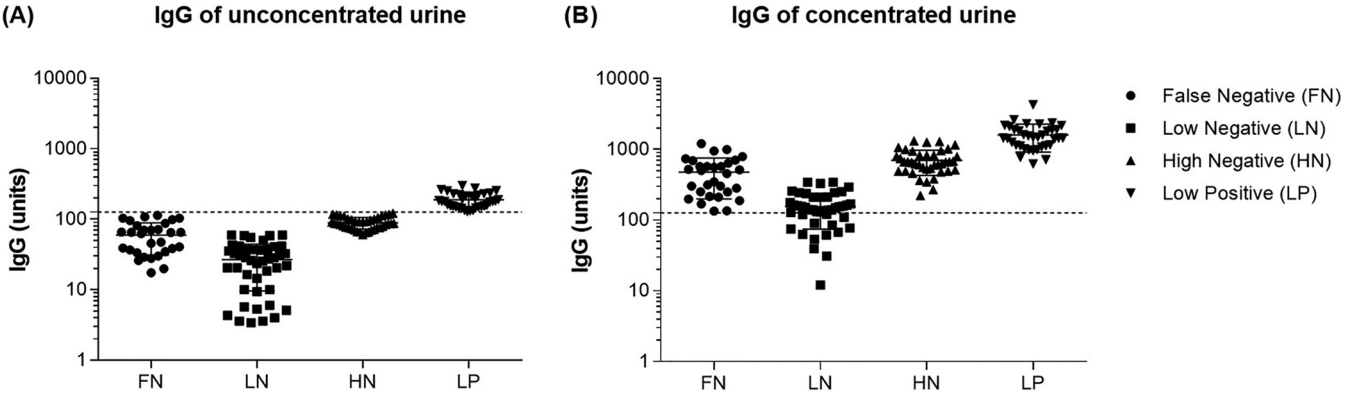

FIG 4 (A and B) Distributions of *Strongyloides*-specific IgG antibody by urine ELISA in 4 diagnostic groups based on the initial urine ELISA in unconcentrated urine (A) and concentrated urine ELISA (B).

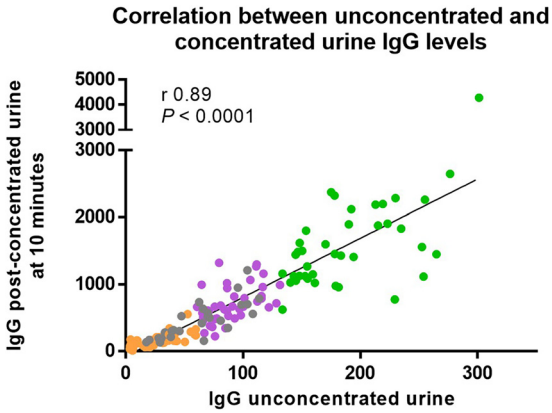

FIG 5 Correlations between *Strongyloides*-specific IgG antibody levels in unconcentrated- and concentrated-urine ELISA in participants in the low-negative (orange), high-negative (purple), low-positive (green), and false-negative (gray) groups.

serum ELISA (19, 26) and by urine ELISA (21). In this study, the addition of a urine-concentration protocol prior to analysis by ELISA improved the diagnostic sensitivity of urine ELISA. In the LN group, which had low IgG antibody levels, urine concentration for ELISA transformed many negative tests to positive tests (68%). Similarly, in the optimization study, 60% of the LN group tested positive after urine concentration. The consistency of the increase in positive results in LN groups by two independent studies suggested the presence of IgG antibody against *S. stercoralis* in urine of only some of the participants. A negative test by urine ELISA, even following urine concentration, may indicate negligible or no IgG antibodies. In the HN group, which consisted of borderline cases, all (100%) tested positive by urine ELISA after urine concentration. In the LP group, concentration of the urine sample significantly increased the IgG levels in all cases. A recent study found that the level of *Strongyloides*-specific IgG in urine was relatively stable over 3 to 30 consecutive days (27), supporting the existence of a low IgG antibody level in urine which is not detectable by a conventional (unconcentrated) urine ELISA.

The group of participants that triggered our interest was the strongyloidiasis false-negative group, which had negative serology but had larvae present in their feces. There were 14 participants in this particular group that had positive *S. stercoralis* larvae in feces, but only a single participant was positive by serum ELISA, and all 14 participants were negative for *Strongyloides*-specific IgG antibody detection by unconcentrated-urine ELISA. By adding the urine-concentration protocol, all 14 participants tested positive by concentrated-urine ELISA. There may be many reasons for false-negative tests using unconcentrated-urine ELISA. Low antibody concentrations may occur

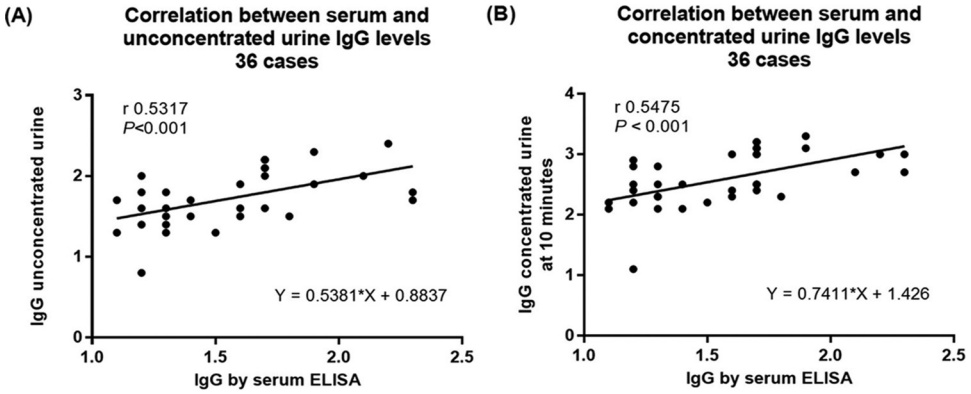

FIG 6 (A and B) Correlation between *Strongyloides*-specific IgG antibody in serum and unconcentrated urine (A) and between serum and concentrated urine (B) by ELISA.

TABLE 4 Frequency of strongyloidiasis according to unconcentrated- and concentrated-urine ELISA in groups of parasite infection as determined by fecal examination (APCT and FECT) (n = 148)

Group[a]	Sample size (n)	Data [no. positive (%)] for:	
		Unconcentrated-urine ELISA	Concentrated-urine ELISA
Group 1 (*S. stercoralis*)	35	7 (20.0)	35 (100.0)
S. stercoralis	31	7 (22.6)	31 (100.0)
S. stercoralis + *O. viverrini*	3	0 (0.0)	3 (100.0)
S. stercoralis + *Taenia* sp.	1	0 (0.0)	1 (100.0)
Group 2 (other parasites)			
O. viverrini	5	1 (20.0)	5 (100.0)
Group 3 (negative)	108	28 (25.9)	94 (87.0)

[a]Group 1, positive for *S. stercoralis* larvae and/or antibody; cut off 126. Group 2, positive for other parasites by fecal examination (FECT). Group 3, negative for *S. stercoralis* antibody and fecal examination; reside in areas of endemicity.

in low-responder individuals or those with a low parasite load. Dilution of urine proportional to various levels of water intake may also affect antibody levels. It is likely that removal of water by the concentration of urine elevated the IgG concentration, which improved the sensitivity of diagnosis of strongyloidiasis in false-negative cases and borderline diagnostic groups (HN and LN). In a comparable circumstance in schistosomiasis, Grenfell et al. (2018) obtained false-negative test results for antigen detection in urine using a point-of-care cathodic circulating-antigen test (POC-CCA); by introducing a phase of urine concentration prior to using the conventional POC-CCA process, they increased the accuracy by up to 60% (28).

Other supporting evidence for the effects of urine concentration was the finding of quantitative correlation between IgG in urine pre- and postconcentration. These correlations were significant overall and also when participants were separated into 4 diagnostic groups, i.e., LN, HN, LP, and FN (data not shown). This observation suggests that IgG present but undetectable in urine samples when first collected can become detectable after urine samples have been concentrated. The observed correlation between urine and serum IgG antibodies in strongyloidiasis in this study was similar to that in our previous reports (21, 27). These correlations suggest that IgG in blood circulation is the source of IgG in urine, but local immune responses cannot be ruled out. It is interesting to note that the samples from the healthy control group (HC) remained negative after urine had been concentrated for 5, 10, or 15 min. Hence, it indicates the true lack of IgG in this group of individuals. Consequently, together with the results in the LN and HN groups, the 10-min centrifugation time was appropriate for the concentration protocol in this study.

Analysis of diagnostic agreement revealed that the kappa value was 0.11 between urine ELISA and fecal examination. The observed low kappa value suggested that the coprological method was less sensitive than the serological method in diagnosed strongyloidiasis. To improve the sensitivity of fecal examination, 3 consecutive days of stool examination was reported and increased the sensitivity of APCT to 85% when it used with repeated fecal samples (27). Due to the limitation of our study, only a single stool was collected for APCT and FECT analysis. It remains a challenging question to estimate the proportion of current or active infection within the group with seropositive diagnosis compared with 3 consecutive days of stool collection for APCT and FECT analysis.

As shown in Table 4, concentrated-urine ELISA detected more positive cases not only in *S. stercoralis* infection but also in other parasitic infections, such as *O. viverrini*. This is interpreted as a consequence of the insensitivity of fecal examination when coinfection with *S. stercoralis* is not detected. Once again, the result indicated the need

for multiple fecal examinations or consecutive fecal examinations over several days by APCT (27). It is not clear whether alternative methods, such as diagnosis by PCR, will help to resolve this problem by supplementing or even replacing the standard fecal examination, such as APCT. Our initial study applying a copro-PCR method for strongyloidiasis revealed that approximately 84% of those seropositive by serum or urine ELISA were also PCR-positive (our unpublished data). It remains a challenging question to estimate the proportion of current or active infection within the group with seropositive diagnosis.

In order to verify the hypothesis of urine concentration in the case of serum ELISA, we increased the sample concentration of serum ELISA in the conventional ELISA protocol (17). The study included serum from false negatives and true negatives who had negative fecal and urine ELISA results. After serum dilution was increased, the result in the true-negative controls remained negative (below the cutoff). On the other hand, the antibody unit in the false-negative group increased, and some of them became positive (our unpublished data). This meant that lowering the serum ELISA dilution can improve the positivity of the ELISA results similar to for urine ELISA. However, the serum dilution of 1:8,000 was reported as the optimal condition for serum ELISA (17). A further study to confirm the result of concentrated serum prior to ELISA is required.

The concentrated-urine ELISA protocol outlined here may be used for strongyloidiasis screening, especially in risk groups with a low immune response, or to improve diagnosis of strongyloidiasis in regions of low prevalence or low endemicity. Remaining questions include whether our current concentrated-urine ELISA can verify if a person is cured after antiparasitic drug treatment. In this regard, in a posttreatment study of schistosomiasis, Grenfell and colleagues demonstrated an improvement of conventional POC in urine antigen assays by including a fast and simple urine-concentration step. This provided not only an increase in accuracy before and after praziquantel treatment, but also preserved its applicability in areas of low-prevalence endemicity (28).

There are several weak points in this study which require consideration for future study. First, in spite of parasitologically positive tests, a considerable proportion of the participants showed false-negative results by urine and serum ELISA, and this indicates the need for standard fecal examination in parallel with immunological methods. It is not clear whether or not the extent of false-negative results depends on transmission levels in a given community. Second, since day-to-day variation of urine dilution can influence the antibody concentration in urine, adjustment by standardization of urine using specific gravity or creatinine levels may assist for better accuracy, reproducibility, and interpretation of ELISA results (29). Third, it needs to be determined whether physical properties of urine, such as turbidity, or the presence of background disease, i.e., diabetes and kidney disease, influence the outcomes of concentrated-urine ELISA. Fourth, cross-reaction studies are limited to date and need to cover more parasite species, i.e., *Trichuris*, filarial worms, hookworms, and coexisting liver flukes (*O. viverrini*), to determine the specificity of the concentrated-urine ELISA. Lastly, the association between *Strongyloides*-specific IgG in serum and urine in different transmission settings is required to justify the use of urine ELISA instead of conventional serum-based ELISA for diagnosis of strongyloidiasis.

In conclusion, the present study has highlighted that the addition of a urine-concentration protocol prior to analysis by ELISA improved diagnostic sensitivity of strongyloidiasis in false-negative cases and diagnostic borderline cases. Concentrated-urine ELISA found a higher proportion of positive cases than did fecal examinations (APCT and FECT). The false-negative cases determined by conventional urine ELISA and serum ELISA became 100% positive by concentrated-urine ELISA. Overall, concentrated-urine ELISA identified 63% more positive cases than did unconcentrated-urine ELISA. Pending further refinement and simplification of the concentration protocol, the concentrated-urine ELISA is potentially useful for diagnosis and screening of strongyloidiasis, especially in risk groups with a low immune response or in low-prevalence areas and also in post drug-treatment follow-up studies. However, this finding on improved diagnostic

sensitivity of strongyloidiasis by an addition of a urine-concentration step prior to analysis by ELISA is considered preliminary evidence. Further studies are required to confirm the result of concentrated-urine ELISA by using a more comprehensive approach, such as serum ELISA and multiple fecal examinations as well as copro-PCR diagnosis. The utility of urine concentration for diagnosis of strongyloidiasis by ELISA may be evaluated in larger sample populations in areas with various transmission levels, in low immune responders, and for monitoring responses to drug treatment.

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We declare no potential conflicts of interest.

All human specimens and the protocols in this study were approved by the Human Ethics Committee of Khon Kaen University, based on the ethics of human specimen experimentation of the National Research Council of Thailand (HE631309), and informed consent was obtained from each subject before specimen collection.

REFERENCES

1. Olsen A, van Lieshout L, Marti H, Polderman T, Polman K, Steinmann P, Stothard R, Thybo S, Verweij JJ, Magnussen P. 2009. Strongyloidiasis: the most neglected of the neglected tropical diseases? Trans R Soc Trop Med Hyg 103:967–972. https://doi.org/10.1016/j.trstmh.2009.02.013.
2. Schar F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P, Odermatt P. 2013. Strongyloides stercoralis: global distribution and risk factors. PLoS Negl Trop Dis 7:e2288. https://doi.org/10.1371/journal.pntd.0002288.
3. Montes M, Sawhney C, Barros N. 2010. Strongyloides stercoralis: there but not seen. Curr Opin Infect Dis 23:500–504. https://doi.org/10.1097/QCO.0b013e32833df718.
4. Ganesh S, Cruz RJ Jr. 2011. Strongyloidiasis: a multifaceted disease. Gastroenterol Hepatol (N Y) 7:194–196.
5. Ericsson CD, Steffen R, Siddiqui AA, Berk SL. 2001. Diagnosis of Strongyloides stercoralis infection. Clin Infect Dis 33:1040–1047. https://doi.org/10.1086/322707.
6. Verweij JJ, Canales M, Polman K, Ziem J, Brienen EA, Polderman AM, van Lieshout L. 2009. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. Trans R Soc Trop Med Hyg 103:342–346. https://doi.org/10.1016/j.trstmh.2008.12.001.
7. Krolewiecki AJ, Ramanathan R, Fink V, McAuliffe I, Cajal SP, Won K, Juarez M, Di Paolo A, Tapia L, Acosta N, Lee R, Lammie P, Abraham D, Nutman TB. 2010. Improved diagnosis of Strongyloides stercoralis using recombinant antigen-based serologies in a community-wide study in northern Argentina. Clin Vaccine Immunol 17:1624–1630. https://doi.org/10.1128/CVI.00259-10.
8. Fernandez-Soto P, Celis-Giraldo CT, Collar-Fernandez C, Gorgojo O, Camargo M, Munoz J, Salas-Coronas J, Patarroyo MA, Muro A. 2020. Strong-LAMP assay based on a Strongyloides spp.-derived partial sequence in the 18S rRNA as potential biomarker for strongyloidiasis diagnosis in human urine samples. Dis Markers 2020:5265198. https://doi.org/10.1155/2020/5265198.
9. Buonfrate D, Requena-Mendez A, Angheben A, Cinquini M, Cruciani M, Fittipaldo A, Giorli G, Gobbi F, Piubelli C, Bisoffi Z. 2018. Accuracy of molecular biology techniques for the diagnosis of Strongyloides stercoralis infection: a systematic review and meta-analysis. PLoS Negl Trop Dis 12:e0006229. https://doi.org/10.1371/journal.pntd.0006229.
10. Sato Y, Takara M, Otsuru M. 1985. Detection of antibodies in strongyloidiasis by enzyme-linked immunosorbent assay (ELISA). Trans R Soc Trop Med Hyg 79:51–55. https://doi.org/10.1016/0035-9203(85)90233-0.
11. Bisoffi Z, Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, Albonico M, Gobbo M, Bonafini S, Angheben A, Requena-Mendez A, Munoz J, Nutman TB. 2014. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. PLoS Negl Trop Dis 8:e2640. https://doi.org/10.1371/journal.pntd.0002640.
12. Bon B, Houze S, Talabani H, Magne D, Belkadi G, Develoux M, Senghor Y, Chandenier J, Ancelle T, Hennequin C. 2010. Evaluation of a rapid enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. J Clin Microbiol 48:1716–1719. https://doi.org/10.1128/JCM.02364-09.
13. Arifin N, Hanafiah KM, Ahmad H, Noordin R. 2019. Serodiagnosis and early detection of Strongyloides stercoralis infection. J Microbiol Immunol Infect 52:371–378. https://doi.org/10.1016/j.jmii.2018.10.001.
14. Corral MA, Paula FM, Gottardi M, Meisel DM, Castilho VL, Goncalves EM, Chieffi PP, Gryschek RC. 2015. Immunodiagnosis of human strongyloidiasis: use of six different antigenic fractions from Strongyloides venezuelensis parasitic females. Rev Inst Med Trop Sao Paulo 57:427–430. https://doi.org/10.1590/S0036-46652015000500010.

15. Tamarozzi F, Longoni SS, Mazzi C, Pettene S, Montresor A, Mahanty S, Bisoffi Z, Buonfrate D. 2021. Diagnostic accuracy of a novel enzyme-linked immunoassay for the detection of IgG and IgG4 against Strongyloides stercoralis based on the recombinant antigens NIE/SsIR. Parasit Vectors 14:412. https://doi.org/10.1186/s13071-021-04916-x.
16. Yunus MH, Arifin N, Balachandra D, Anuar NS, Noordin R. 2019. Lateral flow dipstick test for serodiagnosis of strongyloidiasis. Am J Trop Med Hyg 101:432–435. https://doi.org/10.4269/ajtmh.19-0053.
17. Eamudomkarn C, Sithithaworn P, Sithithaworn J, Kaewkes S, Sripa B, Itoh M. 2015. Comparative evaluation of Strongyloides ratti and S. stercoralis larval antigen for diagnosis of strongyloidiasis in an endemic area of opisthorchiasis. Parasitol Res 114:2543–2551. https://doi.org/10.1007/s00436-015-4458-3.
18. Anderson NW, Klein DM, Dornink SM, Jespersen DJ, Kubofcik J, Nutman TB, Merrigan SD, Couturier MR, Theel ES. 2014. Comparison of three immunoassays for detection of antibodies to Strongyloides stercoralis. Clin Vaccine Immunol 21:732–736. https://doi.org/10.1128/CVI.00041-14.
19. Sithithaworn P, Srisawangwong T, Tesana S, Daenseekaew W, Sithithaworn J, Fujimaki Y, Ando K. 2003. Epidemiology of Strongyloides stercoralis in north-east Thailand: application of the agar plate culture technique compared with the enzyme-linked immunosorbent assay. Trans R Soc Trop Med Hyg 97:398–402. https://doi.org/10.1016/s0035-9203(03)90069-1.
20. Toledo B, Corral MA, Meisel D, Gottardi M, Abdala E, Costa SF, Pierrotti LC, Lescano SAZ, Goncalves EMN, Castilho VLP, Chieffi PP, Gryschek RCB, Paula FM. 2019. Screening of Strongyloides infection using an ELISA test in transplant candidates. Clinics (Sao Paulo) 74:e698. https://doi.org/10.6061/clinics/2019/e698.
21. Eamudomkarn C, Sithithaworn P, Kamamia C, Yakovleva A, Sithithaworn J, Kaewkes S, Techasen A, Loilome W, Yongvanit P, Wangboon C, Saichua P, Itoh M, Bethonoy JM. 2018. Diagnostic performance of urinary IgG antibody detection: a novel approach for population screening of strongyloidiasis. PLoS One 13:e0192598. https://doi.org/10.1371/journal.pone.0192598.
22. Lodh N, Caro R, Sofer S, Scott A, Krolewiecki A, Shiff C. 2016. Diagnosis of Strongyloides stercoralis: detection of parasite-derived DNA in urine. Acta Trop 163:9–13. https://doi.org/10.1016/j.actatropica.2016.07.014.
23. Ruantip S, Eamudomkarn C, Techasen A, Wangboon C, Sithithaworn J, Bethony JM, Itoh M, Sithithaworn P. 2019. Accuracy of urine and serum assays for the diagnosis of strongyloidiasis by three enzyme-linked immunosorbent assay protocols. Am J Trop Med Hyg 100:127–129. https://doi.org/10.4269/ajtmh.18-0569.
24. Koga K, Kasuya S, Khamboonruang C, Sukhavat K, Ieda M, Takatsuka N, Kita K, Ohtomo H. 1991. A modified agar plate method for detection of Strongyloides stercoralis. Am J Trop Med Hyg 45:518–521. https://doi.org/10.4269/ajtmh.1991.45.518.
25. Elkins D, Sithithaworn P, Haswell-Elkins M, Kaewkes S, Awacharagan P, Wongratanacheewin S. 1991. Opisthorchis viverrini: relationships between egg counts, worms recovered and antibody levels within an endemic community in Northeast Thailand. Parasitology 102:283–288. https://doi.org/10.1017/S0031182000062600.
26. Sithithaworn J, Sithithaworn P, Janrungsopa T, Suvatanadecha K, Ando K, Haswell-Elkins MR. 2005. Comparative assessment of the gelatin particle agglutination test and an enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis. J Clin Microbiol 43:3278–3282. https://doi.org/10.1128/JCM.43.7.3278-3282.2005.
27. Ruantip S, Eamudomkarn C, Kopolrat KY, Sithithaworn J, Laha T, Sithithaworn P. 2021. Analysis of daily variation for 3 and for 30 days of parasite-specific IgG in urine for diagnosis of strongyloidiasis by enzyme-linked immunosorbent assay. Acta Trop 218:105896. https://doi.org/10.1016/j.actatropica.2021.105896.
28. Grenfell RFQ, Taboada D, Coutinho LA, Pedrosa MLC, Assis JV, Oliveira MSP, Cruz RR, Almeida A, Silva-Moraes V, Katz N, Coelho PMZ. 2018. Innovative methodology for point-of-care circulating cathodic antigen with rapid urine concentration for use in the field for detecting low Schistosoma mansoni infection and for control of cure with high accuracy. Trans R Soc Trop Med Hyg 112:1–7. https://doi.org/10.1093/trstmh/try014.
29. Wangboon C, Worasith C, Thanan R, Eamudomkarn C, Techasen A, Sithithaworn J, Loilome W, Chamadol N, Pinlaor S, Jumnainsong A, Yongvanit P, Khuntikeo N, Bethony JM, Sithithaworn P. 2019. Evaluation of a short term effect of praziquantel treatment in opisthorchiasis-induced hepatobiliary inflammation by urinary 8-oxodG. Acta Trop 189:124–128. https://doi.org/10.1016/j.actatropica.2018.10.003.