**Capillaria Ova and Diagnosis of Trichuris trichiura Infection in Humans by Kato-Katz Smear, Liberia**

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We examined human stool samples from Liberia for soil-transmitted helminth ova by Kato-Katz smear and by quantitative PCR. Twenty-five samples were positive for *Trichuris trichiura* by smear but negative by quantitative PCR. Reexamination of samples showed that they contained *Capillaria* eggs that resemble *T. trichiura* in Kato-Katz smears.

Kato-Katz smears are the most commonly used diagnostic tool for detecting and quantifying soil-transmitted helminth (STH) infections in field surveys (1). Although this method has some shortcomings, its advantages are field suitability and fast microscopic enumeration of worm eggs. Whereas sensitivity is low for light infections because of the small amount of stool examined (<41 mg), the specificity of Kato-Katz for diagnosis of *Ascaris lumbricoides* and *Trichuris trichiura* infection is considered to be high (2). In contrast, hookworm eggs are difficult to differentiate by morphology, but quantitative PCR (qPCR) enables differentiation among *Necator americanus*, *Ancylostoma duodenale*, and *A. ceylanicum* eggs (3–5).

Among helminth eggs found in human feces, the barrel-shaped eggs of *T. trichiura* worms are considered to be characteristic, with a length of 50–55 µm, a width of 22–24 µm, and clearly protruding bipolar plugs (6). Similar eggs of other members of the *Trichiuridae* family may be differentiated from *T. trichiura* eggs by size and morphology when observed at high magnification, but these eggs have rarely been found in human fecal samples (7–9). Therefore, the presence of eggs of zoonotic members of the *Trichiuridae* family is generally not considered a confounder for detecting *T. trichiura* by Kato-Katz smear.

The Study

To assess the effect of mass drug administration using ivermectin and albendazole for the elimination of lymphatic filariasis on STH prevalence and intensity, we collected stool samples over a period of 3 years in 2 different areas in Foya district (Lofa County) in northwestern Liberia and in Harper district (Maryland County) in southeastern Liberia (10). We examined a single stool sample per subject by microscopy (magnification ×100) with duplicate Kato-Katz smears (41-mg template). We preserved aliquots of randomly selected specimens on FTA cards (GE Healthcare, Little Chalfont, UK) or in RNA later (ThermoFisher, Waltham, MA, USA) and shipped them to Washington University School of Medicine (St. Louis, MO, USA) for analysis by qPCR. Two experienced microscopists (L.G., A.T. Momolu) examined the samples by Kato-Katz smear in both study areas. For detection of STH by qPCR, we extracted DNA from ≈100 mg of stool and tested it as described by Pilotte et al. (5) with a Quantstudio 6 Flex Thermocycler (Applied Biosystems, Carlsbad, CA, USA) and TaqMan Fast Advanced Mastermix (Applied Biosystems). We used the following primers and probes to detect *Schistosoma mansoni* DNA: forward primer 5′-TGTTGGAGTCTTTGTGTTGTGTGTT-3′, reverse primer 5′-CAACATGACTGGGAA-CAGGA-3′, probe 5′-AGGGTTCAAGGTG/ZEN/GTTGTT-3′. We tested 353 stool samples from Foya district by Kato-Katz smear; 163 (72.4%) were positive for *A. lumbricoides* eggs, 231 (65.4%) for hookworm eggs, 27 (7.6%) for *T. trichiura*–like eggs, and 276 (78.2%) for *S. mansoni* eggs. We tested 225 samples from Harper district by Kato-Katz smear; 163 (72.4%) were positive for *A. lumbricoides* eggs, 65 (28.9%) for hookworm eggs, and 51 (22.7%) for *T. trichiura* eggs (Table 1). There was good agreement between the results of the Kato-Katz and qPCR tests for the specimens from Harper (80.5%–91.6%), but generally qPCR had higher sensitivity. Our results were consistent with results previously reported with samples from other areas (3,11). Agreement between the 2 diagnostic tests for samples from Foya ranged from 77.3% to 92.9%, but the sensitivity of the qPCR was unexpectedly low, a finding that was especially true for *Ascaris* and *Trichuris* infection (Table 1). Whereas samples positive for *Ascaris* by Kato-Katz but negative by qPCR had low egg counts, samples positive for *Trichuris* by Kato-Katz...
but negative by qPCR had higher counts; 7 samples contained $\geq 1,000$ barrel-shaped eggs/g of stool (Table 2). We repeated DNA extraction and qPCR and also used an alternative qPCR for *T. trichiura* (3), but these tests did not improve the agreement between microscopy and qPCR results.

To check further whether Kato-Katz–positive, qPCR-negative stool samples contained *T. trichiura* eggs, we examined direct smears of stool samples preserved in RNA later by microscopy (magnification $\times 100$ and $\times 400$) (Figure 1). The samples positive by qPCR contained eggs (6 measured) with typical *T. trichiura* morphology; these eggs had a mean ($\pm SD$) length of $52 \mu m (\pm 2.4 \mu m)$ and width of $25.5 \mu m (\pm 1.3 \mu m)$. In contrast, qPCR-negative samples contained eggs (31 measured) with a mean ($\pm SD$) length of $51.8 \mu m (\pm 1.5 \mu m)$ and width of $32.7 \mu m (\pm 2.1 \mu m)$. The qPCR-negative samples also had less pronounced plugs and a thick, striated shell, features that are consistent with eggs of *Capillaria hepatica* (syn. *Calodium hepaticum*) and some other *Capillaria* species (*Trichuridae*). Eggs of *C. philippinensis* or *C. aerophila* that have been observed in human stool samples previously were either smaller or larger than the *Capillaria* eggs found in Lofa (12,13). Because polar plugs of these eggs are less prominent than those of *T. trichiura*, and because their shapes are sometimes more oval or round, they can also be confused with *A. lumbricoides* eggs by low-power microscopy, especially if only a few eggs were detected (Figure 1).

Members of the subfamily *Capillaridae* are animal parasites with somewhat divergent life cycles, and most

| Site and species | No. positive* | Kato-Katz smear sensitivity, % | qPCR sensitivity, % | McNemar p value |
|------------------|---------------|------------------------------|---------------------|-----------------|
| Foya district, n = 353 | | | | |
| *Ascaris lumbricoides* | 34 | 91.2 | 17.6 | $<0.0001$ |
| Hookworm† | 247 | 93.5 | 83.4 | $<0.0001$ |
| *Trichuris trichiura* | 27 | 100 | 7.4 | $<0.0001$ |
| *Schistosoma mansoni* | 307 | 89.9 | 84.0 | 0.0573 |
| Harper district, n = 225 | | | | |
| A. lumbricoides | 180 | 90.6 | 98.9 | 0.0013 |
| Hookworm† | 99 | 65.7 | 89.9 | 0.0005 |
| *T. trichiura* | 86 | 59.3 | 94.2 | 0.0001 |

*Samples that tested positive by either method.
†Hookworm was *Necator americanus*. No *Ancylostoma duodenale* was detected.

Table 2. Demographics and Kato-Katz and qPCR results for patients positive for *Trichuris trichiura* infection by microscopy, Liberia*.

| Year | Patient no. | Age, y/sex | Village | Demographics | Microscopy, epg | qPCR, cycle threshold |
|------|-------------|------------|---------|--------------|-----------------|----------------------|
| 2014 | P320529 | 45/F | Yallahun | Tt 576 | 24 | Neg |
|      | P320683 | 35/F | Kpombu | 12 | 0 | 0 |
|      | P320694 | 16/M | Kpombu | 24 | 0 | 72 |
|      | P320620 | 15/M | Foya-Dundu | 12 | 120 | 288 |
|      | P320746 | 9/F | Bandenin | 24 | 0 | 0 |
|      | P320452 | 7/F | Felaele | 12 | 0 | 0 |
|      | P320596 | 6/F | Foya-Dundu | 12 | 0 | 90 |
|      | P320656 | 6/F | Kpombu | 120 | 0 | 504 |
| 2016 | P331772 | 36/M | Kpombu | 3,048 | 12 | 24 |
|      | P331921 | 35/M | Felaele | 60 | 0 | 12 |
|      | P331783 | 34/F | Kpombu | 420 | 0 | 0 |
|      | P330724 | 26/M | Keyabendu | 4,224 | 0 | 456 |
|      | P331791 | 6/F | Kpombu | 12 | 0 | 156 |
|      | P331862 | 6/F | Bandenin | 12 | 0 | 168 |
|      | P331983 | 6/F | Bandenin | 36 | 0 | 5,304 |
| 2017 | P341287 | 61/M | Mendikorma | 1,484 | 0 | 0 |
|      | P341282 | 58/M | Mendikorma | 540 | 216 | 0 |
|      | P341284 | 50/M | Mendikorma | 60 | 0 | 132 |
|      | P342148 | 45/M | Keyabendu | 1,368 | 0 | 192 |
|      | P340246 | 39/M | Kamatuhun | 120 | 0 | 216 |
|      | P340307 | 19/F | Bambuloe | 2,028 | 0 | 1,188 |
|      | P340133 | 12/M | Fokolahun | 1,020 | 16,392 | 0 |
|      | P340183 | 9/F | Kpeloe | 72 | 0 | 0 |
|      | P341308 | 9/F | Mendikorma | 36 | 0 | 108 |
|      | P341326 | 9/M | Mendikorma | 456 | 0 | 0 |
|      | P341327 | 6/M | Mendikorma | 2,076 | 0 | 0 |
|      | P340147 | 5/M | Fokolahun | 48 | 0 | 0 |

* $T. trichiura$ infection was confirmed by qPCR in only 2 patients, but 25 had *Capillaria* eggs in their stool. Al, *Ascaris lumbricoides*; epg, eggs per gram of stool; Hk, hookworm; Na, *Necator americanus*; Neg, negative; Sm, *Schistosoma mansoni*; Tt, *T. trichiura*.
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do not infect humans. Pseudoinfections with C. hepatica occur; eggs found in stool are present because they were consumed in infected animal liver. However, actual infections with C. hepatica do not lead to the passing of eggs in stool (9). Other species such as C. philippinensis cause true infections (and autoinfection) with eggs found in stool; the infection is linked to consumption of raw fish. Human capillariasis has not been reported from Liberia, and only isolated case reports have been published from sub-Saharan Africa (7–9). We performed DNA sequencing to better characterize the Capillaria species found in Foya. Using the primers Kt875351.1 (5′-CCCTAGTTGCGACTTTAAACGA-3′) and Capillaria 18S1R (5′-TCCACCAACTAAGAACGGCC-3′), we were able to amplify and sequence a 288-bp portion of the 18S rDNA from T. trichiura qPCR-negative samples that contained only eggs morphologically identified as Capillaria spp. (GenBank accession no. MG859285). The DNA fragment was 100% identical to orthologs of C. hepatica (accession no. MF287972.1), Aonchotheca putorii (C. putorii) (accession no. LC052356.2), and Pearsonema plica (C. plica) (accession no. MF621034.1), Capillaria worm species that have varying life cycles and host species but that are only 95% identical to the ortholog of T. trichiura.

The life cycle and the medical importance of the Capillaria species found in humans in northwestern Liberia remain to be elucidated. In our study some subjects showed high Capillaria egg loads that may indicate a true infection rather than pseudoinfection. However, transient high egg counts have been reported in persons with pseudoinfections (7). Whereas consumption of bush meat in Foya is common, consumption of raw or undercooked fish, which is necessary for transmission of C. philippinensis, is rare.

Conclusions
This study shows that Capillaria eggs similar to those of C. hepatica are not uncommon in stool samples collected in Liberia. These eggs can be misidentified by Kato-Katz smear as T. trichiura or as A. lumbricoides, which can confound results of STH surveys. The misidentification can also lead to an incorrect assumption that anthelmintic treatment was ineffective. Our results also illustrate the value of qPCR for validating Kato-Katz test results and for explaining unexpected findings.

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About the Author
Ms. Fischer is a medical technician and staff scientist at the Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA. For the last 20 years, her research has focused on medical helminthology and tropical medicine.
Infection with Baylisascaris procyonis roundworms is rare but often fatal and typically affects children. Baylisascaris procyonis, the common intestinal roundworm of raccoons, has increasingly been recognized as a source of severe, often fatal, neurologic disease in humans, particularly children. Although this devastating disease is rare, lack of effective treatment and the widespread distribution of raccoons in close association with humans make baylisascariasis a disease that seriously affects public health. Raccoons infected with B. procyonis roundworms can shed millions of eggs in their feces daily. Given the habit of raccoons to defecate in and around houses, information about optimal methods to inactivate B. procyonis eggs are critical for the control of this disease. However, little information is available about survival of eggs and effective disinfection techniques. Additional data provide information on thermal death point and determining the impact of desiccation and freezing on the viability of B. procyonis eggs to provide additional information for risk assessments of contamination and guide attempts at environmental decontamination.

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