MINI-FLOTAC AS AN ALTERNATIVE, NON-INVASIVE DIAGNOSTIC TOOL FOR SCHISTOSOMA MANSONI AND OTHER TREMATODE INFECTIONS IN WILDLIFE RESERVOIRS

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Abstract

Background: Schistosomiasis and food-borne trematodiases are not only of major public health concern, but can also have profound implications for livestock production and wildlife conservation. The zoonotic, multi-host nature of many digenean trematodes is a significant challenge for disease control programmes in endemic areas. However, our understanding of the epidemiological role that animal reservoirs, particularly wild hosts, may play in the transmission of zoonotic trematodiases suffers a dearth of information, with few, if any, standardised, reliable diagnostic tests available. We combined qualitative and quantitative data derived from post-mortem examinations, coprological analyses using the Mini-FLOTAC technique, and molecular tools to assess parasite community composition and the validity of non-invasive methods to detect trematode infections in 89 wild Hubert’s multimammate mice (Mastomys huberti) from northern Senegal.

Results: Parasites isolated at post-mortem examination were identified as Plagiorchis sp., Anchitrema sp., Echinostoma caproni, Schistosoma mansoni, and a hybrid between Schistosoma haematobium and Schistosoma bovis. The reports of E. caproni and Anchitrema sp. represent the first molecularly confirmed identifications for these trematodes in definitive hosts of sub-Saharan Africa. Comparison of prevalence estimates derived from parasitological analysis at post-mortem examination and Mini-FLOTAC analysis showed non-significant differences indicating comparable results between the two techniques ($P = 1.00$ for S. mansoni; $P = 0.85$ for E. caproni; $P = 0.83$ for Plagiorchis sp.). A Bayesian model, applied to estimate the sensitivities of the two tests for the diagnosis of Schistosoma infections, indicated similar median posterior probabilities of 83.1% for Mini-FLOTAC technique and 82.9% for post-mortem examination (95% Bayesian credible intervals of 64.0–94.6% and 63.7–94.7%, respectively).

Conclusions: Our results showed that the Mini-FLOTAC could be applied as an alternative diagnostic technique for the detection of the zoonotic S. mansoni and other trematodes in rodent reservoirs. The implementation of non-invasive diagnostics in wildlife would offer numerous advantages over lethal sampling methodologies, with potential impact on control strategies of zoonotic helminthiases in endemic areas of sub-Saharan Africa and on fostering a framework of animal use reduction in scientific practice.
Background

Digenean trematodes (phylum Platyhelminthes) are characterised by complex life cycles involving replication by asexual reproduction within their intermediate hosts and transmission to vertebrate definitive hosts via ingestion, with sexual reproduction of the hermaphroditic adult parasites in their final infection site [1]. The sole exception is represented by members of the family Schistosomatidae, which are dioecious parasites (i.e. separate sexes) infecting their definitive host via skin penetration [2]. Trematodiasis are of great medical and veterinary importance, responsible for public health issues, economic losses, and conservation concerns [3–6]. Estimates from the World Health Organization show that globally about 220 million people required preventive treatment for schistosomiasis in 2017 [7], while millions of people are suffering one or more food-borne trematodiases [8].

The complex multi-host, zoonotic nature of trematodiases may have a considerable impact on the outcome of disease control programmes in endemic areas [9, 10]. The role of wild small mammals as disease reservoirs is emerging as a public health concern, and the involvement of rodents in the transmission of human agents of schistosomiasis (e.g. *Schistosoma japonicum* and *Schistosoma mansoni*) in different regions of the world is a noteworthy example [11–13]. To date, the characterisation of helminth communities infecting wildlife has largely relied on lethal sampling, severely restricting the host species that can be studied, the adequacy of sampling strategies and sizes, and the scope of the scientific questions that can be addressed [14]. However, diagnostic approaches based on faecal egg count (FEC) techniques alone in wild hosts are inevitably limited to a coarse morphological identification of parasitic elements, often to the taxonomic ranks of either order or family, preventing the fine-scale partitioning of parasite fauna composition [14, 15]. DNA-based methods could contribute significantly to the correct identification of parasitic taxa while implementing non-invasive sampling strategies. Nevertheless, the exclusive application of molecular techniques may under represent parasite community composition and inaccurately depict quantitative estimates of infection if inferences are not properly tested [9, 14].

The diagnostic accuracy and applicability of a range of methodologies have been tested for the improved detection of trematodiases in humans (e.g. rapid tests for circulating antigens, urine and stool microscopy, serological tests, and DNA-based methods) [16–18]. In contrast, our understanding of the epidemiological role that animal reservoirs, particularly wild hosts, may play in the transmission of zoonotic trematodiases is constrained by a dearth of information and standardised, reliable diagnostic tests available [9]. Our aim was to assess trematode infections in wild Hubert's multimammate mice (*Mastomys huberti*) from northern Senegal via the concerted application of post-mortem examination, FEC using the Mini-FLOTAC technique, and molecular analysis. Mini-FLOTAC, combined with Fill-FLOTAC, is a tool based on the flotation of parasitic eggs without requiring a centrifuge (and therefore power supply) for processing [19]. Furthermore, a portion of the faecal samples can be fixed in formalin and stored prior to the analysis, making the method versatile and easy to implement in resource-limited field settings [19–21]. In particular, our objective was to assess the performance of the Mini-FLOTAC as an alternative tool for the detection of *Schistosoma* infections in rodent reservoirs, and therefore its future applicability within non-invasive sampling schemes.

Methods

Post-mortem examination

Between May 2016 and December 2017, sampling of small mammals was conducted at sites in and around the town of Richard Toll (16° 27’ N, 15° 41’ W) and on the shores of Lake Guiers (16° 15’ N, 15° 51’ W), Senegal, following methodologies previously described [13, 22]. At post-mortem examination of *M. huberti*, thoracic and abdominal organs were dissected, scraped, washed with tap water, and observed for the presence of helminths using a glass tray against a black background. The isolated adult digeneans were microscopically identified to the genus level based on their morphology (see identification keys in [23]), counted to quantify infection intensity, and stored in 95% ethanol at −20 °C until molecular analysis. Morphological identification of preserved specimens was obtained after staining in Semichon's carmine, immersion in clearing medium (i.e. ethanol followed by xylene), and mounting on a microscope slide using Canada balsam. For *Plagiorchis* isolates, infection intensity was quantified up to 61 worms per organ; time constraints during fieldwork prevented the integral count of *Plagiorchis* parasites observed in the biliary tract and/or small intestine, therefore the value >61 was used to indicate higher intensities. During post-mortem examinations, faecal material from the necropsied individuals was collected from the rectum (n = 89) and from underneath the wire-mesh live...
trap \((n=8)\) into separate vials, weighted \((0.1-0.7 \, \text{g})\), and stored in 1.5 ml of 10% neutral-buffered formalin.

**Molecular analysis**

After rehydration in nuclease-free water, DNA from individual trematode specimens was extracted using either the Epicentre® MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) or the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. DNA extracts were eluted in 30 μl TE buffer and amplified for the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) and the partial cytochrome c oxidase subunit 1 gene (cox1) of the mitochondrial DNA (mtDNA) using the primer pairs ETTS1 (5'-TGC TTA AGT TCA GCG GGT-3') and ETTS2 (5'-AAC AAG GTT TCC GTA GGT GAA-3') [24], and 2575 (5'-TTT TTT GG GGG CAT CCT GAG GTT TAT-3') and 3021 (5'-TAA AGA AGG AAT ATG AAA ATG-3') [25], respectively. Enzymatic amplification for polymerase chain reaction (PCR) was performed in 25 μl reaction mixtures including PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare UK Limited, Little Chalfont, UK), 0.5 μmol/l of each primer and 2 μl DNA template. Cycling parameters for the ITS region consisted of an initial nucleic acid denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min, with a final extension step for 7 min at 72 °C. Cycling parameters for the cox1 gene consisted of an initial nucleic acid denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 1 min, and 72 °C for 1 min, with a final extension step for 7 min at 72 °C. PCR products were sequenced using the original PCR primers in a 3730xl DNA Analyzer system by Eurofins Genomics (Ebersberg, Germany). Contig assembly and editing were performed with CodonCode Aligner v8.0.1 (CodonCode Corporation, Centerville, MA, USA) and the resulting sequences were compared by alignment with data available in the GenBank database.

**Mini-FLOTAC technique**

Faecal samples were analysed between four and six months after their collection date using the Fill-FLOTAC 2 and Mini-FLOTAC devices [19], together with a flotation solution (FS) made of zinc sulphate heptahydrate \((\text{H}_14\text{O}_{11}\text{SZn})\) and tap water (FS7, see [26] for further details on the different FS types). This FS7 was confirmed to be at a density of 1.35 with a hydrometer (Bran-nan, Cleator Moor, UK). Each faecal sample was fully homogenised in order to fill the Mini-FLOTAC chambers following standard operating procedures [19]. After an average waiting time of 10 min to allow the flotation of parasitic eggs, we performed a double-blind observation of both Mini-FLOTAC ruled grids under an Olympus CX41 microscope equipped with an Olympus DP20 camera, counting all the parasitic eggs we observed. Eggs per gram (EPG) estimates, herein considered a proxy for infection intensity, were calculated following the described protocol [19]: we multiplied the obtained number of parasitic eggs by the multiplication factor, which was derived from dividing the dilution factor by the analysed volume (i.e. 2 ml) in the Mini-FLOTAC chambers (Table 1).

**Statistical analysis**

Statistically significant differences in the proportion of positive individuals were analysed using Pearson’s chi-square test. After the data distribution was assessed as non-normal, significant correlations in the intensity of trematode infections between post-mortem and faecal examinations were analysed using the non-parametric Spearman’s rank correlation \((\rho)\) coefficient. Confidence intervals (CI) at 95% level were calculated for proportions of positive individuals using the Agresti-Coul interval [27]. Statistical tests, considered significant when \(P \leq 0.05\), were implemented in R v3.1.2.

A Bayesian model was applied to estimate the adjusted (true) proportion of individuals positive to Schistosoma infection and the diagnostic accuracy of post-mortem examination and Mini-FLOTAC technique. The model was based on the assumption that the probability \((\pi)\) of a positive test for each technique can be expressed as \(p = \pi \cdot Se + (1 - \pi) \cdot (1 - Sp)\), where \(Se\) represents the true proportion of infection in the population, while \(Se\) and \(Sp\) represent the sensitivity and specificity of the diagnostic techniques, respectively [28]. Prior estimates of the

### Table 1 Grams of faeces, dilution factors, and multiplication factors used to calculate eggs per gram estimates derived from the trematode egg counts

| Grams of faeces | Dilution factor\(^a\) | Multiplication factor\(^b\) |
|----------------|----------------------|--------------------------|
| 0.1            | 150.0                | 75.0                     |
| 0.2            | 75.0                 | 37.5                     |
| 0.3            | 50.0                 | 25.0                     |
| 0.4            | 37.5                 | 18.5                     |
| 0.5            | 30.0                 | 15.0                     |
| 0.6            | 25.0                 | 12.5                     |
| 0.7            | 21.5                 | 10.5                     |

\(^a\) Calculated by dividing the final volume of the solution (15 ml) by the amount of faeces analysed (0.1–0.7 g)  
\(^b\) Calculated by dividing the corresponding dilution factor by the analysed volume of 2 ml
sensitivity (i.e. the proportion of true positives that are correctly identified as such) for post-mortem examination and Mini-FLOTAC technique could not be derived since data, applicable to the surveyed host population, were not available. We used uninformative $\beta$-distribution priors ($\beta \sim (1, 1)$), equivalent to a uniform distribution ranging from zero to one. Specificity (i.e. the proportion of true negatives that are correctly identified as such) of each test was assumed to be 100%. Posterior probabilities were inferred using JAGS v4.3.0 [29] in conjunction with R v3.5.1 (through the rjags and coda packages), implementing two Markov Chain Monte Carlo chains, 200,000 iterations, ‘burn-in’ of 5000, and thinning interval of 40.

Results

Based on the combined morphological and molecular analysis of rDNA and mtDNA data, the trematodes collected at post-mortem were identified as *Echinostoma caproni*, *Plagiorchis* sp., *Anchitrema* sp., *S. mansoni*, and a hybrid between *Schistosoma haematobium* and *Schistosoma bovis*. Overall, these parasites were isolated in 86 out of 89 *M. huberti* (96.6%; 95% CI: 90.6–98.8%), with:

- *Plagiorchis* sp. in the biliary tract and/or small intestine of 78 hosts (87.6%; 95% CI: 79.0–93.1%); *Schistosoma* spp. in the portal system and/or mesenteric vessels of 21 hosts (23.6%; 95% CI: 15.9–33.5%); *E. caproni* in the hepatic parenchyma or small intestine of 15 hosts (16.9%; 95% CI: 10.4–26.1%); and *Anchitrema* sp. in the small intestine of three hosts (3.4%; 95% CI: 0.7–9.9%).

The Mini-FLOTAC analysis identified parasitic eggs in 85 out of 89 individuals (95.5%; 95% CI: 88.7–98.6%), which were morphologically compatible with: *Plagiorchis* sp. in 76 hosts (85.4%; 95% CI: 76.5–91.4%); *S. mansoni* in 21 hosts (23.6%; 95% CI: 15.9–33.5%); *Echinostoma* sp. in 18 hosts (20.2%; 95% CI: 13.1–29.8%); and *Anchitrema* sp. in one host (1.1%; 95% CI: 0–0.7%) (Fig. 1). Results of the combined post-mortem examination, molecular analysis, and Mini-FLOTAC technique are summarised in Table 2. Remarkably, three hosts were positive to *S. mansoni* during the Mini-FLOTAC analysis while their post-mortem examination was negative and vice versa; for *E. caproni*, three hosts were negative at post-mortem whereas their Mini-FLOTAC analysis resulted positive.

Fig. 1 Eggs of *Echinostoma caproni* (A), *Plagiorchis* sp. (B), *Schistosoma mansoni* (C), and *Anchitrema* sp. (D) observed under the microscope during Mini-FLOTAC analysis (scale bars: 100 μm)
### Table 2

Percent prevalence (95% confidence intervals in parentheses) and intensity (median and range in parentheses) of trematode infections in Hubert's multimammate mice (*Mastomys huberti*) at post-mortem examination (infection intensity expressed as parasite counts) and Mini-FLOTAC analysis (infection intensity expressed as eggs per gram)

| Parasite         | Infection | Adults (n = 80) |Juveniles (n = 9) |Total (n = 89) |
|------------------|-----------|----------------|-----------------|---------------|
|                  |           | Males (n = 45) |Females (n = 35) |Males (n = 3) |Females (n = 6) |
| **Plagiorchis sp.** |           |                |                |               |               |
| Post-mortem      | Prevalence (%) | 86.7 (73.4–94.1) | 85.7 (70.1–94.2) | 100 (38.3–100) | 100 (55.7–100) | 87.6 (79.0–93.1) |
|                   | Intensity  | 16 (1–>61)     | 26.5 (2–>61)    | 19 (3–45)     | 21.5 (3–>61)   | 18 (1–>61)      |
| Mini-FLOTAC      | Prevalence (%) | 84.4 (70.9–92.6) | 85.7 (70.1–94.2) | 66.7 (20.2–94.4) | 100 (55.7–100) | 85.4 (76.5–91.4) |
|                   | Intensity  | 4687.5 (92–123,675) | 4,350 (25–134,900) | 2,962.5 (1725–2,475) | 5,212 (610–91,125) | 4,300 (25–134,900) |
| **Schistosoma mansoni** |         |                |                |               |               |
| Post-mortem      | Prevalence (%) | 24.4 (14.1–38.8) | 25.7 (14.0–42.3) | –             | 16.7 (1.1–58.2) | 23.6 (15.9–33.5) |
|                   | Intensity  | 5 (20–35)      | 17 (2–64)       | –             | 2 (na)         | 8 (2–64)        |
| Mini-FLOTAC      | Prevalence (%) | 22.2 (12.3–36.5) | 28.6 (16.2–45.2) | –             | 16.7 (1.1–58.2) | 23.6 (15.9–33.5) |
|                   | Intensity  | 293.5 (50–1237) | 137.5 (15–900)  | –             | 600 (na)       | 262 (15–1237)   |
| **Echinostoma caproni** |     |                |                |               |               |
| Post-mortem      | Prevalence (%) | 20.0 (10.7–34.0) | 143 (5.8–29.9)  | 33.3 (5.6–79.8) | –             | 16.9 (10.4–26.1) |
|                   | Intensity  | 2 (1–32)       | 5 (1–34)        | 1 (na)        | –             | 2 (1–34)        |
| Mini-FLOTAC      | Prevalence (%) | 26.7 (15.8–41.2) | 143 (5.8–29.9)  | 33.3 (5.6–79.8) | –             | 20.2 (13.1–29.8) |
|                   | Intensity  | 135 (18–52,275) | 315 (37–1,375)  | 675 (na)      | –             | 232 (18–52,275) |
| **Anchitrema sp.** |           |                |                |               |               |
| Post-mortem      | Prevalence (%) | 2.2 (0–12.6)   | 5.7 (0.6–19.6)  | –             | –             | 3.4 (0.7–9.9)   |
|                   | Intensity  | 9 (na)         | 12.5 (1–24)     | –             | –             | 9 (1–24)        |
| Mini-FLOTAC      | Prevalence (%) | –             | 2.9 (0–15.8)    | –             | –             | 1.1 (0–0.7)     |
|                   | Intensity  | –             | 750 (na)        | –             | –             | 750 (na)        |

* One pair was formed of *S. mansoni* male and *S. haematobium/S. bovis* hybrid female [13]; however, only eggs with lateral spine, identified as *S. mansoni* eggs, were observed

**Abbreviation:** na, not applicable

The proportion of individuals positive for trematodes was not significantly different between post-mortem examination and Mini-FLOTAC technique, neither when *Plagiorchis* sp. ($\chi^2 = 0.05$, $df = 1$, $P = 0.83$), *S. mansoni* ($\chi^2 = 0.00$, $df = 1$, $P = 1.00$), and *E. caproni* ($\chi^2 = 0.04$, $df = 1$, $P = 0.85$) were considered singularly, nor when they were grouped ($\chi^2 = 0.15$, $df = 1$; $P = 0.70$). The median posterior estimate of the adjusted (true) proportion of individuals positive to *Schistosoma* infection obtained by the Bayesian model in the surveyed *M. huberti* was 28.3% (95% Bayesian credible intervals of 19.4–38.5%). Median posterior sensitivity estimates for Mini-FLOTAC technique and post-mortem examination in the diagnosis of *Schistosoma* infections were 83.1% (95% Bayesian credible intervals of 64.0–94.6%) and 82.9% (95% Bayesian credible intervals of 63.7–94.7%), respectively. Correlation between infection intensities at post-mortem examination (i.e. adult parasite counts) and Mini-FLOTAC analysis (i.e. EPG) was not significant for *Plagiorchis* sp. ($\rho = 0.18$, $P = 0.19$), whereas it was weak for both *E. caproni* ($\rho = 0.51$, $P = 0.053$) and *S. mansoni* (when considering the number of schistosome pairs $\rho = 0.43$, $P = 0.038$; when considering the number of schistosome individuals $\rho = 0.46$, $P = 0.058$). Comparisons for *Anchitrema* sp. could not be made given the small number of infections observed. Similarly, the eight faecal specimens collected from underneath the wire-mesh live trap were not sufficient for meaningful statistical comparisons; however, sensitivity of the Mini-FLOTAC technique was identical to the results obtained on rectal faecal samples collected from the same individual (Table 3).

For all trematode species, representative specimens were archived at the Natural History Museum (London, UK) under the accession numbers 2018.3.7.33–38 (*E. caproni*), 2018.3.7.39–67 (*Plagiorchis* sp.), and 2019.2.13.1–3 (*Anchitrema* sp.). Schistosome trematodes were stored in the Schistosomiasis Collection at the Natural History Museum (SCAN) [30]. Sequencing data were deposited in the GenBank database for *Schistosoma* spp. (accession numbers MF776585-97 for ITS and MF919405-28 for cox1) [13], *Plagiorchis* sp. (accession numbers MH633855-62 for ITS and MH673675-82 for ITS and cox1).
for cox1) [22], and E. caproni (accession numbers MK721181-2 for ITS and MK732350-1 for cox1).

Discussion

The combination of post-mortem examination, Mini-FLOTAC, and molecular analysis was used to assess parasite community composition and FEC as a valid diagnostic method to investigate rodents as reservoirs of zoonotic and non-zoonotic trematodes. The results showed comparable sensitivity estimates for parasitological examination at post-mortem and Mini-FLOTAC, suggesting that this FEC technique could be further implemented in non-invasive sampling strategies targeting trematode infections in rodents. In particular, the diagnostic sensitivity to S. mansoni infections identified the Mini-FLOTAC as a reliable tool for future surveys on rodent reservoirs in the many regions where schistosomiasis is endemic, with the potential to significantly reduce the use of lethal sampling methods. The results showed comparable sensitivity estimates for parasitological examination at post-mortem and Mini-FLOTAC, suggesting that this FEC technique could be further implemented in non-invasive sampling strategies targeting trematode infections in rodents. In particular, the diagnostic sensitivity to S. mansoni infections identified the Mini-FLOTAC as a reliable tool for future surveys on rodent reservoirs in the many regions where schistosomiasis is endemic, with the potential to significantly reduce the use of lethal sampling methods. The results showed comparable sensitivity estimates for parasitological examination at post-mortem and Mini-FLOTAC, suggesting that this FEC technique could be further implemented in non-invasive sampling strategies targeting trematode infections in rodents.
storage time in 10% formalin before processing of the specimens (i.e. between four and six months after collection date). Previous studies and the published protocol for the Mini-FLOTAC advise storage in 5% formalin and for a maximum of approximately one month in order to not impair the sensitivity of the technique [19, 21]. However, our study showed that the diagnostic sensitivity to S. mansoni did not appear to be affected by a longer waiting time before analysis.

The combined morphological and molecular analyses enabled the unequivocal identification of the digenean trematodes isolated at post-mortem examination. The finding of S. mansoni and S. haematobium/S. bovis hybrid has been previously discussed [13]. Similarly, the epidemiology, pathology, and molecular systematics of Plagiorchis sp., a previously undescribed West African lineage, has been documented [22]. To our knowledge, the reports of E. caproni and Anchitrema sp., described herein, are the first molecularly confirmed identifications for these trematodes in definitive hosts of sub-Saharan Africa (see [40–42] for data on gastropod intermediate hosts of E. caproni in the African continent). The life cycle of Anchitrema parasites is largely undetermined since, to date, they have only been recorded in the intestine of various definitive hosts in the tropics and subtropics [23, 43]. Anchitrema sanguineum is the most frequently reported taxon in mammalian hosts but, to date, identifications have been solely based on morphological traits. This species has been sporadically isolated from rats in Egypt [44] and Thailand [45], from the bat Myotis velifer in Mexico [46], and even from the intestinal tract of a domestic dog and a human in Thailand, whose infection was hypothesised to have occurred via oral transmission [43]. In contrast, the biology of E. caproni and other Echinostoma spp. is well studied. These parasites use freshwater gastropods, primarily of the families Planorbidae (e.g. Biomphalaria and Bulinus spp.) and Lymnaeidae, as first intermediate hosts, while fish, molluscs, crustaceans, and amphibians can serve as second intermediate hosts. Infection of definitive hosts (i.e. birds and mammals, including humans), and development into hermaphroditic adult parasites in their intestine and/or biliary tract, occurs by ingestion of metacercariae harboured by the second intermediate host [41, 47–49]. Therefore, echinostomiasis is considered a food-borne zoonosis: whilst the disease does not usually show clinical signs when the infection burden is low, gastrointestinal symptoms and pronounced weight loss can occur with severe infections [47, 50]. Rodents may act as reservoirs of zoonotic Echinostoma spp. by perpetuating the contamination of freshwater bodies with the parasitic eggs via defecation [51, 52]. However, identification of Echinostoma spp. and differentiation between zoonotic and non-zoonotic species can be difficult without a molecular approach due to the morphological similarity among members within the genus [53, 54].

Conclusions
We combined classical and molecular parasitological analyses for species identification and diagnostics testing of zoonotic and non-zoonotic trematodes of wildlife. Our results indicated that the Mini-FLOTAC represents a reliable technique to detect the zoonotic S. mansoni and other parasites in rodent reservoirs. A growing body of information on helminth communities of West African rodents is gradually enhancing our understanding of host use and transmission dynamics [55–57]. To date, the quantification of adult helminths during post-mortem examination remains the gold standard technique for assessing infection in wildlife; as a consequence, non-invasive methods are often untested or sporadically applied [9, 14]. Therefore, additional studies will be necessary to implement the use of the Mini-FLOTAC in non-invasive sampling strategies targeting animal hosts. Future advancements should incorporate FEC diagnostics when obtaining baseline data while testing coprological DNA-based methods. This approach would contribute significantly towards a higher diagnostic throughput and a deeper understanding of the interactions between a parasite and its host community, with potential impact on control strategies of zoonotic helminthiasis and, ultimately, on fostering a framework of animal use reduction in scientific practice.

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Authors’ contributions
SC, EL and JPW conceived and designed the study. SC, CBF, EL, AB, KJM, MS, NDD and KB conducted and coordinated fieldwork. DI, GC and LR provided laboratory reagents, materials, and training. AS, SC and KJM performed the laboratory analyses. SC, AS, KJM and AB analysed the data. SC, EL and JPW drafted the manuscript. All authors provided intellectual input. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article. Parasite specimens and sequencing data from this study are available in the collection of the Natural History Museum (London, UK) and in the
GenBank database, respectively, under the accession numbers listed in this manuscript.

**Ethics approval and consent to participate**

Trapping activities were initiated after explicit consent from local authorities and land owners. Approval for live trapping and euthanasia of small mammals, in compliance with the guidelines of the American Veterinary Medical Association (https://www.avma.org/RB/Policies/Documents/euthanasia.pdf) and the Animal (Scientific Procedures) Act as implemented by the Home Office in Great Britain (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535574/working-with-wild-animals-160706.pdf), was obtained from the Clinical Research Ethical Review Board of The Royal Veterinary College, University of London (reference number 2016 1505).

**Consent for publication**

Not applicable.

**Competing interests**

GC is the inventor and current patent holder of the FILL-FLOTAC and Mini-FLOTAC devices. None of the other authors have any conflict of interest concerning the work reported in this published article.

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**References**

1. Roberts LS, Janovy J, Nadler S. Foundations of parasitology. 9th ed. Boston: McGraw-Hill Education, 2013.
2. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383:2523–64.
3. Thompson RCA, Lembry A, Smith A. Parasites, emerging disease and wildlife conservation. Int J Parasitol. 2010;40:1163–70.
4. Budischak SA, Jolles AE, Ezenwa VO. Direct and indirect costs of co-infection in the wild: linking gastrointestinal parasite communities, host hematolgy, and immune function. Int J Parasitol Wildl. 2012;1:2–12.
5. Lustgarten S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, McCarthy JS, Basáñez MG. A research agenda for helminth diseases of humans: the problem of helmintihesises. PLoS Negl Trop Dis. 2012;6:e1582.
6. Gover CM, Vince L, Webster JP. Should we be treating animal schistosomiasis in Africa? The need for a One Health economic evaluation of schistosomiasis control in people and their livestock. Trans R Soc Trop Med Hyg. 2017;111:244–7.
7. World Health Organization. Schistosomiasis and soil-transmitted helminthiases: numbers of people treated in 2017. Wkly Epidemiol Rec. 2018;93:681–92.
8. Fürst T, Keiser J, Utzinger J, Schuster E. Global burden of human food-borne trematodiases: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12:210–21.
9. Johansen MV, Lier T, Sthithaworn P. Towards improved diagnosis of neglected zoonotic trematodes using a One Health approach. Acta Trop. 2015;141:61–7.
10. Webster JP, Gower CM, Knowles SC, Molyneux DH, Fenton A. One Health - an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evol Appl. 2016;9:313–33.
11. Gentile R, Barreto MG, Gonçalves MM, Soares, D’Andrea PS. The role of wild rodents in the transmission of *Schistosoma mansoni* in Brazil. In: Rokni MB, editor. Schistosomiasis. London: INTECH Open Access Publisher; 2012. p. 231–54.
12. Rudge JW, Webster JP, Lu DB, Wang TP, Fang GR, Basáñez MG. Identifying host species driving transmission of *schistosomiasis* japonica, a multihost parasite system, in China. Proc Natl Acad Sci USA. 2013;110:1457–62.
13. Catalano S, Sène M, Diouf ND, Fall CB, Borlase A, Léger E, et al. Rodents as natural hosts of zoonotic *Schistosoma* species and hybrids: an epidemiological and evolutionary perspective from West Africa. J Infect Dis. 2018;218:429–33.
14. Budischak-SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO. A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Mol Ecol Resour. 2015;15:1112–9.
15. Gassó D, Felu C, Ferrer D, Mentabere G, Casas-Díaz E, Velarde R, et al. Uses and limitations of faecal egg count for assessing worm burden in wild boars. Vet Parasitol. 2015;209:133–7.
16. Keiser J, Duthaler U, Utzinger J. Update on the diagnosis and treatment of food-borne trematode infections. Curr Opin Infect Dis. 2010;23:513–20.
17. Verwey JJ, Stensvold CR. Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. Clin Microbiol Rev. 2014;27:371–418.
18. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect. 2015;21:529–42.
19. Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruyse J, Utzinger J, Rinaldi L. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat Protoc. 2017;12:1723–32.
20. Barda BD, Rinaldi L, Iannidello D, Zepherine H, Salvo F, Sadudzhang T, et al. Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. PLoS Negl Trop Dis. 2013;7:e2344.
21. Barda B, Albonico M, Iannidello D, Arne SM, Keiser J, Speich B, et al. How long can stool samples be fixed for an accurate diagnosis of soil-transmitted helminth infection using Mini-FLOTAC? PLoS Negl Trop Dis. 2015;9:e3698.
22. Catalano S, Nadler SA, Fall CB, Marsh KJ, Léger E, Sène M, et al. Plagiorchis sp. in small mammals of Senegal and the potential emergence of a zoonotic trematodiasis. Int J Parasitol Wildl. 2019;8:164–70.
23. Bray RA, Gibson DJ, Jones A. Keys to the Trematoda, vol. 3. Wallingford: CABI; 2006.
24. Kane RA, Rollinson D. Repetitive sequences in the ribosomal DNA internal transcribed spacer of *Schistosoma haematobia*, *Schistosoma intercalatum* and *Schistosoma matheei*. Mol Biochem Parasitol. 1994;63:153–6.
25. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol. 1992;54:165–73.
26. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J, FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010;5:935–39.
27. Agresti A, Coull BA. Approximate is better than “exact” for interval estimation of binomial proportions. Am Stat. 1998;52:119–26.
28. Branscum AJ, Gardner IA, Johnson VO. Estimation of diagnostic-test sensitivity and specificity through Bayesian modelling. Prev Vet Med. 2005;68:145–63.
29. Plummer M. JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. Proc DSC. 2003;124(125):10.
30. Emery AM, Allan FE, Rabone ME, Rollinson D. Schistosomiasis collection at NHM (SCAN). Parasit Vectors. 2012;5:185.
31. Dinkel A, von Nickisch-Rosenegk M, Bilger B, Merli M, Lucius R, Romig T. Detection of *Echinococcus multilocularis* in the definitive host: coprodagnosis by PCR as an alternative to necropsy. J Clin Microbiol. 1998;36:1871–6.
32. Easton AV, Oliveira RG, Walker M, O’Connell EM, Njenga SM, Mwandawiro CS, et al. Sources of variability in the measurement of *Ascaris lumbricoides* infection intensity by Kato-Katz and qPCR. Parasit Vectors. 2017;10:255.
33. Lu DB, Deng Y, Ding H, Liang YS, Webster JP. Single-sex schistosomiasis infections of definitive hosts: implications for epidemiology and disease control in a changing world. PLoS Pathog. 2018;14:e1006817.
34. Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, et al. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. *PLoS Negl Trop Dis*. 2010;4:e754.

35. Alvelo T, Mediari A, Loytynoja A, Lakkonen J, Jernvall J. Tracking year-to-year changes in intestinal nematode communities of rufous mouse lemurs (*Microcebus rufus*). *Parasitology*. 2015;142:1095–107.

36. Beechler BR, Jolles AE, Budischak SA, Corstjens PL, Ezenwa VO, Smith M, et al. Host immunity, nutrition and coinfection alter longitudinal infection patterns of schistosomes in a free ranging African buffalo population. *PLoS Negl Trop Dis*. 2017;11:e6122.

37. Sène M, Duplantier JM, Marchand B, Hervé JP. Susceptibility of rodents to infection with *Schistosoma mansoni* in Richard-Toll (Senegal). *Parasite*. 1996;3:321–6.

38. D’Andrea PS, Maroja LS, Gentile R, Cerqueira R, Maldonado A, Rey L. The parasitism of *Schistosoma mansoni* (Digenea–Trematoda) in a naturally infected population of water rats, *Nectomys squamipes* (Rodentia–Sigmodontinae) in Brazil. *Parasitology*. 2000;120:573–82.

39. Lu DB, Wang TP, Rudge JW, Donnelly CA, Fang GR, Webster JP. Contrast patterns of schistosomes in a free ranging African buffalo population. *PLoS Negl Trop Dis*. 2017;11:e6122.

40. Morgan JAT, Blair D. Relative merits of nuclear ribosomal internal transcribed spacers and mitochondrial CO1 and ND1 genes for distinguishing among *Echinostoma* species (Trematoda). *Parasitology*. 1998;116:289–97.

41. Grabner DS, Mohamed FA, Nachew M, Méabed EM, Sabry AHA, Sures B. Invasion biology meets parasitology: a case study of parasite spill-back with Egyptian *Fasciola gigantica* in the invasive snail *Pseudosuccinea columella*. *PLoS One*. 2014;9:e88537.

42. Laidemitt MR, Brant SV, Mutuku MW, Mkoji GM, Loker ES. The diverse echiostome community of *Schistosoma mansoni* and soil-transmitted helminths in humans. *Parasitology*. 2010;137:99–110.

43. Huffman JE, Fried B. Echinostomes in veterinary and wildlife parasitology. In: Fried B, Graczyk TK, editors. Echinostomes as experimental models for biological research. Berlin: Springer; 2000. p. 59–82.

44. El-Sokkary MY. *On Architremata sanguineum* (Sonsino, 1894) from wild rats (*Rattus rattus*) caught in Kan-chanaburi Province. *J Trop Med Parasitol*. 1991;14:21–5.

45. Impand P, Waikagul J, Kitikoon V, Limsomboon SA. Report of *Architremata sanguineum* (Sonsino, 1894) from wild rats (*Rattus rattus*) in Chanaburi Province. *J Trop Med Parasitol*. 1991;14:21–5.

46. Caspeta-Mandujano JM, Peralta-Rodriguez JL, Ramirez-Chávez SB, Ramirez-Díaz SE, Tapia-Osorio M, Urbina MGI, et al. Helminths parasites of murciélagos in México. Cuernavaca: Universidad Autónoma del Estado de Morelos; 2017.

47. Fried B, Graczyk TK, Tamang L. Food-borne intestinal trematodiases in humans. *Parasitol Res*. 2004;93:159–70.

48. Toledo R, Esteban JG. An update on human echinostomiasis. *Trans R Soc Trop Med Hyg*. 2016;110:37–45.

49. Lloty WM, Lotty LM, Khalifa RM. An overview of cercariae from the Egyptian inland water snails. *J Coast Life Med*. 2017;7:562–74.

50. Toledo R, Munóz-Antoli C, Esteban JG. Intestinal trematode infections. In: Toledo R, Fried B, editors. Digenean trematodes. Berlin: Springer; 2014. p. 201–40.

51. Jeyarasingam U, Heyneman D, Lim HK, Mansour N. Life cycle of a new echinostome from Egypt, *Echinostoma lei* sp. nov. (Trematoda: Echinostomatidae). *Parasitology*. 1972;65:203–22.

52. Huffman JE. Echinostomes in veterinary and wildlife parasitology. In: Fried B, Graczyk TK, editors. Echinostomes as experimental models for biological research. Berlin: Springer; 2000. p. 59–82.

53. Huffman JE, Fried B. *Echinostoma* and echinostomiasis. *Adv Parasitol*. 1990;29:215–69.

54. Georgieva S, Faltýnková A, Brown R, Blasco-Costa L, Soldánová M, Sitko J, et al. *Echinostoma revolutum* (Digenea: Echinostomatidae) species complex revisited: species delimitation based on novel molecular and morphological data gathered in Europe. *Parasit Vectors*. 2014;7:520.

55. Sall-Dramé R, Brouat C, Bâ CT, Duplantier JM. Variation in cestode assemblages in *Microcercis rubraculus* (Digenea: *Taeniidae*) and echinostomiasis. *Adv Parasitol.* 2017;95:1265–71.

56. Diagne C, Ribas A, Charbonnel N, Dalecky A, Tatard C, Gauthier P, et al. Parasites and invasions: changes in gastrointestinal helminth assemblages in invasive and native rodent species of *Mus musculus* and *Rattus norvegicus* in intertidal habitats of the Mediterranean coast of France. *BMC Med Res Methodol*. 2010;10:67.

57. Ribas A, Diagne C, Tatard C, Diallo M, Poonlaphdecha S, Brouat C. whipworm diversity in West African rodents: a molecular approach and the description of *Trichurus duplicatieri* n. sp. (Nematoda: Trichuridae). *Parasitol Res*. 2017;116:1265–71.