Effect of Incorporation of Bead-Beating during DNA Extraction for Detection of Trichuris trichiura in Stool Samples in Community Settings: A Systematic Review

Divya RSJB Rana[1*], Nischal Pokhrel[2]

[1]Tribhuvan University, Hari Khetan Multiple Campus, Birgunj, Nepal; email: div_rana@yahoo.com

[2]Purbanchal University, B and B Medical Insitutte, Lalitpur, Nepal; email: kneeschalpoks@gmail.com

*Corresponding author

Abstract:

Objectives: This meta-analysis was designed to assess the effect of addition of a bead-beating step during DNA extraction to effectively isolate *Trichuris trichura* DNA for quantitative Polymerase Chain Reaction (qPCR)-based diagnosis. Abstract was reported according to PRISMA-DTA abstract checklist.

Methods:

Eligibility criteria: qPCR-based molecular studies comparing the inclusion of bead-beating step during the DNA extraction from stool samples with extraction without the step were included in the analysis.

Information sources: Studies using real patient samples in community settings were included. PubMed and Google search engine were searched in December 2019.

Risk of bias and applicability: Risk of bias and applicability were assessed using QUADAS-2 checklist.

Synthesis of results: Odds ratio for individual studies were combined to estimate Random Effects Model odds ratio. Additional literature were searched to discuss biochemical nature of helminth eggs.

 doi:10.20944/preprints202004.0419.v2

Results:

Included studies: A total of six independent sub-studies were gathered from two published original articles. Division of the two major studies into six sub-studies was indispensable due to natures of the study carried. 128 of total 192 samples (in all studies) were positive for *Trichiuris trichiura* when bead-beating was used during DNA extraction compared to 108/192 when bead-beating was excluded. Combined odds ratio was 1.66 (95% CI: 1.059 to 2.602). Biochemical nature of helminth eggs was discussed.

Discussions:

Strengths and limitations: Though only two article were included in the study, six exclusive individual sub-studies were analyzed. Inherent differences in the background prevalence of helminth in study population could impact sensitivity of qPCR.

Interpretation: It was found that the inclusion of the bead-beating step during DNA extraction significantly increased the sensitivity of the test.

Others:

Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Registration: Registration was no done in any databases.

Key words: Trichuris trichiura, polymerase chain reaction, DNA extraction, bead-beating, sensitivity

Strengths and Limitations of this study

> While only two studies are included in analysis, these contain a total of six independent sub-studies.

 doi:10.20944/preprints202004.0419.v2

> The sub-studies are different in patient population, sample preservation and processing, and thus can't be combined

> The direction of odds ratio in all of the sub-studies are in one direction, thus the significance of the combined odds ratio is strengthened.

> While sample sizes in individual studies are small to give significant statistical significance, combined odds ratio revealed a statistically significant result.

Introduction:

Soil-transmitted helminths (STH) claim high disease burden especially in the poor parts of the world (1) . The diseases, while being treatable by common deworming medications and preventable with common hygienic standards (2) , are major contributors of worldwide morbidity. Round worm (*Ascaris lumbricoides*, ascariasis), whipworm (*Trichuris trichiura*, trichuriasis) and hookworm (*Ancylostoma duodenale* and *Necator americanus*, ancylostomiasis and necatoriasis) are the three major worms among others that claim the highest disease morbidities. Round worm and whipworm are transmitted by faeco-oral route (3,4) while the hookworm and threadworm (*Strongyloides stercoralis*, strongyloidiasis) are transmitted through penetration of larvae into the open skin (5,6) . Round worm, hookworm and threadworm have tissue-dwelling stages where their specific larval stages leave the gut and penetrate the vascular and solid tissue. Whipworm complete their life cycles in the intestine and in their particular life stage, their heads remain embedded in the linings of the gut.

DNA-based detection of STH in stool samples have proved to be the most sensitive method (7,8) . Quantitative polymerase chain reaction (qPCR) is more consistent method compared to other copro-microscopic techniques and is less affected by the experience of the technologists as personnel with

basic knowledge of PCR are able to perform the experiments with similar results. qPCR has various advantages from detection of presence of worms in stools to follow-up evaluation after various therapeutic interventions to decrease the prevalence (9) . However, high cost of equipment and maintenance of contamination-free conditions may prevent the use of this techniques in resource constraint settings. In spite of that, qPCR carried in multiplex format may be equivalent in cost to conventional microscopy techniques (7) . DNA-based techniques have an additional advantage of the feasibility of carrying tests on later time points if the stool samples are preserved in proper manner (10) .

While the extraction of DNA from stool samples containing round worms and hookworms pose no reported difficulties, the extraction of DNA from *Trichuris* eggs have been reported to require additional handling (11) . Inefficient DNA extraction may lead to decreased *Trichuris* detection sensitivity by qPCR compared to detection by microscopy (9) . Studies have repeatedly pointed out the requirement of bead-beating step during DNA extraction for proper extraction of the *Trichuris* DNAs (7,8) . The bead-beating step is expected to help to break the Trichuris eggs during enzymatic lysis step of DNA extraction reagents, and thus make Trichuris DNA detectable by PCR. This is carried by enclosing stool with lysis buffer and beads of specific size (millimeter or lesser diameters) and type (glass, zirconium, ceramic, garnet, etc.), and oscillating at high speed for given durations. A search for articles which assessed the relevance of bead-beating during DNA extraction was carried and a meta-analysis was carried. While many studies incorporated bead-beating into their DNA extraction methods for *Trichuris* PCR in early 2010s (12,13) , recently few have done direct comparative studies to show the efficacy of inclusion of this step. This systematic review was designed to assess the benefit of including the step for more sensitive detection of *Trichuris* DNA during qPCR. Singleplex or multiplex qPCR for detection of soil-transmitted helminth are usually carried in community settings to determine the prevalence of the helminth and to determine the efficacy of deworming interventions (14) . The research question for this systematic review also envisage the index and reference tests to

have high sensitivity to determine the helminth presence in community setting rather than hospital setting.

Material and Methods:

This study was carried and reported according to the Preferred Reporting Items for a Systematic Review and Meta-analysis for Diagnostic Test Accuracy Studies (PRISMA-DTA) (15). Articles were searched in Pubmed by using "AND" Boolean operator to combine two search fields, "qPCR OR quantitative polymerase chain reaction OR quantitative PCR OR quantitative real-time PCR OR polymerase chain reaction OR PCR" and "Trichuris OR Trichuriasis OR Whip worm OR Whipworm OR Trichuris trichiura". Additional search was carried in Google search engine with search term "trichuris bead beating DNA extraction". Abstracts and/or bodies of the manuscripts were read to identify the comparative studies according to inclusion criteria. Studies using the index and reference tests in community settings were included. The studies should have carried DNA extraction by two methods: one including bead-beating step and, two, without including the bead-beating step. Articles referenced in the selected studies were scanned for more relevant articles but none of the authors of selected articles were contacted for further details. Studies included field-based projects where samples were collected in blanket fashion in suspected population irrespective of presence of any helminth related symptoms in target individuals. Tests should not be carried in individual suspect patients either in the hospital or community setings. Articles using qPCR both as index and reference tests qualified for the review. Index qPCR test preceded by DNA extraction using an additional bead-beating step to break the *Trichuris trichiura* egg. Reference test did not. Data were directly extracted from the selected studies and entered in excel for statistical analyses. Risk of bias and applicability was carried according to QUADAS-2 checklist (16) developed by the QUADAS-2 study group (17) . Additional literature search was carried to study the biochemical structure of helminth eggs.

Statistical Analyses: Odds ratio were calculated for individual studies/sub-studies. Each of the collected stool samples in individual studies were examined by index and reference tests. Samples tested by

 doi:10.20944/preprints202004.0419.v2

index test were regarded as exposed and those tested with reference were regarded as unexposed group to calculae the odds ratio. Random effects model odds ratio was estimated for the meta-analysis. Individual odds ratio of the sub-studies were combined for the meta-analysis. The analysis was carried in MedCalc software which uses Mantel and Haenszel (1959) method for fixed effect model and DerSimonian and Laird (1986) method for random effects modelling (18) . Random effects model was considered more applicable due to heterogeneity in the study as explained in Results.

Data Sharing Statement: No additional data available.

Results:

Search Results: Search through Pubmed resulted in 165 results from inception to Dec 2019 with no barrier to languages. Search through Google resulted in a total of 15 relevant search results. After step-wise sorting (Figure 1), 2 articles from Pubmed and 1 article from Google search were selected for further analysis. Studies that carried experiments to compare the effect of inclusion or exclusion of bead-beating step during DNA extraction from stool on the prevalence of *Trichuris trichiura* were selected. Further reading of the articles led to exclusion of one article for the meta-analysis as it carried DNA extraction in artificially spiked stool samples and did not specifically study the effects of bead-beating in actual field samples. Both of the included studies were carried in community settings which matched with the research question. Study populations were chosen based on prior information that their was some degree of infection prevalence that could be detected by index and reference tests. All of the study participants were apparently healthy and were not included or excluded based on any specific symptoms. While Ayana et al (10) enrolled school children (5-14 years) in Ethiopia, Kaisar et al (19) enrolled mothers (20-37 years) and their babies (1-5 years) in Indonesia. Both of the studies disclosed their funding sources but only Ayana et al stated in the publication that the funders had no role in study design, data collection and analysis, decision to publish, and preparation of the

 doi:10.20944/preprints202004.0419.v2

manuscript. But there was no proof that the funders of Kaisar et al had any input in any of the above activities.

Risk of bias and applicability: There was low-risk of bias and applicability for each of the studies/sub-studies (Figure 2, Supplementary materials). Perhaps due to nature of the diagnostic test, many of the risks could be reduced due to aliquotations. As the patient stool samples could be collected in sufficient amount and aliquoted, both the index and reference tests could be carried on identical samples and, theoretically, at same times. Another factor that reduces the risk of bias was the fact that the index and reference tests consisted of two steps. The second step, qPCR, was identical within each of the studies, and thus the threshold of detection (or analytical sensitivity) was same for the index and reference tests in each of the studies. In all of the studies/sub-studies, the patient samples were taken from specified populations without categorizing according to any symptoms or physical conditions. All of the healthy looking participants were enrolled consecutively. This decreased the overall applicability issue of the review question as the qPCR is intended to be used in community settings.

Qualitative analysis: All together three articles assessed the effect of bead-beating during DNA extraction. Anderson et al (2013) (20) artificially spiked healthy stool samples with *Trichuris trichiura* eggs and carried qPCR to assess the effect of bead-beating. They compared the effect of different types of beads: 0.5 mm glass beads, 0.15 mm Garnet beads, and 0.1 mm zirconium beads using NucliSENS easyMag DNA extraction system (Biomerieux, USA). They concluded (no data reported) that zirconium beads gave best results for DNA isolation. The study found that vortexing without any beads gave comparable results for Trichuris trichiura analytical sensitivity compared to bead beating with zirconium beads beaten for 30 secs at 7,000 oscillations. They reported that the clinical sample showed lower (better) limit of detection compared to the artificially spiked sample hinting the presence of microscopically invisible extra-cellular DNA in clinical stool samples. Study by Kaisar (2017) (19) also assessed the effects of five different kind of beads: 0.5 mm stainless steel,

 doi:10.20944/preprints202004.0419.v2

165 0.5 mm zirconium oxide, 0.7 mm garnet, 0.8 mm garnet and 0.5 mm Yttria-stabilized zirconium oxide.
166 It was found that 0.8 mm garnet gave the best analytical sensitivity and further experiments were
167 carried with this bead by vortexing 3 mins at 1800 rotations per minute. Ayana (2019) (10) used 1.4
168 mm ceramic beads to beat the stool samples for 1 min at 3000 rotations per minute. Just before the bead
169 beating, the stool samples were freeze-thawed to increase the efficiency of cell disruption during bead-
170 beating.

Quantitative analysis: Study by Kaisar et al (2017) divided the 60 collected stool samples into two aliquots and preserved them in fridge or mixed with ethanol and stored at room temperature (19) . Both sets of preserved samples underwent two types of DNA extraction protocols, one including the bead-beating step and the other without. As the samples materials had been altered chemically to some degree due to the preservation methods, we considered the two sets of experiments (frozen and ethanol preserved) as two different sub-studies (Frozen_samp and Ethanol_samp) in this analysis. Both sets of experiment used QIAamp DNA-easy kit from Qiagen, Germany. Overall, the inclusion of bead-beating gave higher positivity rate. 51.7% (31/60) frozen stool samples were positive when bead-beating was used during DNA extraction compared to only 40% (24/60) when bead-beating was not used ($p>0.05$). Similarly, the percentage of positive samples were 55% (33/60) and 45% (27/60) respectively for ethanol-preserved samples ($p>0.05$). Overall, ethanol-preserved samples performed better. Study by Ayana et al (2019) (10) used two different kits to extract DNA. QIAamp DNA Stool Mini kit (S_K) and DNeasy Blood & Tissue kit (BaT_K), both from Qiagen, Germany, were used and the Blood and Tissue kit was reported to perform better. Each method was further divided into one using the bead-beating and the other not. To examine the effect of bead-beating, the stool samples, as assessed microscopically, were divided into 15 fecal egg count (FEC) negative samples, 11 low-intensity FEC positive samples and 10 moderate to heavy intensity FEC positive samples. For the sake of this meta-analysis, the 15 FEC negative (zero value for egg per gram) samples were grouped as one (Zero_FEC_BaT_K or Zero_FEC_S_K for FEC negative/zero samples analyzed by Blood and Tissue kit and Stool Kit respectively) and 21 FEC positive samples (11 low and 10 heavy intensity) as other group (Pos_FEC_BaT_K or Pos_FEC_S_K for FEC positive samples analyzed by Blood and Tissue kit and Stool Kit respectively). This grouping is logical because only a small number (n=15) of the total 159/195 (81.5%) microscopically/DNA *Trichuris* negative samples were used in analysis and combination of all three groups of samples (negative, low intensity and moderate to heavy intensity) could have hamper actual sensitivity estimation. When Blood and Tissue Kit was used 87% (13/15) of

196 the microscopy negative samples were positive by DNA extraction method incorporating bead-beating
197 compared to only 73% (11/15) for method not including bead-beating (p>0.05). The positivity
198 percentages for microscopy positive samples were 100% (21/21) and 95% (20/21) respectively. When
199 the Stool Kit was used, the positivity percentage were 73% (11/15) and 67% (10/15) respectively for
200 microscopy negative samples (p>0.05). For microscopy positive samples, the positivity percentages
201 were 90% (19/21) and 76% (16/21) respectively (p>.05).
202 Random effects model was chosen compared to fixed effect model even when I^2 (inconsistency) was
203 0.00% (95% CI: 0.00-0.00). As the methods of DNA extraction, types of beads, stool preservation
204 methods and level of background prevalence of STH were different in the studies or within the studies,
205 random effects model was considered more appropriate. The random effects model showed a summary
206 odd ratio of 1.66 (95% CI: 1.059 to 2.209) (Table 1, Figure 3). None of the individual sub-studies
207 showed a significant effect of bead-beating while the random effects model showed significant effect.
208 This could be due to small sample sizes in the individual studies. None of the small sub-studies showed
209 a negative effect of bead-beating. Thus, it can be safely concluded that the significant effect in the
210 meta-analysis is due to synergistic effect all the sub-studies whose results directed in one direction.
211 When all the FEC zero and positive samples in Ayana et al were combined as one and reanalyzed
212 (sample sizes of 36 in BaT_K and S_K each) in the meta-analysis (data not shown), comparable
213 significant random effects model results (summary OR of 1.665) was obtained.
214
215 Table 1: Random effects model summary odds ratio

Study	Intervention	Controls	OR	95% CI	z	P	Weight (%)	
							Fixed	Random
Ayana 2019 (Zero_FEC_BaT_K)	13/15	11/15	2.364	0.361 to 15.455			5.73	5.73

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 April 2021 doi:10.20944/preprints202004.0419.v2

Ayana 2019 (Zero_FEC_S_K)	11/15	10/15	1.375	0.286 to 6.603			8.21	8.21
Ayana 2019 (Pos_FEC_BaT_K)	21/21	20/21	3.146	0.121 to 81.744			1.9	1.9
Ayana 2019 (Pos_FEC_S_K)	19/21	16/21	2.969	0.506 to 17.422			6.45	6.45
Kaisar 2017 (Frozen_samp)	31/60	24/60	1.603	0.778 to 3.305			38.63	38.63
Kaisar 2017 (Ethanol_samp)	33/60	27/60	1.494	0.728 to 3.067			39.07	39.07
Total (fixed effects)	128/192	108/192	1.669	1.067 to 2.611	2.246	0.025	100	100
Total (random effects)	**128/192**	**108/192**	**1.66**	**1.059 to 2.602**	**2.209**	**0.027**	**100**	**100**

Biochemical studies of helminth eggs: The eggs of nematodes have been known to be to resist various environmental stresses and chemical. The egg of *Ascaris* alone has shown to remain viable against various acids, alkali and digestive enzymes (21–23) . This extreme resilience is hypothesized to be due to the eggshell, which has to be strong enough to protect the embryo inside (24) . Nematodes

224 eggshells, including those of Hookworm, *Trichuris* and *Ascaris*, in general are made up of 3 layers:
225 outer vitelline layer, middle chitinous layer and inner lipid layer. The middle chitinous layer is a
226 composite layer composed of chitin microfibrils surrounded by protein matrix, which is said to make
227 the egg resistant to mechanical damage (25–27) . The inner lipid layer is impermeable to most
228 chemical (28,29) and maybe a reason for the difficulty in DNA extraction using chemical process. In
229 many nematodes, including *Ascaris*, it consist of ascarosides esters which could be a reason for the
230 impermeability (30,31) , and is said to be responsible for resistance against chemical actions (32) .
231
232
233 **Discussion:**
234 Increasing number of STH studies are using bead-beating steps in the DNA extraction method. While
235 the possible need of this step have been raised earlier (7,8) , very few studies have actually
236 reported the significance of this addition. Though *Trichuris* is considered to be the only STH that
237 require the bead-beating, both Ayana et al (10) and Kaisar et al (19) have found that this could also
238 be also required for hookworms and *Ascaris* respectively . They also reported that the blood and
239 tissue kit gave better result than the stool kit. It may be because stool kits are usually designed for
240 isolation of bacterial DNA in the stools (33) and may not be appropriate for helminth eggs . The sizes
241 of helminth eggs for hookworm, *Trichuris* and *Ascaris* are comparable and the biochemical structures
242 of the egg shells are similar too. While the hookworm eggs are known to be fragile and can rupture
243 within hours of stool collection at room temperature, the *Trichuris* and *Ascaris* eggs remain in soil for
244 longer durations. Present review which includes a meta-analysis shows that inclusion of bead beating
245 makes significant impact on the DNA extraction of *Trichuris* DNA and recommends the use of this
246 techniques.
247 Limitations: Only two published studies qualified for analysis due to paucity of systematic comparitive
248 studies. We divided the two studies into total of 6 sub-studies. It was not possible to keep the sub-

studies combined as the methods of stool preserved, kits used and the cohort of patients studied, if combined, could affect the outcome concluded. Another limitation could be non-inclusion of the cycle threshold data for the PCR which could differentiate the slight changes in the yield of *Trichuris* DNA extracted using various interventions during DNA extraction. Both of the study did not report the limit of detection of the qPCR. Another limitation of the study was the inherent heterogenity in study populations. It has been reported that the qPCR positivity or sensitivity may vary according to intensity of infection in the study population (34) . Thus different study populations differing by background helminth prevalence may give different sensitivities to same qPCR technique and thus bias the analysis. Conclusions: More systematic studies assessing the effect of bead-beating on DNA extraction of *Trichuris* is recommended. As reported by the two studies above, preliminary optimization of the best bead types and/or sizes should also be carried so the impact is clearly concluded. Uniformity in the type of beads, beating conditions, DNA extraction method, primers, kits, PCR conditions, etc. can help in comparative studies from different parts of the world.

Supplementary materials: quadas2 tool kit Rana Pokhrel

Conflicts of interest/Competing interests: None

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: All authors agree for publication

Author contributions: DRSJBR was involved in the conceptualization of the study. DRSJBR and NP carried data curation. DRSJBR did formal data analysis.Both authors wrote original draft preparation and reviewing and editing..

References:

1. Jourdan PM, Lamberton PHL, Fenwick A, Addiss DG. Soil-transmitted helminth infections. Vol. 391, The Lancet. Lancet Publishing Group; 2018. p. 252–65.

2. Campbell SJ, Nery S V., McCarthy JS, Gray DJ, Soares Magalhães RJ, Clements ACA. A Critical Appraisal of Control Strategies for Soil-Transmitted Helminths. Vol. 32, Trends in Parasitology. Elsevier Ltd; 2016. p. 97–107.

3. CDC. CDC - Ascariasis - Biology [Internet]. 2020 [cited 2020 Apr 11]. Available from: https://www.cdc.gov/parasites/ascariasis/biology.html

4. CDC. CDC - Trichuriasis - Biology [Internet]. 2020 [cited 2020 Apr 11]. Available from: https://www.cdc.gov/parasites/whipworm/biology.html

5. CDC. CDC - Hookworm - Biology [Internet]. 2020 [cited 2020 Apr 11]. Available from: https://www.cdc.gov/parasites/hookworm/biology.html

6. CDC. CDC - Strongyloides - Biology [Internet]. 2020 [cited 2020 Apr 11]. Available from: https://www.cdc.gov/parasites/strongyloides/biology.html

7. O'Connell EM, Nutman TB. Review article: Molecular diagnostics for soil-transmitted helminths. Vol. 95, American Journal of Tropical Medicine and Hygiene. American Society of Tropical Medicine and Hygiene; 2016. p. 508–14.

8. Verweij JJ, Rune Stensvold C. Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. Clin Microbiol Rev. 2014;27(2):371–418.

9. Clarke NE, Llewellyn S, Traub RJ, McCarthy J, Richardson A, Nery S V. Quantitative polymerase chain reaction for diagnosis of soil-transmitted helminth infections: A comparison with a flotation-based technique and an investigation of variability in DNA detection. Am J Trop Med Hyg [Internet]. 2018 [cited 2021 Jan 30];99(4):1033–40. Available from: /pmc/articles/PMC6159597/?report=abstract

10. Ayana M, Cools P, Mekonnen Z, Biruksew A, Dana D, Rashwan N, et al. Comparison of four DNA extraction and three preservation protocols for the molecular detection and quantification of soil-transmitted helminths in stool. Cantacessi C, editor. PLoS Negl Trop Dis [Internet]. 2019 Oct 28 [cited 2020 Apr 11];13(10):e0007778. Available from: https://dx.plos.org/10.1371/journal.pntd.0007778

11. Demeler J, Ramünke S, Wolken S, Ianiello D, Rinaldi L, Gahutu JB, et al. Discrimination of Gastrointestinal Nematode Eggs from Crude Fecal Egg Preparations by Inhibitor-Resistant Conventional and Real-Time PCR. Carlow CKS, editor. PLoS One [Internet]. 2013 Apr 19 [cited 2020 Apr 11];8(4):e61285. Available from: http://dx.plos.org/10.1371/journal.pone.0061285

12. Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, et al. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. Am J Trop Med Hyg [Internet]. 2013 Jun [cited 2021 Jan 30];88(6):1041–7. Available from: https://pubmed.ncbi.nlm.nih.gov/23509117/

13. Liu J, Gratz J, Amour C, Kibiki G, Becker S, Janaki L, et al. A laboratory-developed taqman array card for simultaneous detection of 19 enteropathogens. J Clin Microbiol [Internet]. 2013 Feb 1 [cited 2021 Jan 30];51(2):472–80. Available from: http://dx.doi.org/10.1128

14. Farrell SH, Coffeng LE, Truscott JE, Werkman M, Toor J, de Vlas SJ, et al. Investigating the Effectiveness of Current and Modified World Health Organization Guidelines for the Control of Soil-Transmitted Helminth Infections. Clin Infect Dis [Internet]. 2018 Jun 1 [cited 2021 Feb 5];66(suppl_4):S253–9. Available from: https://academic.oup.com/cid/article/66/suppl_4/S253/5020589

15. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies The PRISMA-DTA Statement. JAMA - J Am Med Assoc [Internet]. 2018 Jan 23 [cited 2021 Jan 25];319(4):388–96. Available from: https://jamanetwork.com/journals/jama/fullarticle/2670259

16. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2 Tool [Internet]. 2011 [cited 2021 Jan 31]. Available from: http://www.bristol.ac.uk/population-health-sciences/projects/quadas/quadas-2/

17. Whiting PF. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. Ann Intern Med [Internet]. 2011 Oct 18 [cited 2021 Jan 30];155(8):529. Available from: http://annals.org/article.aspx?doi=10.7326/0003-4819-155-8-201110180-00009

18. MEDCALC. Meta-analysis: odds ratio [Internet]. [cited 2020 Apr 11]. Available from: https://www.medcalc.org/manual/meta-analysis-oddsratio.php

19. Kaisar MMM, Brienen EAT, Djuardi Y, Sartono E, Yazdanbakhsh M, Verweij JJ, et al. Improved diagnosis of Trichuris trichiura by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-Time PCR for intestinal parasites. Parasitology. 2017 Jun 1;144(7):965–74.

20. Andersen LOB, Röser D, Nejsum P, Nielsen HV, Stensvold CR. Is supplementary bead beating for DNA extraction from nematode eggs by use of the nuclisens easymag protocol necessary. Vol. 51, Journal of Clinical Microbiology. American Society for Microbiology Journals; 2013. p. 1345–7.

21. Yoshida S. On the resistance of Ascaris eggs. J Parasitol. 1920;6:132–9.

22. Martin HM. Studies on the Studies on the Ascaris lumbricoides Ascaris lumbricoides [Internet]. 1926 [cited 2020 Apr 11]. Available from: https://digitalcommons.unl.edu/extensionhist

23. Beyhan YE, Yilmaz H, Hokelek M. Effects of acetic acid on the viability of Ascaris lumbricoides eggs: Is vinegar reliable enough to clean the vegetables? Saudi Med J. 2016 Mar 1;37(3):288–92.

24. Wharton DA. The Production and Functional Morphology of Helminth Egg-Shells. Parasitology. 1983;86(4):85–97.

346 25. Wharton DA, Jenkins T. Structure and chemistry of the egg shell of a nematode (Trichuris suis).
347 Tissue Cell. 1978 Jan 1;10(3):427–40.

348 26. Slayter G. Two-Phase Materials - Scientific American [Internet]. 1962 [cited 2020 Apr 11].
349 Available from: https://www.scientificamerican.com/article/two-phase-materials/

350 27. Neville AC. Biology of the Arthropod Cuticle. Springer Berlin Heidelberg; 1975.

351 28. Wharton DA. A Functional Biology of Nematodes. A Functional Biology of Nematodes.
352 Springer US; 1986.

353 29. Barrett J. Biochemistry of Parasitic Helminths. Biochemistry of Parasitic Helminths. Macmillan
354 Education UK; 1981.

355 30. Ludewig AH, Schroeder FC. Ascaroside signaling in C. elegans. WormBook : the online review
356 of C. elegans biology. 2013. p. 1–22.

357 31. Nissen S, Al-Jubury A, Hansen TVA, Olsen A, Christensen H, Thamsborg SM, et al. Genetic
358 analysis of Trichuris suis and Trichuris trichiura recovered from humans and pigs in a sympatric
359 setting in Uganda. Vet Parasitol. 2012 Aug 13;188(1–2):68–77.

360 32. Bartley JP, Bennett EA, Darben PA. Structure of the Ascarosides from Ascaris suum. J Nat Prod.
361 1996;

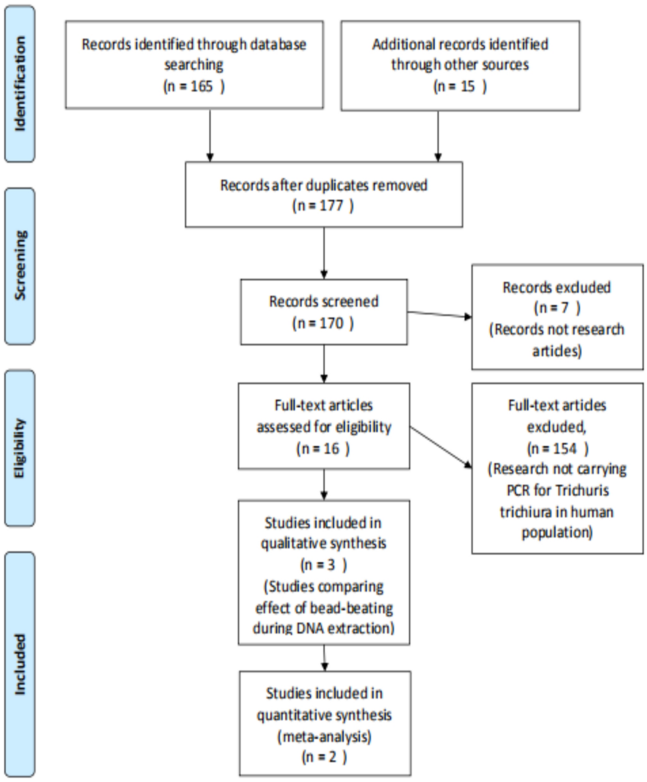
362 33. Fiedorová K, Radvanský M, Němcová E, Grombiříková H, Bosák J, Černochová M, et al. The
363 Impact of DNA Extraction Methods on Stool Bacterial and Fungal Microbiota Community
364 Recovery. Front Microbiol [Internet]. 2019 Apr 17 [cited 2020 Apr 11];10:821. Available from:
365 https://www.frontiersin.org/article/10.3389/fmicb.2019.00821/full

366 34. Cools P, Vlaminck J, Albonico M, Ame S, Ayana M, José Antonio BP, et al. Diagnostic
367 performance of a single and duplicate Kato-Katz, Mini-FLOTAC, FECPAKG2 and qPCR for the
368 detection and quantification of soil-transmitted helminths in three endemic countries. Freeman MC,
369 editor. PLoS Negl Trop Dis [Internet]. 2019 Aug 1 [cited 2021 Feb 5];13(8):e0007446. Available from:
370 https://dx.plos.org/10.1371/journal.pntd.0007446
371

372

 doi:10.20944/preprints202004.0419.v2

373 Figure 1: PRISMA work-flow for selection of studies

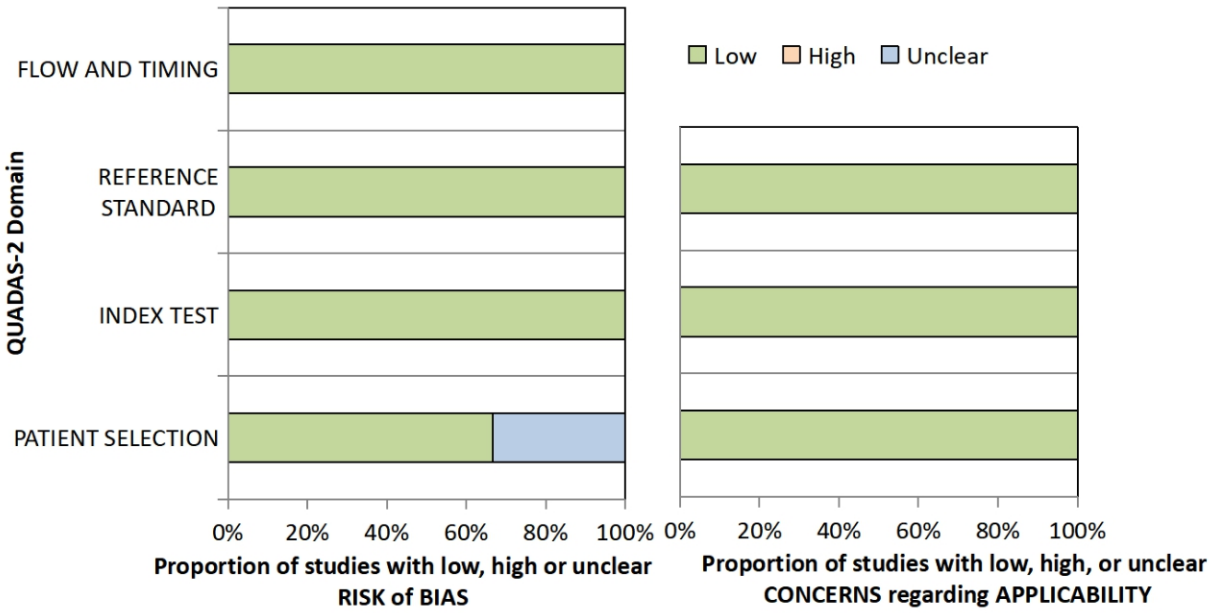


374

375

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 April 2021 doi:10.20944/preprints202004.0419.v2

376 Figure 2: Risk of bias and applicability of included studies

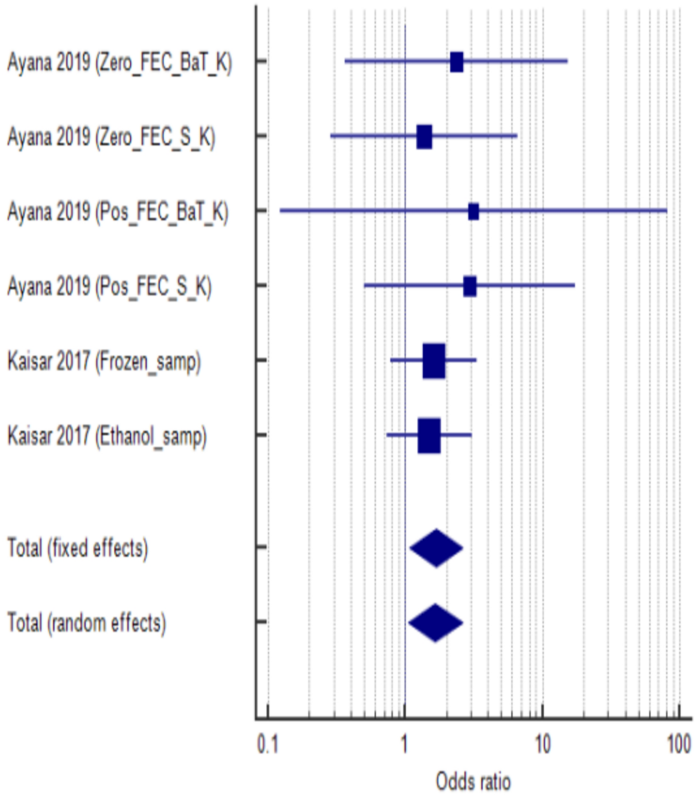


377

378

 doi:10.20944/preprints202004.0419.v2

379 Figure 3: Forest plot for summary odds ratio



380