APPENDICES

APPENDIX 1. PRISMA CHECKLIST

Section/topic	#	Checklist item	Reported on page #
TITLE [Characterization of the environmental presence of hepatitis A virus in Low and Middle-Income Countries: A systematic review and meta-analysis]			Pg. 1
Title	1	Identify the report as a systematic review, meta-analysis, or both.	pg.1
ABSTRACT			Pg. 2
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pg.2-3 line 1-33
INTRODUCTION			Pg. 4
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pg. 3 line 81-90
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 4 line 97-103
METHODS			Pg. 5 line 92
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Pg. 4 line 93
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Pg. 5 line 106
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 5 line 116-119
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 5 line 113
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 6 line 126-132
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 6 line 135-140
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pg. 5 line 109-111
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 6-7 line 154-159
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Pg. 6 line 143-144
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Pg. 6 line 142-152

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			Pg. 7 line 161
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 7 line 162
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 7 line 168
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Pg. 8 line 211
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pg. 8 line 192
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. [from the PRISMA website, added by the authors of the manuscript:] Please note that the published PRISMA checklists contain an error in the wording for Item 21. The item should read: "Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency" in accordance with the text in the Explanation and Elaboration document.	Pg. 7 line 194-209
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pg. 8 line 211
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			Pg. 8-9 line 217-254
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pg. 10 line 255
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg. 10 line 262
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 10 line 267
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg. 11 line 287

Appendix 2: Search strategy used in PUBMED		
Query	**Fields**	**Search term**
#1	All	hepatitis A OR hep A OR hepatitis A virus OR HAV
#2	All	environment OR raw sewage OR treated sewage OR sludge OR water OR surface water OR groundwater OR drinking water OR seawater OR ocean water OR irrigation water OR soil OR crops OR food OR shellfish
#3	All	Afghanistan OR Angola OR Bangladesh OR Benin OR Bhutan OR Bolivia OR Burkina Faso OR Burundi OR Cabo Verde OR Cambodia OR Cameroon OR Central African Republic OR Chad OR Comoros OR Congo, Dem. Rep. OR Congo, Rep. OR Cote d'Ivoire OR Djibouti OR Egypt, Arab Rep. OR El Salvador OR Eritrea OR Ethiopia OR Gambia, The OR Georgia OR Ghana OR Guinea OR Guinea-Bissau OR Haiti OR Honduras OR India OR Indonesia OR Kenya OR Kiribati OR Korea, Dem. People's Rep. OR Kosovo OR Kyrgyz Republic OR Lao PDR OR Lesotho OR Liberia OR Madagascar OR Malawi OR Mali OR Mauritania OR Micronesia, Fed. Sts. OR Moldova OR Mongolia OR Morocco OR Mozambique OR Myanmar OR Nepal OR Nicaragua OR Niger OR Nigeria OR Pakistan OR Papua New Guinea OR Philippines OR Rwanda OR Sao Tome and Principe OR Senegal OR Sierra Leone OR Solomon Islands OR Somalia OR South Sudan OR Sri Lanka OR Sudan OR Swaziland OR Syrian Arab Republic OR Tajikistan OR Tanzania OR Timor-Leste OR Togo OR Tunisia OR Uganda OR Ukraine OR Uzbekistan OR Vanuatu OR Vietnam OR West Bank and Gaza OR Yemen, Rep. OR Zambia OR Zimbabwe
#4	#1 AND #2 AND #3	

APPENDIX 3: DATA EXTRACTION FORM	
Date form completed (dd/mm/yyyy)	
Study ID Number	
Reference citation	
First Author	
Study author contact details	
Publication type (e.g. full report, abstract, letter)	
Notes:	

Study eligibility

Study Characteristics	Eligibility criteria	Eligibility criteria met? Yes	No	Unclear	Location in text or source (pg. & ¶/fig/table/other)
Type of study	Cross-sectional study	☐	☐	☐	
	Ecological study	☐	☐	☐	
Participants	Reports Environmental Source of HAV	☐	☐	☐	
	Study conducted in a LMIC	☐	☐	☐	
Types of outcome measures	Reports Quantifiable HAV concentration	☐	☐	☐	
INCLUDE ☐		EXCLUDE ☐			
Reason for exclusion					
Notes:					

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

CHARACTERISTICS OF INCLUDED STUDIES

	Descriptions as stated in report/paper	**Location in text or source** (pg. & ¶/fig/table/other)
Aim and objectives of the study		
Setting	Country ……..	
	Income Level Low Middle	
	HAV Immunisation program in place Yes No	
	Year HAV Immunisation program 1St initiated ……..	
	Water, sanitation and hygiene (WASH) performance index……	
	Urban Peri-urban Rural	
Study Start Date		
Study End Date		
Notes:		

Outcome 1

	Description as stated in report/paper	Location in text or source (pg. & ¶/fig/table/other)
Outcome name		
Month of data Collection	JAN/…… JUL/…. FEB/…… AUG/…. MAR/…… SEP/…. APR/…… OCT/…. MAY/…… NOV/…. JUN /…… DEC/….	

Number of samples	……	
Outcome definition (HAV quantification criteria)		
Method of HAV detection	RT-PCR RT-qPCR NASBA Other (specify)……..	
Unit of measurement	Genome copies Whole virus Others (specify) ……	
Genotypes detected	I II III IV V VI	
Reported HAV Concentration	……	
Standardised/ Corrected HAV concentration (if reported)	……	
Grade of Quantity/concentration	High Low	
Is outcome/ reported concentration validated?	☐ Yes ☐ No ☐ Unclear	
Limitations of the study		
Imputation of missing data		
Notes:		

Outcome 2

	Description as stated in report/paper		Location in text or source (pg. & ¶/fig/table/other)
Outcome name (Type of environmental source of HAV)			
	Description	HAV concentration	
Source 1			
Source 2			
Source 3			
Source 4			
Source 5			
Source 6			
Source 7			
Source 8			
Source 9			
Source 10			
Source 11			
Notes:			
Study funding sources *(including role of funders)*			
Possible conflicts of interest *(for study authors)*			
Notes:			

APPENDIX 4: RISK OF BIAS ASSESSMENT TOOL		
Risk of bias Item	**Answer**	
	Yes	No
External validity		
Was the study's target environmental source a good representation of the overall HAV environmental presence in the region?		
Were the study samples a true or close representation of the targeted environmental source?		
Was some form of random selection used to select the samples, OR was a logical method used to arrive at the samples selected?		
Was the likelihood of nonresponse bias minimal?		
Internal validity		
Were data collected directly from the environmental source?		
Was an acceptable viral detection criterion used in the study?		
Was the study detection instrument validated?		
Was the same mode of viral detection used for all subjects?		
Was the length of the shortest prevalence period for the parameter of interest appropriate?		
Were the numerator(s) and denominator(s) for the parameter of interest appropriate?		
Summary item on the overall risk of study bias		
LOW RISK OF BIAS: 8 or more "yes" answers. Further research is very unlikely to change our confidence in the estimate. **MODERATE RISK OF BIAS**: 6 to 7 "yes" answers. Further research is likely to have an important impact on our confidence in the estimate and may change the estimate. **HIGH RISK OF BIAS**: 5 or fewer "yes" answers. Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate		

Appendix 5. Summary of risk of bias assessment showing scores and grades for all included studies

	External Validity				Internal Validity						Total score	Risk of Bias Rating
Citation	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10		
Rachida et al., 2016	1	0	0	1	1	1	1	1	1	1	8	Low risk
Osuolale et al., 2015	1	1	1	1	1	1	1	1	1	1	10	Low risk
Adefisoye et al., 2015	1	1	1	1	1	1	1	1	1	1	10	Low risk
Saïd et al., 2014	1	1	1	1	1	1	1	1	1	1	10	Low risk
Chigor et al., 2012	1	1	1	1	1	1	1	1	1	1	10	Low risk
Venter et al., 2007	1	1	1	1	1	1	1	1	1	1	10	Low risk
Nenonen et al., 2009	0	1	0	1	1	1	1	1	1	1	8	Low risk
O'Brien et al., 2017	1	1	1	1	1	1	1	1	1	1	10	Low risk
Katukiza et al., 2013	1	1	1	1	1	1	1	1	1	1	10	Low risk
Kiulia et al., 2009	1	1	1	1	1	1	1	1	1	1	10	Low risk
Jebri et al., 2012	0	1	0	1	1	1	1	1	1	1	8	Low risk
Ouardani et al., 2016	1	1	1	1	1	1	1	1	1	1	10	Low risk
Ouardani et al., 2015	1	1	1	1	1	1	1	1	1	1	10	Low risk
Khelifi et al., 2011	0	0	0	1	1	1	1	1	1	1	7	Moderate risk
Be´ji-Hamza et al., 2014	1	1	1	1	1	1	1	1	1	1	10	Low risk
Amri et al., 2009	1	1	1	1	1	1	1	1	1	1	10	Low risk
Mouna et al., 2010	1	1	0	1	1	1	1	1	1	1	9	Low risk
Khelifi et al., 2006	1	1	1	1	1	1	1	1	1	1	10	Low risk
Elamri et al., 2006	1	1	1	1	1	1	1	1	1	1	10	Low risk
Amdiouni et al., 2017	1	1	1	1	1	1	1	1	1	1	10	Low risk
Benabbes et al., 2012	1	1	0	1	1	1	1	1	1	1	9	Low risk
Guerrero et al., 2011	1	1	0	1	1	1	1	1	1	1	9	Low risk
Ahmad et al., 2018	1	1	1	1	1	1	1	1	1	1	10	Low risk
Ahmad et al., 2016	1	1	1	1	1	1	1	1	1	1	10	Low risk
Ahmad et al., 2015	1	1	1	1	1	1	1	1	1	0	9	Low risk
Khan et al., 2014	0	0	0	1	1	1	1	1	1	1	7	Moderate risk
Chobe et al., 2009	0	0	0	1	1	1	1	1	1	1	7	Moderate risk
Arankalle et al., 2006	0	0	0	1	1	1	1	1	1	1	7	Moderate risk
Umesha et al., 2007	1	1	1	1	1	1	1	1	1	1	10	Low risk
Phanuwan et al., 2006	1	0	0	1	1	1	1	1	1	1	8	Low risk
Chadha et al., 2009	0	0	0	0	1	1	1	1	1	0	5	High Risk
Chitambar et al., 2007	1	1	1	1	1	1	1	1	1	1	10	Low risk
Bai et al., 2019	1	1	1	1	1	1	1	1	1	1	10	Low risk

Item definitions

1.Samples' representation of the region. 2. Sample's representation of the environmental source 3. Random sampling done or not done 4. Likelihood of bias due to non-response 5. Direct collection of samples from source 6. Acceptable detection criterion used 7. Detection instrument validated 8. Same detection techniques used 9. Appropriate shortest prevalence period 10. Appropriate denominators and numerators used in calculation of detection.