ISSN: 2320-5407
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

Manuscript No.: IJAR- 59760

Title: Detection and sociodemographic distribution of rotavirus and hepatitis A virus infections among patients attending a regional hospital in DÃ©dougou, Burkina Faso.

Recommendation:
Accept as it is .....
Accept after Minor revision.....
Accept after Major revision
Do not accept (*Reasons below*)

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity			✓	
Significance			✓	

Reviewer Name: Dr. ANAPANA GOPAL

Reviewer's Comment for Publication.

1. General Comments

The manuscript investigates the detection rates and sociodemographic distribution of rotavirus (RV) and hepatitis A virus (HAV) infections among 150 patients attending the Regional Hospital Center of Dédougou, Burkina Faso, between July 2024 and April 2025. The study addresses an important public health issue, particularly in regions where epidemiological information on enteric viral infections is limited.

The combined investigation of rotavirus antigen and HAV RNA provides useful preliminary information about the occurrence of these infections among patients presenting with gastrointestinal complaints. The inclusion of laboratory-based diagnostic methods and sociodemographic information is a positive aspect of the study.

However, several methodological, statistical, and reporting issues require attention before the manuscript can be considered for publication. In particular, there are inconsistencies between the narrative description and the data presented in Table 1, insufficient detail regarding participant recruitment and laboratory quality control, and limitations in the interpretation of the association between drinking water source and rotavirus positivity. The small number of HAV-positive cases also restricts the strength of the conclusions.

The manuscript has potential value, but substantial revision is required to improve its scientific clarity, methodological transparency, and reliability of interpretation.

2. Content and Originality

1. **Research relevance:** The study addresses an important topic in infectious disease epidemiology and provides preliminary information from the Bankuy region, where published epidemiological data appear to be limited. The simultaneous assessment of

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rotavirus and HAV is relevant to understanding enteric viral infections in the study setting.

2. **Study population and representativeness:** The study includes patients with gastrointestinal complaints attending a single regional hospital. Therefore, the findings represent a hospital-based population rather than the general population of Dédougou or the wider Bankuy region. The authors should consistently describe the findings as detection rates among hospital attendees and avoid broader population-level interpretations.

3. **Original contribution:** The manuscript identifies rotavirus in 18% of participants and HAV in 2.7%. However, the novelty of these findings should be more clearly established by comparing them with previous studies conducted in Burkina Faso and neighbouring West African countries, particularly studies involving adult and mixed-age populations.

4. **Interpretation of rotavirus detection in adults:** A considerable proportion of rotavirus-positive participants belonged to the 25–64-year age group (16/27; 59.3%). This is an interesting observation because rotavirus is commonly associated with severe gastroenteritis in young children. However, the authors should distinguish between the proportion of positive cases occurring in adults and the age-specific detection rate. The fact that more positive cases were observed in adults does not necessarily mean that adults had a higher risk of infection.

5. **Interpretation of HAV findings:** Only four participants tested positive for HAV RNA. Although the manuscript describes their distribution according to age, sex, education, occupation, and water source, these observations are based on a very small number of cases. The authors should avoid drawing epidemiological conclusions from these limited observations.

6. **Environmental risk factors:** The association between drinking water source and rotavirus positivity is potentially important. However, the study did not directly examine water quality, microbial contamination, household water storage, sanitation, or hygiene practices. Consequently, the proposed relationship between water source and viral transmission remains a possible explanation rather than a demonstrated transmission pathway.

7. **Comparison with previous studies:** The comparison with studies reporting rotavirus prevalence among children under five years and HAV seroprevalence among different populations requires more careful interpretation. Detection of viral RNA in stool is not directly equivalent to the detection of anti-HAV antibodies in serum. The authors should clearly distinguish active viral detection from evidence of previous exposure or immunity.

3. Technical Quality

A. Study Design and Sampling

1. The study is described as prospective, but the sampling procedure is not adequately explained. The authors should clarify whether participants were recruited consecutively, randomly, or through convenience sampling.

2. The inclusion criteria require further clarification. The manuscript states that adults aged 18 years or above were included upon written informed consent, while participants below 18 years required parental or guardian consent. However, it does not clearly describe the eligibility and consent procedures for different age groups.

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3. The exclusion criterion stating that individuals with conditions deemed by the investigation team to preclude participation were not included is too general. The specific exclusion criteria should be stated.
4. The manuscript should provide a clear definition of gastrointestinal complaints and explain whether all eligible patients presenting with such complaints were invited to participate.
5. The study population includes participants ranging from 0 to 76 years. The authors should explain how samples and questionnaire information were collected from infants and young children and whether their clinical information was obtained from accompanying caregivers.
6. The sample size of 150 participants should be justified. If a formal sample-size calculation was not performed, this should be acknowledged as a limitation.

B. Laboratory Investigations

1. The rotavirus detection method uses the SD BIOLINE Rota/Adeno Rapid test, which detects group A rotavirus antigen. The authors should consistently use the appropriate terminology, preferably rotavirus A (RVA), throughout the manuscript if this is the specific target of the assay.
2. The manuscript describes the immunochromatographic procedure but does not provide information about the test's diagnostic sensitivity and specificity. These performance characteristics should be reported using the manufacturer's documentation or an appropriate validation study.
3. The authors should clarify whether all stool samples were tested immediately after collection or only after storage. The handling and storage conditions should be described consistently.
4. The HAV detection method requires more detailed technical information. Although the authors provide the primer sequences and thermal cycling conditions, the expected amplicon size is not reported. This information is important for interpreting the agarose gel electrophoresis results.
5. The authors should specify whether positive controls, negative controls, and extraction controls were included during RNA extraction and RT-PCR amplification. The absence of information about these controls makes it difficult to assess the reliability of the molecular detection results.
6. The manuscript should explain how HAV-positive samples were identified from the agarose gel. Representative gel images showing the molecular marker, positive samples, negative controls, and expected amplification product would strengthen the laboratory evidence.
7. The authors should clarify whether the RT-PCR assay was validated for the detection of HAV RNA in stool samples under the conditions used in this study. Information on analytical sensitivity and specificity would improve methodological transparency.
8. The manuscript mentions that approximately 25 g of stool was homogenized in 40 mL of phosphate-buffered saline. Since the initial sample collection is described as approximately 10–50 g, the authors should clarify whether 25 g was used from each collected sample or whether this represents a different preparation procedure.
9. The RNA extraction and storage procedures should be described more clearly, including whether repeated freeze–thaw cycles were avoided and how sample integrity was maintained before molecular analysis.

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10. Molecular characterization or sequencing was not performed. The authors appropriately recommend this for future studies, but should clarify that the current investigation establishes HAV RNA detection rather than providing information about circulating HAV genotypes or genetic diversity.

C. Statistical Analysis

1. The statistical methods mention descriptive analysis, Fisher's exact test, the Mann–Whitney test, and logistic regression. However, the results section does not present the findings of the logistic regression analysis.
2. If logistic regression was performed to identify factors associated with rotavirus infection, the authors should provide the estimated odds ratios, 95% confidence intervals, and corresponding p-values. The variables included in the model and the method used to select them should also be specified.
3. The association between drinking water source and rotavirus positivity is reported as statistically significant ($p = 0.02$). However, Table 3 presents the distribution of positive cases without showing the total number of participants in each water-source category. The authors should provide the complete positive and negative counts for each category so that the underlying comparison can be evaluated.
4. The statement that most rotavirus-positive cases occurred among tap-water users (16/27; 59.3%) describes the distribution of positive cases, not the risk of infection among tap-water users. The authors should distinguish between the proportion of cases and the detection rate within each water-source category.
5. Several water-source categories contain very small numbers of participants, including borehole, tap and bottled water, and borehole and well users. The authors should explain how these sparse categories were handled during statistical testing and whether the reported p-value represents an overall comparison across all categories.
6. The small number of HAV-positive cases ($n = 4$) severely limits statistical power. The authors should present these findings descriptively and avoid implying that the absence of statistical significance demonstrates the absence of an association.
7. The manuscript should clarify whether the reported p-values are based on two-sided Fisher's exact tests for all categorical comparisons and whether the Mann–Whitney test was actually used in the analysis.
8. The statistical analysis should include the number of observations available for each variable and explain how missing or incomplete questionnaire responses, if any, were handled.

4. Language and Presentation

The manuscript is generally understandable and follows a conventional scientific writing style. However, several grammatical, typographical, and formatting problems need correction.

1. In the abstract, the phrase "detection rateand sociodemographic distribution" contains a spacing error. It should be corrected to "detection rate and sociodemographic distribution."
2. The expression "HAVpositive cases" should be corrected to "HAV-positive cases" wherever it occurs.
3. In the Materials and Methods section, the description of the study population is repeated in consecutive sentences. This duplication should be removed.

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4. The RNA extraction paragraph contains duplicated wording: "according to the manufacturer's instructions" appears twice in the same sentence. The sentence should be revised.
5. The expression "feco-oral" should be standardized as "faecal–oral" or "fecal–oral" throughout the manuscript, according to the selected English style.
6. The manuscript alternates between "rotavirus," "RV," and "RVA." The authors should establish consistent terminology, particularly because the rapid test is described as detecting group A rotavirus.
7. The formatting of statistical expressions should be standardized. For example, use consistent spacing in expressions such as "p < 0.05" and "p = 0.02."
8. The use of decimal separators is inconsistent. Table 2 uses commas in confidence intervals (12,2–25,1 and 0,7–6,7), whereas the main text uses decimal points. The authors should use one consistent format.
9. Some table headings and category labels are poorly formatted, including "Livestockfarmer," "Source ofdrinking water," and the broken word "Livestock farmer." These should be corrected.
10. The abstract should be reviewed to ensure that the results and conclusions accurately reflect the limitations of the study, particularly the low number of HAV-positive cases.
A careful language edit and final formatting check are recommended.

5. Structure and Organization

The manuscript follows a conventional scientific structure consisting of an abstract, introduction, materials and methods, results, discussion, conclusion, and references. However, several sections require improvement.
1. **Abstract:** The abstract provides the study objectives, methods, results, and conclusion. However, it should mention that the study was hospital-based and that the small number of HAV-positive cases limits further interpretation.
2. **Introduction:** The introduction provides relevant background information on rotavirus and HAV. However, the research gap should be stated more precisely, particularly regarding the lack of information from the Bankuy region and the need to investigate mixed-age hospital populations.
3. **Materials and Methods:** This section requires substantial improvement. Participant recruitment, eligibility criteria, sample handling, laboratory quality-control procedures, and statistical methods should be described in sufficient detail to allow reproducibility.
4. **Results:** The results are presented in a logical sequence, but the narrative description contains an important inconsistency with Table 1, which must be corrected.

- The text states that the majority of participants had formal education (73.3%).
- However, Table 1 shows that 110 participants (73.3%) had no formal education.

These statements directly contradict each other. The authors should verify the original dataset and correct either the narrative or the table.
5. **Table 1:** The table should be reviewed for accuracy and consistency. All categories should be clearly labelled, and percentages should be checked against the total sample size of 150.
6. **Table 2:** The reported detection rates and confidence intervals should be checked for consistent decimal formatting and presentation.

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7. **Table 3:** The table is difficult to interpret because the positive-case counts are presented without the corresponding negative counts or total participants in each category. The authors should revise it to show the complete distribution and clearly identify the statistical comparisons.
8. **Discussion:** The discussion is generally relevant and compares the findings with previous research. However, it should distinguish descriptive findings from statistically supported associations. The water-source association should be discussed cautiously because water quality was not directly investigated.
9. **Conclusion:** The conclusion appropriately acknowledges several study limitations. However, the language should avoid suggesting that the study establishes environmental transmission pathways. It should emphasize that the findings are preliminary and specific to the hospital-based study population.
10. A separate limitations subsection within the Discussion would improve the manuscript. It should address the relatively small sample size, single-hospital recruitment, possible selection bias, low number of HAV-positive cases, lack of direct water-quality assessment, and limitations of the diagnostic methods.

6. References and Citations

The manuscript includes relevant references on rotavirus epidemiology, HAV infection, vaccine impact, and enteric viral infections in Burkina Faso and other African settings. Nevertheless, the references and in-text citations require careful verification.
1. The authors should verify the accuracy and relevance of all references, particularly those used to support the global disease burden and epidemiological estimates.
2. The introduction cites Vongpradith et al. (2026) and Badjo et al. (2026). Since these are recent publications, the authors should verify their final publication details, bibliographic information, and consistency with the claims made in the text.
3. The statement concerning the estimated global number of deaths from hepatitis A in 2023 is attributed to Franco (2012). A 2012 publication cannot independently establish a 2023 mortality estimate. The authors should cite an appropriate recent source for this statistic.
4. The comparison between the 2.7% HAV RNA detection rate in the present study and the 52% HAV serological findings reported by Traore et al. (2024) requires careful explanation. These measurements represent different diagnostic outcomes and should not be treated as directly comparable prevalence estimates.
5. The authors should ensure that every in-text citation has a corresponding reference-list entry and that all listed references are cited in the manuscript.
6. Author names should be standardized throughout the text and reference list. For example, the manuscript uses both "Traore" and "Traoré" and both "Ouedraogo" and "Ouédraogo."
7. Journal titles, volume numbers, issue numbers, page ranges, article numbers, and DOI links should be checked for accuracy and formatted consistently.
8. The reference to Nakajima et al. (2001) should be checked to ensure that it supports the specific statement made about adult rotavirus detection rates.
9. The authors should ensure that references describing HAV seroprevalence are clearly distinguished from studies investigating active HAV infection through molecular detection.
10. The reference list should be formatted according to the journal's prescribed referencing style.

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7. Overall Recommendation

Minor Revision

The manuscript addresses a relevant public health topic and provides preliminary epidemiological information on rotavirus and hepatitis A virus detection among patients attending a regional hospital in Dédougou, Burkina Faso. The investigation has potential value because it contributes information from a region where published epidemiological data are limited.

However, the manuscript requires substantial revision before it can be considered for publication. The most important concerns are:

1. The inconsistency between the educational-level description in the Results section and the data presented in Table 1.
2. Insufficient detail regarding participant recruitment, sampling procedures, and eligibility criteria.
3. Inadequate reporting of laboratory quality-control procedures for HAV RT-PCR.
4. Incomplete presentation of logistic regression results despite the method being mentioned.
5. Insufficient information in Table 3 to evaluate the association between drinking water source and rotavirus positivity.
6. Overinterpretation risks arising from the very small number of HAV-positive cases.
7. The need to distinguish viral RNA detection from HAV serological evidence when comparing findings with previous studies.
8. Language, formatting, and reference-list inconsistencies requiring correction.

The authors should carefully verify the original dataset, revise the statistical presentation, improve the methodological description, and moderate conclusions that extend beyond the evidence provided. Addressing these issues would substantially improve the scientific quality and reliability of the manuscript.

8. Final Decision

Minor Revision

The manuscript may be reconsidered after minor revisions. The topic is relevant, and the findings could contribute to the epidemiological understanding of enteric viral infections in the Bankuy region of Burkina Faso. However, the authors must address the data inconsistencies, clarify the laboratory and statistical procedures, improve the presentation of results, and ensure that all conclusions are supported by the available evidence.