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OPEN PEER REVIEW

Seroprevalence of Specific Antibodies Against *Toxoplasmagondii* Among Blood Donors in Abadan and Khorramshahr Cities, Southwest Iran

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

0 comments

No public comments in this round.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor in chief

We would like to thank the reviewers for their detailed feedback to improve our submission. We are submitting a revised version of our manuscript entitled "**Seroprevalence of specific antibodies against *Toxoplasma gondii* among blood donors in Abadan and Khorramshahr cities, Southwest Iran**". As a result, the manuscript has undergone a revision with some details indicated below in our response to individual comments.

All comments applied to the paper with descriptions for some of them as following:

Reviewer 1's Comments:

1. Subject: What's the novelty in current article?

Response: The novelty of this study lies in its exploration of the seroprevalence of *Toxoplasma gondii* antibodies (IgG and IgM) in blood donors from Abadan and Khorramshahr cities, located in southwestern Iran. While previous studies have investigated *T. gondii* seroprevalence in various regions, the current study focuses on an under-researched area in Iran. Furthermore, it examines socio-demographic and behavioral risk factors associated with seroprevalence, providing valuable insights into possible transmission routes through blood transfusions in a region that has not been extensively studied in this context.

2. Abstract: Please rephrase according to academic writing. In the Abstract result, you have expressed the Logistic regression analysis. Import the result related to Logistic regression analysis in the result part of your manuscript.

Response: The abstract was rephrased based on academic writing standards. The results from the logistic regression analysis moved to the Results section, as suggested, to align with the manuscript's structure.

3. Are Keywords selected from MESH? Please check again.

Response: We reviewed the keywords selected for the manuscript and corrected them. Now keywords are in line with MESH terms.

4. Introduction: This part has elementary writing. Please rephrase according to academic and scientific writing.

Response: The Introduction section was revised to enhance the clarity and academic tone, ensuring it aligns with the expectations of scientific writing.

5. Materials and Methods:

Explain how the study size was arrived at? If your choice was based on the reported prevalence in some studies, please cite the references. Moreover, express other possible risk factors such as contact with animals (cats), raw/half-cocked meat consumption, raw milk/egg consumption, raw vegetable consumption, and history of blood transfusion.

The study size was determined based on the prevalence data from previous studies and the capacity of the local blood transfusion centers:

Mansouri A, Mojarad MR, Badfar G, Abasian L, Rahmati S, Kooti W, YektaKooshali MH, Soleymani A, Azami M. Epidemiology of Toxoplasma gondii among blood donors in Iran: A systematic review and meta-analysis. Transfusion and Apheresis Science. 2017 Jun 1;56(3):404-9.

We included the references for reported prevalence and further elaborate on the inclusion of risk factors like contact with animals, consumption of raw meat, raw milk, raw eggs, and history of blood transfusion as part of the study's demographic and behavioral investigation.

Regarding risk factors, we designed a checklist according to other articles and asked patients

-Please write the company name rather than kit.

We replaced the term "kit" with the specific company name for the diagnostic tests used in the study.

-Please say which antibody titer value was considered positive and/or negative. In your reported measurements, the antibody titer should be replaced with an OD mount. How did you differentiate the early stage of infection from a past infection according to the antibody titer?

We clarified the antibody titer values considered as positive or negative and provide details on how we differentiated early infection from past infection based on IgM and IgG antibodies, as per the literature and established diagnostic guidelines.

-How did you detect the association between T. gondii seropositivity and the potential risk factors???

We explained in the methodology used to assess the associations between *T. gondii* seropositivity and potential risk factors, including the statistical tests and their interpretations.

-Please improve your text with academic and scientific English.

Whole text were revised and corrected according to academic and scientific English

For example, rephrase: Findings were analyzed by SPSS software (version 19) (SPSS Inc.,Chicago, IL, USA). The chi-square test was applied to access the univariate association between independent variables and outcome. In this survey, P < 0.05 was considered statistically significant:

Was corrected in the text.

6. Results:

-Your findings should relate to demographic information and other possible risk factors, such as contact with animals (cats), raw/half-cocked meat consumption, raw milk/egg consumption, raw vegetable consumption, and history of blood transfusion, respectively.

Response: We discussed this in text completely.

Line89:"between these cases". Between what???

Your sentence in ambiguous. Please express with more details:

Response: Corrected in text

7. Discussion:

-Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

Please compare the results of the literature to your current results. For example, in the sentence" The differences in the seroprevalence of T. gondii antibody may be because of geographic factors", you can discuss the reference data and compare it to your data.

Respond: we compared the results of the literature to your current results in text

Please rephrase all of your manuscript using academic and scientific writing. Please correct the grammatical errors.

We revised the manuscript thoroughly, ensuring that it follows academic and scientific writing conventions and correcting any grammatical errors as pointed out.

2 Revision 1

2 comments · 2 Feb 2025 – 13 Apr 2025

Referee 1 Reviewer

MINOR REVISION 2 Feb 2025

Please refer to the attached file for additional comments and suggestions provided within the text.

Yalda Malekzadegan Reviewer

ACCEPT 13 Apr 2025

In this manuscript, the authors present data on the seroprevalence of specific antibodies against Toxoplasma gondii among blood donors in the cities of Abadan and Khorramshahr, Southwest Iran. However, some points should be considered. (See specific points below.)

This study is a retrospective cross-sectional study, because it was done in 2022.
Some references are not endnotes.
Please mention the probable limitations of the present study.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:

Please refer to the attached file for additional comments and suggestions provided within the text.

Response: All comments were applied within the manuscript.

Reviewer 2:

In this manuscript the authors present data on the seroprevalence of specific antibodies against *Toxoplasma gondii* among blood donors in Abadan and Khorramshahr cities, Southwest Iran. However, some points should be considered. (See specific points below).

This study is a retrospective cross-sectional study, because it was done in 2022.

Response: Thanks for your comment. We would like to clarify that this is not a retrospective study. The data were collected in 2022 through direct sampling and laboratory testing conducted by our research team. However, the manuscript preparation and submission for publication were carried out afterward, which might have caused some confusion regarding the study design. Therefore, the study should be classified as a cross-sectional study rather than a retrospective one.

Some references are not endnotes.

Response: Thank you for your comment and attention. All references were sorted via EndNote.

Please mention to probable limitations of present study.

Response: Was added.

3 Revision 2

4 comments · 1 Jun 2025 – 9 Jun 2025

Referee 2 Reviewer

MINOR REVISION 1 Jun 2025

Dear AE,
Thank you very much for giving me the opportunity to review this revised manuscript.
This cross-sectional study investigates the seroprevalence of T. gondii IgG/IgM antibodies among blood donors in Southwest Iran, addressing a critical gap in transfusion medicine. The authors report a 31.88% IgG seroprevalence, with no significant associations with demographic/behavioral factors. The study is well-structured and methodologically sound and provides actionable data for public health strategies. However, limitations (e.g., reliance on ELISA alone) and minor inconsistencies warrant clarification. The revision (R2) adequately addresses prior reviewer comments, but further refinements are needed.

Methodological Rigor & Clarity
1. Study Design Clarification: The authors correctly note this is a cross-sectional study (not retrospective). To avoid confusion, explicitly state in the Methods: *"This prospective cross-sectional study collected data via direct sampling in 2022."*
2. ELISA Limitations: While the limitation of lacking molecular confirmation is acknowledged (Page 12), quantify its impact. Add: "ELISA's sensitivity/specificity for IgG/IgM in our setting was [X]%/[Y]%, per manufacturer data, reducing false-positive risks."
3. Sample Size Justification: Justify n=345. Was this based on power analysis? If not, add: "Convenience sampling was used due to logistical constraints; future studies should prioritize powered calculations."

Results & Statistical Analysis
* Table 1 Refinement:
4. Merge "IgM positive" and "Both IgG/IgM" columns into one ("IgM+") for clarity, as co-positivity is rare (n=5).
5. Specify if "contact with cats" includes ownership or transient exposure (critical for risk interpretation).
6. Non-Significant Associations: Emphasize clinical relevance over p-values. Add: *"Despite statistical non-significance, the 42.9% IgG seroprevalence in rural donors (vs. 30.9% urban) warrants further study due to potential environmental exposure differences."*

Discussion & Contextualization
7. Geographic Comparisons: The 31.88% IgG rate is higher than those in Turkey/Mexico but aligns with global averages. Contrast with Iranian meta-analyses (e.g., cite Ref 13's national estimate) to highlight regional variability.
8. Risk Factor Paradox: The lack of association with raw meat/cat contact contradicts other studies (Refs 25–27). Discuss potential reasons: "Cultural dietary practices (e.g., thorough meat cooking) or underreported cat exposures may explain discrepancies."
9. Blood Group Analysis: Reiterate that ABO/Rh non-association (Table 2) aligns with global literature (Refs 34–37), reinforcing blood groups' irrelevance to T. gondii susceptibility.

Language & Technical Edits
* Grammar/Syntax:
10. Page 5, Line 24: "The infection is usually occurs" → "usually occurs".
11. Page 6, Line 60: *"May - September 2022 Written"* → Add a comma after "2022."
12. Abbreviations: Define "BTO" (Blood Transfusion Organization) at first use (Page 6, Line 58).

Reviewer Response Assessment
* Retrospective vs. Cross-Sectional: The authors' clarification is adequate.

* EndNote References: Confirmed sorted; no further action needed.
* Limitations Added: The added limitation (ELISA-only) is appropriate but should be expanded (see Point 2 above).

Overall, the manuscript is well-executed and addresses an important public health question. Revisions should focus on clarifying methodological choices, contextualizing non-significant results, and minor technical edits. No additional experiments are needed.

Final Decision: Minor Revision

Kind Regards | Publisher Supervisor

Referee 3 Reviewer ACCEPT 2 Jun 2025

Due to the two prior reviews and the subsequent corrections, I concur with the authors' recent responses and amendments, and I believe the manuscript is now prepared for publication.

Associate Editor ACCEPT 9 Jun 2025

accepted

Editor-in-Chief MINOR REVISION 9 Jun 2025

Public Comment:
Dear AE,
Thank you very much for giving me the opportunity to review this revised manuscript.
This cross-sectional study investigates the seroprevalence of T. gondii IgG/IgM antibodies among blood donors in Southwest Iran, addressing a critical gap in transfusion medicine. The authors report a 31.88% IgG seroprevalence, with no significant associations with demographic/behavioral factors. The study is well-structured and methodologically sound, and it provides actionable data for public health strategies. However, limitations (e.g., reliance on ELISA alone) and minor inconsistencies warrant clarification. The revision (R2) adequately addresses prior reviewer comments, but further refinements are needed.
Methodological Rigor & Clarity
1. Study Design Clarification: The authors correctly note this is a cross-sectional study (not retrospective). To avoid confusion, explicitly state in the Methods: *"This prospective cross-sectional study collected data via direct sampling in 2022."*
2. ELISA Limitations: While the limitation of lacking molecular confirmation is acknowledged (Page 12), quantify its impact. Add: "ELISA's sensitivity/specificity for IgG/IgM in our setting was [X]%/[Y]%, per manufacturer data, reducing false-positive risks."
3. Sample Size Justification: Justify n=345. Was this based on power analysis? If not, add: "Convenience sampling was used due to logistical constraints; future studies should prioritize powered calculations."
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* Table 1 Refinement:
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5. Specify if "contact with cats" includes ownership or transient exposure (critical for risk interpretation).
6. Non-Significant Associations: Emphasize clinical relevance over p-values. Add: *"Despite statistical non-significance, the 42.9% IgG seroprevalence in rural donors (vs. 30.9% urban) warrants further study due to

potential environmental exposure differences."*
Discussion & Contextualization
7. Geographic Comparisons: The 31.88% IgG rate is higher than the rates reported in Turkey/Mexico but aligns with global averages. Contrast with Iranian meta-analyses (e.g., cite Ref 13's national estimate) to highlight regional variability.
8. Risk Factor Paradox: The lack of association with raw meat/cat contact contradicts other studies (Refs 25–27). Discuss potential reasons: "Cultural dietary practices (e.g., thorough meat cooking) or underreported cat exposures may explain discrepancies."
9. Blood Group Analysis: Reiterate that ABO/Rh non-association (Table 2) aligns with global literature (Refs 34–37), reinforcing blood groups' irrelevance to T. gondii susceptibility.
Language & Technical Edits
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12. Abbreviations: Define "BTO" (Blood Transfusion Organization) at first use (Page 6, Line 58).
Reviewer Response Assessment
* Retrospective vs. Cross-Sectional: The authors' clarification is adequate.
* EndNote References: Confirmed sorted; no further action needed.
* Limitations Added: The added limitation (ELISA-only) is appropriate but should be expanded (see Point 2 above).
Overall, the manuscript is well-executed and addresses an important public health question. Revisions should focus on clarifying methodological choices, contextualizing non-significant results, and minor technical edits. No additional experiments are needed.
Final Decision: Minor Revision

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewers' Comments:

Reviewer 1:
Due to the two prior reviews and the subsequent corrections, I concur with the authors' recent responses and amendments, and I believe the manuscript is now prepared for publication.

Response: Thank you.

Reviewer 2:
Dear AE,
Thank you very much for giving me the opportunity to review this revised manuscript.
This cross-sectional study investigates the seroprevalence of *T. gondii* IgG/IgM antibodies among blood donors in Southwest Iran, addressing a critical gap in transfusion medicine. The authors report a 31.88% IgG seroprevalence, with no significant associations to demographic/behavioral factors. The study is well-structured, methodologically sound, and provides actionable data for public health strategies. However, limitations (e.g., reliance on ELISA alone) and minor inconsistencies warrant clarification. The revision (R2) adequately addresses prior reviewer comments, but further refinements are needed.

Methodological Rigor & Clarity
1. Study Design Clarification: The authors correctly note this is a cross-sectional study (not retrospective). To avoid confusion, explicitly state in the Methods: *"This prospective cross-sectional study collected data via direct sampling in 2022."*

Response: Was added in methods.

2. ELISA Limitations: While the limitation of lacking molecular confirmation is acknowledged (Page 12), quantify its impact. Add: "ELISA’s sensitivity/specificity for IgG/IgM in our setting was [X]%/[Y]%, per manufacturer data, reducing false-positive risks."

Response: Was added

3. Sample Size Justification: Justify n=345. Was this based on power analysis? If not, add: "Convenience sampling was used due to logistical constraints; future studies should prioritize powered calculations."

Response: Was added in section 2.2.

Results & Statistical Analysis
* Table 1 Refinement:

4. Merge "IgM positive" and "Both IgG/IgM" columns into one ("IgM+") for clarity, as co-positivity is rare (n=5).

Response: In many published articles, reports were done based on IgG, IgM, and both IgG/IgM antibodies. So we decided to design our tables based on them.

5. Specify if "contact with cats" includes ownership or transient exposure (critical for risk interpretation).

Response: Was added.

6. Non-Significant Associations: Emphasize clinical relevance over p-values. Add: *"Despite statistical non-significance, the 42.9% IgG seroprevalence in rural donors (vs. 30.9% urban) warrants further study due to potential environmental exposure differences."*

Response: Was added in section 3.

Discussion & Contextualization
7. Geographic Comparisons: The 31.88% IgG rate is higher than Turkey/Mexico but aligns with global averages. Contrast with Iranian meta-analyses (e.g., cite Ref 13's national estimate) to highlight regional variability.

Response: Was added.

8. Risk Factor Paradox: The lack of association with raw meat/cat contact contradicts other studies (Refs 25–27). Discuss potential reasons: "Cultural dietary practices (e.g., thorough meat cooking) or underreported cat exposures may explain discrepancies."

Response: Was added.

9. Blood Group Analysis: Reiterate that ABO/Rh non-association (Table 2) aligns with global literature (Refs 34–37), reinforcing blood groups' irrelevance to T. gondii susceptibility.

Response: Was added.

Language & Technical Edits
* Grammar/Syntax:
10. Page 5, Line 24: "The infection is usually occurs" → "usually occurs".

Response: Was corrected.

11. Page 6, Line 60: *"May - September 2022 Written"* → Add comma after "2022."

Response: Was corrected.

12. Abbreviations: Define "BTO" (Blood Transfusion Organization) at first use (Page 6, Line 58).

Response: Was done.

Reviewer Response Assessment
* Retrospective vs. Cross-Sectional: The authors' clarification is adequate.

Response: Was done.

* EndNote References: Confirmed sorted; no further action needed.

Response: Thank you.

* Limitations Added: The added limitation (ELISA-only) is appropriate but should be expanded (see Point 2 above).

Response: Was revised.

Overall, the manuscript is well-executed and addresses an important public health question. Revisions should focus on clarifying methodological choices, contextualizing non-significant results, and minor technical edits. No additional experiments are needed.
Response: Thank you.

4 Revision 3

1 comment · 30 Jun 2025

Editor-in-Chief

ACCEPT 30 Jun 2025

accept