Author's Response to Peer Reviews

Author's Response to Peer Reviews of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study"

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KEYWORDS

impact of climate; seasonal change; frequency; livestock; ticks; Tehran

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Round 1 Review

Reviewer W [1]

General Comments

This paper provides a detailed investigation into the distribution and frequency of tick species infecting livestock and poultry in Tehran province, Iran, with a focus on seasonal and climatic variation. The study highlights the significant economic and epidemiological impact of ticks as ectoparasites and pathogen vectors in livestock.

Response: We sincerely appreciate the time and effort you have taken to review our manuscript [2]. Your feedback is valuable, and we have addressed all of your comments as outlined below.

Specific Comments

Major Comments

1. "Material and methods - Sampling": For the tick sampling in this manuscript, what method did you used in this study (ie, how did you decide which tick samples to include and which samples to exclude)? How did you ensure that the sample is representative of the true distribution of ticks in the study area? The distribution and frequency estimates from this sample might not be extended to the whole tick population if the sampling is biased.

Response: In this study, tick samples were collected from 1623 livestock animals (including chickens, camels, cows, pigeons, dogs, and sheep) infected with ticks. The sampling was carried out using a cross-sectional study design in two different climate regions: mountainous and plain. Livestock were randomly selected based on visible tick infestation, ensuring that the sample represented the true distribution of ticks in the study area, particularly with the help of local veterinary authorities.

Text to be added to the Sampling section of the Methods: "To ensure representative sampling, a cross-sectional study was conducted, covering both mountainous and plain regions.

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Selection of livestock was randomized among those showing visible tick infestation, with veterinary supervision ensuring consistency in sample collection across different geographical zones. The chosen method aligns with established epidemiological studies on tick distribution."

2. "Material and methods - Sampling": Could the author provide the rationale or justification of the choice of "p" and "d" in the sample size calculation?

Response: The parameter p=0.3 was selected based on previous studies on tick prevalence in similar regions of Iran, where an approximate prevalence rate of 30% was observed. The margin of error (d=0.045) was chosen to ensure a 95% confidence level while maintaining a practical sample size for data collection.

Text to be added to the manuscript: "The parameter P(0.3) was selected based on prior studies on tick prevalence in similar regions in Iran, indicating an estimated infestation rate of 30%. The margin of error (d = 0.045) was determined considering a 95% confidence level, ensuring a balance between precision and feasibility of sample collection."

3. "Discussion": I really like the analysis of seasonal trend presented in Table 4 and Figure 4. Could the author elaborate more on this in the Discussion—the general seasonal trend across all species, the reason why you expect some species to be more abundant in warmer versus colder weather, and the implications from the public health perspective?

Response: The analysis revealed that hard ticks like *Rhipicephalus sanguineus* and *Hyalomma marginatum* were more abundant during the spring and summer, likely due to higher temperatures and increased activity of their hosts. In contrast, soft ticks such as *Argas persicus* were more prevalent in the autumn and winter, as they can survive in sheltered environments and colder conditions.

Text to be added to the Discussion section: "The observed seasonal trend aligns with the biological cycles of tick species. Hard ticks such as *Rhipicephalus sanguineus* and *Hyalomma marginatum* exhibited peak abundance in spring and summer due to higher temperatures and increased host activity. Conversely, soft ticks (*Argas persicus*) showed resilience during colder months, likely due to their ability to survive in sheltered environments. This seasonal variability highlights the importance of targeted tick control strategies, particularly in warm seasons when transmission risk of tick-borne diseases is highest."

Minor Comments

1. Line 41: Spell out "\$."

2. *Line 44: Remove "(4)"—duplicated reference number.*

3. Line 51: Remove "(9)"—duplicated reference number.

4. Line 85, "valid diagnostic keys": Could the author be more specific about the "diagnostic key" being used? Adding a sentence to briefly describe the key would be great. Response: To clarify the "valid diagnostic key" used for tick identification, we will specify the exact key used for species identification and provide a brief description of its methodology.

Text to be added to the manuscript (Line 85 of Methods): "The tick species were identified using the diagnostic keys outlined by Jongejan et al. (1987) [3] and Camicas et al. (1998) [4], which provide detailed morphological descriptions and illustrations for the identification of both soft and hard ticks. These keys are widely recognized for their accuracy and reliability in the identification of tick species in the Middle East and neighboring regions."

5. Lines 147-151, "two professional stereo microscopes...in the entomological research": This part should belong to Methods section.

Reviewer AX [5]

General Comments

The manuscript [1] presents a comprehensive study on the seasonal and climatic distribution of ticks in Tehran province, Iran. The research is well structured and provides valuable insights into the diversity and abundance of tick species in different climate zones. The study is relevant to the field of veterinary parasitology and has potential implications for tick control strategies in the region. However, there are several areas where the manuscript could be improved in terms of grammar, sentence structure, and adherence to standard academic writing conventions.

Specific Comments

Major Comments

Grammar and Sentence Structure

Overall clarity: The manuscript is generally clear, but there are instances where the sentence structure could be improved for better readability. Some sentences are overly long and could be broken down into shorter, more concise statements.

For example:

- Original: "The presence of ticks on livestock causes localized lesions at the bite site and systemic lesions, leading to death due to anemia and paralysis due to ticks transmitting various diseases such as theileriosis and babesiosis."
- Suggested revision: "The presence of ticks on livestock causes localized lesions at the bite site and systemic effects, which can lead to anemia, paralysis, and even death. Ticks are also vectors for diseases such as theileriosis and babesiosis."

Subject-verb agreement: There are a few instances where the subject-verb agreement is incorrect.

For example:

• Original: "The distribution of collected ticks (in mountain and plain climates) indicated that out of 806 collected ticks,

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44.78% and 55.21% belonged to the mountainous and plain regions, respectively."

• Suggested revision: "The distribution of collected ticks (in mountainous and plain climates) indicates that out of 806 collected ticks, 44.78% belonged to mountainous regions, while 55.21% were found in plain regions."

Tense consistency: The manuscript occasionally shifts between past and present tense. It is important to maintain consistency, especially in the Results and Discussion sections.

For example:

- Original: "The study is conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."
- Suggested revision: "The study was conducted in two different environments: plains and mountains within 20 selected villages in Tehran Province."

Response: We agree, and several sentences will be broken down for better clarity and conciseness. Below is an example of a revised sentence:

- Original: "The presence of ticks on livestock causes localized lesions at the bite site and systemic lesions, leading to death due to anemia and paralysis due to ticks transmitting various diseases such as theileriosis and babesiosis."
- Revised: "Ticks on livestock cause localized bite-site lesions and systemic effects. They can lead to anemia, paralysis, and even death by transmitting diseases like theileriosis and babesiosis."

Structure and Organization

Abstract: The Abstract is well written and provides a concise summary of the study. However, it could benefit from a brief mention of the key findings related to seasonal variations, as this is a major focus of the study.

Response: Yes, the Abstract will be revised to include more specific quantitative data, such as sample size, species abundance, and seasonal variations.

Revised Abstract (Results section): "Results showed that out of 806 collected ticks, 44.78% were found in mountainous regions and 55.21% in plain regions. The most abundant species was *Rhipicephalus sanguineus* (36.97%), while *Rhipicephalus* (*Boophilus*) annulatus was the least common (0.37%). Seasonal variation indicated peak infestation in spring (60.3%) and lowest in winter (9.5%)."

Introduction: The Introduction provides a good background on the importance of ticks and their impact on livestock. However, it could be strengthened by including more recent references (post-2020) to highlight the current state of research on tick-borne diseases and climate change.

Methods: The Methods section is detailed and well organized. However, the formula used for sample size calculation is not clearly explained. It would be

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helpful to provide a brief explanation of the variables used in the formula (eg, p=0.3 and d=0.045).

Response: A brief explanation of the sample size calculation formula will be added to the Methods section for clarity.

Text to be added to the Sample Size Calculation section of the Methods: "The sample size was calculated using Cochran's formula for prevalence studies. Given an estimated prevalence (p) of 30% and a precision (d) of 4.5%, the final sample size was determined to be 800 ticks, ensuring statistical reliability."

Results: The Results are presented clearly, with appropriate use of tables and figures. However, some of the tables could be simplified for better readability. For example, Table 4 could be restructured to make it easier to compare seasonal activity across species.

Discussion: The Discussion is thorough and compares the findings with other studies effectively. However, it could be improved by discussing the limitations of the study and suggesting areas for future research.

Similarity and Plagiarism

The manuscript appears to be original, with no significant issues of plagiarism detected. However, it is recommended to run the manuscript through a plagiarism detection tool (eg, Turnitin) to ensure that all sources are properly cited and that there is no unintentional duplication of text.

Adherence to Standard Academic Writing

References: The references are generally appropriate and relevant to the study. However, some references are quite old (eg, references from the 1980s and 1990s). It is recommended to include more recent studies to reflect the current state of knowledge in the field.

Add these references to the manuscript:

- Noaman V. Identification of hard ticks collected from sheep naturally infected with Anaplasma ovis in Isfahan province, central Iran. Comp Clin Pathol 2012 Feb 21; 21(3):367-369. [doi: 10.1007/s00580-012-1438-1]
- Noaman V, Abdigoudarzi M, Nabinejad AR. Abundance, diversity and seasonal dynamics of hard ticks infesting cattle in Isfahan province, central Iran. Archives of Razi Institute. 2017 Mar 1;72(1):15-21. [doi: 10.22034/ari.2016.107490]
- Noaman V, Abdigoudarzi M, Nabinejad AR, Heidari MR, Khalilifard M. (2007). Identification of hard ticks of domestic ruminants in two ecological zones of Isfahan province, Iran. Veterinary Journal (Pajouhesh va Sazandegi). 2008;77:88-95.

Response: The following recent references will be added to the manuscript:

- Noaman V. Identification of hard ticks collected from sheep naturally infected with Anaplasma ovis in Isfahan province, central Iran. Comp Clin Pathol 2012 Feb 21; 21(3):367-369. [doi: 10.1007/s00580-012-1438-1]
- 2. Noaman V, Abdigoudarzi M, Nabinejad AR. Abundance, diversity and seasonal dynamics of hard ticks infesting

cattle in Isfahan province, central Iran. Archives of Razi Institute. 2017 Mar 1;72(1):15-21. [doi: 10.22034/ari.2016.107490]

 Noaman V, Abdigoudarzi M, Nabinejad AR, Heidari MR, Khalilifard M. (2007). Identification of hard ticks of domestic ruminants in two ecological zones of Isfahan province, Iran. Veterinary Journal (Pajouhesh va Sazandegi). 2008;77:88-95.

Text to be added to the Discussion section: "Our findings align with previous studies on tick diversity in central Iran (Noaman et al., 2012; Noaman et al., 2017), confirming seasonal variations in tick populations. These studies further support the need for region-specific tick control strategies."

Figures and tables: The figures and tables are well presented and support the findings of the study. However, the legends for some figures (eg, Figure 1>) could be more descriptive. For example, Figure 1 could include a brief explanation of what the "ratio of caught ticks" represents.

The manuscript presents a valuable contribution to the field of veterinary parasitology, particularly in the context of tick distribution and seasonal activity in Tehran province. With some revisions to improve grammar, sentence structure, and adherence to standard academic writing conventions, the manuscript will be suitable for publication in a reputable journal. The manuscript can be considered for publication in JMIRx Bio after major revision.

Response: We trust that these revisions adequately address the reviewer's concerns. Please let us know if any further modifications are required. We look forward to your feedback on the revised manuscript.

Round 2 Review

Reviewer W

Thank you, author, for addressing all my comments and making all necessary changes to the manuscript. I do not have any more comments.

Reviewer AX

General Comments

The revised manuscript titled "Assessing the Influence of Seasonal and Climatic Variations on Livestock

Conflicts of Interest

None declared.

References

- Shen J. Peer review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study". JMIRx Bio. 2025;3:e72765. [doi: <u>10.2196/72765</u>]
- 2. Abbasi E. Assessing the influence of seasonal and climatic variations on livestock tick incidence in Tehran province, Iran: cross-sectional study. JMIRx Bio. 2025;3:e69542. [doi: 10.2196/69542]
- Jongejan F, Zivkovic D, Pegram RG, Tatchell RJ, Fison T, Latif AA, et al. Ticks (Acari: Ixodidae) of the Blue and White Nile ecosystems in the Sudan with particular reference to the Rhipicephalus sanguineus group. Exp Appl Acarol. Nov 1987;3(4):331-346. [doi: 10.1007/BF01193169] [Medline: 3331134]

Tick Incidence in Tehran Province, Iran" has addressed the previous comments and suggestions effectively. The authors have made the necessary revisions to improve the clarity, structure, and overall quality of the manuscript. Below are my final comments.

Strengths

Improved clarity: The Abstract has been revised to be more concise and now includes key findings related to seasonal variation and the most abundant tick species, enhancing readability and impact.

Focused introduction: The Introduction now more clearly highlights the specific gaps in the literature that this study addresses, particularly in the context of Tehran province.

Streamlined methodology: The Methods section has been clarified, with more details on the randomization process and a more concise description of the study area. The inclusion of supplementary tables for geographical coordinates and ecological information is a welcomed addition.

Organized results: The Results section has been streamlined with the use of subheadings, making it easier to follow. All referenced figures and tables are now included, providing a comprehensive view of the findings.

Enhanced discussion: The Discussion section now more effectively focuses on the implications of the findings for tick control strategies in Tehran province. The comparison with studies from other regions has been made more concise, emphasizing key similarities and differences.

Practical conclusion: The Conclusion has been revised to highlight the practical implications of the findings, particularly the need for seasonal tick control measures in different climate zones.

Consistent references: All references are now formatted consistently according to the journal's guidelines, with complete details provided.

Overall recommendation: The manuscript has been significantly improved and is now suitable for publication. I recommend acceptance of the manuscript in its current form.

- 4. Camicas JL, Hervy JP, Adam F, Morel PC. Les Tiques du Monde (Acarida, Ixodida): Nomenclature, Stades Décrits, Hôtes, Répartition. The Ticks of The World (Acarida, Ixodida): Nomenclature, Described Stages, Hosts, Distribution. Paris, France. Éditions de l'Orstom; 1998.
- 5. Noaman V. Peer review of "Assessing the Influence of Seasonal and Climatic Variations on Livestock Tick Incidence in Tehran Province, Iran: Cross-Sectional Study". JMIRx Bio. 2025;3:e72764. [doi: 10.2196/72766]

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