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Tick exposure biomarkers: A One Health approach to new tick surveillance tools

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ABSTRACT

The spread of tick-borne disease (TBD) is escalating globally, driven by climate change and socio-economic shifts, underlining the urgency to improve surveillance, diagnostics, and control strategies. Ticks can transmit a range of pathogens increasing the risk of transmission of human and veterinary diseases such as Lyme disease, tick-borne encephalitis, theileriosis, anaplasmosis, or Crimean-Congo hemorrhagic fever. Surveillance methods play a crucial role in monitoring the spread of tick-borne pathogens (TBP). However, there are shortcomings in the current surveillance methods regarding risks related to ticks. Human-tick encounters offer a novel metric for disease risk assessment, integrating human behavior into traditional surveillance models. However, to more reliably measure tick exposure, a molecular marker is needed. The identification of antibodies against arthropod salivary proteins as biomarkers for vector exposure represents a promising avenue for enhancing existing diagnostic and surveillance metrics. Here we explore how the use of tick saliva biomarkers targeting recombinant proteins and synthetic peptides could significantly improve the assessment of TBD transmission risk and the effectiveness of vector control measures. With focused efforts on creating a biomarker against tick exposure suitable for humans and domestic animals alike, tick surveillance, diagnosis and control would be more achievable and aid in reducing the mounting threat of TBP through a One Health lens.

1. Introduction

Ticks are among the most important vectors of viral, bacterial and parasite pathogens that affect humans and animals worldwide (Moraga-Fernández et al., 2023). Globally, the geographical distribution of ticks has been increasing due to climate, environmental and socio-economic changes that impact both tick populations and their vertebrate hosts (Stachurski et al., 2021). For instance, warmer winters enhance the survival and the duration of the activity period of ticks, thereby increasing the chances of tick exposure and pathogen transmission (Wall and Alasmari, 2021). Notably, the incidence of tick-borne diseases (TBD) has been rising in various regions, such as babesiosis in the USA and tick-borne encephalitis in Europe (Madison-Antenucci et al., 2020; Saegerman et al., 2023). In addition, the occurrence of co-infections caused by ticks could pose an additional risk (Jaenson et al., 2024). As ticks spread globally (Omazic et al., 2023), it will be necessary to monitor their effect on human and domestic animal populations. Migratory birds may be one mechanism through which ticks are being introduced into new areas, as may have been recently the case in the south of France for *Hyalomma* ticks, the main vectors of Crimean-Congo hemorrhagic fever virus, underscoring the importance of vigilant monitoring and risk assessment (Vial et al., 2016; Bernard et al., 2024). In parallel, control efforts focusing on chemical or environmental control show waning worldwide efficacy due to changes in tick populations resistant to pesticides and land use (De Rouck et al., 2023; Wimms et al., 2023). These shifts highlight that tick control will require modern technological advances and cooperation across country borders to combat expansion of ticks and TBD, as well as advances in diagnostic, surveillance, and control measures (Tiffin et al., 2022).

Effective tick control hinges on the ability to accurately detect and monitor tick exposure in wild and domestic animals as well as humans. Current surveillance systems rely on the systematic collection and

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analysis of data regarding disease incidence, geographical distribution, and temporal trends. Despite the increasing threat posed by TBD, the development of biomarkers for tick exposure – a potentially pivotal aspect for early TBD detection and tick management – remains significantly underdeveloped. The lack of reliable biomarkers hampers the precision of these efforts, limiting the ability to identify high-risk areas, detect early outbreaks, and evaluate the success of public health and ecological control interventions.

This review emphasizes the global necessity for intensified research and collaboration to develop molecular tools for tick exposure. By harnessing advancements in biomarker technology, knowledge about changes in TBD, tick geographical distributions, and at-risk populations can increase. Such progress is vital for allocating resources more effectively, devising targeted prevention strategies, and informing both veterinarians and public healthcare providers about TBD risks.

2. Then and now: Determining potential biomarkers for tick bite exposure

Biomarkers using tick saliva offer a promising tool for monitoring the evolving threat posed by ticks and tick-borne pathogens (TBP). Their use can enhance our understanding of host/vector exposure, efficacy of anti-vector control measures, as well as changes in vector-pathogen ecology. In addition, tick exposure biomarkers could be helpful in the diagnosis of TBD in both humans and domestic animals. For example, many patients diagnosed with Lyme disease may have had no knowledge of an antecedent tick bite, and self-reported exposure to ticks is poorly correlated with actual tick exposure while sightings, or encounters, offer a stronger correlation (Hook et al., 2021). The development of a candidate biomarker may aid in addressing the challenges posed by TBD worldwide, ensuring that both health systems and ecosystems are better surveilled to address TBP using a One Health approach. Several attempts have been made to identify suitable antibody responses against tick saliva as biomarkers, though problems around reactivity and specificity remain obstacles to overcome. In addition, an important factor to elucidate in potential biomarker candidates is the immunogenic kinetics that may provide precise windows of exposure based on initial (IgM) or long-term (IgG) antibody production.

Using antibodies against tick saliva components as biomarkers may have first been proposed in 1990 when anti-tick saliva antibodies were found to have a positive association with owning pets and Lyme disease seropositivity (Schwartz et al., 1990, 1991). Since then, several immunogenic tick salivary proteins that have potential as candidates for immune related control and/or surveillance have been identified, as reviewed in (Olajiga et al., 2021; Pham et al., 2021; Ali et al., 2022). Each review highlights different antigenic proteins including calreticulin (CRT), serine protease inhibitors, lipocalins, metalloproteases, salivary proteins (Salps), cement-like proteins and statins among the most prevalent. However, relatively few of the identified immunomodulatory tick saliva components have been tested for their potential as a candidate for biomarker development (Table 1). Further, candidates that have been tested present varying degrees of antigenicity when measured with IgM or IgG. For example, antibodies against the tick protein Rs24p have been suggested as indicators of exposure to *R. sanguineus* in dogs; however, these antibodies were only found for a brief duration following tick infestation (Inokuma et al., 2000). While not suitable for a vaccine, this protein, which induces an immunogenic response over a limited time, could be utilized as a biomarker for determining a potential window of exposure, especially if cross-validated with potential biomarker candidates that induce longer lasting antibody responses (Bonnet et al., 2018).

One of the most studied proteins in this context is CRT, first identified as a secreted protein in the saliva of the ixodid ticks *Amblyomma americanum* and *Dermacentor variabilis* (Jaworski et al., 1995). The immune response against CRT has since been identified as a potential indicator of exposure to ticks from the genera *Amblyomma*, *Dermacentor* and *Ixodes* (Sanders et al., 1999; Alarcon-Chaidez et al., 2006; Vu Hai et al., 2013). For example, CRT from *I. ricinus* was discriminatory between the tick species *Rhipicephalus sanguineus* and *Dermacentor reticulatus* (Vu Hai et al., 2013). However, varying degrees of success have been reported across other tick species with cross-reactivity identified between *I. scapularis* and *A. americanum* (Sanders et al., 1998). Further,

Table 1
A list of tick saliva components, either the full protein sequence or a short peptide region, that have been tested to identify biomarkers of tick bite exposure in different animal sera.

Name	Protein or Peptide	Animal	Tick species	Outcome	Reference
Calreticulin (CRT)	Protein, peptide	Rabbits, humans, mice	*Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*, *Ixodes ricinus*, *Haemaphysalisis longicornis*	AaCRT and DvCRT found in rabbit serum after feeding; recombinant AaCRT indicative of *A. americanum* and *D. variabilis* exposure in rabbits and humans; recombinant IsCRT was recognized in human sera; IrCRT was indicative of *Ixodes* exposure in rabbits but not *R. sanguineus* or *D. reticulatus*; HlCRT was non-immunogenic in mice and rabbits	Jaworski et al. (1995); Sanders et al. (1998); Alarcon-Chaidez et al. (2006); Vu Hai et al. (2013); Zheng et al. (2022)
Rs24p	Protein	Dogs	*Rhipicephalus sanguineus*	Discriminatory between infested and non-infested dogs	Inokuma et al. (2000)
AV422	Protein; Peptide[a]	Rats, humans, *in silico*[a]	*Ixodes ricinus*, *Dermacentor reticulatus*	Recombinant IrAV422 was immunogenic in rats exposed to both *I. ricinus* and *D. reticulatus*; An anti-rIrAV422 response was found in humans and the most immunogenic AV422 T cell epitope was predicted *in silico*	Mihaljica et al. (2017, 2021)
α-Gal modified proteins	Protein	Humans	*Amblyomma americanum*, *Ixodes holocyclus*	Tick proteins with α-Gal modifications from tick saliva illicit specific immune response in humans exposed to ticks	Villar et al. (2021)
Vitogellin-2	Peptide	Rabbits	*Ixodes ricinus*	Second most antigenic peptide tested in rabbits	Bensaoud et al. (2022)
Lipocalin	Peptide	Rabbits	*Ixodes ricinus*	Most antigenic peptide in rabbit serum	Bensaoud et al. (2022)
Microplusin	Peptide	Rabbits	*Ixodes ricinus*	Moderate antigenic response in rabbit serum	Bensaoud et al. (2022)
TIL domain protein	Peptide	Rabbits	*Ixodes ricinus*	Moderate antigenic response in rabbit serum	Bensaoud et al. (2022)
Two Thyropin Domain protein	Peptide	Rabbits	*Ixodes ricinus*	Antigenic response in rabbit serum	Bensaoud et al. (2022)
Secreted protein	Peptide	Rabbits	*Ixodes ricinus*	This peptide was not antigenic in rabbits	Bensaoud et al. (2022)

[a] Only tested *in silico* and remains to be validated *in vivo*.

the kinetics of IgM and IgG antibody responses have not yet been completely determined but long-term IgG antibody production against CRT was documented for at least 17 months after exposure to *I. scapularis*. This finding was in humans and issues around cross-reactivity remain (Alarcon-Chaidez et al., 2006). A biomarker candidate with cross-reactivity across all tick species could be co-opted as a general biomarker for tick exposure though a better candidate is necessary given that CRT was found to be ineffective as a molecular tool in *Haemaphysalis longicornis* (Alarcon-Chaidez et al., 2006; Zheng et al., 2022). While CRT has some potential to be an immunogenic marker, its significant size and the challenges associated with making recombinant proteins render it less ideal for use in quick and cost-efficient diagnostic methods.

Small, highly immunogenic peptides might provide a viable approach for developing rapid and field-applicable diagnostic tools and have been the focus of recent studies. One such candidate, AV422, a salivary component conserved across tick genera, was tested for its immunogenicity as both a recombinant protein and peptide (Mihaljica et al., 2017, 2021). Recombinant AV422 was demonstrated to elicit a robust IgG response in rats and humans while peptides derived from the AV422 protein were predicted to give an informative immunogenic response *in silico* but remain to be tested against tick-exposed serum (Mihaljica et al., 2017, 2021). To find novel peptide candidates, Bensaoud et al. (2022) used quantitative proteomics which identified tick-specific salivary proteins from *I. ricinus*, including protease inhibitors and lipocalins as the most abundant. These proteins were the basis for the generation of six peptides, five of which were tested in rabbits and were found to be antigenic, with lipocalin and vitellogenin peptides having the strongest effect (Bensaoud et al., 2022). Of the above identified potential peptide candidates, further testing is necessary to determine the potential immunological cross-reactivity with other vectors as well as their usefulness in clinical, field and veterinary settings.

Regarding the discovery of alternative biomarkers, recent studies have suggested that instead of antibody responses against foreign proteins as potential biomarkers, non-proteinaceous avenues could be considered such as through the identification of unique host responses to foreign metabolites, genes, or transcripts through new predictive *in silico* tests. These other biomolecules could accurately reveal exposure to a given threat either through detection with antibodies or other basic laboratory-based tests (Ahmad et al., 2023). One such example can be found in alpha-gal syndrome, where a carbohydrate modifying tick saliva proteins elicits a robust IgE antibody response in the host and an allergy to mammalian meat (Villar et al., 2021). Tick proteins with the α-Gal modification were also found to elicit an IgM and IgG response against α-Gal which were significantly higher in tick-exposed patients suggesting the possible use of α-Gal as a molecular marker. Finally, Villar et al. (2021) found the alphagalactome, the tick saliva proteins with α-Gal modifications, to be species-specific when comparing *A. americanum* and *Ixodes holocyclus* ticks indicating that proteins identified through their α-Gal modifications such as cytoglobin-1, 14-3-3 family chaperone and vitellogenin-1, could be potential candidate tick exposure biomarkers. Further, other non-proteinaceous molecules being explored are RNAs and extracellular vesicles (EVs). Several tick RNAs have been identified as elicitors of the host-immune response including long and short non-coding RNAs and microRNAs (Ali et al., 2022; Butler et al., 2023). Exploring EVs may offer a promising angle given their necessity during vector feeding as well as the unique content of their cargo, which can include protein, miRNA, lipids or other biomolecules. These components can be laboratory-tested and provide a unique tick signature that could aid in determining previous tick host exposure (Butler et al., 2023; Venkatesan et al., 2023). While several studies are beginning to explore these possible avenues, more work is necessary before the above components of tick saliva can be used as molecular targets for tools associated with control and surveillance of ticks and TBD.

Table 2
Main protein changes found in tick sialome switching.

Tick species	Sialome switching	Reference
Ixodes scapularis	**Change during the duration of blood meal (first 24 h)** Protease inhibitors (↑) Transporters and/or binding proteins (↑) Ferritin (↑) Hemelipoproteins (↑) Immunogenic enzymes (↑) ribosomal protein-like (↑) Defensin proteins (↑)	Lewis et al. (2015)
Rhipicephalus microplus	**Change during the duration of blood meal** At the beginning (slow and rapid feeding phase): Metalloproteases (+) At the end of the blood meal: Lipocalins (↑) Serpins (↑) Secreted conserved proteins (↓)	da Silva Vaz Junior et al. (2024)
Ixodes ricinus	**Change between ticks fed on naive hosts (rabbit) and twice exposed hosts, and kinetics during blood meal** Immunized host serum from tick SG (after repeat host exposure): Lipocalins (+) Kunitz domain protein (+) Mellaoproteases (+) During blood meal: Most changes occur between 12 and 24 h	Medina et al. (2022)
Ixodes ricinus	**Change between unfed and fed ticks, and between non-infected engorged ticks and *Borrelia afzelii*-infected ticks** Infection with *Borrelia afzelli*: 13 lipocalins (+) 6 Kunitz domain proteins (+) 11 basic tail proteins (+) 16 metalloproteases (+)	Klouwens et al. (2023)
Rhipicephalus bursa	**Change between unfed and fed ticks, and between non-infected engorged ticks and *Babesia ovis*-infected ticks** Infection with *Babesia ovis*: 1 RICIN (+) 1 EVASIN (+)	Couto et al. (2021)

Symbols: (+) compounds found predominately; (↑) increase of expression and/or detection; (↓) decrease of expression and/or detection.

3. The sialome and the immune response: Challenges in tick bite biomarker identification

The exploration of tick saliva composition and its role in the blood-feeding of arthropods has been facilitated by technological advancements and reduced costs. This has led to detailed studies on the composition and functional role of salivary proteins from various tick species (Liu and Bonnet, 2014; Lewis et al., 2015). Targeting saliva is critical for understanding and blocking potential transmission of pathogens as numerous studies have demonstrated that pathogens rely on and may even modulate the vector salivary composition and feeding behavior (see review by Bonnet et al., 2018). While vaccine development has been focused on leveraging immunity against the tick bite, current efforts have been unsuccessful or faced hurdles in robustness, reproducibility or efficacy (Abbas et al., 2023). It has even been demonstrated that some vaccination attempts may enhance efficacy of tick infestations (Almazán et al., 2020). However, failed vaccine candidates against tick bites may serve better as tick exposure biomarkers.

A wealth of data on the tick salivary profiles according to tick species, life-stage and infection with specific pathogens have been generated and compared. Understanding changes in sialomes, or "sialome switching" based on these variables could be considered for future selection and testing of candidate biomarkers (Table 2). Tick saliva composition has been effectively demonstrated to vary based on the life-stage, the infected condition of the tick and the targeted host (Ali et al., 2022; Ribeiro and Mans, 2020). In addition, using mass spectrometry, a recent study followed the changes of the sialome in *Rhipicephalus microplus* across the span of the adult female feeding and found that as the level of engorgement progressed, there were significant fluctuations in the presence of key protein families, including metalloproteases, serpins and lipocalins (da Silva Vaz Junior et al., 2024). Repeated blood-feedings on a vertebrate host were also capable of influencing the sialome as was found in the transcriptome differences between ticks fed on naïve rabbits compared with ticks fed on twice exposed rabbits (Medina et al., 2022). Further, salivary proteins were differently abundant based on the feeding and infectious status of *I. ricinus* ticks, which included unfed ticks, fully-fed ticks or ticks fed and infected with *Borrelia afzelii* (Klouwens et al., 2023). This study suggests that biomarkers specific to either the infected or uninfected status of ticks could be developed to aid in creating more informative diagnostic tests. For example, RICIN and EVASIN potentially indicated the infective status of *Rhipicephalus bursa* when *Babesia ovis* was present in the saliva (Couto et al., 2021). A biomarker in this context, could help indicate exposure to the tick as well as determine if the tick was infected with *Babesia*. While there are many variables to consider affecting the composition of tick saliva, arguably the most important is the first 24-hour window of feeding before possible tick detachment. Following 24 hours of *I. scapularis* feeding, several antigens were identified based on rabbit antibodies raised for detection against exposure to tick saliva (Lewis et al., 2015). Thus, short windows of feeding are sufficient to identify useful biomarker tools.

4. Peptide arrays and advances in biomarker discovery: New avenues for finding antigenic tick saliva peptides

As novel tools are developed in proteomics and bioinformatics, new possible avenues arise for overcoming previously identified hurdles in the identification and development of tick exposure biomarkers. The use of peptide microarrays has emerged as a powerful tool for the serodiagnosis of infectious diseases (Aguilar-Montes de Oca et al., 2022). This technique allows for the high-throughput screening of immunogenic peptides, facilitating the rapid identification of pathogen exposure and indicating the potential to do so with tick species. Microarrays are often paired with classical methods including ELISAs or chemiluminescence to verify the specificity and usefulness of a peptide. Further, using advances in liquid chromatography – mass spectrometry (LC-MS) – may aid in biomarker discovery and serodiagnostics. LC-MS has been further specialized which can allow for tick biomarker discovery through an especially rigorous quality control protocol requiring interdisciplinary collaborations to correctly collect, measure and interpret such small molecules reliably (Rischke et al., 2023).

Serochips have also been created to give a panel of diagnostic peptides that indicate exposure to different TBP based on IgM and IgG epitopes (Tokarz et al., 2018). While this serochip focuses on specific TBP, adding peptides that aid in the identification of tick species would provide further meaningful data in terms of monitoring vector transmission and ecological surveillance. To potentially enhance the immunogenicity of identified single tick saliva peptides, chimeric peptides may be constructed to better identify potential biomarkers (Lu et al., 2015).

Finally, machine learning algorithms have allowed for the prediction of small peptide regions that have high likelihoods of interacting with B cells and MHCII molecules (Fleri et al., 2017). Databases have been constructed based on these methods including the Immune Epitope Database (IEDB), for example, which has constructed a catalog of the predicted most immunogenic amino acid sequences for antibodies and T-cell epitopes (Fleri et al., 2017). However, caution should be taken in regard to over- or underfitting language learning models which may render the predictions either too obtuse or acute, requiring *in vitro* validation to provide biological significance to *in silico* findings (Ng et al., 2023).

5. Conclusions

Continuous innovation in tick biomarker discovery and validation, combined with a growing focus on various tick-associated salivary molecules, holds significant promise for transforming how we monitor and control TBD. Further exploration of candidate biomarkers is necessary to determine their elucidatory capacity in diagnostics, tick-species identification, and transmission of TBP. Specifically, additional studies are needed to determine which antigens could be used to develop IgM or IgG specific biomarkers. Despite their potential, current inexpensive and reliable biomarkers have not been adequately explored, necessitating further research. Biomarkers may play a pivotal role in diagnosing TBD in patients with unclear exposure histories and in surveilling tick populations and control efforts.

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Ethical approval

Not applicable.

CRediT authorship contribution statement

Alexis Dziedziech: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Eva Krupa:** Writing – review & editing. **Kristina E.M. Persson:** Supervision, Writing – review & editing. **Richard Paul:** Conceptualization, Supervision, Writing – review & editing. **Sarah Bonnet:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article.

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