



## Research article

Comparative analysis of *Rhipicephalus* tick salivary gland and cement elementome

Iván Pacheco<sup>a</sup>, Eduardo Prado<sup>b</sup>, Sara Artigas-Jerónimo<sup>a</sup>, José Francisco Lima-Barbero<sup>a,c</sup>, Gabriela de la Fuente<sup>a,c</sup>, Sandra Antunes<sup>d</sup>, Joana Couto<sup>d</sup>, Ana Domingos<sup>d</sup>, Margarita Villar<sup>a,e</sup>, José de la Fuente<sup>a,f,\*</sup>

<sup>a</sup> SaBio, Instituto de Investigación en Recursos Cinegéticos (IREC-CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain

<sup>b</sup> Department of Applied Physics, Faculty of Chemical Sciences and Technologies, Universidad de Castilla-La Mancha, Avda. Camilo José Cela 10, 13071 Ciudad Real, Spain

<sup>c</sup> Sabiotec, Camino de Moledores s/n. 13003, 13071 Ciudad Real, Spain

<sup>d</sup> GHMT - Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical - IHMT, Universidade Nova de Lisboa - UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

<sup>e</sup> Biochemistry Section, Faculty of Science and Chemical Technologies, and Regional Centre for Biomedical Research (CRIB), University of Castilla-La Mancha, 13071, Ciudad Real, Spain

<sup>f</sup> Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

## ARTICLE INFO

## Keywords:

Tick  
Cement  
Salivary gland  
Chemical elements  
Scanning electron microscopy/Dispersive energy spectroscopy  
Elementome

## ABSTRACT

*Rhipicephalus* spp. (Acari: Ixodidae) ticks are obligate hematophagous arthropods, which constitute a model for the study of vector-host interactions. The chemical composition or elementome of salivary glands (SG) and cement provides information relevant for the study of protein-based complex multifunctional tissues with a key role in tick biology. In this study, we characterized the elementome of cement cones in *Rhipicephalus sanguineus* collected from naturally infested dogs and in SG and cement of *R. bursa* collected from experimentally infested rabbits at different feeding stages. The elementome was characterized using scanning electron microscopy (SEM) combined with energy dispersive X-ray spectroscopy (EDS). The results showed the identification of up to 14 chemical elements in the cement, and suggested tick/host-driven differences in the cement elementome between tick species and between SG and cement within the same species. By still unknown mechanisms, ticks may regulate cement elementome during feeding to affect various biological processes. Although these analyses are preliminary, the results suggested that N is a key component of the cement elementome with a likely origin in SG/salivary proteins (i.e., Glycine (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>)-rich superfamily member proteins; GRPs) and other tick/host-derived components (i.e. NAPDH). Future research should be focused on tick elementome and its functional implications to better understand cement structure and function.

## 1. Introduction

Ticks of the *Rhipicephalus* genus (Acari: Ixodidae) are obligate hematophagous arthropods, and a model for the study of ectoparasite vector-host interactions (Dantas-Torres, 2008; Tabor et al., 2017; Antunes et al., 2018). The *Rhipicephalus* genus includes one (e.g. *Rhipicephalus microplus*), two (e.g. *Rhipicephalus bursa*) and three-host (e.g. *Rhipicephalus sanguineus*) tick species (Jonjejan and Uilenberg, 2004; de

la Fuente et al., 2008; Dantas-Torres, 2008; Antunes et al., 2018; Rashid et al., 2019). Brown ear ticks *R. bursa* and brown dog ticks *R. sanguineus* are economically important ectoparasites of a variety of domestic and wild animal species with an impact on livestock industry and dog health worldwide (Dantas-Torres, 2008; Antunes et al., 2018; Paules et al., 2018). *Rhipicephalus sanguineus* are also anthropophilic and vectors of pathogens to humans (Goddard, 1989; Parola et al., 2008). Effective and environmentally friendly control methods, such as vaccines among other

\* Corresponding author.

E-mail addresses: [jose.delafuente@yahoo.com](mailto:jose.delafuente@yahoo.com), [josedejesus.fuente@uclm.es](mailto:josedejesus.fuente@uclm.es) (J. de la Fuente).

<https://doi.org/10.1016/j.heliyon.2021.e06721>

Received 22 July 2020; Received in revised form 4 January 2021; Accepted 1 April 2021

2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

interventions, are required to control tick infestations and tick-borne diseases (de la Fuente et al., 2017; de la Fuente, 2018; Wikel, 2018; Molaei et al., 2019).

The cement is a complex multifunctional protein polymerization substance secreted by most species of the family Ixodidae including *Rhipicephalus* spp. to anchor their mouthparts to the host skin (Suppan et al., 2018). The cement has antimicrobial and adhesive properties and acts to seal the lesion during feeding, while facilitating feeding, pathogen transmission, and protection from mammalian host immune and inflammatory responses (Kazimírová and Štibrániová, 2013; Suppan et al., 2018; Nuttall, 2019). Previous studies of tick cement have revealed its histochemical composition and structure (Bullard et al., 2016, 2019; Suppan et al., 2018; Hollmann et al., 2018; Villar et al., 2020). Recently, the chemical composition or elementome (Ramaswamy et al., 2015) and proteome of tick cement were characterized by SEM-EDS and proteomics approaches in *R. microplus* during various ectoparasite feeding stages (Villar et al., 2020), and results suggested that tick and host derived compounds modulate cement properties throughout tick feeding.

In this study, we aimed at advancing further the study of the elementome in tick cement cones from *R. sanguineus* collected from naturally infested dogs, and in salivary glands (SG) and cement cones from *R. bursa* in experimentally infested rabbits at different feeding stages. The elementome was characterized using SEM-EDS as previously described in *R. microplus* (Villar et al., 2020). The results provided additional support for the use of SEM-EDS to characterize tick elementome.

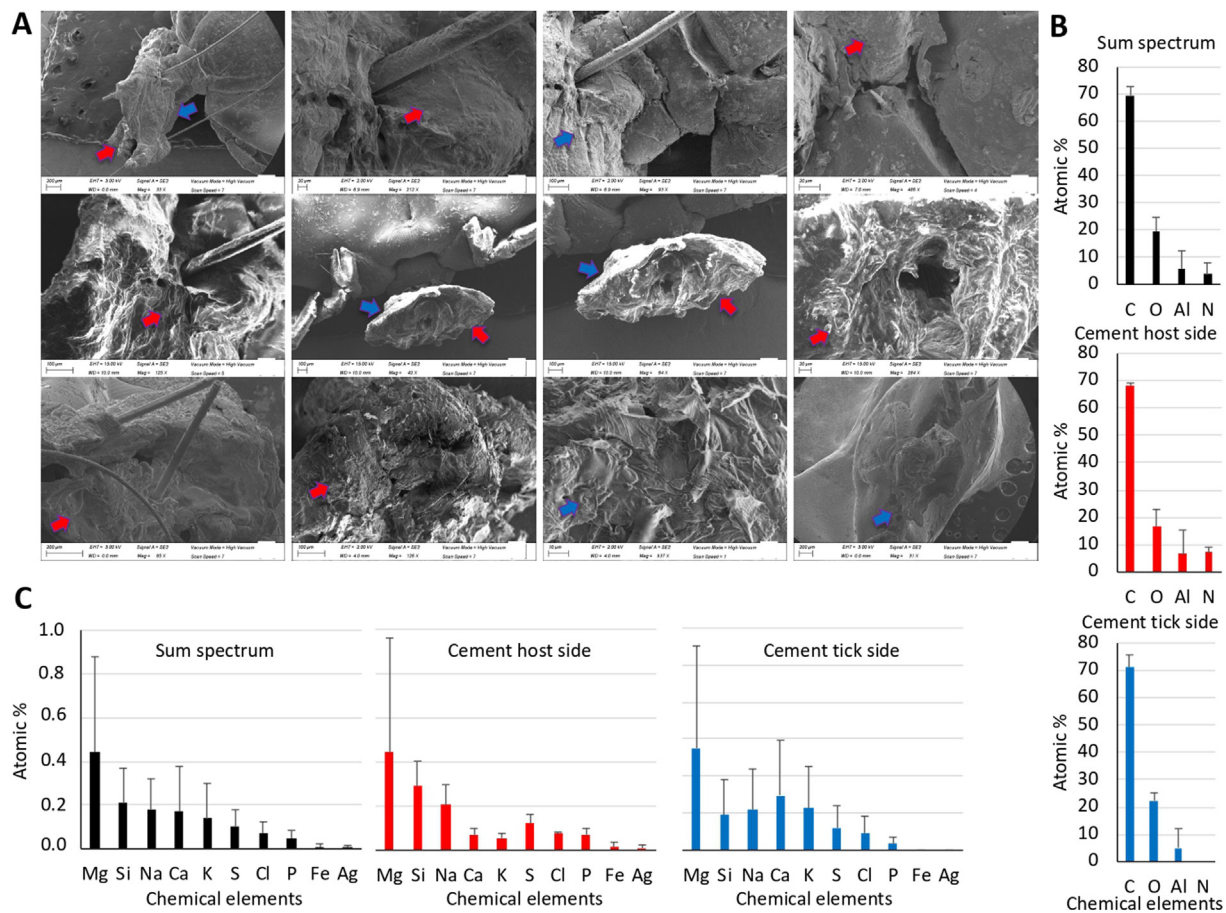
## 2. Materials and methods

### 2.1. Experimental design and rationale of using the *Rhipicephalus* spp. model

Based on a previous study in *R. microplus* (Villar et al., 2020), the analyses were conducted at different levels in two other *Rhipicephalus* spp., *R. sanguineus* and *R. bursa* fed females. *Rhipicephalus sanguineus* specimens were collected from naturally infested dogs at several stages of feeding. These samples were used for the preliminary characterization of the cement elementome by SEM-EDS at tick and host sides of the cement cones, and in comparison with tick exoskeleton palps and dog hair. Then, a controlled study was conducted in *R. bursa* using specimens collected from experimentally infested rabbits at 1–2 days post-attachment (dpa) (T1; slow-feeding period, 35 ± 7 mg), 4–5 dpa (T2, 83 ± 11 mg) and 7 dpa (T3; fast-feeding period, 179 ± 24 mg) for analysis of tick elementome in SG and cement cones by SEM-EDS. In *R. bursa*, cement cones were analyzed at combined tick and host sides for comparison with SG.

### 2.2. Ticks

*Rhipicephalus sanguineus* (Latreille, 1806) females feeding on naturally infested multiple dog breeds were collected in an animal shelter at Ciudad Real, Spain, and morphologically classified according to Dantas-Torres et al. (2013). Adult *R. bursa* (Canestrini and Fanzago, 1878) were



**Figure 1.** *Rhipicephalus sanguineus* tick cement elementome. Chemical elements were identified by SEM-EDA analysis in samples from *R. sanguineus* females collected from naturally infested dogs. (A) Electron microphotographs of tick cement from host side (red arrows) and/or tick side (blue arrows). (B) Representation of tick cement most abundant (>5%) chemical elements. (C) Representation of tick cement less abundant (<1%) chemical elements. Average +S.D. atomic percent of chemical elements was calculated for cement host side (electron image 1/spectrum 1 and electron image 4/sum spectrum), cement tick side (electron image 2/spectrum 5 and electron image 3) and sum spectrum (electron image 1/spectrum 1, electron image 4/sum spectrum, electron image 2/spectrum 5 and electron image 3) (Supplementary Data 1 and 3).

obtained from the colony established at the Instituto de Higiene e Medicina Tropical (IHMT), Lisbon, Portugal. Ticks (groups of 35 females and 20 males) were fed in feeding chambers glued on the shaved dorsum of 3–4 months old Hyla breed rabbits. Animals were maintained and manipulated following protocols compliant with the national and European animal welfare legislation, in frame with DL 113/2013 and Directive 2010/63/EU based on the principle of the three R's: to replace, reduce, and refine the use of animals for scientific purposes. Animal experiments were done with the approval of the Divisão Geral de Alimentação e Veterinária (DGAV), Portugal, under Artº 49, Portaria nº1005/92 from 23<sup>rd</sup> October (permit number 0421/2013).

2.3. Collection of tick SG and cement cones

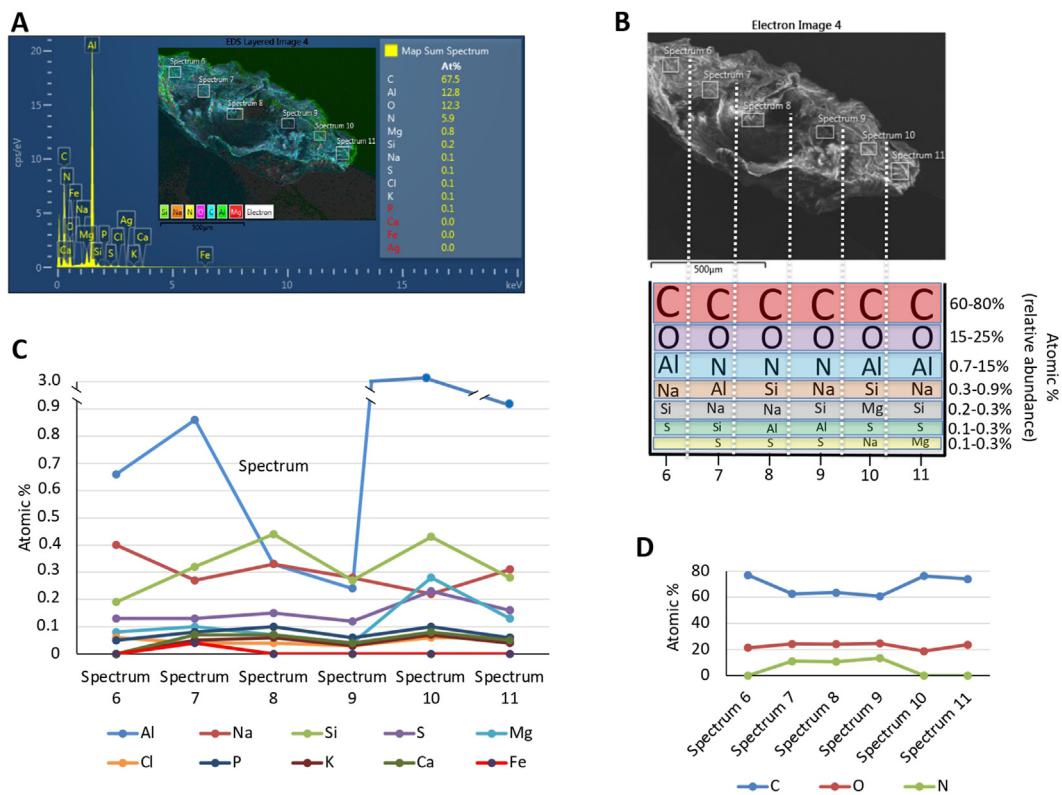
*Rhipicephalus sanguineus* cement cones were collected using soft tissue forceps from mouthparts of manually detached females feeding on naturally infested dogs. Ticks (n = 20) were collected at various feeding times according to scutal index by calculating the ratio of the maximum width of the scutum to the length of the idiosoma (Falco et al., 1996), and cement cones pooled for analysis. Some individual ticks were stored with attached cement cones for SEM-EDS analysis. SG were not extracted from *R. sanguineus* because these ticks were collected from naturally infested dogs at multiple feeding stages, and without knowing the corresponding feeding period as described for the controlled trial using *R. bursa*. *Rhipicephalus bursa* cement cones were collected using soft tissue forceps from mouthparts of manually detached females (3 pools of 5–9 ticks/pool) at 1–2 dpa (T1; slow-feeding period), 4–5 dpa (T2) and 7 dpa (T3; fast-feeding period). SG were extracted from the same dissected ticks as

previously reported (Couto et al., 2020). A mammalian host-derived skin or hairs attached to the cement were removed except in the case of selected *R. sanguineus* samples stored with attached cement cones. However, in *R. bursa* it was difficult to remove rabbit hairs from all samples. Collected cement cones, attached or not to the tick hypostome, were kept in PBS with 1% of protease inhibitor cocktail M221 (VWR Life Science AMRESCO, OH, USA) or RNAlater (Sigma-Aldrich, St. Louis, MO, USA).

2.4. Tick SG and cement elementome physical and chemical parameters

The elementome was characterized using SEM-EDS as previously described (de la Fuente et al., 2020; Villar et al., 2020; Pacheco et al., 2020). *Rhipicephalus bursa* SG and cement samples were dehydrated in an incubator at 37 °C for 24 h prior to analysis with SEM-EDS. The samples were mounted on standard aluminum SEM sample holders with conductive carbon adhesive tabs. The samples were observed and analyzed with a field emission scanning electron microscope (Zeiss GeminiSEM 500, Oberkochen, Germany) operating at high vacuum mode at an acceleration voltage of 15 kV and without metal coating or covering. For the analysis of chemical elements, three area scans per sample were used in an 80 mm<sup>2</sup> EDS detector (Oxford Instruments, Abingdon, United Kingdom). In *R. sanguineus*, chemical elemental composition of cement cones alone or with attached tick palps and dog hair were determined by SEM-EDS under similar conditions. Results are included in Supplementary Data 1–3.

The results of the *R. bursa* SG and cement elementome were compared at different collection time points by One-way ANOVA test (https



**Figure 2.** Variations in the elementome throughout *R. sanguineus* tick cement host side structure. Chemical elements were identified by SEM-EDS analysis throughout tick cement structure on host side in a sample collected from *R. sanguineus* females feeding on naturally infested dogs. (A) Sum spectrum and atomic percent of chemical elements (electron image 4; Supplementary Data 1). (B) Atomic percent relative abundance for elements with >0.1% on each spectrum. (C) Variations in the atomic percentage of less abundant (<3.5% in all spectrums) chemical elements throughout tick cement structure (spectrums 6–11). (D) Variations in the atomic percentage of most abundant (>10% in at least one spectrum) chemical elements throughout tick cement structure (spectrums 6–11). All results are disclosed in Supplementary Data 1 and 3.

://www.socscistatistics.com/tests/anova/default2.aspx;  $p < 0.05$ ,  $n = 2-9$  biological replicates).

### 3. Results

#### 3.1. Cement elementome suggests differences between tick and host sides

A preliminary analysis of the elementome was conducted in *R. sanguineus* cement host and tick sides, and in comparison with dog host hair and tick exoskeleton palps (Figures 1A-1C, 2A-2D, 3A-3C). The elementome was first characterized in cement at host and tick sides (Figure 1A). The results showed high average relative abundance (percent of total atoms in the sample  $>5$  atomic %) of C, O, Al and N, and lower average relative abundance ( $<0.5$  atomic %) of Mg, Si, Na, Ca, K, S, Cl, P, Fe and Ag at cement mammalian host side (Figures 1B and 1C). High average relative abundance ( $>5$  atomic %) of C, O and Al, and lower average relative abundance ( $<0.6$  atomic %) of Mg, Si, Na, Ca, K, S, Cl and P were detected at cement tick side (Figures 1B and 1C). The N was also detected in the cement tick side (Figures 3A-3C). However, elements such as Fe and Ag were detected on the host but not the tick cement side (Figures 1B, 1C, 3A-3C). The analysis of the elementome in cement mammalian host side showed some variations throughout cement structure (Figures 2A-2D). Although at least part of the Al may be a contamination from the aluminum SEM stubs, the relative atomic percentage of C and Al decreased and of N increased while getting closer to the feeding pool (spectrums 8 and 9; Figures 2B-2D).

The elementome was then compared between cement mammalian host side, dog hair and tick exoskeleton palps (Figures 3A-3C). All three

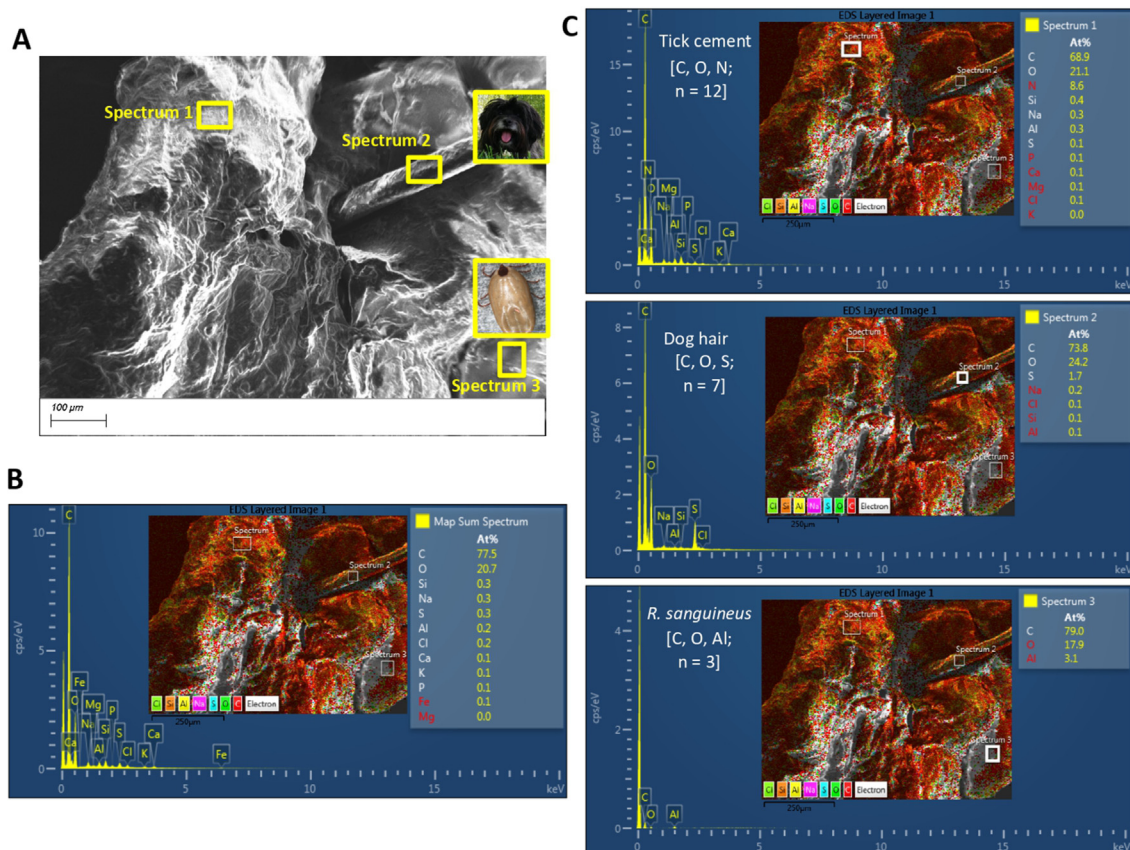
tissues showed high average relative abundance ( $>17$  atomic %) of C and O (Figure 3C). However, cement showed a higher diversity of chemical elements and distinctive higher average relative abundance of N (8.6 atomic %) when compared to dog host hair and tick exoskeleton palps (Figure 3C).

#### 3.2. Salivary gland and cement elementome show differences in the profile of chemical elements during tick feeding

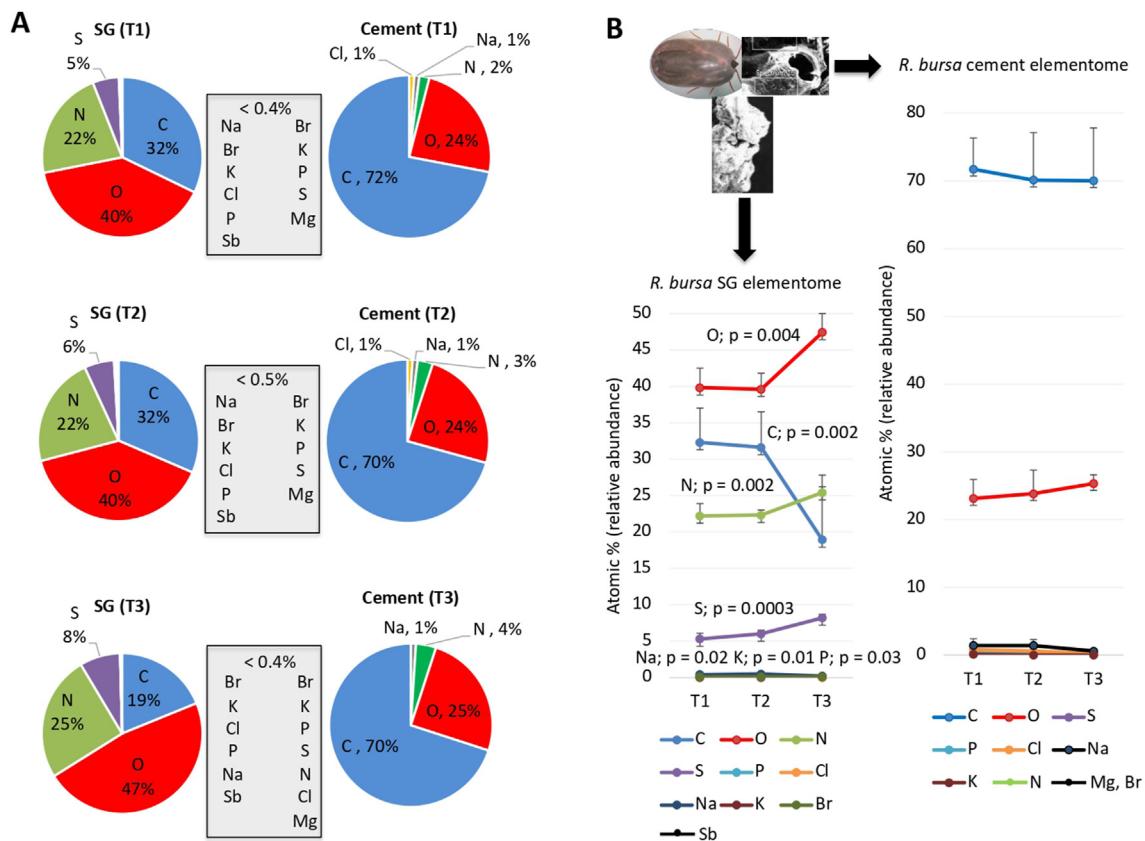
In experimentally fed *R. bursa*, the elementome was characterized in the cement (combined tick and host sides), and in comparison with SG in samples collected at different feeding times (T1-T3; Figures 4A and 4B). At all feeding stages the chemical elements with higher average relative abundance ( $>5$  atomic %) were O, C, N and S in the SG and C, O and N ( $>2$  atomic %) in the cement (Figure 4A). In the SG, the average relative abundance of O, N, S and K increased while for C, Na and P decreased during feeding (Figure 4B). In tick cement, the average relative abundance of chemical elements did not change during feeding (Figure 4B). Consequently, the analysis of the elementome in the cement and SG of *R. bursa* at different time points showed significant variations in the relative abundance of chemical elements and profiles in the SG only (Figures 4A and 4B). Elements K, Mg, Br and Sb were only rarely found in some samples (Supplementary Data 3).

### 4. Discussion

The results of the SEM-EDS analysis suggested differences in the cement elementome when comparing (a) host and tick sides of cement



**Figure 3.** Elementome in *R. sanguineus* cement and palps and dog hair. Chemical elements were identified by SEM-EDS analysis in samples from *R. sanguineus* females collected from naturally infested dogs. (A) Electron image used to compare the composition of chemical elements between tick cement host side (Spectrum 1), dog hair (Spectrum 2) and tick palps (Spectrum 3). (B) Sum spectrum and atomic percent of chemical elements (electron image 1; Supplementary Data 1). (C) Spectrum and atomic percent of chemical elements (electron image 1/spectrums 1-3; Supplementary Data 1). The three most abundant elements ( $>1\%$ ) and the total number of detected elements (n) for each spectrum are shown in brackets.



**Figure 4.** *Rhipicephalus bursa* salivary gland and cement elementome. Chemical elements were characterized by SEM-EDS analysis in samples from tick SG and cement (Supplementary Data 2 and 3). (A) Representation (%) of the chemical elements at different feeding stages (T1-T3). (B) Changes in the representation (%) of the chemical elements at different feeding stages (T1-T3). Only chemical elements with significant differences at different feeding stages (T1-T3) are shown. The composition of chemical elements was compared at different time points by One-way ANOVA test (<https://www.socscistatistics.com/tests/anova/default2.aspx>;  $p < 0.05$ ,  $n = 2-9$  biological replicates).

cones, (b) dog hair and tick cement and exoskeleton palps, and (c) cement and SG (Figures 5A and 5B). These differences may have a tick and/or mammalian host origin with potential functional implications.

Proteins originating in both ticks and hosts are likely the major source of chemical elements in tick SG, saliva and cement (Chinery, 1973; Ayllón et al., 2015; Villar et al., 2016, 2020; Bullard et al., 2016; Hollmann et al., 2018; Kim et al., 2020). For example, the profile of the highly represented Glycine ( $C_2H_5NO_2$ )-rich superfamily member proteins (GRPs) that has been reported in the tick cement (Bullard et al., 2016; Hollmann et al., 2018; Villar et al., 2020) correlate with the relative abundance of C, N, O in the cement (Villar et al., 2020). Recently, the representation of GRPs was shown to decrease in the sialome during feeding of *Amblyomma americanum* females (Kim et al., 2020). These results may explain the decrease in the relative abundance of C during feeding in *R. bursa* SG elementome (Figure 4B). Changes in the elementome during tick feeding may be also explained by physiological mechanisms such as the increase in the intensity of respiratory patterns shown in *R. sanguineus* during feeding (Landulfo et al., 2019).

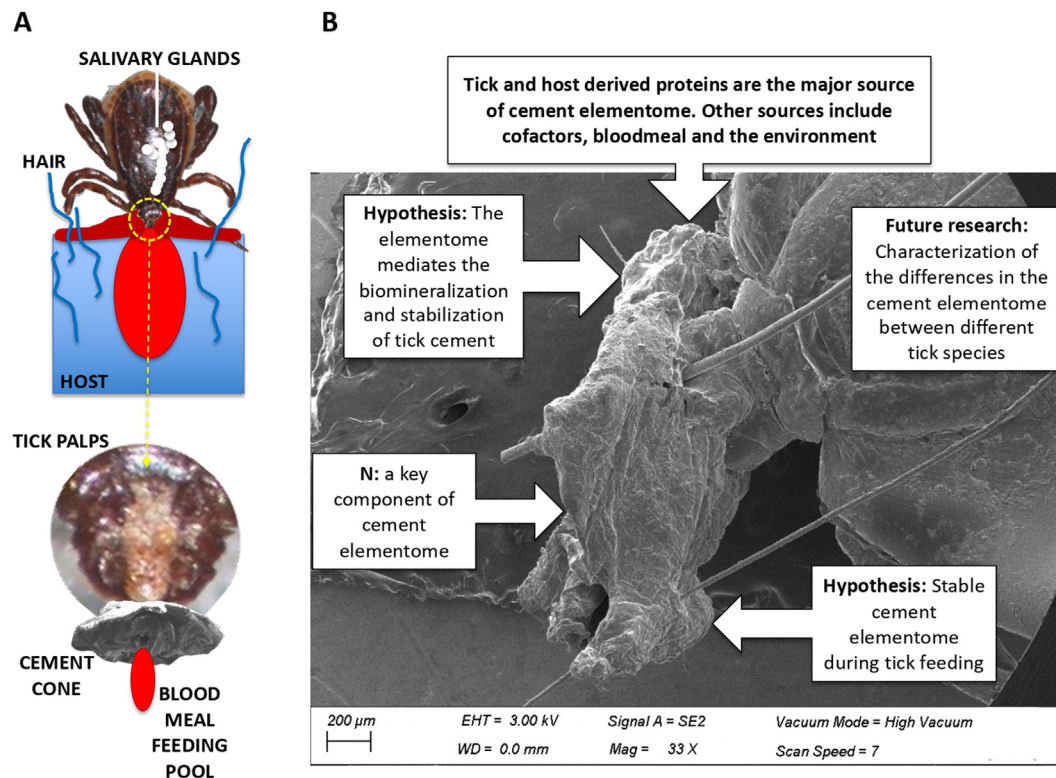
Protein components in the cement may be stabilized by alkaline earth metals such as Ca that has been previously identified in arthropods exoskeleton and cuticle (Vincent, 2002; Gallant and Hochberg, 2017). In our study, Ca was identified in the *R. sanguineus* cement elementome at both tick and host sides suggesting that at least in some tick species it may contribute to biomineralization (process of minerals production) to strengthen and stabilize the cement as proposed for the cuticle in other arthropods (Gallant and Hochberg, 2017). As in other arthropods, Ca is probably obtained by ticks through their bloodmeal (Vohland et al., 2003; Gallant and Hochberg, 2017), which may vary between different tick and mammalian host species (Bradbury et al., 1972) and in response

to stimuli such as pathogen infection (Wang and Wang, 2019). The source of other major biological inorganic elements such as Na, K, Cl and P, which may also contribute to cement biomineralization and coating (Cribb et al., 2010; Gallant and Hochberg, 2017; Suppan et al., 2018; Villar et al., 2020) are probably derived from environmental sources such as water, air and soil.

Tick elementome may be also affected by cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH;  $C_{21}H_{26}N_7O_{17}P_3$ ) (Villar et al., 2020). NADPH is an electron donor that regulates multiple anabolic reactions, including those responsible for the biosynthesis of all major cell components in ticks and in other organisms (Spaans et al., 2015; Alberdi et al., 2019; Della Noce et al., 2019). In ticks, higher levels of NADPH have been associated with response to *Anaplasma phagocytophilum* infection and tolerance to oxidative stress (Alberdi et al., 2019; Della Noce et al., 2019).

Dog hair fibers have been shown to contain C, H, O and N (Ragaisiene et al., 2016), of which C and O were also identified here. However, in our study trace elements such as Se, Cu, Mn, Zn and Co present in mammalian cover hair (BioCheck, 2014) were not identified in the tick SG and cement or in dog hair, thus suggesting that these elements are rare and with a low contribution to tick elementome. Some of the chemical elements identified here in the elementome of SG and cement and in dog hair were recently reported in tick exoskeleton using a similar experimental approach (de la Fuente et al., 2020; Pacheco et al., 2020).

The composition of tick cement elementome contains elements that are common for multiple tick species (Table 1). Nevertheless, although some of these analyses are preliminary, the results suggest interspecific differences between *R. sanguineus* and *R. bursa*. While chemical elements such as Ca, Fe and Ag were found only in *R. sanguineus* cement



**Figure 5.** Summary of the study conclusions and future directions. (A) Representation of the tick and host tissues included in the study. Tick image corresponds to a *R. sanguineus*. (B) Summary of the main conclusions of the study that deserve further attention and research. Electron microscopy image corresponds to *R. sanguineus* tick with exoskeleton palps attached to the cement cone.

**Table 1.** Composition of tick cement elementome in different *Rhipicephalus* species.

Tick species	Cement elementome	References
<i>Rhipicephalus microplus</i>	C, O, N, Na, Cl, S, P, K	Villar et al. (2020)
<i>Rhipicephalus sanguineus</i>	C, O, N, Al, Mg, Na, Si, S, Cl, P, Ca, K, Fe, Ag	This study (Suppl. Data 3)
<i>Rhipicephalus bursa</i>	C, O, N, Na, Cl, S, P	This study (Suppl. Data 3)

Chemical elements identified in tick cement elementome are disclosed from highest to lowest representation according to data available. At least part of the Al may be a contamination from the aluminum SEM stubs.

elementome (Figures 1C, 2C and 3B), Br was found only in *R. bursa* cement and SG (Figures 4A and 4B). These differences could be related to differences between tick hosts, physiology and feeding (i.e. blood-derived Fe sequestration by ferritin to control oxidative stress or Ag potential to reduce wound bioburden while increasing antimicrobial response) (Wilkinson et al., 2011; Hernandez et al., 2018; Cabezas-Cruz et al., 2019). Differences in the elementome composition were also observed between *R. bursa* cement and SG (Figures 4A and 4B), which probably reflects the fact that only a fraction of the molecules present in the SG are secreted into the cement (Bullard et al., 2016, 2019; Suppan et al., 2018; Hollmann et al., 2018; Villar et al., 2020). As shown for exoskeleton elementome, other factors such as pathogen infection (de la Fuente et al., 2020) and geographic origin (Pacheco et al., 2020) of ticks may affect cement chemical composition.

Finally, these results suggested the possibility of focusing on potentially relevant elementome components to further explore their functional relevance. For example, the results suggested that N is a key component of the cement elementome with a likely origin in SG/salivary proteins (i.e., GRPs) and other components (i.e., NADPH). N was identified in *R. sanguineus* cement tick and host sides with higher relative abundance while getting closer to the feeding pool, and in the elementome of *R. bursa* cement and SG. Furthermore, N relative abundance

was higher in the SG than in the cement elementome throughout tick feeding. These results suggested that as previously reported in spider silk (Zhang et al., 2019), limiting N availability outside the feeding pool protects cement from bacterial degradation. Another possibility is that as in other natural biopolymers, N has a role in tick cement piezoelectricity, the electric charge that accumulates in response to applied mechanical stress (Saravanan, 2006). These possible roles for N as for other chemical elements in the cement need to be studied to better understand their functional role in tick biology.

## 5. Conclusions

This study advanced previous studies in *R. microplus* and other tick species (Villar et al., 2020; de la Fuente et al., 2020; Pacheco et al., 2020) using the same SEM-EDS experimental approach and provided the first characterization of the tick SG and/or cement elementome in *R. sanguineus* and *R. bursa*. A limitation of this study which is particularly relevant for the analysis of tick SG elementome is the fact that the EDS method is limited or strongly biased toward the elements on the surface layer. Nevertheless, the results obtained under the same conditions are valid for the analysis of samples at different tick feeding stages.

The results suggest some potentially functionally relevant implications (Figure 5). The cement is a protein-based complex multifunctional biopolymer with a key role in tick biology. Tick and host derived proteins are the major source of cement elementome, together with other sources including cofactors, bloodmeal, and the environment. The cement elementome showed a high diversity with up to 14 chemical elements identified in some samples. By still unknown mechanisms, ticks may regulate cement elementome during feeding to affect various biological processes involved in anchoring to the host skin, sealing the lesion, feeding and pathogen transmission, protecting ticks from bacterial infection or mammalian host immune and inflammatory responses (Kazimírová and Štibrániová, 2013; Suppan et al., 2018; Nuttall, 2019). The elementome mediates biomineralization and stabilization (a process to increase physical stability) of tick cement, a conserved physiological phenomenon in arthropod structures important for feeding and defense (Gallant and Hochberg, 2017). Although these analyses are preliminary, the results suggested that N is a key component of the cement elementome with a likely origin in SG/salivary proteins (i.e., GRPs) and other tick/host-derived components (i.e., NAPDH). The results and main conclusions of this study deserve further attention and research to better understand the structure and function of tick cement.

## Declarations

### Author contribution statement

Iván Pacheco, Eduardo Prado, Joana Couto: Performed the experiments; Analyzed and interpreted the data.

Sara Artigas-Jerónimo, José Francisco Lima-Barbero, Gabriela de la Fuente: Performed the experiments.

Sandra Antunes, Ana Domingos, Margarita Villar: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

José de la Fuente: Analyzed and interpreted the data; Wrote the paper.

### Funding statement

This work was supported by the Consejería de Educación, Cultura y Deportes, JCCM, Spain, project CCM17-PIC-036 (SBPLY/17/180501/000185), and partially funded by Fundação para a Ciência e Tecnologia (FCT) under the project PTDC/CVT-CVT/29073/2017 (UID/Multi/04413/2013). Margarita Villar was supported by the University of Castilla La Mancha, UCLM, Spain, and the Fondo Europeo de Desarrollo Regional, FEDER, EU.

### Data availability statement

Data included in article/supplementary material/referenced in article.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2021.e06721>.

### Acknowledgements

Authors would like to acknowledge Dra. Ana Sofia Santos (Instituto Nacional de Saúde Doutor Ricardo Jorge) for the *R. bursa* picture.

## References

- Alberdi, P., Cabezas-Cruz, A., Espinosa, P.J., Villar, M., Artigas-Jerónimo, S., de la Fuente, J., 2019. The redox metabolic pathways function to limit *Anaplasma phagocytophilum* infection and multiplication while preserving fitness in tick vector cells. *Sci. Rep.* 9, 13236.
- Antunes, S., Couto, J., Ferrolho, J., Rodrigues, F., Nobre, J., Santos, A.S., Santos-Silva, M.M., de la Fuente, J., Domingos, A., 2018. *Rhipicephalus bursa* sialotranscriptomic response to blood feeding and *Babesia ovis* infection: identification of candidate protective antigens. *Front. Cell. Infect. Microbiol.* 8, 116.
- Ayllón, N., Villar, V., Galindo, R.C., Kocan, K.M., Sîma, R., López, J.A., Vázquez, J., Alberdi, P., Cabezas-Cruz, A., Kopáček, P., de la Fuente, J., 2015. Systems biology of tissue-specific response to *Anaplasma phagocytophilum* reveals differentiated apoptosis in the tick vector *Ixodes scapularis*. *PLoS Genet.* 11, e1005120.
- BioCheck, 2014. Analysis of Trace Elements in Cattle. Labor für Veterinärmedizin und Umwelthygiene GmbH. [https://www.biocheck-leipzig.de/images/stories/biocheck/pdf/News\\_2\\_14\\_e.pdf](https://www.biocheck-leipzig.de/images/stories/biocheck/pdf/News_2_14_e.pdf).
- Bradbury, M.W., Crowder, J., Desai, S., Reynolds, J.M., Reynolds, M., Saunders, N.R., 1972. Electrolytes and water in the brain and cerebrospinal fluid of the foetal sheep and Guinea-pig. *J. Physiol.* 227, 591–610.
- Bullard, R., Allen, P., Chao, C.C., Douglas, J., Das, P., Morgan, S.E., Ching, W.M., Karim, S., 2016. Structural characterization of tick cement cones collected from in vivo and artificial membrane blood-fed Lone Star ticks (*Amblyomma americanum*). *Ticks Tick-Borne Dis.* 7, 880–892.
- Bullard, R., Sharma, S.R., Das, P.K., Morgan, S.E., Karim, S., 2019. Repurposing of glycine-rich proteins in abiotic and biotic stresses in the Lone-Star tick (*Amblyomma americanum*). *Front. Physiol.* 10, 744.
- Cabezas-Cruz, A., Espinosa, P., Alberdi, P., de la Fuente, J., 2019. Tick-pathogen interactions: the metabolic perspective. *Trends Parasitol.* 35, 316–328.
- Chinery, W.A., 1973. The nature and origin of the "cement" substance at the site of attachment and feeding of adult *Haemaphysalis spinigera* (Ixodidae). *J. Med. Entomol.* 10, 355–362.
- Couto, J., Villar, V., Mateos-Hernández, L., Ferrolho, J., Sanches, G.S., Santos, A.S., Santos-Silva, M.M., Nobre, J., Moreira, O., Antunes, S., de la Fuente, J., Domingos, S., 2020. Quantitative proteomics identifies metabolic pathways affected by *Babesia* infection and blood feeding in the sialoproteome of the vector *Rhipicephalus bursa*. *Vaccines* 8, 91.
- Cribb, B.W., Lin, C.L., Rintoul, L., Rasch, R., Hasenpusch, J., Huang, H., 2010. Hardness in arthropod exoskeletons in the absence of transition metals. *Acta Biomater.* 6, 3152–3156.
- Dantas-Torres, F., 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Vet. Parasitol.* 152, 173–185.
- Dantas-Torres, F., Latrofa, M.S., Annoscia, G., Giannelli, A., Parisi, A., Otranto, D., 2013. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. *Parasit. Vectors* 6, 213.
- de la Fuente, J., 2018. Controlling ticks and tick-borne diseases...looking forward. *Ticks Tick-Borne Dis.* 9, 1354–1357.
- de la Fuente, J., Estrada-Peña, A., Venzal, J.M., Kocan, K.M., Sonenshine, D.E., 2008. Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Front. Biosci.* 13, 6938–6946.
- de la Fuente, J., Contreras, M., Estrada-Peña, A., Cabezas-Cruz, A., 2017. Targeting a global health problem: vaccine design and challenges for the control of tick-borne diseases. *Vaccine* 35, 5089–5094.
- de la Fuente, J., Lima-Barbero, J.F., Prado, E., Pacheco, I., Alberdi, P., Villar, M., 2020. *Anaplasma* pathogen infection alters chemical composition of the exoskeleton of hard ticks (Acari: Ixodidae). *Comput. Struct. Biotechnol. J.* 18, 253–257.
- Della Noce, B., Carvalho Uhl, M.V., Machado, J., Waltero, C.F., de Abreu, L.A., da Silva, R.M., da Fonseca, R.N., de Barros, C.M., Sabadin, G., Konnai, S., da Silva Vaz Jr., I., Ohashi, K., Logullo, C., 2019. Carbohydrate metabolic compensation coupled to high tolerance to oxidative stress in ticks. *Sci. Rep.* 9, 4753.
- Falco, R.C., Fish, D., Piesman, J., 1996. Duration of tick bites in a Lyme disease-endemic area. *Am. J. Epidemiol.* 143, 187–192.
- Gallant, J., Hochberg, R., 2017. Elemental characterization of the exoskeleton in the whipscorpions *Mastigoproctus giganteus* and *Typopeltis dalyi* (Arachnida: Thelyphonida). *Invertebr. Biol.* 136, 345–359.
- Goddard, J., 1989. Focus of human parasitism by the brown dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae). *J. Med. Entomol.* 26, 628–629.
- Hernandez, E., Kusakisako, K., Talactac, M.R., Galay, R.L., Yoshii, K., Tanaka, T., 2018. Induction of intracellular ferritin expression in embryo-derived *Ixodes scapularis* cell line (ISE6). *Sci. Rep.* 8, 16566.
- Hollmann, T., Kim, T.K., Tirloni, L., Radulović, Ž.M., Pinto, A.F.M., Diedrich, J.K., Yates 3<sup>rd</sup>, J.R., da Silva Vaz Jr., I., Mulenga, A., 2018. Identification and characterization of proteins in the *Amblyomma americanum* tick cement cone. *Int. J. Parasitol.* 48, 211–224.
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, S3–S14.
- Kazimírová, M., Štibrániová, I., 2013. Tick salivary compounds: their role in modulation of host defences and pathogen transmission. *Front. Cell. Infect. Microbiol.* 3, 43.
- Kim, T.K., Tirloni, L., Pinto, A.F.M., Diedrich, J.K., Moresco, J.J., Yates 3<sup>rd</sup>, J.R., da Silva Vaz Jr., I., Mulenga, A., 2020. Time-resolved proteomic profile of *Amblyomma americanum* tick saliva during feeding. *PLoS Neglected Trop. Dis.* 14, e0007758.
- Landulfo, G.A., Li, A.Y., Lima, A.S., Silva, N.C.S., Vale, T.L., Costa-Junior, L.M., 2019. Feeding and respiratory gas exchange of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae). *Exp. Appl. Acarol.* 78, 173–179.
- Molaei, G., Little, E.A., Williams, S.C., Stafford, K.C., 2019. Bracing for the worst - range expansion of the lone star tick in the northeastern United States. *N. Engl. J. Med.* 381, 2189–2192.

- Nuttall, P.A., 2019. Wonders of tick saliva. *Ticks Tick-Borne Dis.* 10, 470–481.
- Pacheco, I., Acevedo, P., Prado, E., Mihalca, A.D., de la Fuente, J., 2020. Targeting the exoskeleton elementome to track tick geographic origins. *Front. Physiol.* 11, 572758.
- Parola, P., Socolovschi, C., Jeanjean, L., Bitam, I., Fournier, P.E., Sotto, A., Labauge, P., Raoult, D., 2008. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Neglected Trop. Dis.* 2, e338.
- Paules, C.I., Marston, H.D., Bloom, M.E., Fauci, A.S., 2018. Tickborne diseases - confronting a growing threat. *N. Engl. J. Med.* 379, 701–703.
- Ragaisiene, A., Rusinaviciute, J., Milasiene, D., Ivanauskas, R., 2016. Comparison of selected chemical properties of fibres from different breeds of dogs and German Blackface sheep. *Fibres Text. East. Eur.* 24, 21–28.
- Ramaswamy, K., Killilea, D.W., Kapahi, P., Kahn, A.J., Chi, T., Stoller, M.L., 2015. The elementome of calcium-based urinary stones and its role in urolithiasis. *Nat. Rev. Urol.* 12, 543–557.
- Rashid, M., Rashid, M.I., Akbar, H., Ahmad, L., Hassan, M.A., Ashraf, K., Saeed, K., Gharbi, M., 2019. A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. *Parasitology* 146, 129–141.
- Saravanan, D., 2006. Spider silk – structure, properties and spinning. *JTATM* 5, 1–20.
- Spaans, S.K., Weusthuis, R.A., van der Oost, J., Kengen, S.W., 2015. NADPH-generating systems in bacteria and archaea. *Front. Microbiol.* 6, 742.
- Suppan, J., Engel, B., Marchetti-Deschmann, M., Nürnberger, S., 2018. Tick attachment cement- reviewing the mysteries of a biological skin plug system. *Biol. Rev. Camb. Phil. Soc.* 93, 1056–1076.
- Tabor, A.E., Ali, A., Rehman, G., Rocha Garcia, G., Zangirolamo, A.F., Malardo, T., Jonsson, N.N., 2017. Cattle tick *Rhipicephalus microplus*-host interface: a review of resistant and susceptible host responses. *Front. Cell. Infect. Microbiol.* 7, 506.
- Villar, M., López, V., Ayllón, N., Cabezas-Cruz, A., López, J.A., Vázquez, J., Alberdi, P., de la Fuente, J., 2016. The intracellular bacterium *Anaplasma phagocytophilum* selectively manipulates the levels of vertebrate host proteins in the tick vector *Ixodes scapularis*. *Parasit. Vectors* 9, 467.
- Villar, M., Pacheco, I., Merino, O., Contreras, M., Mateos-Hernández, L., Prado, E., Barros-Picanco, D.K., Francisco Lima-Barbero, J., Artigas-Jerónimo, S., Alberdi, P., Fernández de Mera, I.G., Estrada-Peña, A., Cabezas-Cruz, A., de la Fuente, J., 2020. Tick and host derived compounds modulate the biochemical properties of the cement complex substance. *Biomolecules* 10, 555.
- Vincent, J.F.V., 2002. Arthropod cuticle: a natural composite shell system. *Composites Part A* 33, 1311–1315.
- Vohland, K., Furch, K., Adis, J., 2003. Contrasting central Amazonian rainforests and their influence on chemical properties of the cuticle of two millipede species – a first study. *Trop. Ecol.* 44, 235–241.
- Wang, B., Wang, X.L., 2019. Species diversity of fecal microbial flora in *Canis lupus familiaris* infected with canine parvovirus. *Vet. Microbiol.* 237, 108390.
- Wikel, S.K., 2018. Ticks and tick-borne infections: complex ecology, agents, and host interactions. *Vet. Sci.* 5, 60.
- Wilkinson, L.J., White, R.J., Chipman, J.K., 2011. Silver and nanoparticles of silver in wound dressings: a review of efficacy and safety. *J. Wound Care* 20, 543–549.
- Zhang, S., Piorowski, D., Lin, W.R., Lee, Y.R., Liao, C.P., Wang, P.H., Tso, I.M., 2019. Nitrogen inaccessibility protects spider silk from bacterial growth. *J. Exp. Biol.* 222 (Pt20), jeb214981.