Ticks and Tick-borne Diseases 14 (2023) 102149

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Letters to the Editor

Regarding the identification of *Rhipicephalus* ticks in the western Mediterranean: a comment on Gago et al. (2022)

Dear Editor,

I read with attention the paper by Gago et al. (2022) that appeared in a recent issue of Ticks and Tick-borne Diseases with the title "Patterns of adult tick parasitization of coexisting European (*Erinaceus europaeus*) and Algerian (*Atelerix algirus*) hedgehog populations in eastern Iberia" (https://www.sciencedirect.com/science/article/pii/S1877959 × 22001509). I would like to call your attention to several concerning issues I found in the paper, namely (i) the lack of reliable identifications of the ticks, (ii) the questionable statistical treatment of weather data, and (iii) the poor Methods section, lacking adequate explanations of the protocols; and (iv) the absence of supplementary material including the database of the study and availability of voucher tick specimens.

The recording of the taxon *Rhipicephalus turanicus* Pomerantzev in eastern Spain did deeply call my attention, since it is now well established that this species is absent in most of the western Mediterranean (Nava et al., 2015, 2018). Although records of a tick alleged to be *R. turanicus* in parts of Spain, Portugal, France, and Switzerland have been reported (Morel and Vassiliades, 1962; Estrada-Peña and Sánchez, 1988; Bernasconi et al., 2002; Estrada-Peña et al., 2004), these identifications are incorrect, and all of them were named by Nava et al. (2018) as *Rhipicephalus turanicus* sensu lato (s.l.). The studies by Estrada-Peña and Sánchez (1988), Estrada-Peña et al. (2004), or Millán et al. (2007) with specimens from Spain did not clarify the status of *R. turanicus* sensu stricto (s.s.) because there is no guarantee that these investigators were working with *bona fide R. turanicus* s.s. (Guglielmone and Nava, 2014). The taxon *R. turanicus* s.s. is morphologically well defined (Filippova, 1997) and phylogenetically represents a lineage independent of other taxa of the *Rhipicephalus sanguineus* group (Dantas-Torres et al., 2013; Chitimia-Dobler et al., 2017; Nava et al., 2018).

The type locality of *R. turanicus* s.s. is Tashkent, Uzbekistan (lectotype), and *bona fide* records of *R. turanicus* s.s. appear to be restricted to localities of Central Asia and southeastern Europe. Findings by several researchers showed that *R. turanicus* s.s. probably has its southern range in Turkey and its western limit west to Israel, as it has been tracked so far. Other studies have confirmed that *R. turanicus* s.s. is absent in Portugal, Italy, or Romania (Dantas-Torres et al., 2017; Coimbra-Dores et al., 2018) supporting the view that previous reports of *R. turanicus* s.s. in countries west of Israel were misidentifications. In conclusion, it can be stated that *R. turanicus* s.l. ticks from the western Mediterranean basin and southern Switzerland do not belong to the taxon *R. turanicus* s.s. (Nava et al., 2018). Records by Gago et al. (2022) cannot be validated because (i) no molecular confirmation has been carried out, (ii) no voucher specimens are available, and (iii) no specific details about the main morphological features were provided, but only references to two published keys that mutually contradict in the diagnosis of both *R. sanguineus* and *R. turanicus*.

I also would like to kindly call the editor's attention about the identity of the alleged *Rhipicehalus sanguineus* collected and identified in this study. The paper by Gago et al. (2022) does not provide information about if the specimens are sensu stricto (according to the rules provided by Nava et al., 2018) or if they belong to the group of cryptic species collectively known as "sensu lato" (which also includes specimens misidentified as *R. turanicus* in Spain but is not the *R. turanicus* s.l. mentioned before). Because the taxon named as *R. sanguineus* s.l. "southeastern Europe lineage" is not formally described, it is not possible to determine if the previous records of *R. turanicus* and/or *R. sanguineus* in Spain correspond to this lineage. Almeida et al. (2017) demonstrated that ticks collected in Portugal had a genetic uniformity, supporting the existence of a well-defined clade consisting of *R. sanguineus* s.l. specimens from Western Europe that are distinct from *R. sanguineus* s.l. from Africa. These authors corroborated the existence of a polymorphic species of the *R. sanguineus* group in Western Europe, which requires to be consensually redescribed. Morphological and molecular studies by Mumcuoglu et al. (2021), resulted in the re-instatement of *Rhipicephalus secundus* Feldman-Muhsam as a valid species, present at least in Israel, Palestinian Territories, Turkey, Albania, and southern Italy; these authors concluded that the species could be absent in Spain (only one sequence was used for comparative purposes). Therefore, the identification (and comparison with already (re)described species of the group) of the *R. sanguineus* s.l. commonly reported in Spain as either "*R. sanguineus*" or "*R. turanicus*" is of high priority. This is important because morphologically close species share the same range.

Results from different phylogenetic analyses (Beati and Keirans 2001; Moraes-Filho et al., 2011; Nava et al., 2012; Dantas-Torres et al., 2013; Liu et al., 2013; Chitimia-Dobler et al., 2017; Bakkes et al., 2020; Hekimoğlu et al., 2016) showed that more than one species were included under the names *R. turanicus* and *R. sanguineus*, respectively. Now that an explicit morphological and genetic differentiation of *R. secundus, R. turanicus* s.s. and *R. sanguineus* s.s. exist (Filippova, 1997; Zemtsova et al., 2016; Nava et al., 2018; Mumcuoglu et al., 2021), it is necessary to carry out additional studies to determine if there is another related taxon not yet formally described and to name the taxon (or taxa) existing in south-western Europe. Meanwhile, authors studying these ticks in the Western Mediterranean should be cautious about their identifications.

The issues mentioned above would be easily resolved by relying appropriately on the existing literature. The study by Gago et al. (2022) lacks the information used to identify the ticks involved, including morphological criteria, data about the stages collected, and/or molecular confirmation of the identifications. Not only is this essential information not presented, but the authors choose to make that material

https://doi.org/10.1016/j.ttbdis.2023.102149
Received 5 October 2022; Received in revised form 1 December 2022; Accepted 5 December 2022
Available online 2 March 2023

unavailable for further research. The sentence that can be read in the paper under the section Data Availability is "The authors are unable or have chosen not to specify which data has been used", which is against the current and necessary tendency towards an open science, a practice commonly adhered by "Ticks and Tick-borne Diseases". In conclusion, regarding the identification of the specimens, I can only modestly inform about the possible unreliability of the identification of these and other ticks mentioned in the paper of reference, such as *Rhipicephalus bursa* Canestrini & Fanzago. This is an exophilic tick found commonly, if not only, on ungulates, but is very similar to *Rhipicephalus pusillus* Gil Collado, a tick that can be found on hedgehogs (Toledo et al., 2009; Satta et al., 2011).

As mentioned above, there are other issues in the article by Gago et al. (2022) that require consideration. The authors determined "the effect of climatic variables on […] the correlation of monthly tick prevalence and mean abundance with the mean temperature, mean maximum temperature, mean minimum temperatures and accumulated precipitation." These statistical analyses were carried out using data from a network of climate recording stations, as stated in the paper. The website used by the authors delivers data of the regional network of climate recording stations. A close look at the available data indicates that the closest stations to the collection sites are located kilometers away. I humbly believe that these weather data are not useful for describing the "abundance" of ticks. One of the collection sites is in a periurban area, making these estimations still more "risky" due to the well-known "heat island" effects of the city (Lehoczky et al., 2017). It has been demonstrated that weather data may change widely even at short distances, and that data from climate recording stations are impractical to model features of the tick's life cycle favoring the use of loggers. The paper by Gago et al. (2022) does not contain any additional information allowing the reader to understand how weather variables were used, or how they were entered into correlation analyses (period of time? sum, median, averaged values?) preventing the verification and/or extension to similar cases. A set of guidelines about how to use weather/climate data in regards to the statistical analysis of tick seasonality has been already published (Estrada-Peña et al., 2013). Nevertheless, a Spearman correlation (as stated to be performed by Gago et al., 2022) is not a recommended technique to be used in studies of external traits impacting the tick abundance (Ogden et al., 2005). A correlation cannot capture the complexities of the effects of climate on the population of ticks together with the host-derived effects. This is of particular importance if the (suspected) species of ticks have an endophilic behavior (i.e., Nava et al., 2018, for *R. sanguineus* s.s.; Estrada-Peña et al., 2018, for *R. pusillus*). Ticks evolved the endophilic behaviour to eliminate the negative effects of the weather conditions "outside" the shelter or nest in which they develop their life cycles (Gray et al., 2013). Therefore, the evaluation of the impact of climate is counterintuitive for an endophilic tick. In any case, I wonder how the effects of climate on the tick population could be measured using only counts of adult specimens, ignoring the larger population of immatures.

I would like to read the reply by the authors of the paper of concern. If not, I kindly suggest the retraction of this paper, because it not only does not present any new contribution to this field of science, but it contains inaccuracies in identification that are impossible to confirm, as well as statistical misuses. If the manuscript is not retracted, or results validated, these incorrect data would remain to other researchers as valid records, perpetuating in future maps of distribution, modeling efforts, or taxonomic studies of this prominent group of ticks.

Data availability

No data was used for the research described in the article.

References

Almeida, C., Simões, R., Coimbra-Dores, M.J., Rosa, F., Dias, D., 2017. Mitochondrial DNA analysis of *Rhipicephalus sanguineus* s.l. from the western Iberian Peninsula. Med. Vet. Entomol. 31, 167–177.

Bakkes, D.K., Chitimia-Dobler, L., Matloa, D., Oosthuysen, M., Mumcuoglu, K.Y., Mans, B.J., Matthee, C.A., 2020. Integrative taxonomy and species delimitation of *Rhipicephalus turanicus* (Acari: Ixodida: Ixodidae). Int. J. Parasitol. 50, 577–594.

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87, 32–48.

Bernasconi, M.V., Casati, S., Péter, O., Piffaretti, J.C., 2002. *Rhipicephalus* ticks infected with *Rickettsia* and *Coxiella* in southern Switzerland (Canton Ticino). Infect. Genet. Evol. 2, 111–120.

Chitimia-Dobler, L., Langguth, J., Pfeffer, M., Kattner, S., Küpper, T., Friese, D., Nava, S., 2017. Genetic analysis of *Rhipicephalus sanguineus* sensu lato ticks parasites of dogs in Africa north of the Sahara based on mitochondrial DNA sequences. Vet. Parasitol. 239, 1–6.

Coimbra-Dores, M.J., Maia-Silva, M., Marques, W., Oliveira, A.C., Rosa, F., Dias, D., 2018. Phylogenetic insights on Mediterranean and Afrotropical *Rhipicephalus* species (Acari: Ixodida) based on mitochondrial DNA. Exp. Appl. Acarol. 75, 107–128.

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.

Estrada-Peña, A., Sanchez, C., 1988. Morfología comparada de *Rhipicephalus sanguineus* y *R. turanicus* (Acarina: Ixodidae). Rev. Ibér. Parasitol. 48, 51–62.

Estrada-Peña, A., Venzal, J.M., Nava, S., 2018. Redescription, molecular features, and neotype deposition of *Rhipicephalus pusillus* Gil Collado and *Ixodes ventalloi* Gil Collado (Acari, Ixodidae). Zootaxa 4442, 262–276.

Estrada-Peña, A., Bouattour, A., Camicas, J.L., Walker, A.R., 2004. Ticks of Domestic Animals in the Mediterranean Region. University of Zaragoza, Zaragoza, p. 131.

Estrada-Peña, A., Gray, J.S., Kahl, O., Lane, R.S., Nijhof, A.M., 2013. Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats. Front. Cell. Infect. Microbiol. 3, 29.

Filippova, N.A., 1997. Ixodid Ticks of Subfamily Amblyomminae. Nauka Publishing House, Moscow, Russia. Fauna of Russia and Neighboring Countries, p. 436.

Gago, H., Ruiz-Fons, F., Drechsler, R., Alambiaga, I., Monrós, J.S., 2022. Patterns of adult tick parasitization of coexisting European (*Erinaceus europaeus*) and Algerian (*Atelerix algirus*) hedgehog populations in eastern Iberia. Ticks Tick Borne Dis. 13, 102148.

Gray, J., Dantas-Torres, F., Estrada-Peña, A., Levin, M., 2013. Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks Tick Borne Dis. 4, 171–180.

Hekimoğlu, O., Sağlam, İ.K., Özer, N., Estrada-Peña, A., 2016. New molecular data shed light on the global phylogeny and species limits of the *Rhipicephalus sanguineus* complex. Ticks Tick Borne Dis. 7, 798–807.

Lehoczky, A., Sobrino, J.A., Skoković, D., Aguilar, E., 2017. The urban heat island effect in the city of Valencia: a case study for hot summer days. Urban Sci. 1, 9.

Liu, G.H., Chen, Y.Z., Song, H.Q., Lin, R.Q., Zhou, D.H., Zhu, X.Q., 2013. Complete mitochondrial genome sequence data provides evidence that dog tick *Rhipicephalus sanguineus* (Acari: Ixodidae) represents a species complex. Int. J. Biol. Sci. 9, 361–369.

Millán, J., Ruiz-Fons, F., Márquez, F.J., Viota, M., López-Bao, J.V., Martín-Mateo, M.P., 2007. Ectoparasites of the endangered Iberian lynx *Lynx pardinus* and sympatric wild and domestic carnivores in Spain. Med. Vet. Entomol. 21, 248–254.

Moraes-Filho, J., Marcili, A., Nieri-Bastos, F.A., Richtzenhain, L.J., Labruna, M.B., 2011. Genetic analysis of ticks belonging to the *Rhipicephalus sanguineus* group in Latin America. Acta Trop. 117, 51–55.

Morel, P.C., Vassiliades, G., 1962. Les *Rhipicephalus* du groupe *sanguineus*: espéces africaines (Acariens: Ixodoidea). Rev. Elev. Méd. Vet. Pays Trop. 15, 343–386.

Mumcuoglu, K.Y., Estrada-Peña, A., Tarragona, E.L., Sebastian, P.S., Guglielmone, A.A., Nava, S., 2021. Reestablishment of *Rhipicephalus secundus* Feldman-Muhsam, 1952 (Acari: Ixodidae). Ticks Tick Borne Dis. 13, 101897.

Nava, S., Mastropaolo, M., Venzal, J.M., Mangold, A.J., Guglielmone, A.A., 2012. Mitochondrial DNA analysis of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) in the Southern Cone of South America. Vet. Parasitol. 190, 547–555.

Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M.B., Szabó, M.P., Guglielmone, A.A., 2015. The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). Vet. Parasitol. 208, 2–8.

Nava, S., Beati, L., Venzal, J.M., Labruna, M.B., Szabó, M.P., Petney, T., Estrada-Peña, A., 2018. *Rhipicephalus sanguineus* (Latreille, 1806): Neotype designation, morphological re-description of all parasitic stages and molecular characterization. Ticks Tick Borne Dis. 9, 1573–1585.

Ogden, N.H., Bigras-Poulin, M., O'callaghan, C.J., Barker, I.K., Lindsay, L.R., Maarouf, A., Charron, D., 2005. A dynamic population model to investigate effects of climate on geographic range and seasonality of the tick *Ixodes scapularis*. Int. J. Parasitol. 35, 375–389.

Satta, G., Chisu, V., Cabras, P., Fois, F., Masala, G., 2011. Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted microorganisms in Sardinia. Italy. J. Med. Microbiol. 60, 63–68.

Toledo, Á., Olmeda, A.S., Escudero, R., Jado, I., Valcárcel, F., Casado-Nistal, M.A., Anda, P., 2009. Tick-borne zoonotic bacteria in ticks collected from central Spain. The Am. J. Trop. Med. Hyg. 81, 67–74.

Zemtsova, G.E., Apanaskevich, D.A., Reeves, W.K., Hahn, M., Snellgrove, A., Levin, M.L., 2016. Phylogeography of *Rhipicephalus sanguineus* sensu lato and its relationships with climatic factors. Exp. Appl. Acarol. 69, 191–203.

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