Veterinary Parasitology: Regional Studies and Reports 7 (2017) 58–61

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

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

Short Communication

Temperate and tropical lineages of brown dog ticks in North America

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ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 21 December 2016
Accepted 13 January 2017
Available online 14 January 2017

Keywords:
Brown dog tick
Phylogeny
Rhipicephalus sanguineus sensu lato
Taxonomy

ABSTRACT

Recent studies document that brown dog ticks, previously considered as *Rhipicephalus sanguineus,* are actually comprised of multiple taxonomic units now referred to as *Rhipicephalus sanguineus* sensu lato (*Rs*sl); two lineages of *Rs*sl have been described in the Americas to date – tropical and temperate. To identify the lineage of *Rs*sl from dogs or premises at multiple sites in the United States and the Caribbean, we evaluated ticks ($n = 191$) collected from several geographic locations ($n = 21$), including Arizona, California, Florida, Hawaii, Illinois, Oklahoma, and Texas in the United States, and from Haiti. All ticks were identified as brown dog ticks by morphologic examination and comparison to standard keys. Sequence analysis of 12S rRNA mitochondrial gene fragments confirmed the presence of both lineages, with the *Rs*sl tropical lineage predominating in Florida, Haiti, Hawaii, and far southern Texas ($n = 9$ locations) and the *Rs*sl temperate lineage present in California, Oklahoma, and Texas ($n = 12$ locations). Mixed populations were not identified although the temperate lineage appeared to separate into two distinct clades. Analysis of additional brown dog tick specimens from the region will allow more complete understanding of the full extent of diversity in the *R. sanguineus* complex and likely has important implications for disease transmission, including zoonotic risk.

1. Introduction

The brown dog tick has a worldwide distribution due to the ubiquity of its primary host, the domestic dog. Globally, this tick has been implicated as a vector of *Anaplasma platys*, *Babesia vogeli*, *Ehrlichia canis*, *Hepatozoon canis*, *Rickettsia conorii*, *Rickettsia massiliae*, and *Rickettsia rickettsii*, although populations appear to vary in their behavior and ability to transmit these different disease agents to dogs and people and not all transmission cycles have been fully confirmed (Dantas-Torres and Otranto, 2015). The taxonomic status of the brown dog tick, which until recently was referred to as *Rhipicephalus sanguineus*, is currently under debate (Nava et al., 2015). At present, the genus *Rhipicephalus* contains >70 recognized species but none are as widespread as brown dog ticks. Recent comparisons of brown dog ticks using morphology and mitochondrial gene sequences have shown that the *R. sanguineus* complex parasitizing dogs in Europe, Asia, South America, and Oceania is actually comprised of several distinct taxonomic units (Dantas-Torres et al., 2013). The two populations described in the Americas to date are currently referred to as *Rhipicephalus sanguineus* sensu lato (*Rs*sl) tropical lineage and *Rs*sl temperate lineage (Levin et al., 2012 and Sanches et al., 2016). Cross-breeding experiments, comparison of vector capacity, engorgement weights, and outbreaks of human disease suggest variation in biologic significance across the taxonomic units (Demma et al., 2005, Eremeeva et al., 2011, Levin et al., 2012 and Moraes-Filho et al., 2015).

Although several comparisons of brown dog ticks from other areas of the world have been reported, published studies on the taxonomic status of brown dog ticks within North America are limited. Colony ticks from Oklahoma were included in a crossbreeding study (Levin et al., 2012), and mitochondrial gene sequences from brown dog ticks from single locations in Arizona (HM138903), California (HM14443), Oklahoma (HM138900), Mexico (HM012572), and St. Kitts (HM138902) are available (Eremeeva et al., 2011 and Levin et al., 2012). More recently, additional brown dog ticks from North America were included in a study of 136 ticks from 23 countries around the world (Zemstova et al., 2016). To further characterize the diversity of brown dog ticks in the United States and the Caribbean, we compared 12S rRNA mitochondrial gene fragments from ticks initially identified as "*R. sanguineus*" by morphology from 21 distinct geographic locations in the region.

2. Material and methods

2.1. Samples

Ticks were collected from the environment or directly from dogs from multiple locations in Oklahoma, Texas, Florida, and California, and single locations in Arizona, Hawaii, Illinois, and Haiti, and stored

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http://dx.doi.org/10.1016/j.vprsr.2017.01.002

in 70% ethanol at room temperature. Ticks were also included from two research colonies independently established from wild tick populations in Oklahoma and California. To evaluate within-population diversity, up to ten adult or nymphal ticks were evaluated from each location. All individual ticks were identified morphologically as brown dog ticks by examination under a dissecting microscope and comparison to standard morphological keys (Keirans and Litwak, 1989).

2.2. DNA extraction

Engorged and nonengorged adults and engorged nymphs were dissected. Nonengorged nymphs were frozen in microcentrifuge vials in liquid nitrogen and crushed with a pestle. Dissected or crushed tick material was digested in tick lysis buffer with proteinase K for 8 h (Halos et al., 2004) and then DNA extracted using a commercial kit according to manufacturer's instructions (illustra™ blood genomicPrep Mini Spin Kit, GE Healthcare, Buckinghamshire, UK). Samples from 4 locations in Texas were sourced from previously extracted brown dog tick DNA stored at —20 °C at Texas A&M University; ticks were morphologically identified as described above prior to nucleic acid extraction.

2.3. PCR and sequencing

A 340–370 bp fragment of the 12S rRNA mitochondrial gene was amplified from each tick as previously described (Szabó et al., 2005). Each reaction consisted of 4 μl of tick genomic DNA and 46 μl of PCR mix containing 2.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3), and 50 mM KCl, 0.2 mM of each dNTP, 25 μM of each primer and 1.25 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA USA). Amplicons were resolved in 2% ethidium bromide-stained agarose gels to confirm expected size, purified using a commercial kit according to manufacturer's instructions (Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA), and submitted with corresponding primers for bidirectional DNA sequencing (Genomics SimpleSeq™, Eurofins, Luxembourg).

2.4. Sequence analysis

Sequences were aligned (MacVector 14.0.0., MacVector Inc., Apex, NC, USA) and compared to one another and to 12S rRNA gene sequences of *R. sanguineus* previously deposited in GenBank. Phylogenetic trees were created using the Maximum Likelihood method based on the Tamara 3-parameter model with MEGA6.06 software with bootstrap values based on 1000 replicates. Within-group pairwise analysis was conducted using the Kimura 2-parameter model. The identity of each sequence was assigned based on similarity to previously reported data for *Rhipicephalus* spp. from dogs (Dantas-Torres et al., 2013).

3. Results

Sequences were obtained from a total of 191 nymph and adult ticks from 21 locations in the United States and the Caribbean (Table 1). A total of 3–10 ticks each were evaluated from multiple locations in California ($n = 3$), Florida ($n = 4$), Oklahoma ($n = 5$), and Texas ($n = 5$). A total of 9–20 ticks each were evaluated from single locations in Hawaii, Illinois, Arizona, and from Haiti. All ticks were confirmed morphologically to be brown dog ticks.

Comparison of 12S rRNA mitochondrial gene sequences identified two lineages of brown dog ticks: *Rs*sl tropical lineage was confirmed from single locations in Arizona, Florida, Hawaii, Illinois, southern Texas (Edinburg), and Haiti, and *Rs*sl temperate lineage was identified from multiple locations in California (3 locations), Oklahoma (5 locations), and Texas (4 locations, Table 1, Fig. 1). No mixed populations of the tropical and temperate lineages were identified at the same location in the present study (Table 1).

Table 1
Geographic origin, annual mean temperature, and identified lineage of brown dog ticks (*Rhipicephalus sanguineus* sensu *lato*) from 21 locations in the United States and the Caribbean.

Geographic origin			Mean annual Temperature (°C)	Number ticks sequenced (n)	Lineage
Country	State	City			
United States	Arizona	Mesa	22.2	10	Tropical
	California	Sacramento	16.1	10	Temperate
		San Diego	17.8	6	Temperate
		Soquel[a]	15.0	10	Temperate
	Florida	Alachua	21.1	9	Tropical
		Melbourne	22.2	5	Tropical
		Naranja	20.0	3	Tropical
		Ormond Beach	21.7	8	Tropical
	Hawaii	Kona	25.6	20	Tropical
	Illinois	Chicago	10.0	10	Tropical
	Oklahoma	Stillwater[a]	15.0	10	Temperate
		Edmond	16.4	10	Temperate
		Little Axe	18.0	10	Temperate
		Perkins	15.0	9	Temperate
		Tulsa	15.9	9	Temperate
	Texas	Dallas	17.9	10	Temperate
		Edinburg	23.3	10	Tropical
		El Paso	18.2	7	Temperate
		Fort Worth	18.5	7	Temperate
		San Angelo	18.6	9	Temperate
Haiti	NA	NA	28.1	9	Tropical

[a] Colony ticks established from local population.

The *Rs*sl temperate lineage could be further delineated into two clusters that shared >97% identity and a similar, overlapping geographic origin (Fig. 1). Within- and between-group pairwise analysis showed complete identity within one cluster to previously published sequences from Arizona and California (HM014443, HM138903) with the exception of three sequences, including two previously reported from Argentina and Portugal (JX206975, KC243806) that differed by 1 or 2 bp, respectively, and one from Oklahoma (Perkins OK-2) that differed by 5 bp. All sequences in the second cluster were identical to one another and differed from those in the first cluster by 2.3–2.6%. Ticks from both temperate lineage clusters were collected from the same premise or removed from the same dog at 7 different locations in California (Sacramento), Oklahoma (Edmond, Perkins, Tulsa), and Texas (Dallas, El Paso, San Angelo) (Fig. 1).

4. Discussion

The data in the present study provide further confirmation that at least two lineages of brown dog ticks are present in North America, including in the United States – *Rs*sl tropical lineage and *Rs*sl temperate lineage. Crossbreeding experiments with ticks from North America (Oklahoma, USA) and those of Mediterranean and African origin suggest these populations represent distinct species that do not produce viable offspring (Levin et al., 2012). Similar, albeit more extensive, genotypic diversity of brown dog ticks has been well established across Europe, Asia, South America, and Oceania (Dantas-Torres et al., 2013). Moreover, experimental transmission studies and surveys of ticks from naturally infested dogs suggest that brown dog tick lineages likely differ in their ability to transmit different pathogens, including *Ehrlichia canis*, *Hepatozoon canis*, and *Anaplasma platys* (Inokuma et al., 2003, Sanogo et al., 2003, Ramos et al., 2013, Demoner Lde et al., 2013, Moraes-Filho et al., 2015). Accordingly, these data may have important implications for relative risk for disease transmission, including zoonotic infections, to dogs and people in the region. Inclusion of sequences from populations of brown dog ticks from more locations in North America, including Canada, Mexico, and the northern United States, would further enhance our understanding of *Rhipicephalus* species diversity and disease risk.