Bacteria associated with Amblyomma cajennense tick eggs

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Abstract

Ticks represent a large group of pathogen vectors that blood feed on a diversity of hosts. In the Americas, the Ixodidae ticks Amblyomma cajennense are responsible for severe impact on livestock and public health. In the present work, we present the isolation and molecular identification of a group of culturable bacteria associated with A. cajennense eggs from females sampled in distinct geographical sites in southeastern Brazil. Additional comparative analysis of the culturable bacteria from Anocentor nitens, Rhipicephalus sanguineus and Ixodes scapularis tick eggs were also performed. 16S rRNA gene sequence analyses identified 17 different bacterial types identified as Serratia marcescens, Stenotrophomonas maltophilia, Pseudomonas fluorescens, Enterobacter spp., Micrococcus luteus, Ochrobactrum anthropi, Bacillus cereus and Staphylococcus spp., distributed in 12 phylogroups. Staphylococcus spp., especially S. sciuri, was the most prevalent bacteria associated with A. cajennense eggs, occurring in 65% of the samples and also frequently observed infecting A. nitens eggs. S. maltophilia, S. marcescens and B. cereus occurred infecting eggs derived from specific sampling sites, but in all cases rising almost as pure cultures from infected A. cajennense eggs. The potential role of these bacterial associations is discussed and they possibly represent new targets for biological control strategies of ticks and tick borne diseases.

Keywords: Amblyomma cajennense, tick eggs bacteria, Staphylococcus, Ixodidae.

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Tick and tick borne disease control has been usually attempted or proposed by the use of a series of strategies aiming mostly on biological events to potentially impair tick feeding, pathogen transmission to mammal hosts (Allen and Humphreys, 1979; Kotsyfakis et al., 2008; Piesman and Eisen, 2008; Rot et al., 2013; Garcia et al., 2014; Schwarz et al., 2014), and in some cases, to restrict tick molting events and pathogen maintenance during tick development (Olds et al., 2012; Calligaris et al., 2013; Doan et al., 2013; Moreno-Cid et al., 2013; Rot et al., 2013). Interestingly, studies on tick egg biology depicting possible strategies to improve egg health, integrity and development when exposed to natural environment, represent a poorly explored venue for the control of tick populations and associated pathogens. In fact, biological events, such as the possible role of the egg microbiome on tick population dynamics or tick development, to our knowledge has never been investigated and no bacteria were previously isolated specifically from tick eggs. However, many studies have described bacteria isolated from adult ticks (Ixodes scapularis, Ixodes ricinus, Dermacentor reticulatus and...
Haemaphysalis concinna) collected in the U.S., part of Europe and Australia (Martin and Schmidtmann, 1998; Murrell et al., 2003; Stojek and Duitkiewicz, 2004; Rudolf et al., 2009; Egyed and Makrai, 2014). In this scenario, we present the isolation and molecular identification of a group of culturable bacteria associated with *A. cajennense* eggs from females sampled in distinct geographical sites in southeastern Brazil. Comparative analyses with other Ixodidae ticks, such as *Anocentor nitens*, *Rhipicephalus sanguineus* and *I. scapularis* is presented. Natural colonization of tick eggs with specific bacterium physiotypes is discussed and it may bring new insights to the control of tick populations and tick-borne diseases.

In this work, actively feeding adult females of *A. cajennense* were retrieved from horses in southeastern Brazil sites, including municipalities with notified spotted fever cases (Brazil government data - Ministério da Saúde, SINAN, http://dr2004.saude.gov.br/sinanweb/ from 2001 to 2015). Sampled ticks were maintained in laboratory until oviposition. Field collected samples of *A. nitens* and *R. sanguineus* actively feeding on horses and dogs were also obtained for comparative analyses. *I. scapularis* tick samples were also analyzed and obtained from U.S. colonies maintained at the Vector-Host Laboratory (Division of Vector-Borne Disease, Center for Disease Control and Prevention, CDC) (Table 1). Tick samples were prepared by initial washing steps, including four successive washes with ethanol 70% (v/v), followed by morphological and taxonomic confirmative analyses. Engorged females were placed in glass vials and maintained at 93% relative humidity, using a saturated solution of KNO$_3$ in a growth chamber at 26 °C and under a photoperiod of 14 h: 10 h (light: dark) until oviposition. The obtained egg masses were washed in ethanol 70% (v/v), air-dried and homogenized in 500 µL of sterile PBS (phosphate buffered saline) with sterile mortar and pestle. Each egg mass, from a single female tick, was split in two to four sub-samples to generate independent egg/PBS homogenates which were individually plated for bacterial isolation.

Bacteria were isolated by direct streaking of 50 µL of the egg/PBS suspensions on LB (Luria-Bertani medium) agar plates, without any enrichment step to prevent competitive selection to occur. Replicas were incubated for 24 h at 30 °C and 37 °C. Bacterial colonies with the same morphology were purified in triplicates and directly inoculated into liquid LB medium for subsequent DNA extraction and storage at -80 °C. Bacterial genomic DNA extraction was routinely performed using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) procedure and eluted in 100 µL of TE buffer. DNA integrity was analyzed by agarose gel electrophoresis (data not shown).

The eubacterial 16S rRNA universal primers 27f, 338f, 907r and 1492r (Lane, 1991) were applied in PCR reactions using the purified bacterial genomic DNAs. PCR conditions included 35 cycles of 94 °C for 1 min, 55 °C for 35 s and 72 °C for 1 min. PCR products from four independent reactions were directly purified with the GFX PCR DNA and Gel band purification kit (GE Healthcare, Buckinghamshire, UK), according to manufacturer’s instructions. Purified PCR products were sequenced using the BigDye Terminator DNA sequencing kit (Applied Biosystems, Foster City, Calif., U.S.A.) and analyzed in a Megabace 1000 DNA sequencer (Amersham Biosciences). Sequences were edited using SeqMan program (DNASTARInc package for Windows platform, 1989-1999), and the identities were obtained by BLAST analyses. Neighbor-joining phylogenetic reconstruction with the Kimura two-parameter correction model was used to obtain a better taxonomic resolution (Kimura, 1980).

A total of 72 morphologically different colonies were visually grouped and isolated from a total of 46 egg mass samples, including 20 samples analyzed for *A. cajennense* from 4 sampling sites located in the states of Rio de Janeiro and Minas Gerais, Brazil, and 26 comparative samples of egg masses from *A. nitens*, *R. sanguineus* and *I. scapularis* species. DNA sequence BLAST analyses grouped all isolates into a total of 17 bacterial types (named as “IsoAC1” to “IsoAC8”, “IsoAN1” to “IsoAN3”, “IsoRS1” and “IsoRS2”, “IsoIS1” to “IsoIS4”) belonging to eight bacterial genera (Table 1), distributed in 12 phylogroups of *Firmicutes* (Bacilli) (70% of *A. cajennense* samples; ~47% of all samples), *Gamma-proteobacteria* (30% of *A. cajennense* samples; ~42% of all samples), *Alpha-proteobacteria* (not in *A. cajennense* but in ~5,5% of all samples) and *Actinobacteria* (not in *A. cajennense* but in ~5,5% of all samples) after 16S rRNA gene sequence analyses (Figure 1). Curiously, most tick egg samples present a restricted culturable bacterial richness, especially *A. cajennense* samples which in some cases yielded isolation of a pure culture of the associated *Staphylococcus scouri* by direct plating of its egg/PBS homogenates. *Staphylococcus* was the most frequent genus of bacteria associated with all tick species tested, occurring in 65% of the *A. cajennense* samples and 45% of all tested egg/PBS homogenates, especially the *S. scouri* (Clade H), which occurred in tick eggs sampled in different states of Brazil, both from Cayenne ticks sampled in the cities of Rio do Ouro (Rio de Janeiro state) and Pouso Alto (Minas Gerais state), and from *A. nitens* obtained in Seropedica (Rio de Janeiro state). *Staphylococcus* spp. were also abundantly detected in a recent metagenomic assessment of bacteria in *R. microplus*, but *S. scouri* was only found associated to adult ticks of this species (Andreotti et al., 2011). Similarly to the isolates cultured from *A. cajennense* eggs, these authors also detected *S. aureus*, other *Staphylococcus* spp., *Serratia marcescens*, *Stenotrophomonas* sp. and *Pseudomonas* sp. in *R. microplus* egg samples.

*Serratia marcescens* (Clade B) and *Stenotrophomonas maltophilia* (Clade D) were the only *Gamma-Proteobacteria* isolated from *A. cajennense* egg
samples and observed coinfecting the eggs from females collected in the Três Rios site. In addition, members of Stenotrophomonas sp. (Clade D) and phylogenetically close to the Cayenne tick isolates, were also observed in association with I. scapularis in the U.S. Ticks can uptake and carry bacteria from their environment or from the skin surface of hosts and some of these bacteria are able to survive and replicate in ticks (Egyed and Makrai, 2014). S. sciuri is in fact considered common colonizers of dogs, cats, found in the skin of cattle and other

| Tick species | Sampling area/hosts | No. ticks laying eggs | Identificationa | Phylogroup (Clade)b | Prevalencec |
|--------------|---------------------|-----------------------|-----------------|---------------------|-------------|
| A. cajennense | Três Rios, RJ, Brazil/horses | 2 | Serratia sp. iso AC1 (EU693533) /100% - Serratia marcescens (KJ806487) | γ (B) | 6/6 |
| | | | Stenotrophomonas sp. iso AC2 (EU693532) /100% - Stenotrophomonas maltophilia (KJ491015) | γ (D) | 6/6 |
| | Rio do Ouro, RJ, Brazil/horses | 2 | Staphylococcus sp. iso AC3 (EU693530) /100% - Staphylococcus sciuri (KJ507203) | F (H) | 3/4 |
| | | | Bacillus sp. iso AC4 (EU693531) /99% - Bacillus cereus (KJ534517) | F (G) | 4/4 |
| | Pouso Alto, MG, Brazil/horses | 2 | Staphylococcus sp. iso AC5 (KP306739) /100% - Staphylococcus sciuri (KJ507203) | F (H) | 4/4 |
| Seropédica, RJ, Brazil/horses | 2 | Staphylococcus sp. iso AC6 (KP306740) /100% - Staphylococcus kloosii (IX102547) | F (I) | 3/6 |
| | | | Staphylococcus sp. iso AC7 (KP306742) /99% - Staphylococcus aureus (HM359234) | F (L) | 6/6 |
| A. nitens | Seropédica, RJ, Brazil/horses | 3 | Staphylococcus sp. iso AN1 (KP306743) /99% - Staphylococcus sciuri (KJ507203) | F (H) | 8/8 |
| | | | Staphylococcus sp. iso AN2 (KP306745) /99% - Staphylococcus saprophyticus (KF254616) | F (J) | 8/8 |
| | | | Enterobacter sp. iso AN3 (KP306734) /99% - Enterobacter aerogenes (KJ631293) | γ (A) | 5/8 |
| R. sanguineus | Boa Esperança, MG, Brazil/dogs | 3 | Pseudomonas sp. iso RS1 (KP306738) /100% - Pseudomonas fluorescens (AB680296) | γ (C) | 2/4 |
| | | | Enterobacter sp. iso RS2 (KP306735) /99% - Enterobacter hormacehi (KF054945) | γ (A) | 4/4 |
| I. scapularis | Laboratory-reared, USA | 4 | Stenotrophomonas sp. iso IS1 (KP306746) /99% - Stenotrophomonas maltophilia (JF681290) | γ (D) | 14/14 |
| | | | Stenotrophomonas sp. iso IS2 (KP306744) /100% - Stenotrophomonas maltophilia (JF681290) | γ (D) | 12/14 |
| | | | Micrococcus sp. iso IS3 (KP306736) /100% - Micrococcus luteus (JX262404) | A (F) | 8/14 |
| | | | Ochrobactrum sp. iso IS4 (KP306737) /100% - Ochrobactrum anthropi (KF956631) | α (E) | 4/14 |
| Total | | 18 | | | 46 |

aIdentification by 16S rRNA gene sequence analyses. The GenBank accession number is given in parenthesis.

bPhylogroup as follows: A = Actinobacteria; F = Firmicutes; α = Alpha-Proteobacteria; γ = Gamma-Proteobacteria.

Clade code “A” to “N” refers to the clusters in the 16S rRNA gene based phylogenetic reconstruction presented in the Figure 1.

cPrevalence= No. positive egg-PBS suspension samples for a specific bacterial isolate/ Total No. of egg_PBS samples analyzed.

Each egg mass from a single female tick was splitted in 2 to 4 samples to generate independent egg_PBS homogenates. See text for details.

Previously annotated as Serratia marcescens strain CS265 in the Genbank.

Previously annotated as Stenotrophomonas maltophilia strain CS266 in the Genbank.

Previously annotated as Staphylococcus sciuri strain CS264 in the Genbank.

Previously annotated as Bacillus cereus strain CS262 in the Genbank.
Figure 1 - Phylogenetic inference of the tick egg associated bacteria using 16S rRNA gene sequences. Neighbor-Joining analysis with Kimura 2-parameter based on the nucleotide sequences was performed. Arrows indicate sequences obtained in the present work, and the GenBank accession codes for other sequences are presented in parenthesis. Sequences were aligned using the ClustalW program (Promega, Madison, WI), and phylogenetic inferences obtained using the MEGA 5.2.2 software. Internal node supports were calculated using bootstrap analyses with 1,000 replicas. Bootstrap values below 70% are not present.

animals (Devriese et al., 1984; Cox et al., 1985; Lilienbaum et al., 1999; Bagcigil et al., 2007; Andreotti et al., 2011; Couto et al., 2011; Garbacz et al., 2013). In this work, several identified bacteria are not typically described as associated with arthropods. The successive washes of the female ticks before oviposition, as previously performed in other tick associated bacteria study (Jutras et al., 2010), and taking the fact that eggs were laid in the laboratory vials and subjected to an additional direct washing step, suggests that these bacteria are not casual soil or other environmental population accidently colonizing the tested egg samples, and could indeed represent active partners participating in specific aspects of tick physiology. It is interesting to mention that all bacterial isolates are able to secrete protease, as determined by clearing zone formation by growths on LB agar plates containing 0.5% casein (Supplementary Material, Figure S1). This suggests that all isolated bacteria could contribute to ticks egg hatching processes, which should be assessed in further work. Also, a growing literature indicates that arthropods containing associated bacteria increase the arthropod-resistance against parasites and/or pathogens (Gravot et al., 2000; Oliver et al., 2003; Hedges and Johnson, 2008; Teixeira et al., 2008; Brownlie and Johnson, 2009; Koehler and Kaltenpoth, 2013; Koehler et al., 2013). The exposed tick eggs are vulnerable to environmental conditions and infection, mostly by soil microorganisms, is expected. Although not elucidated in the present work, we can speculate that the reduced amount of...
bacterial types isolated from the eggs may indicate that these bacteria have outcompeted other bacterial types in these samples. If somehow ticks manage to select specific egg colonizing bacteria and mostly maintain bioactive metabolite producing bacteria, it would in fact represent a strategy for chemical defense to improve success of egg development, hatching and tick population fitness. Interestingly, most isolated bacterial species are members of phylolclades that include known antifungal, bacteriocins and bioactive metabolites-producing bacteria, such as S. sciuri (Clade H), S. maltophilia (Clade D), S. marcescens (Clade B), P. fluorescens (Clade C), M. luteus (Clade F), S. aureus (Clade L), B. cereus/B. thuringiensis (Clade G) (Gardner, 1949; Tagg et al., 1976; Jakobi et al., 1996; Harris et al., 2004; Furushita et al., 2005; Pankeowitz and Hilker, 2006; Banerjee et al., 2011; Gutiérrez-Román et al., 2012; Liu et al., 2013), indicating their possible role on chemical protection of the exposed egg masses. S. marcescens and B. cereus are also known to combat insects and nematodes, organisms that could represent predators of tick eggs (Sikorowski and Lawrence, 1998; Yoshida et al., 2001; Dillon and Charnley, 2002; Nishiwaki et al., 2004). Additionally, the most frequent S. sciuri and S. maltophilia are also known as multidrug resistant bacteria (Stepanovic et al., 2006; Hauschildt et al., 2007; Haenni et al., 2011; Bhargava and Zhang, 2012; Lozano et al., 2012; Huang et al., 2013; Davis et al., 2014; Garcia-León et al., 2014; Harrison et al., 2014) and that would possibly support their high prevalence on A. cajennense egg samples, as a consequence of their potential resistance to competing microorganisms or to other antimicrobial components on eggs surface or in the surrounding environment (Arrieta et al., 2006).

Some bacterial species isolated are also known to be pathogenic to arthropod hosts, as the Clade B S. marcescens also seems to be harmful to ticks, since it was already described that the wax from the eggs of Amblyomma hebraeum has antibiotic activity for their protection specifically against this bacterium (Arrieta et al., 2006). Nymphs, larvae and adult ticks of Amblyomma variegatum also present specific substances against S. marcescens (Pavis et al., 1994). This bacterium species is a known opportunistic pathogen of insects (Sikorowski and Lawrence, 1998) and able to decrease egg hatching time of flies (Romero et al., 2006). Some other bacterial isolates are also representatives of clades that include potential pathogens to ticks, and it raises the possibility that tick egg associated bacteria could also act on trans-generational immune priming, a process of maternal transfer of bacteria to increase the expression of immunity-related genes encoding antibacterial proteins in the emerging larvae, as described for some insects (Freitak et al., 2014). These possibilities should be tested in the future.

It is important to mention that the bacteria presented here were cultured and isolated, not only described at the DNA sequence level. These bacteria, specially S. sciuri, are potential candidates for future paratransgenesis strategies, once they were easily isolated from tick eggs and reported infecting both nymph and adult ixodidae ticks of different species, including R. microplus, Ixodes holocyclus, I. ricinus, Dermacentor reticulatus, Haemaphysalis concina and Amblyomma mambilatrum (Martin and Schmidtmann, 1998; Murrell et al., 2003; Stojeck and Dutkiewicz 2004; Rudolf et al., 2009; Andreotti et al., 2011). In paratransgenesis, arthropod associated bacteria are used as vehicles for expressing foreign genes to kill or reduce pathogen fitness in their arthropod vectors, representing an alternative strategy to reduce pathogen transmission (Beard et al., 1998). Taken together, the present description and isolation of tick eggs associated bacteria offer new targets and tools for biological control strategies of ticks and tick borne diseases.

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References

Allen JR and Humphreys SJ (1979) Immunisation of guinea pigs and cattle against ticks. Nature 280:491-493.

Andreotti R, de Leon AAP, Dowd SE, Guerrero FD, Bendele KG and Scoles GA (2011) Assessment of bacterial diversity in the cattle tick Rhipicephalus (Boophilus) microplus through tag-encoded pyrosequencing. BMC Microbiol 11:e6.

Arrieta MC, Leskiw BK and Kaufman WR (2006) Antimicrobial activity in the egg wax of the African cattle tick Amblyomma hebraeum (Acari, Ixodidae). Exp Appl Acarol 39:297-313.

Bagcigil FA, Moodley A, Baptiste KE, Jensen VF and Guardabassi L (2007) Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet Microbiol 121:307-315.

Banerjee D, Chatterjee S, Banerjee UC, Guha AK and Ray L (2011) Green pigment from Bacillus cereus M(1)(16) (MTCC 5521): Production parameters and antibacterial activity. Appl Biochem Biotechnol 164:767-779.

Beard CB, Durvasula RV and Richards FF (1998) Bacterial symbiosis in arthropods and the control of disease transmission. Emerg Infect Dis 4:581-591.

Belongia EA (2002) Epidemiology and impact of coinfections acquired from Ixodes ticks. Vector Borne Zoonotic Dis 2:265-273.

Bhargava KL and Zhang Y (2012) Multidrug-resistant coagulase-negative Staphylococci in food animals. J Appl Microbiol 113:1027-1036.

Brownlie JC and Johnson KN. (2009) Symbiont-mediated protection in insect hosts. Trends Microbiol 8:348-354.

Calligaris IB, De Oliveira PR, Roma GC, Bechara GH and Camargo-Mathias MI (2013) Action of the insect growth regulator fluzuron, the active ingredient of the acaricide Acatak, in Rhipicephalus sanguineus nymphs (Latreille, 1806) (Acari, Ixodidae). Microsc Res Tech 76:1177-1185.
Couto N, Pomba C, Moodley A and Guardabassi L (2011) Prevalence of meticillin-resistant *Staphylococcus* among dogs and cats at a veterinary teaching hospital in Portugal. Vet Rec 169:72.

Cox HU, Hoskins JD, Newman SS, Turnwald GH, Foil CS, Roy AF and Kearney MT (1985) Distribution of staphylococcal species on clinically healthy cats. Am J Vet Res 46:1824-1828.

Davis JA, Jackson CR, Ferdorka-Cray PJ, Barrett JB, Brousse JH, Gustafson J and Kucher M (2014) Carriage of meticillin-resistant staphylococci by healthy companion animals in the US. Lett Appl Microbiol 59:1-8.

Deviere LA, Nzuambe D and Godard C (1984) Identification and characterization of staphylococci isolated from cats. Vet Microbiol 29:279-285.

Dillon R and Charnley K (2002) Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. Res Microbiol 153:503-509.

Doan HT, Noh JH, Kim YH, Yoo MS, Reddy KE, Jung SC and Kang SW (2013) The efficacy of avermectins (ivermectin, doramectin and abamectin) as treatments for infestation with the tick *Haemaphysalis longicornis* on rabbits in Korea. Vet Parasitol 198:406-409.

Egyed L and Makrai I (2014) Cultivable internal bacterial flora of ticks isolated in Hungary. Exp Appl Acarol 63:107-122.

Freitak D, Schmidtberg H, Dickel F, Lochit G, Vogel H and Vilecinskas A (2014) The maternal transfer of bacteria can mediate trans-generational immune priming in insects. Virulence 15:547-554.

Furushita M, Okamoto A, Maeda T, Ohta M and Shiba T (2005) Production of prodigiosin and chitinases by tropical *Serratia marcescens* strains with potential to control plant pathogens. World J Microbiol Biotechnol 28:145-153.

Haen M, Châtre P, Boisset S, Carricajo A, Bes M, Laurent F and Madec JY (2011) Staphylococcal nasal carriage in calves: multiresistant *Staphylococcus sciuri* and immune evasion cluster (IEC) genes in meticillin-resistant *Staphylococcus aureus* ST398. J Antimicrob Chemother 66:1927-1928.

Harriss AK, Williamson NR, Slater H, Cox A, Abbasi S, Foulds I, Simonsen HT, Leeper FJ and Salmond GPC (2004) The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species-and strain-dependent genome context variation. 2004. Microbiology 150:3547-3560.

Hedges LM and Johnson KN (2008) The induction of host defence responses by *Drosophila* C virus. J Gen Virol 89:1497-1501.

Huang YW, Hu RM and Yang TC (2013) Role of the *pcm-tolCsm* operon in the multidrug resistance of *Szentromahonas maltophilia*. J Antimicrob Chemother 68:1987-1993.

Jakob M, Winkelmann G, Kaiser D, Kempler C, Jung G, Berg G and Bahl H (1996) Maltophilin: a new antifungal compound produced by *Szentromahonas maltophilia* R3089. J Antibiott 49:1101-1104.

Jutras BL, Liu Z and Brissette CA (2010) Simultaneous isolation of Ixodidae and bacterial (*Borrelia* spp.) genomic DNA. Curr Protoc Microbiol 1E.2.1-1E.2.11.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120.

Koehler S and Kaltenpoth M (2013) Maternal and environmental effects on symbiont-mediated antimicrobial defence. J Chem Ecol 39:978-988.

Koehler S, Doubsky J and Kaltenpoth M (2013) Dynamics of symbiont-mediated antibiotic production reveal efficient long-term protection for beewolf offspring. Front Zool 10:e3.

Kotsyfakis M, Anderson JM, Andersen JF, Calvo E, Francischetti IM, Mather TN, Valenzuela JG and Ribeiro JM (2008) Cutting edge: Immunity against a “silent” salivary antigen of the Lyme vector *Ixodes scapularis* impairs its ability to feed. J Immunol 181:5209-5212.

Krause PJ (2002) Babesiosis. Med Clin North Am 86:361-373.
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Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E and Goodfellow M (eds) Nucleic Acid Techniques in Bacterial Systematics. Wiley & Sons, Chichester, pp 115-175.

Lemos ERS (2000) Rickettsial diseases in Brazil. Virus Rev Res 7:7-16.

Lilenbaum W, Esteves AL and Souza GN (1999) Prevalence and antimicrobial susceptibility of staphylococci isolated from saliva of clinically normal cats. Lett Appl Microbiol 28:448-452.

Liu J, Chen P, Zheng C and Huang YP (2013) Characterization of maltocin P28, a novel phage tail-like bacteriocin from Stenotrophomonas maltophilia. Appl Environ Microbiol 79:5593-5600.

Lozano CL, Aspiroz C, Sáenz Y, Ruiz-García M, Royo-García G, Gómez-Sanz E, Ruiz-Larrea F, Zarazaga M and Torres C (2012) Genetic environment and location of the Inu(A) and Inu(B) genes in methicillin-resistant Staphylococcus aureus and other staphylococci of animal and human origin. J Antimicrob Chemother 67:2804-2808.

Martin PA and Schmidtmann ET (1998) Isolation of aerobic microbes from Ixodes scapularis (Acari, Ixodidae), the vector of Lyme disease in the eastern United States. J Econ Entomol 91:864-868.

Mihalca AD, Gerhan CM and Cozma V (2011) Coendangered hard-ticks: Threatened or threatening? Parasit Vectors 4:71.

Moreno-Cid JA, Pérez de la Lastra JM, Villar M, Jiménez M, Pinal R, Estrada-Peña A, Molina R, Lucientes J, Gortázar C, de la Fuente J and SUB/AKR Vaccine Study Group (2013) Control of multiple arthropod vector infestations with tick eggs bacterial isolates. Figure S1 - Detection of protease production by the tick eggs bacterial isolates.

Murrell A, Ito K, Otsuki K, Yamamoto H, Komai K and Matsuda K (2001) Chaperonin turned in the ovoviposition behaviour and larval development of stable flies. Med Vet Entomol 20:115-121.

Romero A, Broce A and Zurek L (2006) Role of bacteria in the oviposition and larval development of stable flies. Med Vet Entomol 20:115-121.

Rot A, Gindin G, Ment D, Mishoutchenko A, Glazer I and Samish M (2013) On-host control of the brown dog tick Rhizophagus sanguineus Latreille (Acari, Ixodidae) by Metarhizium brunneum (Hypocreales, Clavicipitaceae). Vet Parasitol 193:229-237.

Supplementary Material

The following online material is available for this article: Figure S1 - Detection of protease production by the tick eggs bacterial isolates.

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