Ticks and Tick-borne Diseases 8 (2017) 749–756

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Detection and phylogenetic characterisation of novel *Anaplasma* and *Ehrlichia* species in *Amblyomma triguttatum* subsp. from four allopatric populations in Australia

Alexander W. Gofton[a,*], Helen P. Waudby[b], Sophie Petit[c], Telleasha L. Greay[a], Una M. Ryan[a], Peter J. Irwin[a]

[a] School of Veterinary and Life Sciences, Murdoch University, Perth, Australia
[b] Institute for Land, Water, and Society, Charles Sturt University, Albury, Australia
[c] School of Natural and Built Environments, University of South Australia, Adelaide, Australia

ARTICLE INFO

Keywords:
Ehrlichia
Anaplasma
Australia
Amblyomma triguttatum
Tick-borne disease

ABSTRACT

Anaplasma and *Ehrlichia* spp. are tick-borne pathogens that can cause severe disease in domestic animals, and several species are responsible for emerging zoonoses in the northern hemisphere. Until recently, the only members of these genera reported in Australia (*A. marginale*, *A. centrale*, and *A. platys*) were introduced from other continents, through the importation of domestic animals and their associated ticks. However, unique *Anaplasma* and *Ehrlichia* 16S rRNA gene sequences were recently detected for the first time in native Australian ticks, particularly in *Amblyomma triguttatum* subsp. ticks from southwest Western Australia (WA). We used molecular techniques to survey *Am. triguttatum* subsp. ticks from four allopatric populations in southern and western Australia for *Anaplasma* and *Ehrlichia* species, and described here the phylogeny of these novel organisms. An *A. bovis* variant (genotype Y11) was detected in ticks from two study sites; Yanchep National Park (12/280, 4.3%) and Barrow Island (1/69, 1.4%). Phylogenetic analysis of 16S rRNA and *groEL* gene sequences concluded that *A. bovis* genotype Y11 is a unique genetic variant, distinct from other *A. bovis* isolates worldwide. Additionally, a novel *Ehrlichia* species was detected in *Am. triguttatum* subsp. from three of the four study sites; Yanchep National Park (18/280, 6.4%), Bungendore Park (8/46, 17.4%), and Innes National Park (9/214, 4.2%), but not from Barrow Island. Phylogenetic analysis of 16S, *groEL*, *gltA*, and *map1* gene sequences revealed that this *Ehrlichia* sp. is most closely related to, but clearly distinct from, *E. ruminantium* and *Ehrlichia* sp. Panola Mountain. We propose to designate this new species '*Candidatus* Ehrlichia occidentalis'. *Anaplasma bovis* genotype Y11 and '*Candidatus* E. occidentalis' are the first *Anaplasma* and *Ehrlichia* species to be recorded in native Australian ticks.

1. Introduction

Members of the bacterial genera *Anaplasma*, *Ehrlichia*, and '*Candidatus* (*Ca.*) Neoehrlichia' (Family Anaplasmataceae) are obligate intracellular mammalian pathogens that are vectored by ixodids (hard ticks) (Rar and Golovljova, 2011). In nature, these bacteria persist in wildlife reservoir hosts and circulate in mammal-tick-mammal transmission cycles, where ticks act only as vectors and not as reservoirs (Rar and Golovljova, 2011). In mammals, *Anaplasma*, *Ehrlichia*, and '*Ca.* Neoehrlichia' species invade haematopoietic or endothelial cells, where they form and multiply within intracytoplasmic vacuoles (Rar and Golovljova, 2011; Rikihisa, 1991).

Many wildlife reservoirs can sustain asymptomatic or subclinical *Anaplasma, Ehrlichia*, and '*Ca.* Neoehrlichia' infections; however, transmission (via tick-bite) to naïve hosts such as humans and domestic animals can result in serious illness, including anaemia, fever, headache, muscle pains, nausea, rash, and in severe cases, death (Rar and Golovljova, 2011; Rikihisa, 1991). Several species, such as *E. chaffeensis*, *E. ewingii*, *A. phagocytophilum*, and '*Ca.* Neoehrlichia mikurensis', are responsible for emerging zoonotic diseases (Colwell et al., 2011; Paddock and Childs, 2003; Parola et al., 2005; Silaghi et al., 2015; Stuen, 2007).

Abbreviations: Ca, *Candidatus*; 16S, 16S rRNA gene; WA, Western Australia; SA, South Australia; *groEL*, 60 kDa heat shock chaperonin gene; *gltA*, citrate synthase gene; *map1*, major antigenic protein 1 gene; SNP, single nucleotide polymorphism; Mtn, Mountain; NP, National Park

* Corresponding author.
E-mail address: a.gofton@murdoch.edu.au (A.W. Gofton).

http://dx.doi.org/10.1016/j.ttbdis.2017.05.009
Received 4 April 2017; Received in revised form 16 May 2017; Accepted 22 May 2017
Available online 27 May 2017
1877-959X/ © 2017 Elsevier GmbH. All rights reserved.

Until recently, it was unknown whether Australia had any native *Anaplasma*, *Ehrlichia*, or '*Ca*. Neoehrlichia' species (Angus, 1996). Since the arrival of Europeans on the Australian continent nearly 250 years ago, three *Anaplasma* species (*A. marginale*, *A. centrale*, and *A. platys*) have been introduced through the importation of domestic animals and their associated ticks (*Haemaphysalis longicornis, Rhipicephalus australis*, and *R. sanguineus* sensu lato) (Angus, 1996; Callow, 1984). However, in Australia, these introduced *Anaplasma* species have only been reported in domestic animals, are only transmitted by introduced ixodids, and to the authors' knowledge have never been detected in native wildlife or enzootic ixodids (Angus, 1996; Callow, 1984).

Australia has unique tick and mammal fauna that have co-evolved in relative isolation since the breakup of the Gondwana landmass ~100 million years ago (Upchurch, 2008). Indeed, phylogenetic analysis of ixodids has shown some Australian species to be evolutionarily distant from northern hemisphere ixodids (Xu et al., 2003). Importantly, Australia is free from all members of the *Ixodes ricinus/persulcatus* species complex (Barker and Walker, 2014; Roberts, 1970), responsible for the transmission of well-described pathogens, including *Anaplasma*, *Ehrlichia*, and '*Ca*. Neoehrlichia' spp., in other parts of the world.

Recently, bacterial profiling studies of native Australian ixodids based on next-generation bacterial 16S rRNA gene (16S) metabarcoding, have revealed novel *Anaplasma*, *Ehrlichia*, and '*Ca*. Neoehrlichia' species that appear to be unique to Australia (Gofton et al., 2015a; Gofton et al., 2015b). For example, two new species, '*Ca*. Neoehrlichia australis' and '*Ca*. Neoehrlichia arcana', were recently identified in the Australian paralysis tick, *Ixodes holocyclus*, in eastern Australia (Gofton et al., 2015a; Gofton et al., 2015b), and further characterised by PCR and Sanger sequencing (Gofton et al., 2015a; Gofton et al., 2016). Phylogenetic analyses of these '*Ca*. Neoehrlichia' species demonstrated that they are closely related to the emerging zoonotic pathogen '*Ca*. Neoehrlichia mikurensis' that occurs in Europe and Asia (Gofton et al., 2016). '*Candidatus* Neoehrlichia australis' and '*Ca*. Neoehrlichia arcana' were also highly prevalent (8.7% and 3.1%, respectively) in *I. holocyclus* populations from its entire enzootic range (Gofton et al., 2015a; Gofton et al., 2016).

In Gofton et al. (2015a), novel *Anaplasma* and *Ehrlichia* 16S gene sequences were identified in the ornate kangaroo tick, *Amblyomma* (*Am.*) *triguttatum* subsp., from southwest Western Australia (WA). Preliminary analysis of partial 16S sequences from these bacteria suggested they are closely related to *A. bovis* and *E. ruminantium*, respectively, both of which have never been reported in Australia. Although a preliminary analysis of 16S gene sequences has been performed, detailed studies to confirm the taxonomic status of the novel *Anaplasma* and *Ehrlichia* from *Am. triguttatum* subsp. ticks are lacking.

The Australian native ixodid *Am. triguttatum* has several subspecies that are distributed through most of the Australian continent, and parasitise a wide variety of native and introduced fauna. In its enzootic range, this tick frequently bites domestic and companion animals, and is one of the most common ticks to bite people, especially in southwest WA (Gofton et al., 2015a; Greay et al., 2016).

In the present study, molecular techniques were used to identify and characterise *Anaplasma* and *Ehrlichia* species in four allopatric populations of questing *Am. triguttatum* subsp. ticks from southern, southwestern, and north-western Australia.

2. Methods

2.1. Tick collection and identification

Two subspecies of *Am. triguttatum* (*Am. t. triguttatum* and *Am. t. ornatissimum*) were collected from four allopatric populations across South and Western Australia; Yanchep National Park (NP) ($n = 280$), Bungendore Park ($n = 46$), Innes NP ($n = 214$), and Barrow Island ($n = 69$) (Fig. 1). A summary of *Am. triguttatum* subsp. life stages collected at each location is presented in Table 1.

Questing *Am. t. triguttatum* were collected from Yanchep NP and Bungendore Park from October-November 2016 by flagging the ground and low-lying vegetation with a 1 m^2 white flannel cloth stretched between two wooden dowels. Carbon dioxide (dry ice) baits were also used to attract ticks to flagging sites. Questing *Am. t. triguttatum* were collected from Innes NP on the southern tip of Yorke Peninsula, South Australia (SA) in January-December 2006, as part of a survey investigating the seasonal density and distribution of this tick in the area (Waudby and Petit, 2007). Questing *Am. t. ornatissimum* ($n = 69$) were collected opportunistically from the ground at the Barrow Island Nature Reserve from May-June 2016.

After collection, ticks were placed directly into 70% ethanol and stored at ambient temperature until molecular analysis. Ticks were morphologically identified into species, life stage, and sex under a stereomicroscope using standard keys for Australian ticks (Barker and Walker, 2014; Roberts, 1970).

2.2. DNA extraction and PCR

Prior to DNA extraction, the ticks' external surface was decontaminated in 10% sodium hypochlorite, washed in 70% ethanol, rinsed in sterile and DNA-free PBS, and air-dried. Individual ticks were then snap-frozen in liquid nitrogen for 1 min, and pulverised in a 2 ml microtube containing a 5 mm steel bead by beating at 40 Hz for 1 min. DNA was purified from tick homogenates using the DNeasy Blood and Tissue kit (QIAGEN, Germany) following the manufacturer's recommendations. Extraction reagent blank controls were included alongside DNA extractions. DNA extraction, PCR setup and DNA handling procedures were all performed in physically contained and separate dedicated laboratory areas, and PCR and post-PCR procedures were performed in separate dedicated laboratories.

All *Am. triguttatum* subsp. DNA samples were screened for *Anaplasma* and *Ehrlichia* DNA using several PCR assays targeting the phylogenetically informative 16S, 17 kDa heat shock protein (*groEL*), citrate synthase (*gltA*), and Major Antigenic Protein 1 (*map1*) genes (Table S1) (Anderson et al., 1991; Gofton et al., 2016; Kawahara et al., 2004; Liz et al., 2000; Loftis et al., 2006; Paddock et al., 1997; Rar et al., 2010; Weisburg et al., 1991). All PCR assays were performed in 25 μl volumes containing PCR buffer (KAPA Biosystems, South Africa), 2.5 mM $MgCl_2$, 1 mM dNTPs, 400 nM of each primer, and 0.5 U KAPA Taq DNA polymerase (KAPA Biosystems, South Africa). Primary PCRs used 2 μl of genomic DNA as template, and nested and hemi-nested PCRs used 1 μl of primary product as a template. All PCRs included no-template controls. All PCRs were performed with an initial denaturation at 95 °C for 3 min, followed by denaturation, annealing, and extension cycles as outlined in Table S1, followed by a final extension at 72 °C for 5 min.

All PCR products were electrophoresed through 1–2% agarose gels stained with SYBR safe (Invitrogen, USA), and amplicons of the correct length were excised from the gels and purified with the QIAquick gel extraction kit (QIAGEN, Germany) following the manufacturers recommendations. Resulting purified amplicons were sequenced with both PCR primers on an ABI 3730 96 DNA Analyser using Big Dye v3.1 terminators (Life Technologies, USA).

2.3. Phylogenetic analysis

16S, *groEL*, *gltA*, and *map1* nucleotide sequences were aligned with sequences from related *Anaplasma* and *Ehrlichia* species retrieved from GenBank (Benson et al., 2005). Sequences were aligned with MAFFT (Katoh et al., 2002), trimmed to remove terminal gaps, and alignments were refined with MUSCLE (Edgar, 2004). Statistical selection of the most suitable nucleotide substitution model based on the Bayesian information criteria as performed with jModelTest2 (Darriba et al., 2012; Guindon and Gascuel, 2003) for each sequence alignment, and Bayesian phylogenetic reconstructions were produced from alignments using

Download English Version:

https://daneshyari.com/en/article/5546334

Download Persian Version:

https://daneshyari.com/article/5546334

Daneshyari.com