

Rickettsial pathogens and arthropod vectors of medical and veterinary significance on Kwajalein Atoll and Wake Island

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Abstract—Modern surveys of ectoparasites and potential vector-borne pathogens in the Republic of the Marshall Islands and Wake Island are poorly documented. We report on field surveys of ectoparasites from 2010 with collections from dogs, cats, and rats. Five ectoparasites were identified: the cat flea *Ctenocephalides felis*, a sucking louse *Hoplopleura pacifica*, the mites *Laelaps nuttalli* and *Radfordia ensifera*, and the brown dog tick *Rhipicephalus sanguineus*. Ectoparasites were screened for rickettsial pathogens. DNA from *Anaplasma platys*, a *Coxiella* symbiont of *Rhipicephalus sanguineus*, and a *Rickettsia* sp. were identified by PCR and DNA sequencing from ticks and fleas on Kwajalein Atoll. An unidentified spotted fever group *Rickettsia* was detected in a pool of *Laelaps nuttalli* and *Hoplopleura pacifica* from Wake Island. The records of *Hoplopleura pacifica*, *Laelaps nuttalli*, and *Radfordia ensifera* and the pathogens are new for Kwajalein Atoll and Wake Island.

Introduction

Kwajalein Atoll in the Republic of the Marshall Islands houses the U.S. Army Kwajalein Atoll/Regan Test Site, which is inhabited by over 1,000 civilians, contractors, active duty military personnel and their families. Kwajalein Atoll has been occupied by the US military since 1944. Kwajalein Island, the largest island in the atoll, measures over 3.1 km² with an average elevation of 1.8 m above sea level. It is tropical, situated near the equator at 8°43' N, 167°43' E, and receives over 25 cm of rain a year. Wake Island is an atoll and part of the unincorporated territories of the USA. Wake Island is located further north at approximately 19°18' N, 166°38' E. The island is over 3.3 km² with a similar elevation to that of Kwajalein. There are no native human populations on Wake Island but up to several hundred military affiliated personnel work on the island. There is no natural standing or free flowing fresh water on either atoll.

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Surveys of vectors of human and animals diseases were conducted throughout the Pacific after World War II, but very little continued research has been focused on the smaller islands or island nations of the Pacific. Vector-borne diseases have emerged on several occasions on Kwajalein or the neighboring atolls of the Republic of the Marshall Islands. For example, an outbreak of dengue on Kwajalein in 1974 spread throughout the Pacific (WHO 1975). The arthropod fauna of Kwajalein Atoll was reviewed by Sugerman (1972, 1979). Medically significant arthropods included three mosquitoes, *Aedes aegypti* (L.), *Aedes marshallensis* Stone & Bohart, and *Culex quinquefasciatus* Say. In addition, the brown dog tick, *Rhipicephalus sanguineus* (Latreille), and the cat flea, *Ctenocephalides felis* (Bouché), were reported from the atoll (Sugerman 1972). No other ectoparasites of humans or animals were reported. A scorpion, *Isometrus maculatus* (De Geer), was also reported from buildings on the island (Sugerman 1972). Wake Island has historically been free of fleas and most biting arthropods. However, *Ae. aegypti* was established on Wake Island (Rosen et al. 1948). There are no current reports of biting arthropods or ectoparasites on Wake Island. Large populations of *Rattus* spp. are known from Kwajalein Atoll and Wake Island (Spennemann 1997), but their ectoparasites are largely undocumented.

Materials and Methods

We conducted a field survey of ectoparasites that are potential vectors of pathogens of medical and veterinary importance on Kwajalein Atoll from 19 - 23 July 2010. In addition, we spoke with the veterinary clinic staff on Kwajalein Atoll about historic records of parasites, pathogens, ticks, and fleas. Vector-borne pathogens and parasites were rarely documented at the clinic, but local animals on Ebeye Island (Kwajalein Atoll) were suspected of harboring significant tick populations. A walking survey of Ebeye Island revealed numerous tick infested local dogs. Ticks were removed from 2 local dogs with the owner's permission and stored in 95% ethanol. Adult fleas were collected from pets on Roi Namur Island (Kwajalein Atoll) by the veterinary staff and shipped to our laboratory.

Both Kwajalein Atoll and Wake Island have rodent infestations. Rats are routinely trapped by pest control personnel. We examined a freshly killed rat from the housing area on Kwajalein Island. The terminal 1 cm of the tail was clipped off and stored in 95% ethanol. The remainder of the rat was vigorously washed in 35% ethanol for approximately 1 min and carefully examined. All ectoparasites visible on the rat or in the ethanol wash were collected and preserved in 95% ethanol. In addition >100 rodents from Wake Island killed during routine activities in June 2010 were examined for ectoparasites by the local environmental officer. All ectoparasites and the rodent tails from Wake Island were preserved in RNAlater (Qiagen) and shipped to our laboratory for further study.

Arthropods were identified using keys for Micronesian species (Kohls 1957, Hopkins 1961, Wilson 1967, 1972a,b) or compared to reference specimens. Representative voucher specimens of each species were submitted to the Georgia

Southern University Ectoparasite Collection, the US National Tick Collection, or the arthropod collection at the Public Health Command Region - Pacific.

Nucleic acids were extracted from arthropods and rodents. Arthropods and rat tails were washed prior to DNA extraction by rinsing in distilled water for 30 seconds. Total DNA was extracted with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) and resuspended in 10% tris-HCL. DNA was screened for *Anaplasma*, *Babesia*, *Bartonella*, *Coxiella burnetii*, *Ehrlichia*, *Hepatozoon canis*, and *Rickettsia* spp. using real time PCR protocols described by Loftis et al (2006a) or Kledmanee et al. (2009). Positive samples were further characterized by traditional PCR and DNA sequencing.

The 16S rRNA gene was amplified from *Anaplasma/Ehrlichia* positive samples using the primer pairs EC12A and HE3 and RickF1 and RickR4 (Reeves 2005, Reeves et al. 2006). We amplified DNA from the 17 kD antigen gene of samples positive for *Rickettsia* using Primer-1 and Primer-2 as described by Webb et al. (1990) or by pairing Primer-1 and Primer-2 with the real time PCR primers to amplify smaller fragments. Controls for each assay included distilled water as a negative control and synthetic DNA oligonucleotides that corresponded to the primers and probe but have unique sequences as a positive control. The synthetic oligonucleotides can be differentiated from real agents by DNA sequencing.

PCR products were separated by electrophoresis on 4% agarose gels and visualized under ultraviolet light with ethidium bromide. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers, and excess dye was removed by ethanol precipitation. Sequences were determined using an ABI 3700 capillary sequencer (Applied Biosystems, Foster City, California), aligned and assembled with Chromas Lite 2.01 (Technelysium, Australia) and ClustalW (Kyoto University Bioinformatics Center, Japan), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland). DNA sequences from the 16S rRNA gene of a novel *Coxiella* sp. were deposited in GenBank (Accession # HQ116458) as was the 17kD antigenic gene sequence from a *Rickettsia* from Wake Island (Accession # HQ322623).

Results

The following five species of arthropods of medical or veterinary importance were collected during the survey.

Arachnida, Acari

Ixodidae, *Rhipicephalus sanguineus* (brown dog tick), (3 males, 4 females), from dogs, Kwajalein Atoll (Ebeye Island), 21 July 2010.

Laelapidae, *Laelaps nuttalli* Hirst, (1 female) from *Rattus rattus*, Kwajalein Atoll (Kwajalein Island), 20 July 2010; *Laelaps nuttalli* (27 females) from *Rattus exulans*, Wake Island, June 2010.

Myobiidae, *Radfordia ensifera* (Poppe) (1 female) from *Rattus* sp., Wake Island, June 2010.

Insecta

Phthiraptera, Hoplopleuridae, *Hoplopleura pacifica* Ewing, (2 males, 4 females), from *Rattus exulans*, Wake Island, June 2010.

Siphonaptera, Pulicidae, *Ctenocephalides felis*, (cat flea) (3 males), from cats on Kwajalein Atoll (Roi Namur Island), 3 September 2010.

A single *R. sanguineus* from Kwajalein Atoll (Ebeye Island) tested positive for *Anaplasma/Ehrlichia* by real time PCR. Two fragments of the 16S rRNA gene were amplified from that sample using independent PCR protocols. A 730 bp fragment of the 16S rRNA gene amplified with the EC12A/HE3 primer pair matched that of *Anaplasma platys* (GenBank Accession FJ686112). A 1140 bp fragment of 16S rRNA was amplified using the Rick1F/Rick4R primer pair and was a 98% match for the *Coxiella* symbiont of *R. sanguineus* (GenBank Accession D84559). One *C. felis* from Kwajalein Atoll (Roi Namur Island) tested positive for a spotted fever group *Rickettsia*. The 390 bp DNA sequence for the 17kD antigenic gene was 100% identical to *Rickettsia* sp. cf1and5 and other *Rickettsia* from fleas (GenBank Accessions AY953286, DQ166937). There are other identical DNA sequences in GenBank from uncultured *Rickettsia* detected in fleas. A pool of 27 *L. nuttalli* and 2 *H. pacifica* from Wake Island tested positive by real time PCR for *Rickettsia* in duplicate assays. However, the traditional PCR primers did not amplify enough DNA to sequence. Nested PCR with the real time PCR primers allowed amplification of a 76 bp fragment of the 17kD antigenic gene. The fragment was a 94% match for the 17 kD antigenic gene of *Rickettsia conorii* str. Malish 7 (complete genome GenBank Accession # AE006914) and a 100% match with the predicted protein sequence of *Rickettsia heilongjiangensis* (GenBank Accession # BAJ09683). All other assays were negative.

Discussion

Rhipicephalus sanguineus and *C. felis* were previously reported from Kwajalein Atoll (Sugerman 1972). US Army veterinary clinic personnel regularly examine animals and reported no populations established on Kwajalein Island. However, dogs on Ebeye and Roi Namur Islands are infested. Most of the local workers take the ferry between Ebeye and Kwajalein daily creating an avenue for introducing ectoparasites. Both *C. felis* and *R. sanguineus* are vectors of rickettsial and parasitic agents to humans and domestic animals.

Ctenocephalides felis is primarily an ectoparasite of medium sized mammals including cats and dogs; however, it will feed on humans and can transmit a wide range of pathogens. *Rickettsia* sp. cf 1 and 5 or related agents with identical gene sequences were previously reported from *C. felis* and other fleas in the USA, Asia, and Africa (Loftis et al. 2006b, Reeves et al. 2005). This *Rickettsia* is of unknown pathogenicity but some assays fail to differentiate it from *Rickettsia felis* (Reeves et al. 2005).

Unlike most ticks, *R. sanguineus*, can survive and breed successfully indoors. Dogs are the primary host for this tick, but it can feed on humans. It is the primary vector of *Rickettsia conorii*, the agent of Mediterranean spotted fever, in Europe and

Asia (e.g. Psaroulaki et al. 2003). *Rhipicephalus sanguineus* is a vector of canine ehrlichiosis, anaplasmosis, babesiosis, and hepatozoonosis (Shaw et al. 2001). The detection of *A. platys*, an agent of canine cyclic thrombocytopenia (Shaw et al. 2001), in a tick from Ebeye indicates the canine disease is circulating on Kwajalein Atoll. *Anaplasma platys*, infects dog platelets and often co-infects dogs along with *Ehrlichia canis*. Symptoms of canine cyclic thrombocytopenia can range from asymptomatic to a fatal hemorrhagic disease.

Two species of ectoparasitic mites *L. nuttalli* and *R. ensifera* and a louse *H. pacifica* were collected from rats. All three species are among the most widespread ectoparasites of peridomestic rats throughout much of the world. Traub et al. (1978) implicated *H. pacifica* as a vector of *Rickettsia typhi*, the agent of murine typhus. Neither *L. nuttalli* nor *R. ensifera* have been implicated as vectors of *R. typhi*. The DNA in the ectoparasites was poorly preserved and partially degraded, possibly related to their storage in RNA later. However, the detection of a spotted fever group *Rickettsia* from the combined pool of *L. nuttalli* and *H. pacifica* from Wake Island indicates that a *Rickettsia* is present there. This *Rickettsia* might be transmitted by the ectoparasites but they could have acquired it in a blood meal or it could be an endosymbiont. Other potential vectors should be examined including bird ticks, fleas, other mites, or lice. A longer DNA sequence or a live isolate could determine the species present. The threat to human health is unknown. Kwajalein Atoll and Wake Island support large populations of *Rattus* spp. Large scale surveillance of rats for ectoparasites and rodent-borne disease has not been conducted.

Overall the arthropod vectors on Kwajalein Atoll and Wake Island have apparently changed little since the surveys by Sugarmann (1972, 1979). However, we have demonstrated that some ectoparasites harbor rickettsial agents on Kwajalein Atoll and Wake Island. They could transmit these agents to humans or domestic animals but further study is needed to assess the risk of transmission. Our survey is preliminary and a larger survey of ectoparasites is needed to fully characterize the agents present on Wake Island or Kwajalein Atoll.

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