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Invertebrate Vectors, Parasites, and Rickettsial Agents in Guam

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Abstract—We conducted a 3-week field study of ectoparasites of humans and domestic animals throughout Guam. Thirteen species of ectoparasitic arthropods were collected. Ectoparasites of medical or veterinary significance included the ticks *Rhipicephalus sanguineus* and *Rhipicephalus microplus*, fleas *Ctenocephalides felis* and *Xenopsylla cheopsis*, and the head louse *Pediculus humanus capitis*. Polymerase chain reaction based screening for rickettsial and protozoan pathogens detected pathogens in eight arthropods. These included *Anaplasma platys, Anaplasma marginale, Coxiella burnetii, Babesia canis vogeli,* and *Hepatozoon canis*.

Guam is a tropical Pacific island with a history of vector-borne disease outbreaks. Based on unpublished epidemiological data from the Guam Public Health Department, there have been epidemics and introductions of numerous vector-borne tropical diseases throughout the history of the island. Infected individuals

arrive in Guam from neighboring islands, Asia, Australia, and the Americas and present at hospitals. For example, an outbreak of Japanese encephalitis occurred in the Commonwealth of the Northern Marianas Islands in 1990 (Paul et al. 1993), and some infected individuals traveled to Guam for treatment. Other diseases such as murine typhus and tick-borne rickettsial diseases are inevitably present on Guam and remain unreported or misdiagnosed.

Ectoparasite-borne rickettsial diseases have historically caused significant morbidity and mortality for militaries throughout the world (Moe & Pedersen 1980). Projected military buildups on Guam increase the chances of human and vector exposure. There is no active surveillance of vector-borne diseases at major ports of entry, major military installations, National Guard facilities, or by the Guam Public Health Department.

Most published reports of vectors and pathogens on Guam are 30–60 years old (Alicata 1948, Kohls 1957, Hammon et al. 1958, Hopkins 1961, Wilson 1967, 1972a, 1972b) and probably no longer accurate. Continuous surveillance for vectors is critical for protecting human and veterinary health. New exotic species and associated pathogens can be introduced on planes or ships when tourists and their pets travel to Guam. For example, exotic black flies (Simuliidae) were discovered on Guam in 2010 (Reeves & Adler 2011). Thousands of tourists travel to Guam annually, and when even small percentages are infected with pathogens, the risk for introducing disease is significant. For example, a Japanese tourist with schistosomiasis was documented traveling to Guam when he presented at a hospital for complications (Matsuda et al. 1999). Unfortunately, many vector-borne diseases can be misdiagnosed as better known diseases such as measles, rubella, hepatitis, or nonspecific "flu-like" illnesses. Outbreaks of introduced diseases are possible, so continued vigilance is needed to detect and prevent epidemics. The combination of a year-round tropical climate, introduced pathogens, established vectors, and lack of medical surveillance could be disastrous. With plans for a continued military buildup on Guam, an active surveillance system is critically needed.

We conducted a 3-week vector and pathogen survey in March 2010 on military bases and civilian sites throughout the island and collected a wide range of arthropods of medical and veterinary significance. New records of mosquitoes, black flies, and biting midges are reported elsewhere (Nunn et al. 2011, Reeves & Adler 2011, Rueda et al. 2011, Swanson & Reeves 2011). We report on the ectoparasites and molecular surveillance of associated pathogens.

Materials and Methods

Ectoparasites were collected by hand by U.S. Department of Defense, Department of Agriculture, and Guam Public Health Department personnel at veterinary clinics during routine examinations or from dead animals trapped by pest control personnel or found on roads. Head lice were collected by Guam Public Health Department personnel from humans. Specimens were identified by comparison to reference materials or with the taxonomic keys and published records for fleas, lice, and ticks (Wilson 1967, 1972a, 1972b, Hopkins 1961, Kohls 1957).

Initially each ectoparasite was macerated with a sterile razorblade and crushed with a Teflon pestle before digesting the remains with Protinase K. Total DNA was extracted from individual or pooled ectoparasites (Table 1) with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) and resuspended in 10% tris-HCL and nuclease free water. Extracts were screened for DNA from *Anaplasma, Bartonella, Coxiella burnetii, Ehrlichia,* and *Rickettsia* by real time polymerase chain reaction (PCR) following the protocols described by Loftis et al. (2006). Ticks from dogs were tested for *Babesia* spp. and *Hepatozoon canis* using the PCR assays described by Kledmanee et al. (2009). Fleas, mites, and chewing lice were tested for filarial nematodes using the PCR assays described by Nuchprayoon et al. (2005). Positive samples were further characterized by sequencing DNA from PCR amplicons. Controls included a distilled water negative control and a positive control consisting of unique synthetic DNA oligonucleotides that corresponded to the primers and probe. These oligonucleotides have sequences unlike any known agent and can be differentiated from real agents by DNA sequencing if positive control contamination was suspected. We amplified DNA from the 17 kD antigen gene of *Rickettsia* using Primer-1 and Primer-2 (Webb et al. 1990), the bacterial 16S rRNA gene of *Anaplasma* and *Ehrlichia* using the EC12A and HE3 primers (Reeves et al. 2006a), and the *gltA* gene of *Bartonella* using the BhCS.781 and BHCS.1137 primers (Norman et al. 1995). In addition, we amplified the 16S rRNA gene from selected samples with the RickF1 and RickR4 primers described by Reeves (2005). 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).

Voucher specimens for the ectoparasites collected were deposited in the Georgia Southern University Parasite Collection or the U.S. National Tick Collection. No DNA sequences were deposited in GenBank, because there were no novel genotypes.

Results

We collected 13 species of blood-feeding or ectoparasitic arthropods (Table 1). The majority of the specimens were collected on domestic animals. Some of these are unlikely to be vectors of either human or veterinary diseases. Rickettsial and protozoan pathogens were detected in eight arthropods (Table 1). These pathogens included *Anaplasma platys, Anaplasma marginale, Coxiella burnetii, Babesia canis vogeli,* and *Hepatozoon canis*. *Coxiella burnetii* is the agent of Q fever, a potentially significant disease in humans and animals. The other pathogens were primarily veterinary.

Table 1. Ectoparasites and blood-feeding arthropods collected on Guam with associated pathogens

Location	Date (2010)	Host	Family	Species	Number Collected	Pathogens Detected (Positive Samples)
Barrigada	23 February	*Rattus rattus*	Pulicidae	*Xenopsylla cheopsis*	7	None
Barrigada	23 February	*Rattus rattus*	Macronyssidae	*Ornithonyssus bacoti*	1	None
Yigo	25 February	*Sus scrofa*	Ixodidae	*Rhipicephalus microplus*	1	None
Yigo	25 February	*Sus scrofa*	Haematopinidae	*Haematopinus suis*	1	None
Agana Heights	5 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	11	*Anaplasma platys* (1), *Hepatozoon canis* (1)
Dededo	5 March	*Gallus gallus*	Menoponidae	*Menacanthus stramineus*	2	None
Yigo	8 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	46	*Babesia canis* (1), *Anaplasma platys* (1), *Coxiella burnetii* (2)
Yigo	8 March	*Canis lupus familiaris*	Pulicidae	*Ctenocephalides felis*	8	None
Yigo	8 March	*Felis catus*	Pulicidae	*Ctenocephalides felis*	1	None
Yigo	8 March	*Felis catus*	Trichodectidae	*Felicola subrostratus*	10	None
Yigo	8 March	*Capra hircus*	Trichodectidae	*Bovicola caprae*	1	None
Inarajan	6 March	*Gallus gallus*	Menoponidae	*Menacanthus stramineus*	3	None
Talofofo	6 March	*Bubalus bubalis*	Haematopinidae	*Haematopinus tuberculatus*	8	None
Dededo	8 March	*Canis lupus familiaris*	Trichodectidae	*Trichodectes canis*	5	None

Dededo	8 March	*Canis lupus familiaris*	Pulicidae	*Ctenocephalides felis*	9	None
Santa Rita	9 March	*Canis lupus familiaris*	Pulicidae	*Ctenocephalides felis*	1	None
Talofofo	6 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	3	*Coxiella burnetii* (1)
Yona	9 March	*Canis lupus familiaris*	Pulicidae	*Ctenocephalides felis*	6	None
Yona	9 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	2	None
Yona	9 March	*Gallus gallus*	Menoponidae	*Menacanthus stramineus*	7	None
Santa Rita	10 March	*Cervus marianus*	Ixodidae	*Rhipicephalus microplus*	22	None
Dededo	11 March	*Homo sapiens*	Pulicidae	*Ctenocephalides felis*	4	*Coxiella burnetii* (1)
Mangilao	11 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	4	None
Mangilao	11 March	*Capra hircus*	Trichodectidae	*Bovicola caprae*	8	None
Santa Rita	11 March	*Bubalus bubalis*	Ixodidae	*Rhipicephalus microplus*	1	None
Talofofo	11 March	*Bubalus bubalis*	Ixodidae	*Rhipicephalus microplus*	2	None
Yigo	11 March	*Bos taurus*	Ixodidae	*Rhipicephalus microplus* *Anaplasma marginale*	17 2	None
Yigo	11 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	4	None
Mangilao	15 March	*Canis lupus familiaris*	Pulicidae	*Ctenocephalides felis*	10	None
Mangilao	15 March	*Canis lupus familiaris*	Ixodidae	*Rhipicephalus sanguineus*	5	None
Dededo	16 March	*Gallus gallus*	Menoponidae	*Menopon gallinae*	3	None
Ordot	21 April	*Homo sapiens*	Pediculidae	*Pediculus humanus capitis*	16	None

Discussion

Two tick species were collected on Guam. The most significant for humans and military working dogs was *Rhipicephalus sanguineus*. It is a vector of parasitic and rickettsial agents to humans and dogs. *Rhipicephalus sanguineus* is the primary vector of *Rickettsia conorii,* the agent of Mediterranean spotted fever, to humans in Europe, Africa, and Asia (e.g., Psaroulaki et al. 2003). Dogs are the main host for this tick; however, it can feed on humans. Unlike most ticks, *R. sanguineus* can survive and breed successfully indoors. Foci of *R. sanguineus* that fed on humans were reported on several U.S. military bases (Goddard 1989).

Rhipicephalus microplus, a cattle tick, was collected from deer, cattle, water buffalo, and feral pigs. This one-host tick is a vector of numerous bacterial and parasitic pathogens of livestock. Because of the economic damage caused by bovine babesiosis and anaplasmosis, this tick is among the most significant ectoparasites of livestock in the tropics and subtropics worldwide (Camus & Montenegro-James 1994).

A blood-feeding mite, *Ornithonyssus bacoti*, was collected. This mite is primarily an ectoparasite of rodents; however, it can bite humans. Its vectoral capacity for rickettsial agents is poorly studied. In Egypt, it was associated to both *Rickettsia* and *Bartonella* spp. of unknown pathogenicity (Reeves et al. 2007). The mite can be found on Guam, but with small sample sizes we couldn't determine if it harbored any pathogens. Previous surveys of rats on Guam also reported populations of *Laelaps echidninum* (Alicata 1948). Our sampling of rodents was small and inevitably underrepresents the ectoparasite fauna of rodents in Guam. Reeves et al. (in press) reported a spotted fever group *Rickettsia* from *Laelaps nuttalli*, a rodent mite, found on rats in Micronesia.

Several other species of ticks and ectoparasitic mites occur on Guam. However, their significance as vectors of pathogens to humans or wildlife is poorly studied and unknown. The bird tick, *Carios capensis,* was reported on Guam (Kohls 1957). Several potentially pathogenic *Rickettsia, Coxiella*, and *Borrelia* spp. were reported from *C. capensis* in the eastern U.S. (Reeves et al. 2006c). Two species of endemic *Amblyomma* are known from Guam, but they were not collected or tested for pathogens.

The flea collections revealed a limited diversity with two nearly cosmopolitan species of fleas: the cat flea *(Ctenocephalides felis)* and Oriental rat flea *(Xenopsylla cheopsis)*. Hopkins (1961) reported a questionable collection of a bat flea, *Ischnopsyllus indicus*, from Guam, but we were unable to collect this species.

Xenopsylla cheopsis is an ectoparasite of peridomestic rats and is a vector of murine typhus and plague (Hopkins 1961, Farhang-Azad et al. 1985). Hopkins (1961) previously reported *X. cheopsis* from Guam. The second species, *Ctenocephalides felis*, is an irritating pest of humans and domestic animals. It is a vector of several zoonotic pathogens including *Bartonella henselae, Dipetalonema reconditum, Dipylidium caninum, Rickettsia felis,* and *Rickettsia typhi* (Farnell & Faulkner 1978, Azad 1990, Chomel et al. 1996, Reif & Macaluso 2009) and numerous other pathogens and parasites of animals. *Ctenocephalides felis* is possi-

bly the most significant ectoparasite collected during this survey because it readily feeds on humans and transmits a wide variety of pathogens. A novel spotted fever group *Rickettsia* was reported from cat fleas from the Marshall Islands, which are close to Guam (Reeves et al. in press).

Four species of chewing lice and three species of sucking lice were collected (Table 1). No pathogens or parasites were detected in the lice. The chewing lice *Bovicola caprae, Felicola subrostratuss,* and *Menacanthus stramineus* and sucking louse *Haematopinus tuberculatus* are generally not considered vectors of human or animal pathogens. The pig louse, *Haematopinus suis*, can transmit swinepox virus and *Mycoplasma parvum* (Durden 2002), but it is unlikely to be a major vector. *Trichodectes canis*, a chewing louse of dogs, is an intermediate host for *D. caninum*, the dog tapeworm (Melnikow 1869), but fleas are more likely to be the primary hosts for the dog tapeworm. Neither fleas nor lice were tested for *D. caninum*.

There was a collection of human head lice, *Pediculus humanus capitis*. This ectoparasite is widespread in Guam. *Pediculus humanus capitis* is not traditionally considered a vector of pathogens, but recent molecular studies in Nepal indicate that head lice might play a role in the transmission of *Bartonella quintana,* the agent of trench fever (Sasaki et al. 2006). However, the molecular association between head lice and bacterial pathogens is far from proof that they can be vectors. All three species of human lice could be present on Guam. Pubic lice, *Pthirus pubis,* are less frequently reported due to social stigmas. Body lice, *Pediculus humanus humanus,* are more common in cold regions. The body louse is a vector of *Bartonella quintana* and *Rickettsia prowazekii*. These agents circulate in urban homeless populations in the continental U.S. (Comer et al. 2001, Reeves et al. 2008) and could be introduced to Guam. Several additional species of lice should be present on Guam. These include the lice of house mice and peridomestic rats. Lice from *Rattus* spp. are associated with rickettsial pathogens (Reeves et al. 2006b).

The results of molecular screening (Table 1) demonstrate that several potential vectors infected with pathogens were present on Guam. We briefly discuss the relevance of the pathogens detected.

Two DNA sequences amplified from from dog ticks were identical to those of those of *Anaplasma platys* in GenBank. *Anaplasma platys* is widely distributed in the tropics and subtropics where *R. sanguineus* has been incriminated as the probable vector (Inokuma et al. 2000, Sanogo et al. 2003). Prevention of *A. platys* by tick control has not been demonstrated, which raises some doubt that the sole-source of transmission is tick-bite (Shaw et al. 2001). *Anaplasma platys*, an agent of canine cyclic thrombocytopenia (Shaw et al. 2001), infects dog platelets. Infected dogs can be asymptomatic or suffer a fatal hemorrhagic disease. Coinfections with *Ehrlichia canis* possibly lead to severe disease (Sanogo et al. 2003). *Ehrlichia canis* was reported by several small animal veterinarians on Guam during our site visits (unpublished data).

DNA sequences identical to *Anaplasma marginale* came from several tick pools. It is endemic in livestock throughout the topics and subtropics and causes bovine anaplasmosis, which is an economically significant disease of livestock

(Shimada et al. 2004). *Anaplasma marginale* can be maintained in chronically infected animals, is vertically transmitted in ticks, and persists even on small islands (Camus & Montenegro-James 1994, Shimada et al. 2004). *Anaplasma marginale* is not pathogenic to humans.

DNA sequences of the IS1111 gene of *C. burnetii*, the agent of Q fever, were sequenced from pools of ticks and fleas. Clinical Q fever in humans varies from a mild fever to pneumonia, hepatitis, or death (Hartzell et al. 2007, Woldehiwet 2004). Numerous arthropods including ticks, flies, and fleas have all been associated with *C. burnetii* in other locales (Spyridaki et al. 2002, Loftis et al. 2006, Nelder et al. 2008). Ectoparasites probably acquire the bacterium when feeding on blood, feces, or infected fluids. We used a quantitative PCR assay described by Loftis et al. (2006) for *C. burnetii*. The number of bacteria in the samples was low, with 5-50 bacteria/μL in the positive samples. However, the infectious dose for Q-fever is 1 bacterium (Hatchette et al. 2001), and even small numbers of bacteria are potentially significant. Arthropods can be vectors and reservoirs of *C. burnetii* (Spyridaki et al. 2002), but the vector competence of *R. sanguineus* and *C. felis* is not well characterized.

We screened ticks for *Babesia* and *Hepatozoon* spp., which are pathogenic protozoans. Both *Babesia canis vogeli* and *Hepatozoon canis* were detected in dog ticks. DNA sequences were identical matches to several in GenBank. They are transmitted by the same vector, *R. sanguineus*, and can co-infect susceptible animals (Kledmanee et al. 2009). In addition, *Hepatozoon canis* often co-infects dogs infected with *E. canis* or *Leishmania* spp. (Shaw et al. 2001). There are no known vectors of *Leishmania* spp. in Guam (Quate 1959); however, based on serologic tests, *E. canis* was reported by several small animal veterinarians on Guam (unpublished data). *Babesia* spp. are transmitted by ticks while feeding, but *Hepatozoon* spp. are transmitted when the vector is consumed by the potential host. Dogs infected with either agent can become chronically infected or die (Baneth et al. 2003, Shaw et al. 2001).

The ectoparasite fauna of Guam is limited when compared to that of larger Pacific islands, mainland Asia, Australia, or North America. However, we collected several parasites that feed on both domestic animals and humans. Pathogens were detected in several of these. The survey was limited in scope and we did not sample all animal species known from Guam. Rickettsial pathogens are known from ectoparasites in other Micronesian nations (Reeves et al. in press). Additional studies with larger surveys could present a more complete assessment of the threat of parasitic and vector-borne pathogens to humans and domestic animals on Guam.

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