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Survey for selected pathogens in Philippine deer (*Rusa marianna*) from Guam, Marianna Islands, USA

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ABSTRACT

Philippine deer (*Rusa marianna*), native to the Philippine Islands, were introduced to Guam in the late 1700's. Dense populations have become established throughout the island where they cause damage to native plant communities resulting in habitat degradation. In addition, cervids can serve as reservoirs for important pathogens of livestock and people. From February–March 2015, blood, tissue and ectoparasite samples were collected from 132 free-ranging Philippine deer on Guam. Data from 10 deer sampled in 1997 were also analyzed. Deer were negative for antibodies to many of the pathogens assessed including epizootic hemorrhagic disease virus, parainfluenza 3 virus, bovine viral diarrhea virus, bovine herpesvirus 1, and *Brucella* spp.; however, two (2%) and nine (7%) deer were seropositive for bluetongue virus and *Toxoplasma gondii*, respectively. Five (4%) deer had low titers (1:100) to *Leptospira interrogans* serovars Bratislava (n = 4), Canicola (n = 2), and Icterohaemorrhagiae (n = 1). None of the kidney samples from *Leptospira*-seropositive deer were immunohistochemically positive for leptospires. No nematodes or trematodes were detected in lungs, abdomen, abomasum or liver. A few deer had 1–4 *Cooperia* spp. in the small intestine, although very small nematodes may have not been captured by the #100 mesh used for screening. Of the 105 deer evaluated for ectoparasites, 90.5% were infested with *Rhipicephalus microplus*. Tick burdens were generally high and classified as low (< 500 ticks) (59% of infested deer), medium (500–1000 ticks) (22%), and high (> 1000 ticks) (19%). Molecular testing of blood samples for *Babesia* spp. was negative, but 11 (8%) deer were positive for *Anaplasma* spp. Sequence analysis revealed that deer were infected with three species of *Anaplasma* including *A. marginale*, *A. phagocytophilum*, and an *Anaplasma* sp. similar to *A. platys*. Finding *A. marginale*, *T. gondii*, *Leptospira* and heavy burdens of ticks in Philippine deer is of economic and public health importance.

1. Introduction

Wildlife species have been implicated in the transmission and maintenance of many emerging infectious diseases around the world (Miller et al., 2012; Gortázar et al., 2016). Cervids in particular are reservoirs and hosts for many pathogens and vectors of importance to human and livestock health (Rhyan and Spraker, 2010). However, data on the role of wildlife in pathogen transmission is poorly understood in many regions of Southeast Asia, notably Guam, a United States territory and part of the Mariana Islands.

Guam is 540 km^2 and has two distinct geological regions; a coral limestone plateau in the northern half of the island and a mixture of volcanic hills and valleys in the southern half. The current population is 174,445 and residents live in numerous small villages (largest village has only 45,000 residents), primarily in the central and northern parts of the island. Farming activities occur throughout the island and are primarily subsistence, small-scale operations with various crops and a variety of livestock (goats, cattle, swine, horses, and chickens) (Duguies et al., 2000). Nearly a third of the island's land (~16,000 ha) is included in several U.S. military bases, which have limited access and

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Table 1
Pathogens, diagnostic assays and diagnostic laboratories used for pathogen surveillance for Philippine deer from Guam.

Pathogen type	Pathogen	Assay[a]	Diagnostic laboratory[b]	No. positive/no. tested (%)[c]			
				AAFB 1997	AAFB 2015	NGB 2015	Total
Viruses	Epizootic hemorrhagic disease virus	AGID	UGAVDL	0/10	0/57	0/70	0/137
	Bluetongue virus	AGID	UGAVDL	0/10	0/57	2/70 (3)	2/137 (1.5)
	Parainfluenza 3 virus	SN	UGAVDL	0/10	0/57	0/70	0/137
	Bovine viral diarrhea virus	SN	UGAVDL	0/10	0/57	0/70	0/137
	Bovine herpesvirus 1 (infectious bovine rhinotracheitis)	SN	UGAVDL	0/10	0/57	0/70	0/137
Bacteria	*Brucella* spp.	Card test	UGAVDL	0/10	0/57	0/69	0/136
	Leptospira interrogans	MAT, IHC	UGAVDL	0/10	3/57 (5)	2/69 (3)	5/136 (4)
	Anaplasma spp. (all species detected)	PCR	SCWDS	N.D.[d]	5/57 (9)	6/70 (9)	11/127 (8)
	A. platys-like sp.	PCR	SCWDS	N.D.	4/57 (7)	6/70 (9)	10/127 (8)
	A. marginale	PCR	SCWDS	N.D.	1/57 (2)	0/70	1/127 (0.8)
	A. phagocytophilum	PCR	SCWDS	N.D.	0/57	1/70 (1)	1/127 (0.8)
Parasites	*Babesia* spp.	PCR	SCWDS	N.D.	0/57	0/70	0/127
	Toxoplasma gondii	MAT[e]	USDA	N.D.	7/57 (12)	2/70 (3)	9/127 (7)

[a] AGID: agar gel immunodiffusion; SN: serum neutralization; IHC: immunohistochemistry; MAT (*Leptospira*): microscopic agglutination test; MAT (*Toxoplasma*): modified agglutination test; PCR: polymerase chain reaction.
[b] SCWDS: Southeastern Cooperative Wildlife Disease Study; UGA VDL: University of Georgia Veterinary Diagnostic Laboratory; USDA: United States Department of Agriculture.
[c] Blood and serum was not collected from every individual.
[d] N.D., not done.
[e] As described by Dubey and Desmonts (1987).

residents.

The introduction of exotic animals on Guam, most notably the brown tree snake (*Boiga irregularis*), wild pigs (*Sus scrofa*) and Philippine deer (*Rusa marianna*), has led to extensive predation and habitat destruction resulting in population reductions or extirpation of many bird species (Savidge, 1987). In addition, pathogens in these introduced ungulate species may be a concern. The diseases of domestic species on Guam and feral animals such as pigs, deer, and water buffalo (*Bubalus bubalis*) are poorly studied. The last survey, focused on domestic animals, was conducted in 1999 and although animals were found to generally be in good health, several diseases and parasites were identified (Duguies et al., 2000). However, a recent survey of introduced wild pigs on Guam revealed exposure to several pathogens of importance (e.g., *Leptospira*, *Brucella*, and *Toxoplasma gondii*) that also may be found in cervids or the domestic animals present on Guam (Cleveland et al., 2017). In the 1770's, Philippine deer were introduced to Guam and populations have been expanding due to decreased hunting (Wiles et al., 1999) and are now a concern due to habitat destruction, but also because they are hosts for ticks (*Rhipicephalus microplus*) of agricultural concern (Reeves et al., 2012; Vander Velde and Vander Velde, 2013).

Introduced pigs and deer pose a risk to human, livestock, and other wildlife in numerous ways. In addition to wildlife habitat destruction and disease concerns described above, these species pose a human risk due to hunting accidents due to both legal and illegal (poaching) hunting. Also, deer pose concerns to cultural resources (e.g., deer alter the distribution and abundance of local medicinal plants and facilitate the establishment of invasive plant species that may destroy archeological sites). In 2015, management actions were undertaken to reduce these concerns through wild deer and pig population control methods within fenced areas of two military bases on Guam. During this program, we conducted a comprehensive pathogen surveillance study of the Philippine deer to investigate their potential role in the maintenance or transmission of pathogens of economic and public health concern.

2. Materials and methods

2.1. Sites

Andersen Air Force Base (AAFB) is a 4135 ha military installation in northern Guam (13.5875°N, 144.9244°E). Forested portions of the base contain high quality native habitat, some of which is included in the Guam National Wildlife Refuge. The Naval Base Guam Naval Munitions Site (NBG NMS) is centrally located on the island and covers approximately 5723 acres (13.44000°N 144.65250°E).

2.2. Sample collection

Samples were collected from March to April 2015. Well-trained marksmen humanely dispatched Philippine deer using suppressed 0.223 caliber rifles with scope attachments from a vehicle or from the ground over bait. During removal, shooters only fired if the situation met the following criteria: 1) there was certainty that the animal would be dispatched and not escape, 2) if other animals were nearby, every animal had a high probability of being dispatched, and 3) it was safe to dispatch the animal. The shooting methods followed the American Veterinary Medical Association's guidelines for humane euthanasia of animals (AVMA, 2013) and animal and sample collection procedures were reviewed and approved by UGA's Institutional Animal Care and Use Committee (A2014 09-021).

Immediately after euthanasia, blood samples were collected via cardiocentesis and placed into ethylenediaminetetraacetic acid and plain tubes (Greiner Bio-one, Monroe, NC, USA). Clotted blood was centrifuged at 1250*g* for 15 min and serum was removed. Whole blood and serum were frozen at − 20 °C until diagnostic testing. Each animal was weighed, sexed, aged by tooth eruption and wear, and reproductive and antler status was recorded. Ticks and parasites from the lung, liver, and gastrointestinal tract were collected, counted, and preserved in 95% ethanol for identification. Tick loads were categorized into low (< 500 ticks), medium (500–1000 ticks), and high (> 1000 ticks).

2.3. Diagnostic testing

Necropsies were conducted on 101 deer; however, serum, blood, and ectoparasites were collected from an additional 31 deer that were not necropsied. Information on pathogen screening performed on deer as well as diagnostic assays and diagnostic laboratories used are listed in Table 1. The entirety of the abomasum and intestinal contents were screened through a fine mesh screen (#100 mesh, 149 μm) and concentrated materials were preserved in 70% ethanol until they were examined under a dissecting scope for parasites which were identified