ZOONOTIC AND INFECTIOUS DISEASE SURVEILLANCE IN ECUADOR: *EHRLICHIA CANIS*, *ANAPLASMA PHAGOCYTOPHILUM*, *BORRELIA BURGDORFERI*, AND *DIROFILARIA IMMITIS* PREVALENCE RATES IN CANINES

Implications for Special Operations Forces Medical Operations, Personnel, and Canines

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ABSTRACT

Vector-borne diseases (VBD) make up a large number of emerging infectious and zoonotic diseases. Ticks, fleas, and mosquitoes are effective vectors parasitizing canines, making dogs adequate reservoirs for zoonoses. The U.S. military deploys personnel and government-owned animals around the world with possible risk of exposure to VBD. Canine VBD have veterinary and public health significance for the host nations as well as for the U.S. troops and its working animals deployed in the theater of operations. These factors make disease surveillance a great importance. The objective of this work was to survey canines from the cities of Manta and Guayaquil in Ecuador to determine prevalence of heartworm disease (*D. immitis*), ehrlichiosis (*E. canis*), Lyme disease (*B. burgdorferi*), and anaplasmosis (*A. phagocytophilum*). Canine blood samples (1-3ml) collected from the cities of Manta (n=50) and Guayaquil (n=50) were tested on site using a SNAP® 4Dx® Test Kit. Prevalence for single or multiple disease status was calculated for each city. In the city of Manta the overall prevalence of diseases was 78%; 52% for *E. canis* alone, and 26% for co-infection with *E. canis* and *A. phagocytophilum*. The overall prevalence for the city of Guayaquil was 88%; 40% for *E. canis* alone, 22% for *A. phagocytophilum* alone, and 26% for co-infection with *E. canis* and *A. phagocytophilum*. Neither heartworm disease nor Lyme disease was detected in any sample. In conclusion, this study showed the extensive presence of *E. canis* and *A. phagocytophilum* in both cities in Ecuador, emphasizing the value of surveillance for zoonotic diseases to determine disease prevalence and risk assessments, as well as to implement control measures.

INTRODUCTION

Vector-borne diseases (VBD) are rapidly emerging and globally distributed.[1,2] In recent years, large numbers of the emerging infections and zoonotic (animal disease that can be transmitted to humans) diseases are described to be caused by arthropod-borne pathogens.[2] Ticks, fleas, mosquitoes, and phlebotomine sand flies are ectoparasitic arthropods and effective vectors of a large number of pathogens. Canine populations are susceptible to arthropod parasites, making dogs adequate reservoirs for infectious animal and zoonotic diseases such as heartworm disease (*Dirofilaria immitis*), ehrlichiosis (*Ehrlichia canis*), Lyme disease (*Borrelia burgdorferi*), and anaplasmosis (*Anaplasma phagocytophilum*).[3-8] The U.S. military deploys personnel and government-owned animals to different regions of the world with possible risk of exposure to vector-borne diseases.[7-11] Because of veterinary and public health significance and the impact of canine vector-borne diseases in susceptible populations in the host nations as well as in U.S. troops and animals, surveillance of such disease is of great importance for current and future military deployments in order to decrease disease exposure risk and occupational and environmental hazard.[10,12-16] The objective of this work was to survey canines from the cities of Manta and Guayaquil in Ecuador in order to determine prevalence of heartworm disease, Lyme disease, ehrlichiosis, and anaplasmosis.

MATERIAL AND METHODS

Canine blood samples were collected in the Ecuadorian port cities of Manta and Guayaquil (Figure 1). The city of Manta is lo cated in the province of Manabí in the central western Ecuador (0º 57' 0.08" S 80º 42' 58.3" W), and the city of Guayaquil is located in the province of Guayas (2º 11' S 79º 53' W) on the banks of the Guayas River. Guayaquil was selected for this study because it is Ecuador's largest and most populated city and Manta because of the U.S. troop and civilian contingency assigned to the U.S. Military Base there. A sample each of n=50 canines from Manta and Guayaquil were included (n=100). Dogs volunteered by local veterinary clinics and pet grooming facilities were included. Samples also included free roaming dogs collected by veterinary clinics' staff or brought into the clinic for a veterinary exam by a community member (Figure 2). A 1-3cc blood sample was obtained by venipuncture of the cephalic vein from each canine, and the sample was divided and placed into a tube with EDTA and a tube without additives. Each sample (whole blood) was tested on site for heartworm disease (*D. immitis* antigen), ehrlichiosis (antibody to *E. canis*), Lyme disease (antibody to *B. burgdorferi*), and anaplasmosis (antibody to *A. phagocytophilum*), using a SNAP® 4Dx® Test Kit (IDEXX Laboratories, Inc., Westbrook, ME), following the manufacturer's instructions.[17] SNAP® 4Dx® is a rapid assay test system using enzyme-linked immunoabsorbent assay (ELISA). After testing, samples were transported under cold conditions to a refrigerated unit and store at -20ºC. Results from each blood sample were recorded as positive or negative for any of the diseases tested. In addition, the number of positive or negative samples for each individual disease, as well as the samples positive to more than one disease agent were recorded.

Statistical Analysis

Local prevalence for single or multiple disease status was calculated as the proportion of positive samples from the total of the samples tested on each city. Odd ratios (statistical odd is referred to the odd of an event occurring calculated by dividing the probability of the event by the probability of the event not occurring) for single or multiple diseases among cities were compared by logistic regression analysis using the PROC LOGISTIC function of the Statistical Analysis System (SAS 9.2).[18]

Results

In Manta the overall prevalence for the tested diseases was 78% (Table 1). Dogs positive for *E. canis* as a single infection or in co-infection with *A. phagocytophilum* were identified (Table 1). However, from the tested dogs no antibody positive animals for *A. phagocytophilum* alone or single infection were found (Figure 3, Table 1).

The overall prevalence for the tested diseases was 88% in Guayaquil (Table 2). Forty percent of samples were positive for *E. canis* and 22% for *A. phagocytophilum*. In addition, 26% of the samples were positive to co-infection with *E. canis* and *A. phagocytophilum* (Table 2, Figure 4). In both cities, all blood samples tested were negative for heartworm (*D. immitis*) antigen and Lyme disease (*B. burgdorferi*) antibody (Tables 1 and 2). The proportion of antibody and/or antigen positive samples by organism within the total positive samples by city is shown in Table 3, with *E. canis* having the higher proportion in both cities. Logistic regression analysis

Figure 1: *Local children and dogs from the cities of Manta and Guayaquil, Ecuador. A-Shows a dog from Manta. B-Same dog with a high burden of ticks. C-Ticks on tub after bathing and grooming. D and E-Children and pet dogs from Manta. F-Dogs from Guayaquil.*

Table 1: *Number of positive samples to antigen or antibody in canine blood samples collected in the city of Manta, Ecuador.*

Causative organism	Antigen or antibody positive/all tested dogs	Prevalence
At least ≥ 1 organism	39/50	78%
Ehrlichia canis (total)	39/50	78%
Anaplasma phagocytophilum (total)	13/50	26%
Ehrlichia canis (alone)	26/50	52%
Anaplasma phagocytophilum (alone)	0/50	0%
E. canis + *A. phagocytophilum* (co-infection)	13/50	26%
Borrelia burgdorferi	0/50	0%
Dirofilaria immitis	0/50	0%

Figure 2: *Map of the country of Ecuador in South America, showing the port cities of Manta and Guayaquil.*

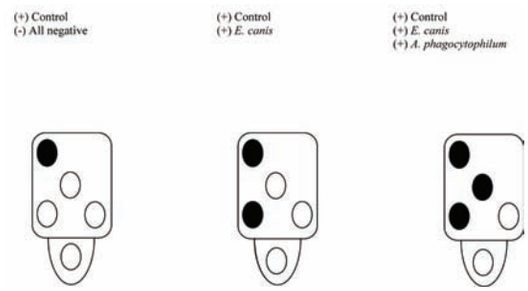

Figure 3: *Depiction of on-site test results observed and recorded in the city of Manta.*

showed that the odds of being positive to *E. canis* and/or *A. phagocytophilum* are 0.5 times larger for a dog in the city of Guayaquil than for a dog in the city of Manta. However, the odds of being positive to *E. canis* are 1.8 times larger for dogs in the city of Manta than the odds of being positive for dogs in the city of Guayaquil. For *A. phagocytophilum*, the odds for a dog in the city of Manta were 0.6 times least likely to be positive than the odds for a dog in Guayaquil of being positive to *A. phagocytophilum.*

Discussion

Both Manta and Guayaquil are coastal cities at sea-level with hot, humid year-round tropical climates. Tropical humid climates provide an adequate environment for the presence of vectors such as ticks and mosquitoes.[19, 20] In South America, the presence of arthropod vectors such as *Rhipicephalus sanguineous* (brown dog tick), *Ixodes spp* ticks, and *Aedes albopictus* and *Anopheles spp* mosquitoes have been reported.[21,22,23] Presence of these arthropod vectors is of significance since *R. sanguineus* is the primary vector for *E. canis,* and *Ixodes spp* ticks are vectors for *A. phagocytophilum* and *B. burgdorferi*.[24-26] Also, *Aedes spp* and *Anopheles spp* mosquitoes are capable of transmitting *D. immitis,*[8] and these mosquito genera have been reported in Ecuador.[27]

Table 2: *Number of positive samples to antigen or antibody in canine blood samples collected in the city of Guayaquil, Ecuador.*

Causative organism	Antigen or antibody positive/all tested dogs	Prevalence
At least ≥ 1 organism	44/50	88%
Ehrlichia canis (total)	33/50	66%
Anaplasma phagocytophilum (total)	24/50	48%
Ehrlichia canis (alone)	20/50	40%
Anaplasma phagocytophilum (alone)	11/50	22%
E. canis + *A. phagocytophilum* (co-infection)	13/50	26%
Borrelia burgdorferi	0/50	0%
Dirofilaria immitis	0/50	0%

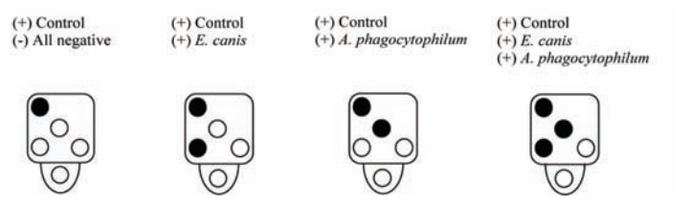

Figure 4: *Depiction of o*n-site test results observed and recorded in the city of Guayaquil, Ecuador.

Table 3: *Proportion of antibody/antigen positive samples by organism within total positive samples by city.*

	Manta		Guayaquil	
Causative organism	**antibody (+) / total (+)**	**%**	**antibody (+) / total (+)**	**%**
At least ≥ 1 organism	39/39	100	44/44	100
Ehrlichia canis (total)	39/39	100	33/44	75
Anaplasma phagocytophilum (total)	13/39	67	24/44	55
Ehrlichia canis (alone)	26/39	67	20/44	45
Anaplasma phagocytophilum (alone)	0/39	0.0	11/44	25
E. canis + *A. phagocytophilum* (co-infection)	13/39	33	13/44	30
Borrelia burgdorferi	0/39	0.0	0/44	0.0
Dirofilaria immitis	0/39	0.0	0/44	0.0

In general, VBD cause impairment of health status in humans and animals, leading to disruption of activities and losses of millions of dollars worldwide annually.[1] For example, in dogs *A. phagocytophillum* causes fever, depression, myalgia, anorexia, and lameness,[28] and in humans its clinical signs include fever, headache, lethargy, myalgia, elevated liver function enzymes, and reduction of platelets.[26,29] Similar clinical signs are reported in *E. canis* infections. [29-31] In addition, the time from exposure to onset of clinical signs for both *A. phagocytophillum* and *E. canis* in dogs and humans is between one to four weeks,[32,33] potentially leading to an immediate (incubation period ≤ 15 days) and delayed impact (incubation period ≥ 15 days) on military operations.[27] The potential impact on military operations will be beyond onset of clinical signs because treatment with antibiotics will take approximately two weeks with an additional convalescence period extending beyond 30 days post infection.[34]

All samples collected in this surveillance were negative for *B. burgdorferi* antibody and *D. immitis* antigen. It was an unexpected finding, since the presence of vectors (mosquitoes and ticks) and diagnostic evidence for the etiologic agents for these two diseases have been reported in Ecuador and other neighboring and regional countries such as Brazil, Colombia, Peru, and Argentina.[21-23,35-44] Heartworm disease caused by *D. immitis* has been reported in the Galapagos Islands of Ecuador and in the Amazon regions of the neighboring countries of Colombia, Peru, and Brazil, [35-38, 42] and similar findings have been reported for Lyme disease cause by *B. burgdorferi*.[39,45] However, in a surveillance study in Chile where 1056 dogs were sampled, no infection was detected for *D. immitis*,[44] and *B. burgdorferi* was not confirmed in suspected Chilean human clinical cases using ELISA, indirect fluorescent antibody (IFA), and Western Blot Analysis.[47] In another study in Southern Chile, *B. burgdorferi* was not detected by polymerase chain reaction (PCR) in 62 wild ticks collected from wild rodents and cervidae (deer).[48] It is possible that natural barriers or ecological conditions such as the ocean separating the Galapagos Islands

from the mainland, the Andes Mountains, or other geographical factors could maintain Manta and Guayaquil free of *B. burgdorferi* and *D. immitis* even when the climatic conditions and vectors might be present. Despite those possibilities, the presence of favorable climatic conditions and vectors make VBD more likely to be present.[49] Therefore, while this study did not detect evidence of their presence, heartworm and Lyme diseases may be present in the cities of Manta and Guayaquil, possibly at a low prevalence. Screening for diseases with truely low prevalence is more difficult, because low prevalence results in a decrease of a predictive value of a positive test result, leading to a requirement to increase the sample size (number of animals) needed in order to detect disease-positive animals.[50] For *D. immitis* additional diagnostic problems are encountered, because sensitivity is crucially influenced by the number of adult female parasites.[51] In general, it has been suggested that co-infection with two or more VBD, like in this report, could lead to more complex immunological effects, confounding and complicating even more the diagnosis of vector-borne pathogens.[3]

Conclusion

The findings of this surveillance study showed the presence of *E. canis* and *A. phagocytophilum* in the cities of Manta and Guayaquil in Ecuador. This finding emphasizes the value of surveillance for zoonotic diseases to determine disease prevalence and risk assessments, as well as indication for implementing control measures. Studies such as this are valuable tools for U.S. military conventional or Special Operation Forces (SOF) units who will deploy or are already deployed. Special Operations Forces commanders, medical planners, public health and preventive medicine personnel and medics should take note, as they specifically must plan and ensure comprehensive preventive measures are implemented for all U.S. personnel in the areas of operation (AO) and for educating local civilian communities.

An additional goal of this work was to increase the SOF medic's knowledge and awareness of zoonotic and infectious disease through surveillance studies in animals. The findings emphasize the critical need for continual and aggressive field surveillance for zoonotic and infectious diseases present in animals within specific AOs.[52] Vector-borne diseases have the capabilities of significantly impairing mission accomplishment by affecting force health. The SOF medics should use this value-added asset to protect themselves, teammates, and deployed forces from disease, including military working animals. In addition, by working with local health officials, they can use this information to protect the health of local military personnel and civilians.

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