Leptospiroseresis is considered the most widespread of all zoonoses\(^1\) and an emerging infectious disease by the World Health Organization (WHO).\(^2\) Pathogenic Leptospiroseres species diversity in correspondingly diverse natural mammalian communities in the humid tropics is\(^3,4\) suggested an ancient co-evolutionary host-parasite history, with humans and later domestic animals as accidental hosts.

The Hawaiian Islands provide a rare Leptospirosis study opportunity. The archipelago has a limited number of Leptospirosi epidemic species, nearly all of which are human commensals (e.g., Rattus spp., Mus spp.). Recent research has shown high Leptospirosis species diversity in correspondingly diverse natural mammalian communities in the humid tropics.\(^3,4\) Extensive trapping efforts typically are confined to one site or at most two districts. Difficulty in making inter-island comparisons of leptospirosis infections in non-domestic host populations is further compounded because data were not collected concurrently on multiple islands.

Because maintenance of this pathogen is reliant on non-human hosts, public health prevention efforts have typically focused on animal control measures in conjunction with public education to increase awareness of common exposure risks. Understanding specific patterns of host-serovar associations assists in informing public health efforts by providing insight into which animal carriers are associated with the Leptospirosis variant of interest.

Using a large-scale dataset composed of 15,171 animals collected over a period of 14 consecutive years, with 8 years of concurrent trapping across Oahu, Kauai, and Hawaii islands, this retrospective summary represents the largest and longest study of leptospirosis among non-domestic animal populations in Hawaii and 1) provides an update on leptospirosis in animals on Oahu and Hawaii since Higa and Fujinaka’s\(^11\) island-wide studies on the islands of Hawaii and Oahu, respectively; trapping efforts typically were confined to one site or at most two districts. Difficulty in making inter-island comparisons of leptospirosis infections in non-domestic host populations is further compounded because data were not collected concurrently on multiple islands.

Because maintenance of this pathogen is reliant on non-human hosts, public health prevention efforts have typically focused on animal control measures in conjunction with public education to increase awareness of common exposure risks. Understanding specific patterns of host-serovar associations assists in informing public health efforts by providing insight into which animal carriers are associated with the Leptospirosis variant of interest.

**MATERIALS AND METHODS**

**Animal sampling.** As part of a statewide initiative for leptospirosis monitoring and surveillance by the Hawaii State Department of Health (HDOH) Vector Control Branch, five primary animal reservoirs from Oahu, Kauai, and Hawaii islands were trapped and tested for evidence of leptospirosis infection: mongoose (HA), mouse (MM), brown rat (RN), roof rat (RR), and the Polynesian rat (RE). Animal trapping on Oahu was conducted from 1990 to 2003, whereas trapping on the islands of Hawaii and Kauai was conducted from 1991 through 1998. Trapping was opportunistic and was conducted at residential or business sites in response to rodent pest complaints or at field sites (e.g., waterfalls, streams, or tarp plots) temporally associated with a confirmed human case.

Live captures were brought to a HDOH Vector Control facility and killed by carbon monoxide gas then immediately

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**Abstract.** We describe the geographic distribution and variation in host-pathogen specificity for *Leptospiroseres* small mammal collected concurrently from three Hawaiian Islands over a period of 14 years: 1990–2003. Four serogroups (Icterohaemorrhagiae, Ballum, Sejroe, and Australis) were identified from the 15,171 animals tested. Serogroup prevalence differed across host species and islands (P < 0.0001 for each), but not across years. The host associations and biogeographic patterns of *Leptospiroseres* in Hawaii indicate a pathogen community shaped by ecological factors.
weighed, sexed, and dissected. Harvested homogenized kidneys were used to inoculate Ellinghausen-McCullough-Johnson-Harris culture media followed by incubation at room temperature in the absence of ambient light. Cultures were inspected weekly for 6 weeks by dark field microscopy (HDOH, unpublished data).

**Serogroup identification.** Serogroup identification of isolates was performed at the HDOH Vector Control Branch (Halawa Valley, Oahu) by the microscopic agglutination test (MAT), which screens the live unknown cultured isolate against a panel of rabbit antisera selected for the Pacific region and obtained from the U.S. Center for Disease Control and Prevention (CDC), Atlanta, GA (Table 1). In the case of cross-reactions of an unknown isolate to multiple antisera, identification was determined according to the antisera with the highest titer (i.e., greatest dilution) reaction. When a particular serovar became temporarily unavailable, a different serovar of the same serogroup was used in its stead. Therefore, identification is accurate only to the serogroup level.

Isolates were considered of undetermined serogroup if the kidney culture contained leptospires as well as other bacteria (mixed) despite the presence of fluorouracil in the growth media, or were of insufficient quantity for use in the MAT (insufficient growth), or did not match any of the known antisera in the panel (unable to type).

**Statistical analyses.** Leptospiral serogroup distributions were measured within and across host species, and within and across all three islands. Calculations of summary prevalence proportions used counts of all leptospiral culture positive animals, including those of undetermined serogroup, in the numerator, and counts of all trapped animals (both infective and non-infective animals) in the denominator. The \( \chi^2 \) test was used to test the frequency distributions in host and island contingency tables.

A generalized estimating equations (GEE) model with the identity link function was performed using the PROC GENMOD procedure in SAS version 9.2 (SAS Institute, Cary, NC) to examine the differences in leptospiral serogroup prevalence between serogroups, host species, or islands. For these analyses, the prevalence proportions for each serogroup were calculated relative to the frequencies of hosts and islands. Pairwise comparisons and linear contrasts were used to compare the differences of leptospiral serogroup prevalence proportions among hosts and islands. The Bonferroni procedure was used to adjust the \( P \) values to control the family-wise error rate at 5%. All comparisons with adjusted \( P \) values of \( \leq 0.05 \) were considered significant.

The GEE model with the identity link function was also used to examine the temporal changes in leptospirosis infection prevalence across years adjusted for serogroup, host species, and island. To enable comparisons across the explanatory variables (i.e., serogroup, island, host species, trap year), prevalence proportions were calculated using the total count of animals trapped as the denominator.

### RESULTS

**Overall prevalence.** Table 2 provides a summary of leptospiral prevalence by serogroup, host species, and island. A total of 15,171 animals were tested, with 2,766 animals found to harbor culture-positive *Leptospira* in their kidneys (18.2%). Host-specific prevalence ranged from 11.4% for RE to 26.7% for RN. Leptospiral infection prevalence varied significantly among the five host species (\( \chi^2 = 250.2, \text{df} = 4, P < 0.0001 \)). Overall prevalence also differed significantly across the main Hawaiian islands with the highest prevalence on Oahu (25.1%), followed by Kauai (10.9%), and Kauai (10.3%) (\( \chi^2 = 523.2, \text{df} = 2, P < 0.0001 \)). Of the 2,766 culture positive animals, four leptospiral serogroups were identified. Icterohaemorrhagiae was the most common (37.7%), followed by Ballum (28.4%), Sejroe (20.1%), and Australis (0.4%). Undetermined serogroups comprised 13.4% (371 animals) of the *Leptospira* culture positives. Undetermined samples were not included in further counts and statistical analyses. Therefore, 2,395 out of a total of 2,766 culture-positive results (86.6%) were considered in additional tests of host and island distributions (Table 2).

**Serogroup prevalence by host species.** Four serogroups were identified in the carrier species studied: Sejroe, Ballum, *Icterohaemorrhagiae*, and *Australis*. The Serogroup prevalence by host species is presented in Table 1.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Culture positives (N = 2,766)</th>
<th>Serogroup Leptospira* (N = 2,395)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sejroe</td>
<td>555 (20.1)</td>
<td>555 (23.2)</td>
</tr>
<tr>
<td>Ballum</td>
<td>786 (28.4)</td>
<td>786 (32.8)</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>1,043 (37.7)</td>
<td>1,043 (43.5)</td>
</tr>
<tr>
<td>Australis</td>
<td>11 (0.4)</td>
<td>11 (0.5)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>371 (13.4)</td>
<td></td>
</tr>
</tbody>
</table>

*The following strains were discontinued from the panel when either the antisera was depleted and not replaced, or the type culture used for quality control was lost: Bataviae Van Tienen (1996a), Mini georgia LT 117 (1996a), Autumnalis autumnalis Akiyama A (2003).*

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**Table 1**

Panel of 10 reference strains* of *Leptospira* used by the Hawaii State Department of Health Vector Control Branch in the microscopic agglutination test (MAT)

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Serovar</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Canicola</td>
<td>canicola</td>
</tr>
<tr>
<td>2</td>
<td>Australis</td>
<td>australis</td>
</tr>
<tr>
<td>3</td>
<td>Autumnalis</td>
<td>autumnalis</td>
</tr>
<tr>
<td>4</td>
<td>Ballum</td>
<td>ballum</td>
</tr>
<tr>
<td>5</td>
<td>Bataviae</td>
<td>bataviae</td>
</tr>
<tr>
<td>6</td>
<td>Sejroe</td>
<td>hardjo</td>
</tr>
<tr>
<td>7</td>
<td>Mini</td>
<td>georgia</td>
</tr>
<tr>
<td>8</td>
<td>Icterohaemorrhagiae</td>
<td>copenhageni</td>
</tr>
<tr>
<td>9</td>
<td>Pomona</td>
<td>pomona</td>
</tr>
<tr>
<td>10</td>
<td>Pyrogenes</td>
<td>pyrogenes</td>
</tr>
</tbody>
</table>

*Culture-positive samples with undetermined serogroup are not included in this set of counts.*
Icterohaemorrhagiae, and Australis (Figure 1). All four serogroups were observed in mongooses (HA), brown (RN), and roof rats (RR). All serogroups except Australis were identified in mice (MM) and Polynesian rats (RE). Icterohaemorrhagiae, Sejroe, and Ballum were found in all host species tested.

**Serogroup dominance by host species.** Although the host range of the four leptospiral types was broadly inclusive of all animal carrier species tested in this study, each species appeared to be strongly associated with one predominant serogroup and to a lesser degree with multiple secondary serogroups. The most common serogroup was found to differ by host species, with Sejroe the primary serogroup in mongoose (73.7%), Ballum in mice (82.2%), and Icterohaemorrhagiae in rats (87.4% RN; 69.8% RR, 55.2% RE) (Figure 1).

Statistical tests of relative serogroup prevalence showed evidence of host specificity. Within each species, linear contrasts between the numerically dominant serogroup and each of the other serogroups observed in that species were highly significant ($P < 0.0001$ for all comparisons).

**Serogroup distribution by island.** Serogroups differed in composition and relative prevalence across islands. All four serogroups were documented on Oahu and Hawaii, whereas leptospiral diversity was narrower on Kauai caused by the absence of Sejroe (Figure 1).

Tests of serogroup prevalence by islands showed a significant association between island affiliation and serogroup prevalence. For each island, pairwise comparisons among serogroups showed highly significant differences in prevalence proportions ($P < 0.0001$).

**Geographic distribution of leptospiral host associations.** Host-specific associations apparent in the pooled all-islands dataset remained primarily consistent when examined at the island level (Figure 2). Sejroe is the dominant serogroup in mongooses (HA) on the islands in which mongooses are established (i.e., Oahu and Hawaii). Ballum is the dominant serogroup in mice regardless of which island was examined. Icterohaemorrhagiae is the primary serogroup for all rats (RN, RR, RE) in the pooled data, as well as for the majority of rats on each island.

Statistical tests under the GEE model of host specificity patterns for each island were, in general, consistent with the pooled dataset. For each island, there is a significant difference in the distribution of relative prevalence across hosts overall (Kauai: $\chi^2 = 3566.8$, $df = 9$, $P < 0.0001$; Oahu: $\chi^2 = 18417.1$, $df = 12$, $P < 0.0001$; Hawaii: $\chi^2 = 11080.4$, $df = 12$, $P < 0.0001$). Detailed scrutiny of each host pairwise comparison for Ballum and Icterohaemorrhagiae are consistent for the pooled dataset and for each island’s dataset. The only exceptions to host specificity trends in the pooled dataset are for Australis and Sejroe host comparisons on Kauai. The only animals on Kauai found to harbor Australis were brown rats (RN, 2.8% prevalence); hence, comparisons of Australis prevalence between all other host species on Kauai were not significant. Similarly, Sejroe was absent from all animals tested on Kauai hence comparisons of prevalence for this serogroup were not significant.

**Temporal analyses.** After adjusting for serogroup, host species, and island, changes in prevalence across years were found to be not significant ($\chi^2 = 13.2$, $df = 13$, $P = 0.43$).

**DISCUSSION**

The application of an epidemiological approach (i.e., epizootiology) that includes ecological and evolutionary considerations can help provide insights into disease factors that may influence the dynamics of zoonotic diseases such as leptospirosis. Host associations and biogeography are two important factors that can have direct effects on the patterns of infectious zoonotic disease.

Host effects such as the preferential association of a serogroup with a particular animal species (i.e., host specificity) were found to be significant in this study. Each serogroup was associated with one primary host species and multiple ancillary hosts. The patterns of host specificity in the Hawaiian Islands are consistent with leptospiral serogroup-host associations that have been generally observed worldwide, for example *Rattus* spp., are known carriers of Icterohaemorrhagiae and mice of Ballum.17–19 Sejroe in Hawaii is common only in mongooses, which indicates this animal is the likely sole primary maintenance host for Sejroe, although other animal species are susceptible. Ballum was observed to be relatively common in all four rodent species but not mongooses.
Because the mongooses are phylogenetically quite distant from rodents, and *Rattus* and *Mus* are considered closely related genera, Ballum may be less well adapted to mongooses as a host. Australis was not observed in mice despite a large sample size of tested animals (\( N = 3,171 \)) therefore indicating that mice may not be susceptible to Australis. *Icterohaemorrhagiae* appears to be the least host specific because it was amply represented in all animals. Host specificity has been attributed to host-related biological compatibility factors, genetic factors mediating resistance, and age-related immunological factors.20

Host availability was a second mechanism by which hosts were found to contribute to the pattern of serogroup prevalence in Hawaii. The availability of appropriate maintenance hosts appears to determine which leptospiral serogroups were present. A serogroup is not likely to be found on an island if its primary maintenance host is absent. For example, in this study no evidence of Sejroe was found on Kauai, one of three islands in Hawaii (along with Lanai and Niihau) where mongooses have not been established. Furthermore, the apparent absence of Sejroe on the island of Kauai indicates that the other four major animal carriers, although susceptible to Sejroe, are either not compatible reservoir hosts capable of maintaining this serogroup in the long term or that Sejroe has not yet been introduced to this island.

In regards to biogeography, there was a significant effect of insularity on leptospiral community richness, serogroup abundances, and prevalence. Community richness as indicated by serogroup diversity was found to be lower on Kauai than on Hawaii and Oahu, with one less serogroup on Kauai as compared with the other islands. The dominant serogroup also differed across islands. Analyses of prevalence within and across islands highlight the uniqueness of each island’s leptospiral profile.

Our data show that leptospiral infection prevalence proportions in this study varied significantly across serogroups, host species, and islands, but not across years. Of note, a significant temporal trend in infecting serogroups has been identified in humans in Hawaii with a decrease in cases related to infections with *Icterohaemorrhagiae* and an increase in infections related to Australis from 1974 to 2008 (\( P < 0.0001 \) for each). The increasing impact of Australis may be related to the increasing population of feral swine and their impingement into urban population centers. Studies are planned to

investigate the contribution of feral swine on human leptospirosis in Hawaii.

Because the prevalence data in this study are based on opportunistic trapping as opposed to a systematic survey, extrapolations and generalizations from the results should be made judiciously. Trap efforts varied widely with uneven sample sizes between islands and animal species and greater sampling efforts in urban centers than rural sites. Another caution is that infection prevalence in animals may not necessarily translate directly into risk predictors for humans because contact rate, persistence of the bacteria in the environment, and other factors determine the probability of pathogen transmission.20

Leptospiral diversity and prevalence can be affected by a number of environmental influences21; this study illustrates how two ecological factors, host-pathogen interactions and geography, can shape the community ecology of a pathogen. An understanding of the mammalian reservoirs of leptospirosis can provide a basis for the management of disease risks by targeting potential transmission sources and pathways.19

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