LEPTOSPIRES IN WILDLIFE FROM TRINIDAD AND GRENADA

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ABSTRACT: Serum samples from 894 wild animals (representing 31 species) from Trinidad and Grenada were examined by the microscopic agglutination test for leptospiral antibodies; 198 were positive. These included 39 bats, 88 mongooses, six opossums, 10 peridomestic rodents, 15 forest rodents, 10 lizards, and 30 toads. Thirteen pathogenic serogroups were involved. Thirty-nine *Leptospira* isolates were reported from mongooses, opossums, rodents and toads.

INTRODUCTION

Most human cases of leptospirosis are thought to result from contact with domestic pets, livestock, or water contaminated with urine from infected domestic or wild animals. Although the universal role of peridomestic and field rodents, particularly *Rattus* spp., in the transmission of leptospirosis to man and his domestic animals is well known, comparatively little is known about the importance of other wild species in the transmission of the organism and the contamination of the environment. However, there are a few recent reports on leptospirosis in Caribbean wildlife (Everard et al., 1976; Green et al., 1978; Damude et al., 1979a; Everard et al., 1980). In addition, Galton and Sulzer list the isolates found throughout the world up to July 1973 (Galton, 1966; Sulzer, 1975).

Detailed epidemiological investigation is needed to find the animal sources not only of epidemics, but of small foci of the disease and single sporadic cases. This paper presents data obtained mainly between 1975 and 1979 on the leptospiral strains found in some wild animals from Trinidad and Grenada which may help such studies in the southeastern Caribbean, where the disease is more common in man than is generally realized (Everard et al., 1976; Damude et al., 1979b; Everard et al., 1979b; Everard et al., 1979c).

MATERIALS AND METHODS

The mongooses were caught in wood and wire-mesh box traps, while the bats were caught by mist netting or in a butterfly net on a long pole. The amphibians were hand-caught either at night or during the day from places of hiding, the vultures were shot, and the monkeys were zoo specimens. Most of the other animals were caught in small to medium Havahart traps placed in their normal habitat with appropriate bait. The majority of the animals were killed by excess ether anesthesia, though some of the Grenada mongooses were killed by injection of 1 mg succinyl choline chloride in 0.5 cc distilled water. A few animals were narcotized with ether and later marked-and-released or kept captive. Because many of the animals also were investigated for arboviruses, rabies or salmonellae, it was not always possible to undertake *Leptospira* isolation and serology from the same animal.

Blood was collected from narcotized or moribund animals by cardiac puncture. A few drops of blood were used directly when isolation was attempted; otherwise it was allowed to coagulate in a vial and then rimmed and centrifuged. The sera were labelled and stored at −20 C until needed; those from Grenada were taken under cold storage to Trinidad. All sera were screened at the PAHO/WHO Caribbean Epidemiology Centre (CAREC) in Trinidad, by the Leptospira Microscopic Agglutination Test (LMAT) (Galton et al., 1965; Cole et al., 1973; Sulzer and Jones, 1976). Routinely, the following live *Leptospira* antigen serovars were used to represent 12 serogroups: *ballum, canicola, pyrogenes, icterohaemorrhagiae* and/or *copenhageni* and/or *mankarso, bataviae, grippotyphosa, autumnalis* and/or *fort-bragg* and/or *djasiman, georgia* and/or *wolffi* (Hebdomadis), *shermani, javanica, tarassovi* and *panama.* Occasionally, *andamana, australis, celledoni, cynopteri* and *pomona* antigens were used, so that 17 serogroups could be represented. Those sera which contained agglutinins at a titer of ≥100 were considered to be positive and were titrated to the end point.

In the early part of the study cultures of small animals were made from an inoculum of 1 mm diameter cores of kidney tissue taken aseptically after heat searing the surface of the organ. Later, in an improved aseptic technique, the animal was washed thoroughly with a detergent/hypochlorite mixture (32 g Diversol [Diversey Corporation (East Carib-

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bean) Ltd., P. O. Box 315, Port-of-Spain, Trinidad] per liter of distilled water giving 1,000 parts of available chlorine/10⁶). The ventral surface was wiped with a gauze swab soaked in alcohol, and the skin was cut and pinned back. The scissors and forceps used for this incision were reused on all animals processed on the same day, being decontaminated in Diversol between each use. The body wall was wiped with an alcohol swab, and sterile instruments were used to cut the musculature, displace the viscera and remove the kidneys into a sterile plastic petri dish. Another set of sterile instruments was used to cut the kidney into pieces and inoculate one piece into each of four bottles containing medium. One mongoose kidney provided four pieces of tissue inoculum, but both kidneys of smaller animals were used. The number of bottles was reduced when numerous animals had to be processed on successive days. In a few cases urine was taken directly from the bladder.

The techniques used in the culture of leptospires were those described by Sulzer and Jones (1976), Turner (1968) and Turner (1970). Prior to 1976, Stuart's or Fletcher's media, or 3–5% tryptose phosphate broth (all with 8–10% inactivated rabbit serum) in semisolid or liquid form were used for kidney cultures and for maintaining the cultures in serial passage. After 1976, Ellinghausen, McCullough, Johnson and Harris (EMJH) semisolid or liquid medium was used (Ellinghausen and McCullough, 1965; Johnson and Harris, 1967). Five-fluorouracil (Johnson and Rogers, 1964) at a concentration of 100–300 μg/ml, and/or discs impregnated with 25–30 μg neomycin sulfate, were added to each culture vial to inhibit the growth of bacterial contaminants. Three to 10 ml (or more) of EMJH medium was used in clean, sterile screw-capped Bijou, Universal or McCartney vials. In all cases kidney tissue was cultured but, in addition, occasionally up to three drops of whole blood and/or up to three drops of urine were inoculated separately into vials of medium using aseptic techniques.

In Trinidad, cultures were incubated at 28–34 C; in Grenada, where no incubator was available, ambient room temperature (26–30 C) was used. The cultures were examined approximately weekly by dark field microscopy. Nearly all were kept for 6 wk unless grossly contaminated, and some were kept for 8 wk or more. Cultures containing leptospires were subcultured in fresh medium and decontaminated as necessary using standard techniques (Sulzer and Jones, 1976). Established cultures were sent to the *Leptospira* Reference Laboratory at the Centers for Disease Control (CDC) for serotyping by the cross-agglutinin-absorption method involving the preparation of homologous antisera in rabbits (Kmety et al., 1970; Sulzer and Jones, 1976). Isolates from Grenada were first taken to CAREC and established in subculture before being sent to CDC. Duplicate cultures were kept at CAREC and identified to serogroup by screening the unknown culture against standardized rabbit antisera prepared from known *Leptospira* antigens (Sulzer and Jones, 1976).

RESULTS

Tables 1 and 2 list the wild animals examined serologically for leptospiral agglutinins on Trinidad and Grenada, respectively. The serogroups found were designated according to the one giving the highest titer. In the case of the very few animals which had similar titers to more than one serogroup, the more common group was recorded. Where the numbers of animals were adequate the percentages seropositive have been given. The highest titers recorded from each group of animals on both islands were: bats 1:800, mongooses 1:12,800, opossums 1:800, forest rodents 1:400, peridomestic rodents 1:1,600, lizards 1:1,600, and toads 1:1,600. Tables 1 and 2 also show the proportional distribution of maximally elevated titers to each serogroup; combining the results for the two islands, agglutinins to Icterohaemorrhagiae (31%), Hebdomadis (13%), Autumnalis (13%), Javanica (11%) and Panama (8%) were the most common.

Analyzing the 71 positive mongoose sera from Grenada, only 15 of the 45 sera designated Icterohaemorrhagiae reacted exclusively to *copenhageni* and *icterohaemorrhagiae* antigens. The remaining 30 gave multiple reactions comprising 13 to Canicola, 11 to Autumnalis (*fortbragg* and/or *autumnalis*), 11 to Javanica, seven to Shermani, seven to Pyrogenes, four to Bataviae, two to Tarassovi and two to Panama. Several of these titers were high compared to the Icterohaemorrhagiae titer of the same sample. Thus, one mongoose gave titers of *copenhageni* 1:6,400, *icterohaemorrhagiae* 1:6,400, *pyrogenes* 1:3,200, *canicola* 1:3,200, and *bataviae* 1:400; while another mongoose gave titers of 1:6,400 to *icterohaemorrhagiae*, 1:1,600 to *copenhageni*, 1:3,200 to *fort-bragg*, and 1:3,200 to *autumnalis*. Of the 15 sera which reacted exclusively to Icterohaemorrhagiae antigens, six gave titers in the range 1:100–1:200, six in the range 1:400–1:800, and three gave titers of 1:≥1,600. Of the Icterohaemorrhagiae titers of the 30 sera which gave multiple reactions, there were three at 1:12,800, 10 at 1:6,400, one at 1:3,200, six at 1:1,600, three at 1:800, three at 1:400, two at 1:200, and two at 1:100. The titers of the eight sera recorded as Autumnalis (*autumnalis* and *fort-bragg*) ranged be-

TABLE 1. Leptospiral agglutinins in wild animals from Trinidad.

Species (no. tested)	Ictero-haemorrhagiae	Autumnalis	Hebdomadis	Canicola	Javanica	Panama	Pyrogenes	Tarassovi	Ballum	Bataviae	Cynopteri	Total (%) seropositive
					Serogroup							
Carollia perspicillata (19)					1						2	2 (11)
Phyllostomus hastatus (48)		1	7		2			2			2	13 (27)
Pteronotus davyi (15)					2							2 (13)
Molossus major (20)						2	1					5 (25)
Artibeus jamaicensis (15)												0 —
Mormops sp. (3)												0 —
Glossophaga sp. (8)												0 —
Micronycteris sp. (5)												0 —
Herpestes auropunctatus (37)	1			6	1	2	4		2	1		17 (46)
Didelphis marsupialis (22)										1		1 (5)
Marmosa mitis (73)			1		2				1			4 (5)
Marmosa fusca (7)												0 —
Caluromys philander trinitatis (14)			1		1							1 (7)
Rattus rattus (32)	2	2										5 (16)
Rattus norvegicus (7)		1			2							3 —
Rattus sp. (unidentified) (7)												0 —
Mus musculus (7)	2											2 —
Proechimys guyannensis (37)			1		3		4					8 (22)
Nectomys squamipes (17)			1		1				1		1	4 (24)
Heteromys anomalus (4)		1										1 —
Oryzomys capito (7)				1	1							2 —
Rhipidomys couesi (2)												0 —
Zygodontomys brevicauda (1)												0 —
Akodon urichi (1)												0 —
Cebus sp. (2)												0 —
Coragyps atratus (6)												0 —
Tupinambis nigropunctatus (12)	2	1			1				1			5 (42)
Ameiva ameiva (4)	4											4 —
Iguana iguana (1)		1										1 —
Bufo marinus (80)	2	7	5		2	1		3				20 (25)
Hyla minuta (2)												0 —
Total (515)	13	14	16	7	19	5	9	5	5	2	5	100 —

TABLE 2. Leptospiral agglutinins in wild animals from Grenada.

Species (no. tested)	Serogroup												
	Icterohaemorrhagiae	Autumnalis	Hebdomadis	Canicola	Javanica	Panama	Pyrogenes	Tarassovi	Ballum	Bataviae	Shermani	Grippotyphosa	Total (%) seropositive
Anoura sp. (61)	1	1	5	1		3					1		13 (21)
Glossophaga sp. (52)		1	1										4 (8)
Herpestes auropunctatus (200)	45	8	3	1	2	6	5	1	1	1	2	2	71 (36)
Bufo marinus (66)	2					2		1		1	1		10 (15)
Total (379)	48	11	9	1	2	11	5	2	1	2	4	2	98 —

tween 1:100 and 1:3,200; two of them showed no cross-reactions and six had cross-reactions to mainly Icterohaemorrhagiae. The six sera designated Panama showed no cross-reactions and gave titers between 1:100 and 1:1,600. Three of five Pyrogenes sera reacted only to this group (titer range 1:100–1:400) and two showed cross-reactions. The two Shermani (1:400 and 1:100 titers), two Javanica (1:100 and 1:400 titers), the single Bataviae (1:400) and two Grippotyphosa sera (1:100 and 1:200) reacted to only the designated serogroup. An analysis of the 17 seropositive mongooses from Trinidad showed similar patterns.

Of the 53 isolates listed from wild animals on Trinidad and Grenada (Everard et al., 1980), 14 are reported in full elsewhere (Everard et al., 1976; Green et al., 1978). The remaining 39 (21 from Trinidad and 18 from Grenada) are shown here in Table 3 according to host species and island. On Grenada dense growth of leptospires was seen by dark field microscopy in 46 of 461 attempted cultures. These were: mongoose (*Herpestes*) 31/190 (16%), bats 0/121, rodents 13/91 (14%), toad (*Bufo*) 2/58 (3.5%), and opossum (*Didelphis*) 0/1. However, due to technical problems, only 18 isolates survived.

DISCUSSION

Geologically, Trinidad and Tobago are South American in origin with a fauna and flora largely of mainland stock. Listed from Trinidad are over 25 species of amphibia (Kenny, 1969), 22 lizards (Underwood, 1962), 37 or 38 snakes (Boos, 1975), 40 species of terrestrial mammals (Alkins, 1979) and 58 species of bats (Goodwin and Greenhall, 1961). Grenada has only five species of amphibians, eight species of lizards, six species of snakes and 12 mammalian species (exclusive of bats) of which seven are rodents (Groome, 1970). Only *Dasyprocta* (Agouti) among rodents, and possibly *Marmosa mitis* and *Dasypus novemcinctus* among the remaining five terrestrial mammal species are thought to be indigenous to Grenada (De Vos et al., 1956). Progressing northwards, the islands show an increasingly sparse land fauna, the conspicuous terrestrial vertebrates other than lizards being largely those introduced species, notably *Rattus*, *Mus*, the toad *Bufo marinus*, and the mongoose (*Herpestes auropunctatus*) which have survived well because of their adaptability and, usually, a lack of inter-specific competition. We

believe that these introduced species can be an important source of human leptospiral infection because they form large populations and live in close proximity to man. Although *Mus* and *Rattus* spp. are generally regarded as the primary carriers of wildlife leptospirosis to man and animals, it should be recognized that the toad is equally common in yards and gardens and that the mongoose is very frequently found in semiurban and urban habitats where there is adequate grass or thicket cover.

Table 1 shows that while 19% of peridomestic rodents and 22% of forest rodents in Trinidad were positive for leptospiral agglutinins, the proportion for *Herpestes* on that island was 46%. In a study of mammals in California, 89% of carnivores and 60% of rodents were seropositive; the higher proportion of seropositives in carnivores than rodents seems to be a general trend (Cirone et al., 1978). However, it is common for rodents to be seronegative while excreting leptospires, and some of the seronegative animals in our study may well have been currently infected. The proportions of rodents with past or current infections are therefore probably higher than shown in Table 1. Two forest rodents of possible importance are *Nectomys squamipes*, which is likely to come into contact with leptospires since it is semi-aquatic, and *Proechimys*. Australis (*australis*) has been isolated from *N. squamipes* in Brazil, while several serogroups and numerous serovars have been isolated from *Proechimys semispinosus* in the Panama Canal Zone (Galton, 1966; Sulzer, 1975). Also in Panama, isolates have been obtained from *Zygodontomys* and *Heteromys*.

Our findings confirm those of a previous study in which the importance of *Herpestes auropunctatus* in the epidemiology of leptospirosis in the Caribbean was stressed (Everard et al., 1976). That study found 35% of Grenadian mongooses examined with antibodies to at least three serogroups (Icterohaemorrhagiae, Pomona and Canicola), while 33 to 51% of Trinidadian mongooses examined were seropositive, with agglutinins to Canicola predominating, but those to Icterohaemorrhagiae and Pomona also encountered. The proportions of seropositive mongooses in the present study (36% on Grenada and 46% on Trinidad) are consistent with these earlier findings. The predominance of agglutinins to Icterohaemorrhagiae in Grenadian mongooses and Canicola in Trinidad mongooses is still evident, but in the present study agglutinins to Canicola were not found in Grenadian mongooses, and Pomona was not included in our antigen pool. The high titers that were found in many mongoose sera could indicate recent or current infection.

Of the 17 isolates obtained from Trinidadian and Grenadian mongooses (Everard et al., 1980), six have been reported fully elsewhere (Everard et al., 1976; Green et al., 1978). The five Bataviae (*brasiliensis*) and one Tarassovi (*atchafalaya*) isolates recorded here show that the mongoose may be a wildlife source of human infections by these serogroups in the area. The fact that five Bataviae isolates were obtained from mongooses in Grenada yet only one of 71 specimens was seropositive to this serogroup deserves comment; although Bataviae was designated the infecting group in only one case, it was a secondary reactor in four other cases with titers between 1:100 and 1:400. Further, serological tests may be negative or show very low titers for the infecting serogroups in some animals (Minette, 1964). Alternatively, there may have been a shift in predominance or, since mongoose trapping took place throughout the island, geographical variation. The isolation of Panama (*mangus*) from *Herpestes* recorded, for the first time, the existence of this serogroup in the southeast Caribbean (Green et al., 1978). It is now known to be one of the five serogroups most commonly found in man, livestock and wildlife in the area.

The mongoose has been incriminated as a carrier of leptospires elsewhere. *Leptospira icterohaemorrhagiae* and Hebdomadis (*jules*) have been isolated from *Herpestes* in Jamaica (Sulzer, 1975). In Hawaii, 32% of specimens examined were positive by both isolation and serology techniques (Minette, 1964). Also in Hawaii, *canicola*, Hebdomadis (*sejroe*) and *icterohaemorrhagiae* have been isolated from *Herpestes* (Galton, 1966). In St. Croix, two samples of 21 mongooses each were examined and 11 and 14 specimens were found seropositive to Hebdomadis antigens (Nellis and Everard, 1983). On Barbados, 12 of 19 mongoose sera (63%) were positive, 10 of them to Autumnalis (*fort-bragg*); *fort-bragg* was also isolated from a mongoose (Damude et al., 1979a). Bataviae (*djatzi*) and *icterohaemorrhagiae* have been isolated from *Herpestes* on Puerto Rico (Galton, 1966). Hebdomadis (*maru*) and Po-

TABLE 3. *Leptospira* serovars isolated from wildlife in Trinidad and Grenada.

Host	Serovars isolated in Trinidad (nos. of isolates in parentheses)	Serovars isolated in Grenada (nos. of isolates in parentheses)
Herpestes auropunctatus	*canicola* (1)	*copenhageni* (4) *brasiliensis* (5) *atchafalaya* (1)
Marmosa fuscata	*lanka* (1)	
Marmosa mitis	*lanka* (1) *ballum* (3)	
Caluromys philander trinitatis	*ballum* (1)	
Rattus norvegicus	*copenhageni* (1)	*copenhageni* (1)
Rattus rattus	*copenhageni* (1) *ballum* (2) *lanka* (1)	*copenhageni* (4) *ballum* (1)
Proechimys guyannensis	*copenhageni* (1)	
Bufo marinus	*autumnalis* (8)	*navet* (1) *peruviana* (1)

mona (*kennewicki*) have been found in *Herpestes urva* in Taiwan (Tsai and Fresh, 1971), and Javanica in *Herpestes javanicus* from Indonesia (Galton, 1966). *Icterohaemorrhagiae* has been isolated from *Herpestes* sp. in Egypt, and Australis (*australis*) from *Mustela nivalis* in Nicaragua (Sulzer, 1975).

Although the proportions of *Bufo* found seropositive in Trinidad and Grenada were only 25% and 15%, respectively, the large numbers in which these animals are found make them an important reservoir of leptospires. Their ability to excrete the organism is substantiated by the 10 isolates obtained (Table 3). Of importance is the fact that both isolation and serologic studies in Trinidad show the predominance of Autumnalis and Hebdomadis, to which agricultural workers are commonly exposed. Galton (1966) and Sulzer (1975) do not list isolates from *Bufo*, but Babudieri et al. (1973) obtained two similar isolates from 112 *Bufo marinus* in the Philippines which they named Bufonis *carlos* (new serogroup and serovar). Coghlan (pers. comm.) found that these two lines of *carlos* were agglutinated by Autumnalis antiserum and so may prove to be in this group. The isolation of Australis (*peruviana*) from a Grenadian toad is the first record of this serogroup in the southeast Caribbean and warrants the routine inclusion of Australis antigen in preliminary screening tests. Australis (*peruviana*) has been isolated from cattle in Peru (Galton,

1966). Tarassovi/Bataviae (*navet*) was first isolated from man in Trinidad during 1971 (Green et al., 1978). Its isolation from a toad in Grenada points to a possible wildlife host for this strain. Three toads from Trinidad seropositive for Tarassovi (Table 1) help to substantiate this.

Forest rodents, opossums, bats and lizards probably have less impact on the transmission of leptospires to man and his animals than peridomestic rats, mongooses and toads. However, *Ameiva* lizards are common in gardens in Trinidad where cats are few, and Iguanas are often found near human habitations. Bats, in particular *Molossus* and *Artibeus*, often inhabit house eaves, as do *Didelphis* and *Marmosa* spp. occasionally. Although only 5% of opossums in the present study were seropositive (Table 1), two Autumnalis and four Ballum isolates were obtained from them (Table 3). Isolates have also been obtained from *Didelphis* in Panama (Sulzer, 1975). Their ability to excrete leptospires is not in doubt. We did not examine any *Dasypus* specimens, but the armadillo, *Chaetophractus*, has been shown to be an important reservoir of pathogenic leptospires in Argentina (Myers et al., 1977).

The predominant serogroup in bats on both islands was Hebdomadis, and the absence of Hebdomadis cultures may reflect the fact that this group is a difficult one to isolate. However, the lack of success with 121 attempts to isolate leptospires from bats in Grenada and 86 attempts in Trinidad also suggests that bats are not persistent carriers. Indeed, there are few reports of bat isolates from elsewhere, and Galton (1966) and Sulzer (1975) list only Canicola (*schuffneri*) and Cynopteri (*cynopteri*) from *Cynopterus* bats in Indonesia. Serologic evidence of Cynopteri was found in Trinidad bats and *Nectomys* (Table 1), and *cynopteri* has been isolated from *Didelphis* and *Proechimys semispinosus* in Panama (Galton, 1966). The importance of bats in the epidemiology of leptospirosis is not known but it might well be substantial in damp or wet caves where these animals live in dense colonies.

Among 66 hospital cases of leptospirosis recorded in Trinidad between 1977 and 1980, and other individuals who had serologic evidence of previous infection, agglutinins to serogroups Icterohaemorrhagiae, Autumnalis, Hebdomadis, Canicola and Panama were most commonly found, and agglutinins to Bataviae, Tarassovi,

Ballum, Grippotyphosa, Pyrogenes, Shermani and Javanica also were recorded. Agglutinins to Autumnalis and Hebdomadis were frequently as common as those to Icterohaemorrhagiae, if not more so, among certain categories of Trinidadian rural workers (Everard, unpublished data). The presence of all these serogroups except Autumnalis, Shermani and Javanica in Trinidadian humans has been confirmed by isolation (Everard et al., 1980). From Grenada, among 45 human seropositive current and old cases, Icterohaemorrhagiae, Panama and Canicola were the three serogroups most frequently found, but agglutinins to Pyrogenes, Autumnalis, Grippotyphosa, Ballum, Bataviae, Javanica and Shermani also were recorded (Everard et al., 1979). The exposure of humans in Trinidad and Grenada to at least 10 serogroups suggests infection from multiple hosts and environmental sources.

During 1978 in the United States, there were 108 cases of leptospirosis reported in humans (Centers for Disease Control, 1979). Seven of them were thought to originate from wild animal sources, 16 from livestock, seven from domestic pets, 24 from infected water, two from multiple animal species, and 32 from multiple animal species and water. Twenty were from an unknown source. Seventy-five percent of wild animals examined by Cirone et al. (1978) in the United States had leptospiral antibodies. The most commonly found agglutinins were to serogroups Pomona, Autumnalis, Pyrogenes, Icterohaemorrhagiae and Australis.

Domestic pets and livestock are major disseminators of leptospires into both urban and rural environments. The importance of dogs has been confirmed in Port-of-Spain, Trinidad, where leptospires were isolated from the kidney tissues of 10 of 50 (20%) strays examined, and 53 of 96 strays (55%) were seropositive, with Canicola (40%), Icterohaemorrhagiae (30%), Pyrogenes (15%), Hebdomadis (6%) and Tarassovi (6%) being the five serogroups to which agglutinins were most frequently encountered (Everard et al., 1979a). Unpublished serologic studies from Trinidad and Grenada indicate that among cattle, pigs, sheep, goats and equids, agglutinins to the following serogroups are encountered most frequently: Icterohaemorrhagiae (33%), Autumnalis (26%), Hebdomadis (10%), Panama (6%), Canicola (5%) and Pyrogenes (5%). This sequence closely par-

allels that observed in hospital patients from Trinidad. It also seems that wild animals are an important source of contamination of the environment by leptospires and therefore a probable cause of human infection.

Zoonoses in wildlife vertebrates which are not transmitted by arthropod vectors or through direct contact need other compensating factors for successful transmission of high endemicity. The presence of soil moisture or free-standing water provides this factor for leptospires. The expansion of agrarian economies in the southeast Caribbean with their emphasis on irrigation and water management has led to continually changing patterns of land usage and development, and to a more favorable environment for leptospires. The ability of efficient wildlife carriers of leptospires (particularly mongooses, toads, and peridomestic rodents) to contaminate with large numbers of pathogenic leptospires the soil and water resources of rural communities should be especially recognized.

ACKNOWLEDGMENTS

LITERATURE CITED

ALKINS, M. E. 1979. The Mammals of Trinidad. Department of Zoology, Occasional Paper No. 2, University of the West Indies, St. Augustine, Trinidad, 75 pp.

BABUDIERI, B., E. R. CARLOS, AND E. T. CARLOS. 1973. Pathogenic leptospira isolated from toad kidneys. Trop. Geogr. Med. 25: 297–299.

Boos, H. 1975. Check list of Trinidad snakes. J. Trin. Field Nat. 1975: 22–28.

CENTERS FOR DISEASE CONTROL. 1979. Leptospirosis Surveillance, Annual Summary 1978. Centers for Disease Control, Atlanta, Georgia, 8 pp.

CIRONE, S. M., H. P. RIEMANN, R. RUPPANER, D. E. BEHYMER, AND C. E. FRANTI. 1978. Evaluation of the hemagglutination test for epidemiologic studies of leptospiral antibodies in wild mammals. J. Wildl. Dis. 14: 193–202.

COLE, J. R., C. R. SULZER, AND A. R. PURSELL. 1973. Improved microtechnique for the leptospiral microscopic agglutination test. Appl. Microbiol. 25: 976–980.

DAMUDE, D. F., C. J. JONES, AND D. M. MYERS. 1979a. A Study of leptospirosis among animals in Barbados W.I. Trans. R. Soc. Trop. Med. Hyg. 73: 161–168.

————, ————, H. ST. C. WHITE, AND D. M. MYERS. 1979b. The problem of human leptospirosis in Barbados. Trans. R. Soc. Trop. Med. Hyg. 73: 169–177.

DE VOS, A., R. H. MANVILLE, AND R. G. VAN GELDER. 1956. Introduced mammals and their influence on native biota. Zoologica 41: 163–194.

ELLINGHAUSEN, H. C., AND W. G. McCULLOUGH. 1965. Nutrition of *Leptospira pomona* and growth of 13 other serotypes: Fractionation of oleic albumin complex (OAC) and a medium of bovine albumin and polysorbate 80. Am. J. Vet. Res. 26: 45–51.

EVERARD, C. O. R., E. P. I. CAZABON, D. W. DREESEN, AND C. R. SULZER. 1979a. Leptospirosis in dogs and cats on the island of Trinidad: West Indies. Int. J. Zoonoses 6: 33–40.

————, AND G. M. FRASER-CHANPONG. 1979b. Serological evidence of leptospirosis in Caribbean schoolchildren. Trans. R. Soc. Trop. Med. Hyg. 73: 591–593.

————, ————, L. J. BHAGWANDIN, S. ALI, AND A. G. D. BUDHLALL. 1979c. Leptospirosis in patients from Grenada, West Indies. Trans. R. Soc. Trop. Med. Hyg. 73: 303–305.

————, A. E. GREEN, AND J. W. GLOSSER. 1976. Leptospirosis in Trinidad and Grenada, with special reference to the mongoose. Trans. R. Soc. Trop. Med. Hyg. 70: 57–61.

————, C. R. SULZER, L. J. BHAGWANDIN, G. M. FRASER-CHANPONG, AND A. C. JAMES. 1980. Pathogenic *Leptospira* isolates from the Caribbean islands of Trinidad, Grenada and St. Vincent. Int. J. Zoonoses 7: 90–100.

GALTON, M. M. 1966. Leptospiral serotype distribution lists, July 1966. U.S. Dept. of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia, 130 pp.

————, C. R. SULZER, C. A. SANTA ROSA, AND M. J. FIELDS. 1965. Application of a microtechnique to the agglutination test for leptospirosis antibodies. Appl. Microbiol. 13: 81–85.

GOODWIN, G. G., AND A. M. GREENHALL. 1961. A review of the bats of Trinidad & Tobago. Bull. Am. Mus. Nat. Hist. 122: 187–302.

GREEN, A. E., C. R. SULZER, C. O. R. EVERARD, AND W. L. JONES. 1978. Four new *Leptospira* serotypes from Trinidad. West Indian Med. J. 27: 117–126.

GROOME, J. R. 1970. A Natural History of the Island of Grenada, W.I. Caribbean Printers, Trinidad, 115 pp.

JOHNSON, R. C., AND V. G. HARRIS. 1967. Differentiation of pathogenic and saprophytic leptospires. J. Bacteriol. 94: 27–31.

————, AND P. ROGERS. 1964. 5-fluorouracil as a selective agent for growth of leptospirae. J. Bacteriol. 87: 422–426.

KENNY, J. S. 1969. The Amphibia of Trinidad. Studies on the Fauna of Curaçao and other Caribbean Islands. 29: 1–78.

KMETY, E., M. M. GALTON, AND C. R. SULZER. 1970. Further standardization of the agglutinin-absorption test in the serology of leptospires. Bull. W.H.O. 42: 733–738.

MINETTE, H. P. 1964. Leptospirosis in rodents and mongooses on the island of Hawaii. Am. J. Trop. Med. Hyg. 13: 826–832.

MYERS, D. M., A. C. CAPARO, AND J. P. MORENO. 1977. Isolation of serotype *hardjo* and other leptospirae from armadillos in Argentina. Bull. Pan. Am. Health Organ. 11: 131–139.

NELLIS, D. W., AND C. O. R. EVERARD. 1983. The biology of the mongoose in the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands. 195: 1–162.

SULZER, C. R. 1975. Leptospiral serotype distribution lists, supplement to 1966 Publication, July 1966 to July 1973. U.S. Dept. of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia, 70 pp.

————, AND W. L. JONES. 1976. Leptospirosis—Methods in laboratory diagnosis. U.S. Dept. of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia, H.E.W. Publ. No. (CDC) 79-8275, 40 pp.

TSAI, C. C., AND J. W. FRESH. 1971. Leptospirosis in the Hebdomadis and Pomona serogroups from mongooses in Taiwan. Trop. Geog. Med. 23: 201–203.

TURNER, L. H. 1968. Leptospirosis II—Serology. Trans. R. Soc. Trop. Med. Hyg. 62: 880–899.

————. 1970. Leptospirosis III—Maintenance, isolation and demonstration of leptospires. Trans. R. Soc. Trop. Med. Hyg. 64: 623–646.

UNDERWOOD, G. 1962. Reptiles of the Eastern Caribbean. Caribbean Affairs (New Series) Publication, Department of Agriculture, University of the West Indies, St. Augustine, Trinidad, 192 pp.