Pesq. Vet. Bras. 1(1):19-29, jan./mar. 1981

Leptospira interrogans in several wildlife species in Southeast Brazil[1]

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ABSTRACT.- Cordeiro F., Sulzer C.R. & Ramos A.A. 1981. [***Leptospira interrogans* in several wildlife species in southeast Brazil**.] *Pesquisa Veterinária Brasileira 1(1):19-29*. Proj. Sanidade Animal, Embrapa/UFRRJ, Seropédica, RJ 23890-000, Brazil.

A leptospiral host-serovar relationship in Southeast Brazil is described. Of the 43 animal species examined, 8, of the Orders *Rodentia and Marsupialia,* were identified as carriers of leptospires. The serovar *pomona* was found in 6 of the 8 carrier species. The "four-eyed" opossum (*Philander opossum*) has been shown to be a carrier of the serovars *ballum* and *grippotyphosa.* The serovar *australis* was found in a water rat *(Nectomys squamipes).* The serovar *mangus,* of the serogroup *Panama,* was found in an opossum *(Didelphis albiventris).*

INDEX TERMS: *Leptospira interrogans,* serovars (serotypes), wildlife species, *Rodentia, Marsupialia*, Brazil.

RESUMO.- [**Leptospira interrogans em diversas espécies de animais silvestres na Região Sudeste do Brasil.**] De 43 espécies de animais examinados, 8 pertencentes às Ordens *Rodentia* e *Marsupialia*, foram identificadas como portadoras de leptospiras. O sorovar *pomona* foi encontrado em 6 das 8 espécies portadoras: A cuíca *(Philander opossum)* foi identificada como portadora dos sorovares *ballum* e *grippotyphosa.* O sorovar *australis* foi encontrado em rato d'água *(Nectomys squamipes).* O sorovar *mangus,* do sorogrupo *Panama,* foi encontrado em um gambá *(Didelphis albiventris).*

TERMOS DE INDEXAÇÃO: *Leptospira interrogans,* sorovares (sorotipos), animais silvestres, *Rodentia, Marsupialia,* Brasil.

INTRODUCTION

Wild animals are known to be carriers of the causal agent of leptospirosis (*Leptospira interrogans*), and some of these animals may be natural hosts for pathogenic leptospires (Alston & Broom 1959). Infection is believed to be widespread among wild animals which transmit the organism to domestic animals and to man. The host-parasite relationships in leptospirosis apparently are complex. In addition to a wide array of hosts, several serovars are also involved in the *Leptospira interrogans* complex (U.S. Dept of Health, Education and Welfare 1966, 1975). By 1967, 130 serovars distributed over 18 serogroups had been identified, many of them isolated from wild animals (World Health Organization 1967).

From the epidemiological point of view it is important to know which animal species are reservoirs and which serovars are involved in a particular area of study. Some serovars are known to have an elective affinity for certain species, so called primary hosts, in which they cause a mild disease with little damage to the host. Primary hosts can harbor the spirochaetes in their kidney tubules and shed them intermittently into the urine for long periods.

The most common pathogenic serovars in Brazil are: *icterohaenaorrhagiae,* isolated from man (Corrêa 1969/70), dog (Castro et al. 1962), cattle (Rosa et al. 1961), and swine (Rosa et al. 1970); and *pomona,* found in cattle (Freitas et al. 1957) and swine (Rosa et al. 1973). Several other serovars have been recovered from human patients (Corrêa 1969/70, Corrêa et al. 1904, 1965/67) and from animals (Guida 1948, 1958, Guida et al. 1959). In addition, antileptospiral agglutinins have been detected in serological surveys (Castro et al. 1962, Rosa et al. 1969/70, Cordeiro et al. 1974, 1975a).

The following serovars have been recovered from wild animals: *icterohaenaorrhagiae, ballum, grippotyphosa, wolffi and szwafizak.* The wild animals involved were: norwegian rat (*Rattus norvegicus*), South American field mouse (*Akodon arviculoides*), roof rat (*Rattus rattus*), opossum (*Didelphis marsupialis*), wild guinea pig (*Cavia aperea*), four-eyed opossum (*Philander opossuna*), water rat (*Nectomys squamipes*), rice rat (*Oryzomys eliuris* and *O. ratticeps*), burrowing mouse (*Oxymycterus quaestor*), and cane rat (*Zygodontomys basiurus*) (Castro et al. 1961, Corrêa et al. 1965/67, Rosa 1970). Five new serovars have been isolated and identified in Brazil: *brasiliensis,* from the opossum; *guaratuba,* from lhe four-eyed opossum; *guaicurus* and *goiano,* from cattle; and *guidae,* from swine (Wolff & Bohlander 1960, Rosa 1970,

[1] Accepted for publication on August 20, 1980.

Based on a thesis submitted by F. Cordeiro in partial fulfillment of the requirements for the Master in Preventive Veterinary Medicine degree at the School of Veterinary Medicine, University of California, Davis, Ca 95616, USA, in August 1977.

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Rosa et al. 1975, U.S. Dept of Health, Education and Welfare 1975). Most of this research has been done in the State of São Paulo.

In Itaguaí County, State of Rio de Janeiro, the house mouse *(Mus muscutus brevirostris)* has been found to be an important reservoir of leptospires, particularly of the serovars *ballum* and *tropica* (Cordeiro 1970, Cordeiro & Silva 1974). In a serological survey among dairy cattle from 28 counties in this State about 22% of the sera were positive; the serovars mainly involved were *wolffi, tarassovi, grippotyphosa, pomona and bratislava* (Cordeiro et al. 1975b). Therefore, it seemed desirable to evaluate the host-serovar relationships in the region. In this report the bacteriological, serological, and epidemiological findings concerning the natural occurrence of leptospires among several wildlife species in the southeastern region of Brazil, from 1968 to 1974, are described.

MATERIALS AND METHODS

Origin of the material

Most of the animals used in this study were trapped live inthe field, some in the store rooms of several farms, and a few were shot in the field. Those animais came from 25 counties in the States of Rio de Janeiro, São Paulo and Minas Gerais, located in Southeast Brazil (Fig.1, Table 1). Most of these areas consist of low growing vegetation and natural pastures, with some forest. The altitude varies from 5 to 800m. The annual medium temperature is 23°C in the plains and 18ºC in the mountains. The annual rainfall is about 1304 mm, and the relative air humidity is 75%. The 43 species examined, belonging to 12 orders and totaling 1,064 animals, are described in Table 2. These animals were classified in the Instituto de Biologia of the Universidade Federal Rural do Rio de Janeiro.

Table 1. Distribution of the wild animais captured in several counties of Southeast Brazil, 1968-1974

County		Wild animals captured	
Nº	Name	Total	Genera
1	Miracema	6	*Rattus*
2	Sta. Maria Magdalena	6	*Rattus, Cavia, Philander*
3	Campos	6	*Rattus, Didelphis*
4	Cordeiro	9	*Rattus, Didelphis, Nectomys, Akodon*
5	Bom Jardim	2	*Rattus*
6	Nova Friburgo	6	*Rattus, Philander*
7	Conceição de Macabú	25	*Akodon, Rattus, Nectomys*
8	Macaé	48	*Philander, Rattus, Nectomys, Akodon*
9	Casimiro de Abreu	21	*Rattus*
10	Cabo Frio	1	*Philander*
11	Rio Bonito	9	*Rattus, Oxymycterus, Akodon, Nectomys*
12	Três Rios	26	*Mus, Rattus, Akodon, Didelphis, Bufo*
13	Duque de Caxias	5	*Molossus, Desmo dus*
14	Rio de Janeiro	16	*Mus, Desmodus, Rattus*
15	Itaguaí	676	*35 genera; most Akodon, Mus, Rattus*
16	Vassouras	19	*Bufo, Leptodactylus, Akodon, Didelphis, Rattus, Coendou*
17	Valença	61	*Rattus, Didelphis, Mus, Akodon, Cavia, Stombus, Bufo*
18	Barra do Piraí	3	*Rattus, Oryzomys*
19	Resende	9	*Didelphis, Rattus*
20	Bananal	37	*Rattus, Didelphis, Akodon, Mus, Oryzomys*
21	Pindamonhangaba	44	*Akodon, Rattus, Bufo, Oryzomys, Mus*
22	São José dos Campos	1	*Didelphis*
23	Jaboticabal	11	*Didelphis, Tupinambis, Galictis*
24	Belmiro Braga	13	*Rattus, Mus, Oxymycterus*
25	Mar de Espanha	4	*Didelphis, Akodon*
Total		1,064	

Fig. 1. Part of southeast Brazil showing several counties where the wild animais were trapped. (Counties are identified in Table 1).

Bacterial methods

The animals captured live were anesthetized with ether and bled by cardiac puncture. They were killed by exsanguination. Kidneys and a piece of liver were removed aseptically and ground in a mortar with buffered saline (pH 7.4). The urine was collected by bladder puncture. Blood, urine, and a suspension of kidney and liver tissues were inoculated into tubes of Fletcher's semisolid and Korthof's liquid media, according to the method of Sulzer and Jones, and incubated at 30ºC. The tubes were inspected for growth of leptospires weekly, for 40 days. One weanling guinea pig was inoculated intraperitonially with 1 ml of tissue suspension from each sample of kidney and liver. The temperature of each guinea pig was measured from the 3rd to the 10th day after inoculation. When guinea pigs' anal temperature was 40ºC or higher the animal was exsanguinated, and the same material was used for isolation attempts. The same procedure was used with the guinea pigs that did not show temperature until the 10th day post-inoculation.

Serological methods

Three hundred and twenty eight serum samples were collected from the animais and stored frozen at -20ºC until tested by the microscopic agglutination test (MAT). Fifteen live cultures of leptospires (Table 3) were used as antigens. The antigens were grown for 7 days in Korthof's or Stuart's media, according to the method described by Sulzer and Jones. A titre of 1:40 or higher with at least 50% agglutination was considered a positive reaction.

Hyperimmune sera were prepared from 17 isolated strains by inoculating into 4 kg rabbits live cultures grown in Fletcher's semi solid medium. The rabbits were inoculated into the marginal vein of the ear with a weekly schedule of injections of ml, 2ml, 4ml, and 4ml. Seven days after the last inoculation, a blood serum sample was taken from each rabbit and tested for antibodies with the homologous strain by the MAT. If the titre was 1:12,800 or higher, the rabbit was exsanguinated, and the blood serum was collected and stored at -20ºC.

Preliminary identification of 19 of the 37 isolated strains considered in this study (Table 4) were made by the MAT; 17 of them were tested by the cross-agglutination screening procedure in the MAT, with leptospiral serovars and immune sera representative of all known pathogenic serogroups (Table 3). These strains, as well as the reference strains, were adapted to Korthof's, Stuart's or Ellinghausen's liquid media for use as antigen. The antigens were tested for density and purity before being used. Satisfactory antigens were considered to be those without contamination and with density of about 100 organisms per microscopic field, 450X, with dark-field condenser, and without "breed nests".

Serovars were determined according to the methodology recommended by Kmety et al. 1970. Cross agglutinin-absorption tests were performed on 17 strains and several serovars were detected in the preliminary identification.

Table 2. Wildlife species examined in search of *Leptospira interrogans* in the southeastern region of Brazil, 1968-1974

Order	Species	Common name	Total examined
Rodentia	*Akodon arviculoides*	South American field mouse	307
	Cavia aperea	Wild guinea pig, preá	8
	Coendou insidiosus	Coandú	1
	Euryzygomatomys guiara	Bamboo rat	1
	Mus musculus	House mouse	173[a]
	Nectomys squamipes	Water rat	42
	Oryzomys nigripes	Rice rat, calunga	10
	Oxymycterus hispidus	Swine rat	15
	Rattus norvegicus	Norway rat, sewer rat	21
	Rattus rattus	Roof rat	189
Marsupialia	*Didelphis albiventris*	Opossum, gambá	92
	Philander opossum	Four-eyed opossum	35
Chiroptera	*Artibeus lituratus*	Fruit bar	63
	Carollia perspicillata	Fruit bar	14
	Desmodus rotundus	Vampire bat	8
	Glossophaga soricina	Fruir and nectar bat	18
	Molossus molossus	lnsectivorous bat	3
	Molossus ater	Insectivorous bat	3
	Myotis nigricans	Insectivorous bat	2
	Noctilio leporinus	Fish eater bat	1
	Sturnira lilium	Fruit bat	2
	Vampyrops lineatus	Fruit bar	1
Camivora	*Galictis cuja*	Ferret	3
Edentata	*Euphractus sexcinctus*	Yellow hand armadillo	1
Falconiformes	*Heterospizia meridionalis*	Leather coat falcon	1
Strigiformes	*Tyto alba*	White owl	1
	Otus choliba	Spix screech owl	1
Cuculiformes	*Crotophaga ani*	Black anu	1
Gruiformes	*Aramides* sp.	Shore bird, saracura	2
Salientia	*Bufo crucifer*	Toad	1
	Bufo ictericus	Cururú toad	21
	Leptodactylus ocellatus	Frog	7
	Stombus boiei	Buli frog	1
Squamata	*Ameiva ameiva*	Calango	1
	Hemidactylus mabouia	Lagartixa	1
	Mabuia sp	Vibora	2
	Tupinambis teguixin	Lizard	3
	Ophiodes striatus	Glass snakc	1
	Leimadophis poecilogyrus	Grass snake	2
	Mastigodryas bifossatus	Jararacuçu do brejo	2
	Oxyrhopus petola	Snake	1
	Philodryas schotti	Parelheira snake	1
	Liophis miliaris	Water snake	l
Total			1,064

[a] Results of most of these examinations were previously reported (Cordeiro 1970, Cordeiro & Silva 1974).

Statistical methods

For statisticai analysis the data were grouped by species (Table 5) and the Chi-square test (Remington 1970) was performed to determine whether the presence of leptospires and animal species were independent variables.

The formula used was

$$X^2 = \sum^{k} \frac{(Oi - Ei)^2}{Ei}, \; i = 1$$

The relationship between the MAT and the procedure for isolating leptospira was determined in the same manner to test the hypothesis that the results of the two procedures are independent.

The sensitivity and specificity of the isolation procedure, as well as the agreement, when compared with the MAT, were determined according to the formla below:

	MAT		
	+	-	
	+ N++	N+-	N+.
Isolation	- N- +	N - -	N - .
	N.+	N. -	N.. = sample size

$$\text{Sensitivity} = \frac{N++}{N.+}$$

$$\text{Specificity} = \frac{N--}{N.-}$$

$$\text{Agreement} = \frac{(N++)+(N--)}{N..}$$

RESULTS

Of the 43 wild animal species examined (Table 2), 8 were identified as carriers of leptospires. They are Order Rodentia: *Mus musculus*, *Akodon arviculoides*, *Rattus rattus*, *Rattus norvegicus, Nectomys squamipes* and *Oryzomys nigripes;* Order Marsupialia*: Didelphis albiventris* and *Philander opossum.* Samples of these species totaled 869, of which 99 (11.39%) were found to be infected (Table 5). One hundred and twenty seven strains of leptospires were isolated from several tissues and body fluids of these animais (Table 6). The largest number of isolations were from renal tissue followed by urine, liver, and blood. Multiple isolates were sometimes obtained from different tissues and body fluids of the same animal (Table 7).

Of the 37 strains considered in this paper, 18 were preliminarily classified by the MAT, as follows: 9 belonging to Pomona serogroup, 3 to Javanica, 2 to Ballum, 1 to Panama, 1 to Australis, and 2 to Grippotyphosa. The Aa-14 strain does not react within our present serogroups. It must be checked to determine if it will infect animais. The origins of these strains are shown in Table 4. The other 18 strains were lost in the consecutive transferring, some by contamination and some because they did not grow satisfactorily in the media. Table 8 shows the distribution of the isolated strains by counties and the animal species and serogroups involved.

The prevalence of leptospiral antibodies found in the wild animal sera is shown in Table 9. Of 328 serum samples tested, 21 (6.4%) were positive (1:40 or higher). *Tarassovi* was the prevailing serovar in 5 counties: Bananal, Macaé, Campos, Belmiro Braga, and Casimiro de Abreu. The highest titres were for the serovars *pomona* and *panama* (1:320), in Itaguaí and Cordeiro counties, respectively.

According to the results of the cross agglutination screening test and the absorption tests (Tables 10, 11), the strains Aa-1, Aa-12, Dm-1, Mm-1, Ns-2, On-1, On-2 and Rr-4 were identical to *pomona* serovar; the strain Ns-1 to *australis;* the strain Po-1 to *ballum;* the strains Po-2 and Po-3 to *grippotyphosa,* and the strain Dm-8 to *mangus* in the *Panama* serogroup. The strains Aa-3 and Aa-4, identical to each other, and Rr-5 are new *javanica* serovars. The strain Aa-14 has tentatively been proposed as a new serogroup, but the pathogenicity needs to be completely established before this can be considered final. The strains Aa-9 and Aa-10 did not grow.

Table 3. Leptospiral serovars used as antigen in the microscopic serum-agglutination test and cross-agglutination screening test

Serogroup	Serovar	Strain
Icterohaemorrhagiae	*icterohaemorrhagiae*[a]	RGA
	copenhageni[b]	M 20
	mankarso[b]	Mankarso
Celledoni	*celledoni*[b]	Celledoni
Canicola	*canicola*[a, b]	Hond Utrecht IV
Ballum	*ballum*[b]	Mus 127
	castelloni[a]	Castellon 3
Pyrogenes	*pyrogenes*[a, b]	Salinem
	alexi[b]	Hs 516
Cynopteri	*cynopteri*[b]	3522 C
	butembo[a, b]	Butembo
Autumnalis	*auturnnalis*[a, b]	Akiyami A
	fort-bragg[b]	Fort Bragg
	sentot[b]	Sentot
	djasiman[b]	Djasiman
Australis	*australis*[b]	Ballico
	bratislava[a]	Jez bratislava
Pomona	*pomona*[a, b]	Pomona
Grippotyphosa	*grippotyphosa*[a, b]	Moskva V
Hebdomadis	*mim*[a]	Sari
	georgia[b]	LT 117
	wotffi[a, b]	3705
	borincana[b]	HS 622
Bataviac	*bataviae*[a, b]	Van Tienen
Tarassovi	*tarassovi*[a, b]	Perepelicin
Panama	panama[a, b]	CA 214 K
Shermani	*shermani*[b]	LT 821
Semaranga	*patoc*[b]	Patoc I
Andamana	*andamana*[b]	CH II
Javanica	*javanica*[a, b]	Veldrat Batavia 46

(a) Microscopic serum- agglutination test (MAT).
(b) Cross-agglutination test.

Table 4. Preliminary identification of some strains isolated from wildlife in Southeast Brasil, 1968-1974

Strain Nº	Origin of the material			Isolation date	Preliminary classification serogroup
	Animal reg. nº	Place of capture	Isolated from		
Aa-1[a]	125	Seropédica	Urine	08/01/69	Pomona
Aa-3	377	"	Kidney	01/21/70	Javanica
Aa-4	468	"	Blood	06/10/70	Javanica
Aa-9	645	"	Guinea pig	08/31/70	Ballum
Aa-10	660	Itaguaí	Kidney	08/21/70	Pomona
Aa-12	676	Macuco	Kidney	09/14/70	Pomona
Aa-14	1,000	Bananal	Urine, Kidney	11/05/73	?
Dm-1	264	Seropédica	Liver	11/10/69	Pomona
Dm-8	1,031	"	Kidney	06/23/74	Panama
Mm-1	14	"	Kidney	02/10/69	Pomona
Ns-1	592	"	Kidney	07/15/70	Australis
Ns-2	593	"	Nidney, Urine	07/09/70	Pomona
On-1	605	"	Livel	07/15/70	Pomona
On-2	682	Barra do Piraí	Kidney	10/07/70	Pomona
Po-1	598	Seropédica	Kidney	07/28/70	Ballum
Po-2	1,046	"	Kidney	05/17/74	Grippotyphosa
Po-3	1,049	"	Kidney	11/17/74	Grippotyphosa
Rr-4	635	Itaguaí	Kidney	09/07/70	Pomona
Rr-5	967	Seropédica	Kidney	05/16/73	Javanica

[a] Aa = *Akodon arviculoides*, Dm = *Didelphis albiventris* (previously identified as *D. marsupialis*), Mm = *Mus musculus*, Ns = *Nectomys squamipes*, On = *Oryzomys nigripes*, Po = P*hilander opossum*, Rr = *Rattus rattus*.

Table 5. Occurrence of *Leptospira interrogans* in several wildlife species in southeast Brazil, 1968-1974

Species	Total examined	Total found infected	% positives	Serovar found
Mus musculus[a]	173	63	36.41	*ballum, pomona, tropica*
Akodon arviculoides	307	14	4.56	*pomona,* 2 new serovars
Rattus rattus	189	5	2.64	*pomona,* 1 new serovar
Didelphis albiventris	92	8	8.69	*pomona, mangus*
Philander opossum	35	3	8.57	*grippotyphosa, ballum*
Nectomys squamipes	42	2	4.76	*australis*
Rattus norvegicus	21	2	9.52	not identified
Oryzomys nigripes	10	2	20.00	*pomona*
Total	869	99	ll.39	9 serovars 7 serogroups

[a] Results of most of these examinations were previously reported (Cordeiro 1970, Cordeiro & Silva 1974).

The Chi-square value calculated in contingency (Table 12) of leptospiral isolation results and identification of the animal species (X2 = 139.51) was significant ata level less than 0.1%. The calculated value for the Chi-square, in Table 13, (X^2 = 8.9987) must be considered significant at a level less than 1%. The agreement between the isolation and identification procedures was 89%. The sensitivity and specificity of the leptospiral isolation procedure, when compared with the MAT were 24% and 94%, respectively.

Table 6. Distribution of leptospiral isolations from wildlife of Southeast Brazil, according to specics and substrates

Wildlife species	Substrate					
	Kidney	Urine	Liver	Blood	Guinea pig	Total
Mus musculus	58	7	5	4	7	81
Akodon arviculoides	7	2	2	2	2	15
Didelphis albiventris	6	2	4	1	1	14
Rattus rattus	5	-	-	-	-	5
Philander opossum	3	-	-	-	1	4
Nectomys squamipes	2	1	-	-	-	3
Rattus norvegicus	1	1	-	-	1	3
Oryzomys nigripes	1	-	1	-	-	2
Total	83	13	12	7	12	127

Table 7. Multiple isolations obtained from different substrates of the same animal or passage in guinea pig

Substrates	Total of isolations
Kidney and urine	6
Kidney and guinea pig	5
Kidney and liver	4
Kidney and blood	1
Kidney, guinea pig and urine	2
Kidney, urine, blood and guinea pig	1
Kidney, urine, liver and blood	1
Liver and guinea pig	1
Blood and guinea pig	1

DISCUSSION

This study has demonstrated that the fauna in parts of the southeast Brazil, ias the are the States of Rio de Janeiro, São Paulo, and Minas Gerais, include several carriers of leptospira. The rate of infected animals found in the sample of involved species (11.39%) is significant when compared with the results of Santa Rosa (4%) (Rosa et al. 1970). If however, we consider only the samples of the counties where infected animals were found, the rate would be 17.31% (Table 8). It must represent a high potential of infection for domestic animals, particularly since the species recognized as carriers are widely distributed over the Brazilian territory.

The 8 species pointed out (Table 5) belong to the Orders Rodentia and Marsupialia, what reaffirms the previously observed carrier condition among rodents and marsupials. With the exception of the so called "commensals" (genera *Rattus* and *Mus),* the other species have already been recognized as carriers of several leptospiral serovars in the State of São Paulo. The observed serovars, however, are dissimilar. Serovar *grippotyphosa* showed the highest prevalence, but serovar *pomona* was prevalent in the State of Rio de Janeiro. It was found in 6 of the 8 carrier species. This serovar was isolated from aborted foetus of cows and sows of the States of São Paulo and Santa Catarina (Freitas et al. 1957, Rosa et al. 1973). In addition, it has been considered to be responsible for great losses in cattle-and-swine-raising operation in several countries (Cordeiro et al. 1975). Cattle and swine probably play an important role in mantaining the spirochaete and in spreading the disease among domestic animals. Antibodies against *pomona* have been observed in several serological surveys in bovines, swine, and equines in this region (Freitas et al. 1957, Rosa et al. 1969/70, Cordeiro et al. 1974, 1975a).

In the State of Rio de Janeiro the marsupial four-eyed opossum has been shown to be a carrier of serovar *grippotyphosa.* The same serovar was isolated from opossum and from several rodents in the State of São Paulo. Antibodies against it were detected in bovines, equines and swine. Several human cases of leptospirosis due to this serovar have been described in the Brazilian literature.

Serovar *ballum* was also found in the four-eyed opossum, and in the South American field mouse, as well as in the house mouse (Cordeiro 1970, Cordeiro & Silva 1974). The water rat was shown to be a carrier of serovar *australis,* which is known to be responsible for disease among sugar cane cutters in Australia (Alston & Broom 1958). Antibodies against it have been found in cattle, swine, dogs, and man (Corrêa et al. 1964, Rosa et al. 1969/70, Cordeiro et al. 1975a).

Some consideration must be given to the source of leptospira isolations. The kidney was demonstrated to be the most important tissue for the isolations (65%), which must be interpreted to mean that the infected animals were in the carrier state. However, urine (10%), liver (9%), blood (5%) and passage in guinea pigs (9%) contributed to the number of isolated strains.

Out of the 25 counties sampled, 11 had animal carriers in their fauna. The largest number of isolations were from animals from Itaguaí County. The largest sample, however, was from this County. Most of the counties were located in the State of Rio de Janeiro, but specimens from animals from 2 of the 4 counties sampled in the State of São Paulo, and from 1 of the 2 in Minas Gerais were positive.

The low prevalence of serum reactors (6.4%) among the 328 wild animal samples may be due to the fact that many were healthy non reacting carriers and that probably some in the carrier state were not producing antibodies. Antibody titres in the blood tend to decrease after the disease. The leptospires can, however, nest in the renal tubules without stimulating a detectable antibody response in the blood. Another consideration is that just 5 of the 24 sera from which isolations were obtained were positive in the MAT. Of these, 4 had antibodies for the same isolated serovar. The prevalent serovar *(tarassovi)* in the MAT was not isolated from any animal, but the only strain that had a low titre (1:100) in the screening test with this serovar was Aa-14.

The association observed between the results of leptospiral isolation and animal species estimated by the Chisquare value 139.51 ($P < 0.1\%$) and by the total isolations obtained from each species could reflect differences between species in Southeast Brazil, related to the ability to "carry" leptospires.

This was particularly true in the State of Rio de Janeiro. In addition, ecological and climatic factors could interfere in the distribution and propagation of the spirochaetes among the wild animals.

The association observed between the results of the isolations and the MAT estimated by the Chi-square value 8.9987 ($P < 1\%$) does not lead to the conclusion that an animal with leptospiral antibodies must necessarily harbor leptospires in its tissues. In other words, antibodies could be present and leptospires absent and vice versa, depending on when the samples were taken.

The percentage of sensitivity (24%) found shows that the isolation of leptospires from an animal is not necessarily associated with the presence of antibodies. The specificity (94%) and the agreement (89%) permit us to say that, despite the low sensitivity found, the leptospiral isolation procedure is a satisfactory one and the only tool for the identification of the animal carriers.

Table 8. Distribution of leptospiral isolations from wildlife by counties in Southeast Brazil, 1968-1974

County		Total of animals	Species involved	Total of isolations	Serogroup involved
Nº	Name				
1	Miracema	6	*Rattus rattus*	1	Not done
4	Cordeiro	1	*Akodon arviculoides*	1	Pomona
14	Rio de Janeiro	7	*Rattus rattus*	1	Not done
15	ltaguaí	144	*Mus musculus*	62	Pomona, Ballum
	"	260	*Akodon arviculoides*	11	Pomona, Ballum, Javanica
	"	30	*Didelphis albiventris*	5	Pomona, Panama
	"	31	*Rattus rattus*	4	Pomona, Javanica
	"	6	*Philander opossum*	3	Ballum, Grippotyphosa
	"	30	*Nectomys squamipes*	2	Pomona, Australis
	"	19	*Rattus norvegicus*	1	Not done
	"	5	*Oryzomys nigripes*	1	Pomona
16	Vassouras	1	*Akodon arviculoides*	1	Not done
17	Valença	19	*Didelphis albiventris*	1	Not done
18	Barra do Piraí	1	*Oryzomys nigripes*	1	Pomona
19	Resende	3	*Didelphis albiventris*	1	not done
20	Bananal	5	*Akodon arviculoides*	1	May be a new serogroup
21	Pindamonhangaba	2	*Mus musculus*	1	Not done
25	Mar de Espanha	2	*Didelphis albiventris*	1	Not done
	Total	572		99 =	17.31%

Table 9. Prevalence of leptospiral antibodies in wildlife sera from several counties in Southeast Brazil, 1968-1974

County	Sera			Serovars and reciprocal titres
	Examined	Positive	%	
ltaguaí	182	10	5.49	*pomona* = 320, *icterohaemorrhagiae* = 160, *tarassovi* - 160, *grippotyphosa* = 40
Bananal	31	4	12.90	*tarassovi* = 80
Macaé	19	3	15.79	*tarassoui* = 80
Cordeiro	8	1	12.50	*panama* = 320
Campos	6	1	16.67	*tarassovi* = 40
Belmiro Braga	2	1	50.00	*tarassovi* = *40*
Casimiro de Abreu	1	1	100.00	*tarassovi* = 160
13 other counties	79			
Total	328	21	6.4	

Table 10. Agglutination reactions of 17 leptospiral strains with antisera against 26 leptospiral serovars of the screening battery

Antiserum	Reciprocal titre against antigen																	
	Homol-ogous	Aa-1	Aa-3	Aa-4	Aa-12	Aa-14	Dm-1	Dm-8	Mm-1	Ns-1	Ns-2	On-1	On-2	Po-1	Po-2	Po-3	Rr-4	Rr-5
ballum	6,400	-[a]	50	-	-	-	-	-	-	-	-	ND	-	1,600	-	-	-	-
canicola	25,600	-	-	-	-	-	-	-	-	-	-	ND	-	200	-	-	-	-
copenhagen	12,800	100	-	200	100	-	100	400	100	400	-	ND	-	200	-	-	-	6,400
bataviae	25,600		-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-	-
grippotyphosa	12,800	-	-	-	-	-	50	-	50	-	-	ND	-	-	6,400	6,400	-	-
pyrogenes	12,800	-	200	-	-	-	-	-	-	100	-	ND	-	100	-	-	-	1,600
autumnalis	25,600	100	-	-	1,600	-	800	200	200	800	400	ND	400	-	-	200	400	-
pomona	51,200	6,400	-	-	51,200	-	6,400	-	6,400	200	6,400	6,400	6,400	-	-	-	12,800	-
wolffi	6,400	-	-	-	100	-	-	-	-	-	-	ND	-	-	-	-	-	-
australis	6,400	50	-	-	-	-	100	200	-	3,200	-	ND	-	-	400	-	-	-
tarassovi	6,400	-	-	-	-	100	-	-	-	-	-	ND	-	-	-	-	-	-
georgia	51,200	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-	
ja vanica	6,400	-	6,400	6,400	-	-	-	-	-	-	-	ND	-	-	-	-	3,200	3,200
celledoni	6,400	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	400	400
sentot	3,200	-	-	-	400	-	-	200	-	-	200	ND	-	-	-	200	-	-
djasiman	25,600	-	-	-	800	-	-	-	-	-	400	ND	-	-	-	100	-	-
borincana	12,800	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-	-
cynopteri	6,400	-	-	-	50	-	-	-	-	-	-	ND	-	-	-	-	-	-
butembo	12,800	ND	-	ND	50	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
alem	6,400	ND	-	ND	-	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
panama	12,800	ND	-	ND	-	-	ND	6,400	ND	ND	-	ND	ND	ND	-	ND	ND	-
shermani	12,800	ND	-	ND	-	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
patoc	6,400	ND	-	ND	-	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
andamana	51,200	ND	-	ND	-	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
fort-bragg	12,800	ND	-	ND	-	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	-
mankarso	6,400	ND	-	ND	100	-	ND	-	ND	ND	-	ND	ND	ND	-	ND	ND	800

[a]- = negative at 1:50 initial dilution.
ND = not done.

Table 11. Results of the cross agglutinin-absorption tests on 13 leptospiral isolated strains

Antiserum	Absorbed with	Reciprocal of titer against antigen			
		Homologous		Absorbing strain	
		Before	After	Before	After
pomona	Aa-1	6,400	200	6,400	-
Aa-1	*pomona*	12,800	-	6,400	-
pomona	Aa-12	51,200	400	25,600	400
Aa-12	*pomona*	25,600	-	6,400	-
pomona	Dm-1	6,400	100	1,600	50
Dm-1	*pomona*	12,800	100	6,400	100
mangus	Dm-8	25,600	200	51,200	100
Dm-8	*mangus*	51,200	100	25,600	200
pomona	Mm-1	6,400	400	3,200	200
Mm-1	*pomona*	6,400	50	6,400	-
australis	Ns-1	3,200	400	3,200	100
Ns-1	*australis*	6,400	100	1,600	-
pomona	Ns-2	6,400	-	3,200	-
Ns-2	*pomona*	6,400	-	6,400	-
pomona	On-1	6,400	400	3,200	-
On-1	*pomona*	6,400	100	3,200	-
pomona	On-2	6,400	400	6,400	100
On-2	*pomona*	3,200	-	6,400	-
ballum	Po-1	1,600	-	800	-
Po-1	*ballum*	1,600	100	800	50
grippotyphosa	Po-2	12,800	-	6,400	-
Po-2	*grippotyphosa*	6,400	-	6,400	-
grippotyphosa	Po-3	6,400	200	6,400	50
Po-3	*grippotyphosa*	12,800	400	12,800	100
pomona	Rr-4	12,800	400	6,400	200
Rr-4	*pomona*	6,400	-	3,200	-

- = negative at 1:50initial dilution.

Table 12. Contingency table of leptospiral isolations and animal species

Species	Leptospiral isolations		Total
	Positive	Negative	
Mus musculus	63	110	173
Akodon arviculoides	14	293	307
Rattus rattus	5	184	189
Didelphis albiventris	8	84	92
Philander opossum	3	32	35
Nectomys squamipes	2	40	42
Rattus norvegicus	2	19	21
Oryzomys nigripes	2	8	10
Total	99	770	869

X^2= 139.51; P<0.1%.

Table 13. Contingency table of the microscopic agglutination test and the isolation procedure results

		MAT		Total
		+	-	
Leptospiral isolations	+	5	19	24
	-	16	288	304
Total:		21	307	328

X^2= 8.9987; P< 1%; $\frac{293}{328}$ = 89% in agreement; $\frac{5}{21}$ = 24% sensitivity; $\frac{288}{307}$= 94% specificity.

+ = positive, - = negative.

Acknowledgments.- The authors thank Professor Adriano Lucio Peracchi, from the Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, 23890-000 Seropédica, R J, Brazil, for his assistence in classifying the animais. Thanks are also due to Vania Lucia Baêta Andrade, Luiz Dias de Castro Junior and Luiz Soares da Costa for t h e i r technical assistance.

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