Animal leptospirosis in the Federated States of Micronesia

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Abstract

Results of previous studies of leptospirosis on the island of Kosrae, and Pohnpei suggested that the prevalences to be among the highest in the world. This study was to broaden

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udies indicated the need to broaden
the islands of the FSM. In November,
expanded leptospirosis research to
uk, and Yap began. The objective of
was to document the prevalence of
and to better understand the risk
n infection.

eptospirosis

cterized by two broad categories of
d; and; non-host adapted. These
cal signs, epidemiology, and control
d leptospirosis occurs when a special
lationship exists between the infect-
cular host species. This relationship
mal becoming a carrier of leptospiro-
d of time, and maintains the infection
es of host adapted infections are, *L.*
dogs, *L. pomona* and *L. bratislava*
L. hardjo infections in cattle. Animals
tions are mildly affected, and gener-
of illness. The only clinical evidence
rosis in swine may be abortion when
d with a host adapted serovar during

assistance for the development of a control program for leptospirosis. Assistance was provided by a team of investigators from the Hawaii Department of Health, and Australia. Over the past several years the investigators traveled to Kosrae documenting the presence of leptospirosis in humans and animals.[3,4] Their findings indicated that leptospirosis was a serious health risk on Kosrae, and suggestions for the control of leptospirosis were submitted to the FSM Department of Health Services.

In 1993, investigators from the Pacific Basin Medical Officers Training Programme (PBMOTP), conducted a cross sectional survey in Pohn
the scope of the investigation into the other islands in the Federated States of Micronesia and centered on documenting the prevalence of the disease in animals. Swine, dogs, and rodents were selected for the survey because of their close contact with the human inhabitants. Serum samples were collected and then tested for leptospirosis by the Microscopic Agglutination Test (MAT). In addition to serological testing, attempts were made to isolate leptospirosis organisms from the kidneys and urine of pigs and rodents.

The MAT indicated that the serological prevalence of leptospirosis is high in each animal species tested. Leptospires were isolated from cultures taken in Pohnpei, and have been typed to serogroup. Isolate results as to serovar typing have been atypical, and may be due to the isolates being a previously unidentified serovar.

Animal leptospirosis surveys have been conducted in in the Pacific. Results from such surveys indicate that leptospirosis is likely to be the most serious disease threat to humans in these oceanic regions. Suggestions for leptospirosis control is made based on these results.

Introduction

Efforts to document leptospirosis in the Federated States of Micronesia (FSM), began on the island of Kosrae in 1991. In 1990, 9 people from Kosrae were evacuated to Hawaii because of acute kidney failure.[1-5] One death occurred before an airlift was possible. These incidents in 1990 prompted the FSM. Department of Health Services, to seek

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The primary epidemiological consideration of a host adapted infection is that the host becomes an asymptomatic carrier which periodically sheds millions of organisms in the urine over extended periods of time. This scenario creates a maintenance cycle of exposure leading to widespread infection among animals in the area, and a marked leptospire contamination of the environment as well. When a high prevalence of uncontrolled host adapted infection occurs, a significant public health risk develops. Human infection can occur via contact with the environmen t or carrier animals.

Non host adapted leptospirosis is considered an accidental infection, which occurs when exposed to leptospires adapted to, and shed by, another species of animal. Animals with non-host adapted leptospirosis usually become clinically ill, and then respond with a vigorous immune response. This host response is effective in clearing the leptospire from the animal, resulting in a short term carrier state with limited urinary shedding of the organism. For this reason non host adapted infection is limited to sporadic cases, and will not be maintained in a group of animals as long as the source of the non adapted leptospires is removed.

The immunologic aspects of host adapted leptospirosis should be considered when using serologic results from the MAT to assess the risk of human infection. The IgM antibody is the major antibody detected by the MAT[5], and may be relied upon as evidence of active or recent infection in an animal. An important limitation however, is that a diagnostic level of IgM antibody does not persist in the serum for extended periods of time. Such is the case in swine with a chronic host-adapted infection of moderate duration. The MAT has severe limitations in the diagnosis of abortion, and in the identification of renal or genital carriers of leptospirosis.[7] Infected animals may have MAT titers below the widely accepted minimum significant titer of 1:100. Leptospires may evade the immune system by becoming sequestered in the renal tubules and uterine lumen or in immunologically protected tissues, such as the eyes and brain.[6] When this occurs the immune system is no longer stimulated to produce IgM antibody of IgG antibody and the levels of IgM gradually subside to undiagnostic levels. Swine and other animals with this category of disease are a major source for the spread of leptospirosis to man and other animals.[8] During the first month of infection, these animals exhibit intense and constant urinary shedding of up to 100 million organisms / ml of urine.[9,10] Then later in the disease, as a chronic carrier, significant levels of organisms are intermittently shed in the urine for extended periods of time. [8,9,11,] In host-adapted cases such as these, the results of the MAT are not a reliable indicator for the presence of leptospirosis.

Materials and methods

Swine, dogs, and rodents where selected for leptospirosis testing because they are considered to be the primary mammalian species inhabiting the islands, and are in close contact with humans. Fruit bats due to their endangered status, and difficulty in capture, were not included in the survey. Cattle, goats, and horses are carriers of leptospirosis, but were excluded from the study because they are very rare in the FSM. Cats are plentiful, but are resistant to leptospirosis, and are normally not considered as carriers of the disease.

The leptospirosis survey in each state included serological testing of the three principal animal species using the MAT. On Pohnpei attempts were made to recover leptospire isolates for the purpose of serovar identification.

Sample Determination. Existing municipal boundaries were used to divide each island into sampling areas. The number of samples collected in each locale varied according to practical circumstances, such as animal availability, population, time available, and transportation logistics.

Porcine serum collection. Swine serum samples collected from confined pigs represent the most frequently sampled group of animals in the leptospirosis study. The pigs were restrained with the use of a hog snare, and 10 to 15 ml. of blood was removed from the right brachecephalic vein using a Vacutainer blood collection apparatus. The samples were chilled in an ice chest and transported to the lab for serum separation. After centrifugation the samples were frozen and stored pending shipment to the Leptospirosis Reference laboratory in Australia.

Canine serum collection. Dogs in the FSM are unconfined, and have wary dispositions making them the most difficult group of animals to sample. The majority of dogs sampled were located in close proximity to the pig farms. The animals were restrained, and 3 to 5cc whole blood was collected from the cephalic vein. The serum was separated and stored frozen until shipment.

Rodent serum collection. The majority of rodents tested were rats. A small number of mice were sampled as well. All rodents were trapped live, using wire netted traps. The most successful traps were set around households and pig pens. Although traps were set in more isolated areas, the traps were stolen. The rodents were taken to the local laboratory, and anesthetized. Rats were identified as one of three species, *Rattus rattus* (roof rat), *Rattus norvegicus* (Norway rat), and *Rattus exulans* (Polynesian rat). Mice were identified as one of two species, *Mus domesticuls*, (house mouse) and *Mus musculis* (musk mouse). Cardiac blood was collected from anesthetized rodents using a glass pipette. Serum was separated and stored frozen until shipment.

Serological testing

Porcine, canine, and rodent serum were submitted to the WHO Leptospirosis Reference Laboratory, Brisbane, Australia. All samples with IgG and IgM levels of 1:100 were considered positive for leptospirosis.[3]

Table 1. Leptospire identified from Pohnpei

Municipality	Species	MAT	Culture Result	Serogroup
Sokehs	Roof rat	negative	K+,U+	Ballum
Sokehs	Roof rat	negative	K-, U+	Australis
Sokehs	Norway rat	negative	K-,U+	Ballum
Sokehs	Roof rat	Australis	K+,U+	Australis
Sokehs	Polynesia rat	Australis	K-, U+	Australis
Sokehs	Norway rat	negative	K-, U+	Australis
Sokehs	Roof rat	negative	K-,U+	Australis
Sokehs	Roof rat	negative	K+,U+	Australis
Nett	Norway rat	negative	K+,U+	Australis
Kolonia	Roof rat	negative	K+,U+	Australis
Kolonia	Norway rat	Australis	K+,U+	Australis
Kolonia	Roof rat	negative	K+,U+	Australis
Kolonia	Roof rat	Australis	K+,U+	Australis
Kolonia	Roof rat	negative	K+,U-	Australis
Kolonia	Pig	No result	K+,U+	Australis
Kolonia	Pig	No result	K+,U+	Australis

Note. Abbreviations used: K = Kidney, U = Urine

Leptospire Isolation. Pohnpei was the only island where investigators were able to successfully isolate Leptospires from rats, and pigs. The majority of isolates were cultured from the rats.

Opportunities to culture pig kidneys and urine were limited to cultural events considered appropriate for investigators to interfere with the customary slaughter of pigs. Successfully isolating Leptospires from pigs slaughtered in the customary fashion was quite challenging. The pigs were processed on the ground either in dry dusty conditions, or wet muddy environments, along with a bountiful supply of flies.

Culture technique for isolation of Leptospires from porcine urine and kidney tissue. When swine were processed for the Kamadipw feast a survey crew aseptically remove a kidney from the freshly killed carcasses. The pig's abdomen was prepared for aseptic laparotomy by scrubbing the area with betadine surgical scrub and alcohol. The laparotomy was performed with sterile instruments. An assistant using sterile gloves spread open the abdominal wall, allowing another person to insert a sterile gloved hand into the abdominal cavity and manually remove one of the kidney. Immediately after removal of the kidney it was placed on another sterile gloved hand, and the glove was reflected over the kidney sealing it in a relatively sterile environment. The specimen was placed in a cooler and taken to the laboratory.

In the laboratory the kidney was prepared for the harvest of renal tissue intended for culture. A modified sterile plastic 3cc syringe barrel was used to remove 1 to 2 grams of kidney tissue. Sawing a 3cc plastic syringe barrel at a 45 degree angle creates a beveled opening that is capable of incising renal tissue, and allows for the removal of the sample. To prepare the kidney for culture a portion of the renal capsule is reflected exposing the underlying parenchyma. A hot spatula was used to sear the kidney in the exposed area. The syringe barrel was inserted into the seared portion of the kidney and 1 gram of underlying tissue removed. The renal tissue was placed in a small sterile plastic whirl bag containing 10 ml. of phosphate buffered saline (PBS) with 200 u gms. of 5-flourouracil, and then macerated to thoroughly disperse the sample within the PBS solution. The plastic bag containing the specimen was allowed to hang at an angle long enough to separate the fluid from the tissue. When separation occurred, a 3cc syringe with a 23 to 25 gauge needle was used to penetrate the plastic bag just above the fluid level. The needle was then placed 1/4 inch below the supernatant, and a 1 ml. aliquot removed. The 1 ml. sample was added to 9ml. of the PBS solution producing a 1 : 10 dilution. From this, 1/2ml. was placed in 5cc EMJH media or 5cc PMS media and allowed to incubate for up to 8 weeks. After 48 hours each of these tubes was also subcultured and diluted by another factor of 10. During incubation, cultures were examined under a dark field microscope every 5 to 7 days to detect the presence of leptospires.

Table 2. Pohnpei leptosporosis serologic results 1995-1996

Species	MAT	(IgG,IgM=100)	Positive	Serology
				62 bratislava (pb)
				11 bratislava
				2 australis
Swine	256	81	32	1 ballum
				1 canicola
				1 panama
				1 zanoni
				16 bratislava (pb)
				11 australis
Dogs	57	2	51	1 bratislava
				1 copenhageni
Rats	86	7	8	7 australis
Sokehs				
				1 australis
Swine	53	3	6	1 bratislava (pb)
				1 bratislava
Dogs	7	6	86	3 bratislava (pb)
				3 australis
Rats	32	1	3	1 australis
Kolonia				
Dogs	1	0		
	1	0		
Rats	27	5	19	5 australis
Nett				
Swine	48	2	4	2 bratislava(pb)
Dogs	3	1	33	1 bratislava
Rats	6	1	17	1 australis
U				
Swine	59	20	34	18 bratislava(pb)
				2 bratislava
Dogs	6	1	17	1 bratislava
Rats	9	0		

Species	MAT	(IgG,IgM=100)	Positive	Serology
Madolenihmw				
				20 bratislava(pb)
				4 bratislava
Swine	53	27	51	1 copenhageni
				1 ballum
				1 canicola
				6 australis
Dogs	21	11	52	4 bratislava (pb)
				1 copenhageni
Rats	6	0		
Kitti				
				4 bratislava
				australis
Swine	43	29	67	1 copenhageni
				1 panama
				1 zanoni
Dogs	19	10	53	8 bratislava(pb)
				2 australis
Rats	6	0		
Sapwuahfik				
				3 canicola
Swine	9	7	78	2 bratislava
				1 bratislava
				1 pomona
Dogs	2	1	50	1 bratislava (pb)
Nukuoro				
				2 canicola
Swine	15	4	27	1 bratislava(pb)
				1 javanica
Mwoakilloa				
				4 bratislava (pb)
				2 bratislava
				2 celledoni
Swine	63	13	21	2 canicola
				2 panama
				1 cynopteri
Kapingamarangi				
				1 bratislava
Swine	20	6	30	2 bratislava (pb
				1 canicola

Table 3. Kosrae leptospirosis serologic results 1996

Species	MAT	(IgG,IgM=100)	Positive	Serology
Whole of Kosrae				
Swine	145	40	28	22 bratislava 4 panama 3 bratislava (pb) 2 bratislava (dp) 2 zanoni 2 copenhageni 1 grippotyphosa 1 cynopteri 1 pomona 1 djasiman 1 szwajzak
Tafunsak				
Swine	40	11	28	6 bratislava 2 bratislava 1 pomona 1 cynopteri 1 grippotyphosa
Lelu				
Swine	40	10	28	6 bratislava 2 bratislava 1 pomona 1 cynoteri 1 grippotyphosa
Malem				
Swine	31	6	19	3 bratislava 1 copenhageni 1 panoma 1 djasiman
Utwa				
Swine	40	12	30	9 bratislava 1 bratislava (pb) 1 bratislava (db) 1 zanoni

Table 4. Chuuk leptospirosis serologic results 1996

Species	MAT	(IgG,IgM=100)	Positive	Serology
Whole of Chuuk				
Swine	141	59	42	30 bratislava 8 copenhageni 5 bratislava (pb) 4 bratislava (db) 3 robinsoni 3 panama 2 pomona 2 zanoni 1 australis 1 canicola
Dogs	9	6	67	3 copenhageni 1 bratislava 1 bratislava (pb) 1 hardjo
Rats	2	0		
Weno				
Swine	69	29	42	14 bratislava 4 bratislava (pb) 3 bratislava (db) 3 panama 2 pomona 1 australis 1 canicola 1 robinsoni
Dogs	2	2	100	1 copenhageni 1 bratislava
Rats	2	0		
Uman				
Swine	72	30	42	16 bratislava 8 copenhageni 2 zanoni 2 robinsoni 1 bratislava pb) 1 bratislava (dp)
Dogs	6	4	67	2 copenhageni 1 bratislava 1 hardjo

Table 5. Yap leptospirosis serologic results 1996

Species	MAT	(IgG,IgM=100)	Positive	Serology
Swine	135	51	34	31 bratislava 6 copenhageni 6 panama 2 pomona 2 zanoni 2 bratislava (pb) 1 bratislava (dp) 1 bulgarica
Rats	22	1	5	1 bulgarica
Colonia / Dalipebineaw				
Swine	35	12	34	6 bratislava 2 copenhageni 1 pomona 1 panama 1 bratislava (pb) 1 bratislava (db)
Rats	22	1	5	1 burgarica
Namgil				
Swine	30	8	27	7 bratislava 1 bulgarica
Tomil / Gagil / Ramuu				
Swine	37	12	32	8 bratislava 2 panama 1 copenhageni 1 zanoni
Maap				
Swine	33	19	58	10 bratislava 3 panama 3 copenhageni 1 bratislava (pb) 1 zanoni 1 pomona

Urine for culture, was collected by cystocentesis at the time the kidney was removed. A 1 ml. urine sample was added to 9ml. PBS producing a 1:10 dilution and 1/2ml. of this solution placed in EMJH media. Each tube was subcultured after 48 hours incubation, and all cultures were monitored for growth over a 2 month period.

Culture technique for isolation of Leptospires from rodents. After capture, the rodents were transferred to large heavy gauged transparent plastic bags. This allowed the investigators to safely grasp the rodent and present if for the injection of an anesthetic agent. Sodium pentobarbital, or the combination of Ketamine and Xyalazine were used with great success. The drugs provided a predictable and profound analgesia allowing the animal to be sampled in a humane manner. The anesthetized rats were placed on dissecting boards for laparotomy. Kidney tissue and urine were aseptically removed. The samples were then placed into 5ml. of EMJH media or PMS media and subcultured immediately, and after 48 hours.

Results

Because of cross agglutination reactions among serovars and serogroups, accurate interpretation of MAT results must also be based on information received by culture and isolation of leptospirosis organisms. Twenty six positive cultures were obtained from Pohnpei and have been identified as to serogroup. For the last two years extensive testing, in an effort to identify the isolates as to serovar, has resulted in speculation that a previously unidentified serovar has been isolated from rats and pigs on Pohnpei. The organism in question belongs to the serogroup Australis. However, because cross agglutination is extensive in the Australis serogroup, the identification of the serovar is still in question. At this time further attempts at identification are underway, and final identification may not be available until the Spring of 1998. Table 1 represents the culture and isolation results.

Leptospirosis, prevalence and serovar identification in the FSM

The most common serovars identified by the MAT belong to the Australis serogroup, these serovars are bratislava (pig biotype), bratislava, and australis. It is likely that the organisms identified by the MAT may not be present, and this represents the serologic reaction of the unidentified serovar obtained from the isolate cultures. Until the information is available, the serologic results are reported and interpreted as received.

The MAT is considered the standard serologic test for the diagnosis of leptospirosis. The was used to detect IgG and IgM antibodies against the following serovars

1. pomona
2. hardjo
3. tarassovi
4. grippotyphosa
5. celledoni
6. copehhageni
7. australis
8. zanoni
9. robinsoni
10. canicola
11. kermastos
12. szwajizak
13. medanensis
14. bulgarica
15. cynopteri
16. ballum
17. bataviae
18. djasiman
19. javanica
20. panama
21. shermani
22. bratislava

22a. dog biotype (db)
22b. pig biotype (pb)

Discussion

The Leptospirosis survey provided an opportunity to observe a large percentage of the pigs, and many of the dogs some of the islands of the FSM. The lack of severe clinical signs of leptospirosis, and the high frequency of positive

serologic results, suggest that host adapted leptospirosis is entrenched in the FSM animal populations. Serologic results also suggest that some leptospires appear to have become host adapted to more than one host species. Such may be the case with the leptospire for which the serological reaction *L. bratislava*, and *L. bratislava* (pig biotype) was found with high frequency in clinically normal dogs and pigs.

Rodents have always been identified with the spread of leptospirosis, particularly, the serovars which cause severe clinical infections in human. Rodents play a significant role in the leptospirosis problem in the FSM[2,5]. Hospital records indicate that Weils disease, a severe form of leptospirosis attributed to rats, does occur frequently in Pohnpei and Kosrae. In addition, the serologic results suggest that rodents may share host adapted serovars with pigs and dogs. This is a question, however, that needs to be clarified with further research. The serologic prevalence of leptospirosis in dogs was for the most part documented on the island of Pohnpei where investigators were able to test a significant number of animals[2,3,4]. The serologic prevalence of 51% is extremely high, and dogs should be considered to play a major role in the spread of the disease. Unlike the majority of pigs, which are confined, dogs roam and urinate over a broad area. This promotes environmental contamination with leptospires, especially near households and pig pens.

The high prevalence of host adapted infections among the animals studied indicates the health risk FSM residents from leptospirosis. The source of the infection should be considered to be both from the environment heavily contaminated with leptospires, and through contact with the carrier animals. The following suggestions are offered for formulating a leptospirosis control program.

Suggested leptospirosis control program

Mitigation of the effects of leptospirosis can be accomplished through the following control measures:

1. The systematic reduction of the number of leptospires in the environment through:
   a) vaccination of animals;
   b) periodic administration of antibiotics to the animal population; and
   c) eradication of the rodent population;

2. A public education campaign, to increase public awareness of:
   a) the risk of infection;
   b) the symptoms; and
   c) the benefits of early treatment.

Given the significant health risk of leptospirosis in the FSM, and the practical considerations of implementing an effective vaccination program, and the time it will take to institute other control measures, it is imperative that a public education campaign to increase public awareness be implemented at the earliest possible date.

Vaccination. To confer protective immunity and reduce urinary shedding, a leptospirosis vaccine must contain the serovars present within the locality, and must be administered every 6 months. Human vaccination is occasionally used to control leptospirosis in some circumstances, but the detrimental side effects of the vaccine preclude the routine use of human vaccination as a control method. Animal vaccination is an accepted and proven method for controlling leptospirosis. Vaccination has been shown to significantly reduce shedding of leptospires by animals when administered at least twice a year.

At this point, it may be feasible to develop a vaccine which contains the major causative serovars present on the islands. Isolates have been obtained and are maintained at the Leptospirosis Reference Laboratory. These isolates may be used to manufacture vaccines that will be efficacious for use in FSM. In 1995, Grand Laboratory, in South Dakota, USA quoted a price of $US360 to produce 1500 doses of a trivalent leptospirosis vaccine.

In making the decision whether or not to initiate a vaccination program in the FSM, some practical considerations must be addressed. The question of who will be willing and or able to bear the financial commitment to develop a vaccine, and administer it must be resolved. To be effective a leptospirosis vaccination must be administered twice yearly, and dogs as well as pigs should be vaccinated. Considering the life style of the dogs in the FSM this is a considerable task. The enormous logistic necessary for regular animal and human vaccination will be major constraints.

Rodent Eradication. In addition to a vaccination initiative, a rodent eradication program must also be initiated in order for there to be an effective reduction in the number of leptospires in the environment.

Antibiotics. Periodic administration of antibiotics will also help to reduce urinary shedding of leptospires in animals. However, many of practical considerations related to vaccination also apply to this method of control. Antibiotic use to control leptospirosis is a hit or miss expensive proposition at best.

Public Education and Awareness. The significant public health risk of leptospirosis in FSM, and the recent price paid in human lives and suffering, a vital determining factor to be considered in the selection of a control plan is whether it can be put into place quickly and at minimal expense. Probably the single-most effective control measure would be the institution of a nationwide public information, education, and awareness program. While the practical considerations of vaccination and other means of control are being worked out,

a public awareness campaign should be conducted. A nationwide public information program is the most practical and most beneficial control measure available. Such a program should not be considered as the only means of control, but could be implemented immediately with environmental health and personal hygiene initiatives.

Intervention with antibiotics in the early stages of the disease will ameliorate the symptoms within 24 hours. An informed public, seeking treatment when signs first develop, will reduce the severity of the disease. The objective of the public information program should be to present basic information about leptospirosis so that:

a) the risk of infection;
b) the symptoms; and
c) the benefits of early treatment

become common knowledge. Island media should be utilized to provide public service announcements. Posters and brochures should be distributed. A "Leptospirosis Awareness Month" should be declared.

A special effort should also be made to inform tourists visiting the islands in a non-alarming way, that there is risk of leptospirosis, and what precautions should be taken to avoid infection. When the tourist leaves and develops the disease elsewhere, they must tell the physician where they have been.

Conclusion

The results of this study underscore the need to develop a control plan for the systematic reduction of leptospirosis. The presence of wide spread infection in the animals that are in close contact with humans, and a heavily contaminated environment combine to create an extremely dangerous situation in the FSM. For the past seven years researchers and advisors have suggested that control plans be implemented, yet no meaningful plan has been forthcoming. Funds must be generated and dedicated for the implementation of effective control measures for this disease at the earliest possible date.

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