POST-FLOODING SURVEILLANCE OF LEPTOSPIROSIS AFTER THE ONSLAUGHT OF TYPHOONS NESAT, NALGAE AND WASHI IN THE PHILIPPINES

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Abstract. Due to the tropical climate of the Philippines, the country experiences at least 20 typhoons annually. Numerous cases of leptospirosis are reported as aftermaths of typhoons, especially after heavy flooding. In September 2011, two storms hit the upper regions of the country while in December of the same year, a storm struck the southern part of the Philippines. These storms resulted in widespread flooding in the affected areas. Due to this, surveillance of leptospirosis among residents of Regions I, III and X were conducted. A total of 190 persons (symptomatic and asymptomatic) participated in the post-flooding surveillance on leptospirosis. Ninety-four of the 190 participants were MAT-positive. Fifty-nine of the seropositives were symptomatic. Results also showed that symptomatic and asymptomatic males, farmers (agriculture), and those in the productive age group (21-40 years old), were the most affected in both the symptomatic and asymptomatic cases. The most common infecting *Leptospira* serovars were Patoc, Semaranga, Grippotyphosa, and Ratnapura, Copenhageni, and Poi in all the subjects.

Keywords: leptospirosis, post-flooding, Typhoons Nalgae, Nesat, and Washi, Philippines

INTRODUCTION

From its time of recognition, leptospirosis was considered as an occupational zoonotic disease associated with exposure to urine of animals containing leptospires, either by direct contact or indirectly through mud and surface waters contaminated with urine (Vinetz, 2001; Bharti *et al*, 2003; ILS and WHO, 2003). In the Philippines, leptospirosis is very common although most cases are underreported due to its nonspecific signs and symptoms and scarcity of laboratories that may aid physicians in diagnosing cases (Masuzawa *et al*, 2001; Yanagihara *et al*, 2007). In the rural areas, infection is usually due to occupational exposure. However, due to the country's tropical climate, flooding is also a major source of infection especially during the wet season, especially in urban areas. Outbreaks of

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leptospirosis in the Philippines are usually reported during the rainy months, June to November (Masuzawa *et al*, 2001; Yanagihara *et al*, 2007).

Leptospirosis in humans may either cause mild symptoms like fever, headache, malaise, myalgia, etc. (Faine *et al*, 1999; Vinetz, 2001; Bharti *et al*, 2003; ILS and WHO, 2003). But, if diagnosed and treated late, may cause severe symptoms like renal and hepatic failure, pulmonary hemorrhage, and even death. In the Philippines, several studies on human leptospirosis, mostly serosurveillance, have been conducted (Famatiga, 1971; Arambulo *et al*, 1972; Famatiga *et al*, 1972; Basaca-Sevilla *et al*, 1981; Padre *et al*, 1988; Masuzawa *et al*, 2001; Yanagihara *et al*, 2007; Amilasan *et al*, 2012). These and other unpublished studies have reported the following symptoms commonly observed from leptospirosis cases: fever, abdominal pain, headache, arthralgia, myalgia, conjunctival suffusion, oliguria and/or proteinuria, renal failure, jaundice. MAT-positivity or seropositivity ranged from 21% to 70% (Basaca-Sevilla *et al*, 1981; Padre *et al*, 1988; Masuzawa *et al*, 2001; Yanagihara *et al*, 2007). Reported case-fatality rates or mortality rates also varied ranging from 8-14% (Yanagihara *et al*, 2007; Amilasan *et al*, 2012).

An average of 20 storms hit the Philippines every year. In September 2009, two successive strong typhoons hit the country submerging 80% of Metro Manila (McCurry, 2009). Due to these strong typhoons followed by flood, 2,000 people were suspected to have been infected with leptospirosis. And, of this number, 175 died due to the said infection. In another study (Amilasan *et al*, 2012), researchers reported that 471 persons were hospitalized in a government-owned hospital due to an outbreak of leptospirosis in 2009. Fifty-one of the 471 hospitalized patients died due to the severity of infection and delay in treatment. In September 2011, Typhoon Pedring (International name: Nesat) hit 35 provinces in the country. Five days later, Typhoon Quiel (International name: Nalgae) further damaged 17 out of the 35 provinces. After two months, a strong tropical storm, Sendong (International name: Washi), struck the southern part of the country. These storms resulted in widespread flooding and because of this, the study was implemented in order to conduct surveillance of leptospirosis in the hard-hit areas such as Regions I, III, and X.

In preventing and controlling leptospirosis, identification of the prevailing serovars that are endemic in a certain geographic area is important. This will be helpful in developing preventive and control measures against the said infection. One of the measures is that of developing a local serovar-specific vaccine. Vaccination may prevent carrier state and shedding of pathogenic leptospires in animals, and serve as an effective prophylaxis for humans to prevent severe cases of leptospirosis and even death.

MATERIALS AND METHODS

Study area and study population

An enhanced post-flooding surveillance in selected regions was conducted due to the flooding brought by strong typhoons in 2011. Regions I (Ilocos Region) and III (Central Luzon Region) were chosen as study areas after the said regions experienced consecutive typhoons, Pedring and Quiel, in September. Region X (Northern Mindanao Region), on the other hand, was included in the enhanced surveillance after the area was hit by Typhoon Sendong in December. Samples

from Regions I and III were collected from November 2011 until March 2012 while samples from Region X were collected from December 2011 until February 2012.

The participants were divided into symptomatic (hospital) patients and asymptomatic (community) participants. Hospital participants were suspected leptospirosis cases that were admitted in the hospital and had clinical manifestations of the said infection (hereinafter alternatively referred to as symptomatic or hospital patients). Community participants (hereinafter alternatively referred to as community or asymptomatic participants) were those persons residing in the same area and had possibly the same exposure as the symptomatic patients. A total of 190 participants from Regions I, III, and X were included in the study. From Region I, 76 persons joined the study. Forty-six of them came from hospital referrals while 30 were asymptomatic participants. Forty-three subjects were from Region III and consisted of 9 hospital patients and 34 community participants. Lastly, all 71 participants from Region X were from hospital referrals.

A structured questionnaire was used in order to get the socio-demographic characteristics of the subjects. This was administered through one-on-one interview with the subjects or with their guardian and given in English or the vernacular.

Microscopic agglutination test

Serum samples were obtained from both the hospital and community participants. The subjects' sera were used for serological diagnosis of leptospirosis of the participants using the microscopic agglutination test (MAT). The sera were made to react with live antigens of different leptospiral serovars. After a 2- to 4-hour incubation period, the serum-antigen mixtures were checked for agglutination under a darkfield microscope. A titer ≥1:400 in a single serum sample was considered MAT-positive (Masuzawa *et al*, 2001; ILS-WHO, 2003).

A panel composed of 18 strains belonging to 17 serovars was used in this study. Three of the antigens are local isolates *ie*, strains LT 398 (Kmety and Dikken, 1993), LT 101-69 (Kmety and Dikken, 1993), and UP-BL-FR13 (Yanagihara *et al*, unpublished data). Of these 18 strains, 16 are pathogenic and 2 are non-pathogenic strains (strains Veldrat Semarang 173 and Patoc I).

Rapid test kit

A qualitative rapid test kit for detecting *Leptospira* IgG and IgM by BioLine© (Standard Diagnostics: Gyeonggi-do, Korea) was also used to diagnose leptospirosis among the subjects. This rapid test kit is based on immunochromatographic lateral flow method wherein 5 μl of the patient serum was added in the test kit and made to react with the assay diluent. After 20 minutes of incubation, the appearance of a purple "G" or "M" line in the test kit would indicate the presence of *Leptospira* IgM and/or IgG antibodies specific for *L. interrogans* species only.

Culture

Whole blood and urine samples were collected from the symptomatic and asymptomatic participants. One, two, and three drops of whole blood and urine were each inoculated in modified Korthof's medium with 5-fluorouracil (5-FU) and kept at 30°C (ILS-WHO, 2003; Villanueva *et al*, 2010). These cultures were examined for growth of leptospires weekly for 3 months. Negative cultures were only declared after the three-month observation period.

Ethical considerations

Collection and analyses of the different samples from humans was reviewed

Table 1
Distribution of MAT results of post-flooding surveillance samples from symptomatic patients.

Region	MAT results			MAT-positivity (%)
	MAT-positive	MAT-negative	Total	
Region I	24	22	46	52.2
Region III	6	3	9	66.7
Region X	29	42	71	69.1
Total	59	67	126	46.8

Table 2
Distribution of MAT results of post-flooding surveillance samples from asymptomatic participants.

Region	MAT results			MAT-positivity (%)
	MAT-positive	MAT-negative	Total	
Region I	16	14	30	53.3
Region III	19	15	34	55.9
Region X	0	0	0	0.0
Total	35	29	64	54.7

and approved by the University of the Philippines Manila Research Ethics Board (UPM-REB). The patients or their guardian were given thorough verbal and written explanations of the study either in English or the vernacular. After the explanation, informed consents were obtained, and samples were collected.

RESULTS

Socio-demographic profile of hospital patients and community participants and detection of leptospiral antibodies through microscopic agglutination test (MAT)

A total of 190 persons participated in the post-flooding surveillance on leptospirosis. One hundred twenty-six of them were patients with symptoms of leptospirosis and hospitalized while 64 were asymptomatic or participants from the communities where the hospitalized participants reside. Ninety-four (94) out of 190 (49.5%) participants had antibodies against *Leptospira* species. From the 126 symptomatic patients, 59 were MAT-positive (46.8%) (Table 1). On the other hand, leptospiral antibodies were detected in 35 out of 64 (54.7%) asymptomatic participants (Table 2).

Most of the MAT-positive symptomatic patients fall under the physically dynamic age bracket of 21-30 years old (28.8%), were mostly of males (35.7%), and working as agricultural farmers (25.4%) (Table 3). Seropositivity was also high among students and unemployed (11.9%).

Since the samples were collected after flooding events, it was not surprising to see that the most common contact of the

Table 3
Socio-demographic profile of symptomatic patients according to MAT results.

Category	MAT-positive (59)	MAT-negative (67)
	n (%)	*n* (%)
Age group (years)		
0-10	0 (0)	0 (0)
11-20	13 (22.0)	16 (12.7)
21-30	17 (28.8)	18 (14.3)
31-40	12 (20.3)	13 (10.3)
41-50	10 (17.0)	8 (6.4)
51-60	0 (0)	5 (4.0)
61-70	3 (5.1)	5 (4.0)
>70	0 (0)	1 (0.8)
Not known	4 (6.8)	1 (0.8)
Sex		
Male	45 (35.7)	51 (40.5)
Female	11 (8.7)	15 (12.0)
Not known	3 (2.4)	1 (0.8)
Occupation		
Outdoor		
Worker (construction, manual labor)	5 (8.5)	6 (9.0)
Vendor (sidewalk, push cart or kariton)	1 (1.7)	0 (0.0)
Driver (jeepney, tricycle, pedicab)	3 (5.1)	4 (6.0)
Farmer (agriculture)	15 (25.4)	11 (16.4)
Farmer (livestock)	1 (1.7)	0 (0.0)
Fisherman	1 (1.7)	0 (0.0)
Military and police	0 (0.0)	2 (3.0)
Delivery services	0 (0.0)	0 (0.0)
Abattoir worker/butcher	0 (0.0)	0 (0.0)
Sewage worker	0 (0.0)	0 (0.0)
Animal care	0 (0.0)	0 (0.0)
Other (barangay patroller, watcher, scavenger)	1 (1.7)	0 (0.0)
Indoor		
Worker (factory, utility, etc)	1 (1.7)	1 (0.8)
Vendor (sari-sari store, market, etc)	0 (0.0)	0 (0.0)
Office and clerical work	0 (0.0)	1 (0.8)
Medical professionals and health workers	0 (0.0)	0 (0.0)
Other indoor non-office occupations	0 (0.0)	0 (0.0)
Other (domestic helper, teacher)	2 (3.4)	9 (13.4)
Student	7 (11.9)	9 (13.4)
Stopped school	0 (0.0)	0 (0.0)
Housewife	6 (10.2)	4 (6.0)
Retired	1 (1.7)	0 (0.0)
Unemployed	7 (11.9)	10 (14.9)
Not known	8 (13.6)	10 (14.9)

Table 4
Proportion of *Leptospira* serovars possibly infecting MAT-positive symptomatic patients according to place/region.

Serovar	Reg I (46)	Reg III (9)	Reg X (71)	Total (126)
	n (%)	*n* (%)	*n* (%)	*n* (%)
Hardjo	0 (0.0)	0 (0.0)	2 (6.9)	2 (3.4)
Pomona	1 (4.2)	0 (0.0)	1 (3.5)	2 (3.4)
Semaranga	5 (20.8)	4 (66.7)	15 (51.7)	24 (40.7)
Icterohemorrhagiae (Ictero 1)	1 (4.2)	1 (16.7)	1 (3.5)	3 (5.1)
Patoc	16 (66.7)	5 (83.3)	25 (86.2)	46 (78.0)
Australis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hebdomadis	0 (0.0)	0 (0.0)	2 (6.9)	2 (3.4)
Ratnapura	3 (12.5)	0 (0.0)	2 (6.9)	5 (8.5)
Losbanos	0 (0.0)	0 (0.0)	2 (6.9)	2 (3.4)
Copenhageni	0 (0.0)	4 (66.7)	7 (24.1)	11 (18.6)
Icterohemorrhagiae (RGA)	1 (4.2)	0 (0.0)	0 (0.00)	1 (1.7)
Autumnalis	1 (4.2)	0 (0.0)	1 (3.5)	2 (3.4)
Grippotyphosa	1 (4.2)	0 (0.0)	2 (6.9)	3 (5.1)
Pyrogenes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Poi	3 (12.5)	5 (83.3)	12 (41.4)	20 (33.9)
Canicola	1 (4.2)	0 (0.0)	0 (0.0)	1 (1.7)
Manilae	0 (0.0)	1 (16.7)	0 (0.0)	1 (1.7)
Tarassovi	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	24 (52.2)	6 (66.7)	29 (40.9)	59 (46.8)

symptomatic patients was immersion/wading in floodwater (Fig 1). However, it is noteworthy that the percentage of MAT-negative symptomatic patients with exposure to floodwater is higher than those who tested positive (46.0% *vs* 35.7%). Contacts through wounds and abrasions were also common among the hospital patients.

The most prevalent *Leptospira* serovars seen among the 59 MAT- positive hospital patients were Patoc (78.0%), Semaranga (40.9%), Poi (33.9%), and Copenhageni (18.6%) as shown in Table 4. For Regions I and X, Patoc had the highest MAT positivity among the 18 serovars used (66.7% and 86.2%, respectively), followed by serovar Semaranga. For Region III, serovars Poi and Patoc tied as the top infecting serovars (83.3%).

Table 5 shows that, similar to MAT-positive symptomatic patients, most of the MAT-positive asymptomatic participants were from the age groups 21-30 (34.3%). High seropositivity was also observed among asymptomatics in the 31-40 age bracket (31.4%). Most were males (60.0%) and working as farmers (91.4%). With majority of the positive participants being farmers, the most common type of contact was occupational (50%), with contact to possibly contaminated environment such as rice paddies (48.4%) (Figs 2 and 3). Also as stated earlier, with the samples collected during post-flooding, immersion in floodwaters was also noted as one of the major types of contact.

Table 5
Socio-demographic profile of asymptomatic participants according to MAT results.

Category	MAT-positive (35)	MAT-negative (29)
	n (%)	*n* (%)
Age group (years)		
0-10	0 (0.0)	0 (0.0)
11-20	4 (11.4)	5 (17.2)
21-30	12 (34.3)	4 (13.8)
31-40	11 (31.4)	8 (27.6)
41-50	5 (14.3)	6 (20.7)
51-60	3 (8.6)	5 (17.2)
61-70	0 (0.0)	1 (3.5)
>70	0 (0.0)	0 (0.0)
Not known	0 (0.0)	0 (0.0)
Sex		
Male	21 (60.0)	17 (58.6)
Female	14 (40.0)	12 (20.3)
Not known	0 (0.0)	0 (0.0)
Occupation		
Outdoor		
Worker (construction, manual labor)	0 (0.0)	0 (0.0)
Vendor (sidewalk, push cart or kariton)	0 (0.0)	0 (0.0)
Driver (jeepney, tricycle, pedicab)	0 (0.0)	0 (0.0)
Farmer (agriculture)	32 (91.4)	22 (75.9)
Farmer (livestock)	0 (0.0)	0 (0.0)
Fisherman	0 (0.0)	0 (0.0)
Military and police	0 (0.0)	0 (0.0)
Delivery services	0 (0.0)	0 (0.0)
Abattoir worker/butcher	0 (0.0)	0 (0.0)
Sewage worker	0 (0.0)	0 (0.0)
Animal care	0 (0.0)	0 (0.0)
Other (barangay patroller or watcher, scavenger)	0 (0.0)	0 (0.0)
Indoor		
Student	1 (2.9)	2 (6.9)
Stopped school	0 (0.0)	0 (0.0)
Housewife	1 (2.9)	1 (3.5)
Retired	0 (0.0)	1 (3.5)
Unemployed	1 (2.9)	1 (3.5)
Not known	0 (0.0)	0 (0.0)

For the asymptomatic participants, Patoc (65.7%), Semaranga (45.7%), Grippotyphosa (37.1%), and Ratnapura (31.4%) were the most prevalent *Leptospira* serovars (Table 6). Serovar Grippotyphosa was found to be the predominant serovar in Region I while Patoc was the most frequently occuring serovar in Region III.

Table 6
Proportion of *Leptospira* serovars possibly infecting MAT-positive asymptomatic participants according to place/region.

Serovar	Reg I (30)	Reg III (34)	Total (64)
	n (%)	*n* (%)	*n* (%)
Hardjo	0 (0.0)	0 (0.0)	0 (0.0)
Pomona	0 (0.0)	0 (0.0)	0 (0.0)
Semaranga	5 (31.3)	11 (57.9)	16 (45.7)
Icterohemorrhagiae (Ictero No. 1)	1 (6.25)	1 (5.3)	2 (5.7)
Patoc	8 (50.0)	15 (78.9)	23 (65.7)
Australis	0 (0.0)	0 (0.0)	0 (0.0)
Hebdomadis	0 (0.0)	0 (0.0)	0 (0.0)
Ratnapura	2 (12.5)	9 (47.4)	11 (31.4)
Losbanos	0 (0.0)	0 (0.0)	0 (0.0)
Copenhageni	1 (6.3)	2 (10.5)	3 (8.6)
Icterohemorrhagiae (RGA)	2 (12.5)	0 (0.0)	2 (5.7)
Autumnalis	0 (0.0)	0 (0.0)	0 (0.0)
Grippotyphosa	13 (81.3)	0 (0.0)	13 (37.1)
Pyrogenes	1 (6.3)	6 (31.6)	7 (20.0)
Poi	1 (6.3)	6 (31.6)	7 (20.0)
Canicola	1 (6.3)	6 (31.6)	7 (20.0)
Manilae	1 (6.3)	2 (10.5)	3 (8.6)
Tarassovi	0 (0.0)	0 (0.0)	0 (0.0)
Total	16 (53.3)	19 (55.9)	35 (54.7)

Table 7
Summary of SD BioLine© LeptospiraIgG/IgM rapid test results.

Region	Positive			Negative	Total
	IgG + only	IgM+ only	IgG and IgM +		
Region I	2	16	5	53	76
Region III	1	8	2	32	43
Region X	4	8	14	45	71
Total	7	32	21	130	190

Leptospira IgG/IgM Rapid Testing (Rapid ImmunochromatographicTest)

Leptospira IgG/IgM Rapid Testing was performed on serum samples of symptomatic and asymptomatic participants in the enhanced post-flooding surveillance on leptospirosis. Sixty of the 190 samples tested positive, of which 7 (11.7%) were IgG positive indicating past exposure or infection with leptospirosis (Table 7). Thirty-two (53.3%) were only IgM positive, whereas 21 (35.0%) were both IgM and IgG positive. Positivity in IgM or IgM and IgG indicates acute or recent infection.

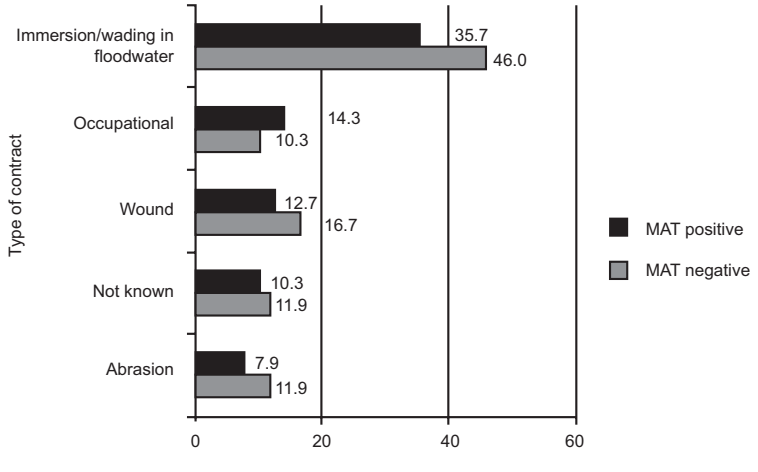

Fig 1–Type of contact of the symptomatic patients according to MAT results.

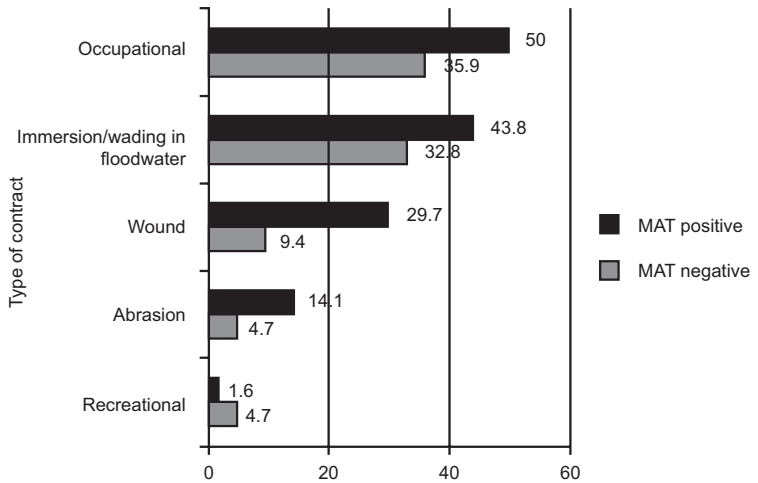

Fig 2–Type of contact of asymptomatic participants according to MAT results.

Isolation of leptospires

Leptospires were isolated from the blood of ten symptomatic patients from Region I. No leptospires were isolated from the samples that came from Regions III and X. Leptospires were not isolated from any urine sample as well. Serotyping and genotyping of the isolates were previously reported (Villanueva *et al*, 2014). Serotyping revealed that six of the participants were infected with *L. interrogans* serogroup Grippotyphosa while four were infected *with L. interrogans* serovar Pyrogenes. Although all isolates were pathogenic based on *fla*B-PCR and typing results, only four out of the eight isolates that underwent further pathogenicity testing were lethal to 4-week old Golden Syrian Hamsters at a 10^5 and 10^7 infective dose.

DISCUSSION

Leptospirosis is a zoonosis that has plagued the Philippines for decades now (Yanagihara *et al*, 2007). A number of reports on human and animal leptospirosis in the Philippines have been published proving the endemicity of the country to this infectious disease.

Results of this study showed that 94 participants had antibodies against *Leptospira* species (49.5%). Fifty-nine of them were hospital patients while 35 were community participants. Most of the participants fall under the physically dynamic age brackets of society and were mostly males. The higher seropositivity in males compared to females were also reported (Padre *et al*, 1988; Yanagihara *et al*, 2007; Amilasan *et al*, 2012). In the present study, it was also

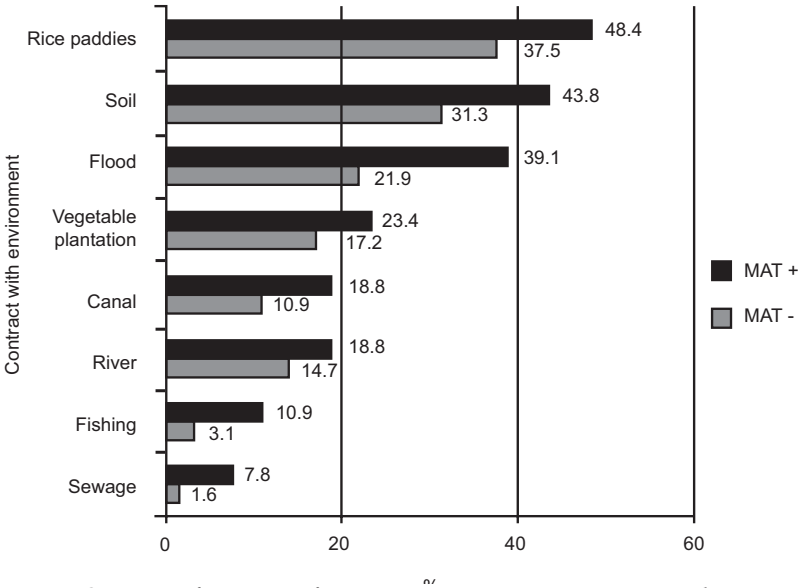

Fig 3–Sources of contact of asymptomatic participants according to MAT results.

observed that agricultural farmers had the highest MAT-positivity in both asymptomatic and symptomatic participants compared to the rest of the occupations. This profile is very common in rural areas of the country where agriculture and livestock are the main sources of income. Because of their occupation, most of their contact with leptospires happened in rice paddies and soil aside from floodwater. The MAT- positivity among students and unemployed can solely be attributed to their exposure to floodwater due to their lack of occupational exposure to leptospires.

The most common contact experienced by the symptomatic patients was immersion in floodwaters. However, the number of MAT-positives among the flood-exposed patients turned out to be lower than the MAT-negatives (35.7% and 46.0%, respectively). The number of organisms present in the environment and the duration of contact with the contaminated environment are both important factors for a disease to have clinical manifestation on its host (Faine *et al*, 1999). More virulent organisms in floodwater and prolonged wading increase the chance of acquiring the infection. The presence of wounds and abrasions also significantly increase the probability of getting infected because these can serve as portals of entry of the leptospires into the host. It is possible that the MAT-negative patients who were exposed to leptospires may have taken early antibiotic treatment, which eliminated leptospires so quickly that antibodies were not detected anymore. For the asymptomatic participants, occupationally-exposed individuals had higher MAT-positivity and the sources of contact of these persons were rice paddies, soil, etc. This is not surprising since most of the respondents in this study were farmers. Padre *et al* (1988) had similar findings in their survey in three rice-farming villages in Bulacan and Nueva Ecija. They also reported that MAT-positivity was high among rice farmers due to their occupational exposure.

In previous reports, antibodies against serovars Manilae, Autumnalis, Sejroe, Bataviae, Grippotyphosa, Hyos, Australis, Pomona, Javanica, Cynopteri, Icterohaemorrhagiae, Pyrogenes, Hardjo, Fort Bragg, Shermani, Poi, Tarassovi, and Losbanos were detected in human sera (Famatiga, 1971; Famatiga *et al*, 1972; Padre *et al*, 1988; Masuzawa *et al*, 2001; Yanagihara *et al*, 2007; Amilasan *et al*, 2012). In the

present study, prevalent *Leptospira* serovars detected among the MAT- positive symptomatic patients were mostly against serovars Patoc and Semaranga, which are both nonpathogenic. On the other hand, antibodies against both pathogenic and non-pathogenic serovars were detected among asymptomatic participants. Serovar Patoc was the most common serovar, and this may be due to its cross-reactivity to several pathogenic serovars. The high titers observed for Patoc, which is a non-pathogenic strain, may be explained by the fact that positive reactions for saprophytic strains are found to manifest earlier than the pathogenic strains (Babudieri, 1971). Reactivity of the serum samples from the subjects of this study could also indicate infection caused by other pathogenic serovars that were not included in the panel of serovars used in the study. Prevalence of certain serovars in particular regions such as Grippotyphosa for Region I, and Ratnapura for Region III, Losbanos in Region X was also detected. Antibodies against serovar Manilae, one of the local serovars isolated in the Philippines, was detected in sera of participants from Regions I and III only. The present findings on the prevailing infecting serovars among the participants suggest that the *Leptospira* serovars we had several decades ago are still the causative agents of leptospirosis in humans until now.

MAT is the reference test for leptospirosis (ILS-WHO, 2003). However, this test is laborious, needs technical expertise, and cannot be used at bedside. There are currently a number of rapid diagnostic tests that are commercially available and can be used at bedside. For this study, SD BioLine© (Standard Diagnostics: Gyeonggi-do, Korea) *Leptospira*IgG/IgM Rapid Test was used. Sixty of the 190 participants in this study were positive by using this kit. The positivity in MAT was higher compared to this rapid test (47.4% *vs* 31.6%). However, it should be noted that the antigens used for the two tests were different thus may explain the low sensitivity of the test kit obtained. The rapid test kit utilized in the study only had the pathogenic strain *L. interrogans* antigen coating (SD BioLine insert, 2011) whereas the panel of live antigens for the Microscopic Agglutination Test was composed of representative serovars from four pathogenic species: *L. interrogans, L. kirschneri, L. meyeri* and *L. borgpetersenii,* as well as the non-pathogenic species *L. biflexa*. Due to this wider range of detecting serovars for MAT, a higher positivity may be expected compared to the test kit. Variability of the modes of exposure as well as environmental factors and prevailing *Leptospira* serovars in the Philippines against the conditions by which the test kits were manufactured may also be a factor for the obtained results. Thus, it is advisable that rapid test kits alone should not be the sole basis of a positive/negative laboratory confirmation for leptospirosis as may be the practice with some hospitals or institutions; and that there is a necessity for a customized *Leptospira* Rapid Diagnostic Test Kit for use in the Philippine setting that will detect locally prevalent serovars. Furthermore, clinical and laboratory diagnosis, together with the history of exposure of the cases, should go hand in hand in diagnosing suspected leptospirosis cases.

Leptospires were isolated from the blood of ten participants from Region I. Leptospiremia only occurs during the first week up to ten days of infection (ILS and WHO, 2003). After this, leptospires are already cleared from the blood and can only be detected in certain cells like

kidneys and bladder, or urine. Since leptospires were still detected from the blood of the subjects, it is possible that they are currently infected.

Leptospiral shedding in the urine occurs 1-3 weeks after onset of symptoms and can persist up to months depending on the bacterial load (ILS and WHO, 2003). The absence of leptospiruria may be due to the acidity of human urine. The pH of normal human urine may range from 4.5-8.0. Leptospires do not survive well in acidic urine thus recovery of live leptospires becomes impossible when culture is not done immediately after voiding. Contamination of specimen may also inhibit the growth of leptospires in the urine culture. Lastly, despite the presence of leptospires in urine, antibiotic treatment can kill leptospires. This leads to shedding of dead leptospires that can no longer be cultured.

All isolates were pathogenic based on characterization by polymerase chain reaction and serotyping. However, not all isolates caused death in experimentally infected golden Syrian hamsters after 21 days. Hamsters that survived became carriers of the bacteria due to the presence of organisms in their kidneys and/or urine cultures after the 21-day infection period. This is supported by Faine *et al* (1999) who reported that surviving animals may become carriers in 14-28 days after inoculation.

Results from this enhanced post-flooding leptospirosis surveillance showed the persistence of certain pathogenic *Leptospira* such as serovars Grippotyphosa, Losbanos, Manilae, and so forth, through the decades. This further strengthens the need for a vaccine that should contain these circulating serovars in the country. This study also suggested that, indeed, occupational exposure such as farming as well as immersion/wading in floodwater place an individual at high risk of acquiring leptospirosis. It is a known fact that the Philippines is a flood-prone country, and many areas are still dependent on agriculture. Preventive and control measures should be directed against these identified sources of exposure. Lastly, seropositivity was observed not only in persons that had clinical manifestations of the infection but also in asymptomatic individuals. Further studies need to be done in order to do a more comprehensive surveillance on asymptomatic individuals also since Philippines is endemic for leptospirosis and there are many possible sources of infection (that is, rodents, other animals, and contaminated environment).

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