usually combined with a short-duration digestion method (less than 4 hr), permits the recovery of live larvae, but the extent of recovery is not satisfactory for estimating the parasite burden. Additionally, since less than half of the skeletal muscle is usually employed for the digestion (Abdel-Hameed, 1984), the precise number of migrated larvae cannot be determined. In contrast, the digestion method alone permits a fairly good recovery, although a large amount of sediment remains after digestion, making the counting of larvae using stereoscopic microscopy quite time consuming. In the present report, we describe an improved method for recovering and counting larvae derived from skeletal muscle. The method is based on extended incubation in digestive fluid, followed by alcohol.

Female BALB/c mice weighing 28-30 g were infected with 300 embryonated eggs of *T. canis* according to the method of Oshima (1961). All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. Three wk later, skeletal muscle from each mouse was minced with 150 ml of artificial gastric juice (0.5% of 1:10,000 pepsin and 0.7% hydrochloric acid, pH 1.5). After mixing

well with a blender, the minced tissue was divided into 3 equal parts. The first portion was incubated in digestive fluid for 4 hr at 37 C with vigorous agitation. The mixture was then sieved with a wire mesh (mesh diameter: 1.0 mm) and the fluid was centrifuged at 320 g for 5 min. The total digestion time was 4 hr (Method 1). Larval counting was performed on the resulting sediment using stereoscopic microscopy. Since undigested tissues remained on the mesh after sieving, these materials were re-incubated with digestive fluid for an additional 44 hr. They were vigorously agitated and prepared for counting in the same manner as above. The second portion of minced tissue was incubated in digestive fluid for 24 hr with vigorous agitation. The solution was centrifuged as above and the sediment was re-incubated in 50 ml of fresh digestive fluid for an additional 24 hr. No filtration with wire mesh was performed. Thus, the total digestion time was 48 hr (Method 2). Larval counts in the whole sediment were performed as above. The third portion was prepared in the same manner as the second portion, but 10 ml of 50% ethanol in distilled water was added to the sediment after the second 24 hr incubation step (Method 3). The number of larvae in the sediment was then

counted.

Digestion period (hr)			
4		48	
Sediment	Undigestive material	Ethanol treatment	
		−	+
9.5±3.0	1±0.9		
10.5±3.7		23.2±8.3*	26.3±8.5*

Six mice were used for this experiment. Table I shows the number of larvae recovered with each procedure. There was a significant difference in larval recovery between the 4 hr digestion group and the 48 hr digestion group ($p<0.01$). Although ethanol treatment did not significantly affect recovery, we were able to find the larvae more easily in the ethanol-treated samples. The use of alcohol in the final step has the advantages that lipid droplets, which are insoluble in trypsin-based digestive fluid, are soluble in alcohol, and that alcohol acts as a surface tension depressant that facilitates the identification of larvae. This is reflected in the time required to complete counting one of a single sample: with ethanol treatment, counting took 16.7±2.5 min (mean ± SD); without ethanol treatment, counting took 33.8±7.5 min. For comparison, with the sample digested for

4 hr without ethanol, counting took 91.2±14.1 min. From the undigested material, we were able to find larvae after additional incubation for 20 hr and 24 hr with freshly prepared digestive fluid, suggesting that a 4 hr incubation was insufficient for the digestion of skeletal muscle.

We further assessed whether this recovery technique can be carried out by an inexperienced person (T.N.). Six BALB/c female mice were orally administered albendazole (100 mg/Kg/day) suspended in olive oil for 5 days, beginning 1 day before inoculation. Six control animals were given only olive oil. Three wk after inoculation, the mice were killed and their skeletal muscle tissue was digested with the Method 3, under the guidance of an experienced researcher (Z.J.). Larvae migrating to the brain were counted by squash preparation (Abdel-Hameed, 1984). At the beginning of the experiment, it took almost 3 hr to complete the counting from just 1 skeletal muscle sample, but this soon fell to 30 min. The average recovery from skeletal muscle was 56.8±4.8% in the control group versus 29.8±9.8% in the albendazole group. In skeletal muscle, 104.5±3.5 larvae were found in the control group versus 50.7±22.3 in the albendazole group, indicating that

prophylactic treatment can reduce the larvae in skeletal muscle (Table 2).

Table 2. Number of larvae recovered from mice inoculated with 300 *T. canis* eggs.

Albendazole		Control	
Skeletal muscle	Brain	Skeletal muscle	Brain
50.7±22.3	38.8±12.9	104.5±3.5	66±11.8

*Six BALB/c mice were treated with 100 mg/Kg/day of albendazole suspended in olive oil for five consecutive days beginning from 1 day before inoculation

†Six control mice were given only olive oil.

Larval recovery from skeletal muscle was performed by the Method 3.

The improved method described here requires substantially less operator time (being more than 5-fold faster) to count larvae and the recovery rate is 3-fold higher than that of our previously reported methods (Horiuchi, et al., 2005; Satou, et al., 2005). However, the larvae recovered are no longer alive, which is likely due to the much longer incubation time required. Therefore, while this method would be suitable for measuring the efficacy of treatments that act before larval migration, it would not allow measurement of the active larval tissue burden.

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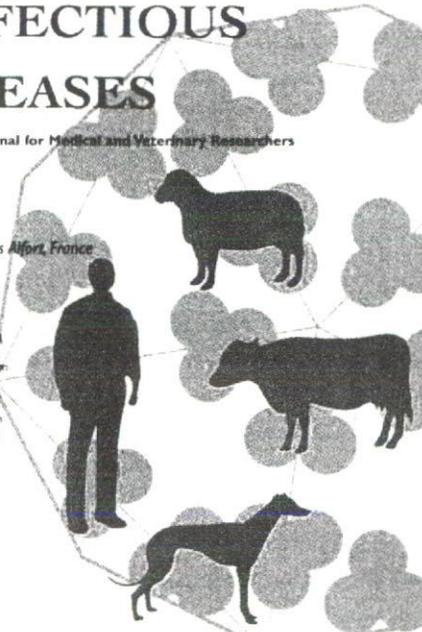
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Current status of leptospirosis in Japan and Philippines

Yasutake Yanagihara[a], Sharon Y.A.M. Villanueva[a],
Shin-ichi Yoshida[a], Yoshihiro Okamoto[b],
Toshiyuki Masuzawa[b,*]

[a]Department of Bacteriology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1,
Higashi-ku, Fukuoka 812-8582, Japan
[b]Laboratory of Microbiology and Immunology, Faculty of Pharmacy, Chiba Institute of Science,
Shiomi-cho 3, Choshi, Ciba 288-0025, Japan

Abstract

Leptospirosis is an acute febrile illness with a wide variety of clinical manifestations and is encountered throughout the world, prominently in tropical areas with high rainfall. In this paper, we review the current status of leptospirosis in two Asian countries, Japan and Philippines, which have quite different situations in terms of economy, environment, infrastructures and prevailing infectious diseases. In Japan, until 1960, more than 200 deaths due to leptospirosis had been reported yearly. After 1960, the number of reported cases had rapidly decreased. Now, leptospirosis cases notified every year were less than 20. After the early 1960s, modernization of agriculture was introduced, at the same time, inactivated vaccine against *Leptospira* was applied for humans and there was good maintenance of infrastructures such as water works and sewage systems.

In Philippines, on the other hand, leptospirosis patients tend to be frequently found in flood-prone areas of urban setting such as Metro Manila, which was found to be the endemic foci of leptospirosis. Morbidity in a rural area (Cabatuan, Iloilo) was 147 cases per 100,000 populations. From 1998 to 2001, about 70% of 1200 suspected leptospirosis patients in Philippines were serologically positive. The average age of patients was 32 years old where

*Corresponding author. Tel./fax: +81 479 30 4685.
E-mail address: masuzawat@cis.ac.jp (T. Masuzawa).

87% of the cases were males and 70% were outdoor workers. Case fatality rate was found to be 12–14%. The estimated major serovars in Metro Manila and neighboring provinces were Manilae, Losbanos, Tarassovi, Poi and an unknown serovar. Outbreaks of leptospirosis in the Philippines are thought to be associated with heavy rainfall, rapid urbanization (dramatic increase in populations), deforestation, increasing number of flood-prone areas, poor infrastructures and many others.

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Résumé

La leptospirose est une maladie fébrile aiguë avec une grande variété de manifestations cliniques. Elle sévit dans le monde entier particulièrement dans des régions tropicales à fortes précipitations. Cet article passe en revue l'état actuel de la leptospirose dans deux pays asiatiques, le Japon et les Philippines, qui ont des situations économiques, environnementales, infrastructurelles et d'expansion infectieuse tout à fait différentes. Jusqu'en 1960, plus de 200 cas de leptospirose avaient été rapportés annuellement au Japon. Après 1960, le nombre de cas rapportés avait rapidement diminué. A nos jours, moins de 20 cas de leptospirose sont signalés chaque année. Après le début des années soixante, avec la modernisation de l'agriculture, un vaccin inactivé contre la *Leptospira* avait été faite chez les humains et accompagné d'un bon entretien des infrastructures telles que la canalisation d'eau usée et systèmes d'égouts.

Aux Philippines d'autre part, les patients de leptospirose tendent à être fréquemment localisés dans des milieux urbains prompts à l'inondation tels que le métro de Manille. Le métro de Manille s'est avéré être le centre endémique de la leptospirose. D'après nos analyses, les morbidité dans le secteur rural (Cabatuan, Iloilo) est de 147 cas pour une population de 100.000 habitants. De 1998 à 2001, environ 70% de 1.200 patients suspectés de leptospirose aux Philippines se sont révélés positif au test sérologique. L'âge moyen des patients est de 32 ans dont 87% des cas de sexe masculins et 70% des ouvriers exerçant dans un environnement non protégé. Le taux de mortalité s'est avéré être de 12–14%. Les principaux serovars estimés dans le métro de Manille et les provinces voisines étaient Manilae, Losbanos, Tarassovi, Poi et un serovar inconnu. On pense les manifestations de leptospirose aux Philippines peuvent être associé aux fortes précipitations, à l'urbanisation rapide (augmentation dramatique des populations), au déboisement, à l'augmentation du nombre de secteurs propices a l'inondation, a la pauvreté des infrastructures et beaucoup d'autres facteurs.

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1. Introduction

Leptospirosis is an acute febrile illness with a wide variety of clinical manifestations and is encountered throughout the world, most prominently in tropical areas with high rainfall [1]. The estimated annual number of leptospirosis cases is 350,000–500,000 according to World Health Organization (WHO)–International Leptospirosis Society (ILS) survey [2]. However, the disease has non-specific

signs and symptoms that are similar to those of malaria, dengue hemorrhagic fever, hepatitis, typhoid fever and the like. Thus, it remains under-diagnosed and therefore underestimated [3]. The confirmation of cases, usually through laboratory tests, is either not done or not possible especially in resource-poor countries. Bharti et al. [4] have emphasized in their review that leptospirosis has emerged as a globally important infectious disease in the past decade. They wrote that worldwide, leptospirosis occurs in urban areas of developed and developing countries, as well as in rural regions. Mortality due to leptospirosis remains significant. This may be attributed to several factors such as delays in diagnosis due to lack of resources and knowledge of the disease as well as due to the pathogenicity of some leptospiral strains or the genetic make-up of the host's immune system. Furthermore, pulmonary hemorrhage due to leptospirosis has been recently reported. It is often lethal and is considered to be a major manifestation of leptospirosis but its pathogenicity remains to be unclear. The major drugs for leptospirosis treatment ar

Leptospirosis is treatable with antibiotics and chemotherapeutics, although an early diagnosis is important because clinical symptoms can develop rapidly and the disease can be fatal to humans and a variety of animals. However, the major drugs for leptospirosis treatment are difficult to implement especially in developing countries.

Among Asian countries located in tropical areas, such as India, Thailand, Philippines, Indonesia, etc., leptospirosis is a potentially serious problem and many people have been infected with *Leptospira*. Laras et al. [5] reported that Southeast Asian countries such as Lao PDR, Vietnam, Cambodia and Indonesia have areas where leptospirosis is endemic among both humans and animals. Seventy percent of serovars of the major pathogenic species have been found in Southeast Asia. Hurstbridge, Bataviae and Icterohaemorrhagiae tonkini LT 96-69 were reported to be the predominant leptospiral serogroups identified in patients with clinical jaundice. Bataviae, on the other hand, was the most frequently reported serogroup among patients with non-malarial febrile illness. Pyrogenes and Hurstbridge were found to be the principal serogroups among patients with hemorrhagic fever. McBride et al. [6] reported that leptospirosis has become an important public health problem in Asia and Latin America. They attributed the transmission of the disease in developing countries to poverty, especially poor sanitation.

This review briefly summarizes the current status of leptospirosis and the pathogen, *Leptospira* in Japan and Philippines.

2. Current status of leptospirosis in Japan

2.1. Discovery of Leptospira and its classification

In 1915, Inada and Ido isolated the spirochetes from a patient suffering from an acute febrile illness with jaundice and named the bacterium *Spirochaeta icterohaemorrhagiae* [7]. Soon, the same spirochetes were independently discovered by two other research groups [8,9] in Germany. Noguchi proposed the genus name

Leptospira (thin spirals) in 1918. The species name, *interrogans*, was given by Stimson who found the spirochetes in the kidney tubules of patients who died of yellow fever and named the bacteria *Spirochaeta interrogans* because the spirochetes resembled the interrogation mark.

Until 1989, the genus *Leptospira* was divided into two species, i.e., *L. interrogans*, pathogenic species isolated from patients and reservoir animals and *L. biflexa*, nonpathogenic and saprophytic species isolated from the environment. Both species can be further subdivided into more than 200 serovars and 60 serovars, respectively, based on results of agglutination and cross-absorption tests using specific antisera. The 200 serovars are traditionally grouped into several serogroups which consist of antigenically related serovars. The serogroups do not have any taxonomic standing, and to date, the serogroup concept has not been used. Yasuda et al. [10] proposed a genetic classification of *Leptospira* based on DNA–DNA relatedness, describing 10 genomospecies of *Leptospira*. Since then, additional genomospecies have been added [1,11].

2.2. Leptospirosis in Japan, past and present

Until 1960, more than 200 deaths of leptospirosis were reported every year in Japan. After 1960, this number decreased rapidly. Now, leptospirosis is a notifiable infectious disease in Japan under the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients with Infections (the Infectious Diseases Control Law) that was enacted in 2003. The number of cases per year is less than 20 (Table 1). Between November 2003 and April 2005, 20 cases were reported. Before the early 1960s, more than 200 deaths were reported among leptospirosis patients and most of them were farmers working in rice fields. After the early 1960s, the mechanization of agriculture was introduced, and farmers started using rubber boots while working in the field. At the same time, an inactivated vaccine against *Leptospira* was developed. For these reasons, the number of cases in Japan decreased dramatically. Instead of infections during farming, infections were caused by a variety of recreational activities, such as swimming, diving, canoeing, etc. in rivers in Japan. Some cases (Case nos. 3, 18 and 20) were imported from Southeast Asian countries. Furthermore, we demonstrated that some patients were infected by contact with animal imported from foreign countries (described later). In Japan, serovars Icterohaemorrhagiae, Copenhageni, Autumnalis, Hebdomadis and Australis have been recognized as major agents of leptospirosis in humans. Furthermore, Canicola and Hardjo were also found. Serovars Grippotyphosa, Javanica, Castellonis and Rachmati have been isolated from patients and in domestic and wild animals in the Okinawa islands.

2.3. Prevalence of Leptospira in wild rodents in Japan

After the 1960s, the importance of leptospirosis was forgotten in Japan. Although the rate of infection decreased with the introduction of modern agricultural methods, the pathogen and infected reservoir animals may still exist. To elucidate the current

Table 1
Leptospirosis reported in Japan (November 2003–April 2005)

Case no.	Year	Sex	Age	Prefecture in exposed	Exposure	Infecting serovar
1	2003	Female	70	Tokushima	Work in rice field	Hebdomadis
2	2004	Male	30	Tokyo	Sewerage work	Icterohaemorrhagiae-Copenhageni
3		Male	30	Indonesia (imported)	Not determined	Sejroe
4		Male	20	Okinawa	Activity in river	Hebdomadis
5		Male	40	Okinawa	Sewerage work	Hebdomadis
6		Male	40	Okinawa	Activity in river	Hebdomadis
7		Male	30	Okinawa	Sewerage work	Hebdomadis
8		Male	40	Okinawa	Sewerage work	Hebdomadis
9		Male	40	Okinawa	Sewerage work	Autumnalis/Rachmati
10		Male	50	Kyushu	Activity in river and mountain	Hebdomadis
11		Female	80	Chiba	Work in rice field	Icterohaemorrhagiae-Copenhageni
12		Male	60	Ehime	Work in flooding river	Australis
13		Male	60	Kanagawa	Work on extermination of rodents	Canicola
14		Male	40	Nagasaki	Work in rice field	Autumnalis
15		Male	60	Nagasaki	Farmwork	Hebdomadis
16		Male	70	Kagoshima	Farmwork	Canicola
17		Male	50	Okinawa	Contact with dog and goat	Javanica
18		Male	20	Thailand (imported)	Not determined	Australis
19		Female	30	Chiba	Contact with dog	Not determined
20	2005	Female	40	Malaysia (imported)	Rafting	Hebdomadis/Poi/Pyrogenes

The Infectious Disease Surveillance Center (IDSC) was notified of these cases between November 2003 and April 2005. Case nos. 3, 18 and 20 were imported from East Asia.

status and to evaluate the risk of infection, a field survey for *Leptospira* in small wild mammals has been done from Hokkaido, the northernmost island, to Okinawa, the southernmost island. Forty-five of 1,101 small wild mammals captured between 2003 and 2005 were infected with *Leptospira* (prevalence 4.1%). The infected species were *Rattus norvegicus*, *Apodemus speciosus*, *A. argenteus*, and *Suncus murinus*. The animals were captured in rice fields, domestic animal farms, sewers, garbage dumps, etc. The result suggests that small mammals carrying *Leptospira* live among humans and the risk of infection is still present. The Ministry of Health, Labor and Welfare in Japan listed leptospirosis as a category 4 infectious disease in 2003 and the current status of leptospirosis can be monitored.

2.4. Leptospirosis caused by contact with imported animals

To evaluate the risk of a variety of infectious diseases caused by imported animals, we examined the infections of *Leptospira* resulting from contact with imported animals (26 species, 512 animals). *Leptospira* DNA was detected in 31 animals (11 species, prevalence rate 4–60%, average 6.1%) by *flaB*-PCR. Furthermore, *Leptospira kirschneri* was isolated from the kidneys of 5 of 10 African dormice (*Graphiurus murinus*) and 5 of 10 southern flying squirrels (*Graucomys volans*). These findings suggest that leptospires (some serovars may not be even present in Japan) might have invaded Japan from foreign countries via imported animals.

Although there is a risk of infection with *Leptospira* from contact with imported animals, most animals imported to Japan do not undergo any quarantine procedure except for dogs, monkeys, bats and mastomys which are inspected for rabies virus, hemorrhagic fever virus, Lassa virus, etc. To date, more than 2 million animals have been imported as pets or companion animals from foreign countries into Japan. Some of them are domesticated and some are wild.

We diagnosed leptospirosis in two patients exposed to southern flying squirrels imported from the United States [12]. In this study, to determine the source of infection, *Leptospira* spp. were isolated from the animals in their company and the DNA of the isolates were sequenced.

The cases: An animal trading company in Shizuoka, Japan imported 106 southern flying squirrels from Miami, Florida on March 27, 2005. Before patient 1, a 29 year old man, became ill, the workers dressed casually and touched the animals with bare hands in their routine work. On April 22, 2005, patient 1, who handled a variety of exotic animals at the company, was hospitalized with fever (temperature 40 °C), headache, chills, nausea, vomiting, jaundice and uremia. These symptoms are similar to those of locally acquired leptospirosis. He was treated with an intramuscular injection of streptomycin (2 mg/day) for 7 days, which is the recommended treatment for leptospirosis in Japan [13]. On June 1, 2005, patient 2, a 28-year-old man who worked at the same company, was hospitalized in the same hospital with fever (temperature 39 °C), headache, chills, nausea, vomiting, jaundice and uremia. The patient had been in contact with imported animals. He recovered with intramuscular injections of streptomycin (2 mg/day) for 3 days, followed by treatment with oral amoxicillin for 3 days. *Leptospira* DNA was detected in serum samples from patient 1 and whole blood from patient 2 by PCR of the flagellin gene (*flaB*) [14]. The sequences detected in the two patients were identical and showed a high degree of similarity to *L. kirschneri*. Convalescent-phase serum samples from both patients reacted to *L. kirschneri* strain Moskva V.

To determine the validity of the association between animals held by the company and leptospirosis, exotic animals (75 animals, 7 species) housed in the company were tested. Five of 10 kidney cultures from southern flying squirrels were culture positive. *Leptospira* isolates from a blood culture of patient 2 showed *flaB* and DNA gyrase B (*gyrB*) sequences identical to those of the animal isolates. Further comparative characterization revealed these isolates to be genetically identical and from the result of a cross agglutination test, both isolates were identified as serovar Grippotyphosa.

Serovar Grippotyphosa commonly causes canine leptospirosis and infects a variety of domestic and wild animals in the United States. In Japan, serovar Grippotyphosa is distributed in the southernmost islands, the Okinawa archipelago, but not on Honshu Island, the main island. The patients did not travel to Okinawa or a foreign country before the disease onset. Our findings support the conclusion that the patients were infected with *L. kirschneri* serovar Grippotyphosa by contact with southern flying squirrels. Similarly, in the United States, humans have acquired monkeypox infection from pet prairie dogs, which had been infected by exotic African rodents. These findings show that exotic pets represent a substantial hazard. The leptospirosis cases reported here may serve as a warning against importing exotic animals.

3. Leptospirosis in Philippines

Philippines is an archipelagic country situated in Southeast Asia. Several studies on leptospirosis in Philippines have been done in the past and certain areas were reported to be endemic for this infectious disease. However, in WHO publication, "Leptospirosis worldwide, 1999 and 2001" [15], there was no description of leptospirosis incidence rates in Philippines. Moreover, there seemed to be no systematic survey system in Philippines with regards to this zoonosis.

The background of leptospirosis in Philippines and results of a study done by the authors from 1998 to 2001 will be discussed in the next sections.

3.1. Historical overview of Leptospirosis in Philippines

In 1932, Lissner [16] first isolated *Leptospira* in Philippines from a patient with Weil's disease. Aragon et al. [17] showed that the common *Leptospira* serovars isolated from wild rat kidneys were Pyrogenes, Bataviae, Javanica and a new serovar Manilae, named after the city of Manila where the rats were caught. Carlos et al. [18–20] reported that 20 of 27 sera from water buffaloes in Philippines showed significant antibody titers to serovars Tarassovi, Sejroe and Poi. Furthermore, antibody against 10 serovars, namely Bataviae, Icterohaemorrhagiae, Javanica, Grippotyphosa, Pyrogenes, Canicola, Cynopteri, Autumnalis, Hebdomadis and Tarassovi was found in sera from dogs in Manila. In another study, six isolates obtained from dogs belonged to serogroups Autumnalis, Icterohaemorrhagiae, Grippotyphosa and Pyrogenes and one from a cat was identified as Grippotyphosa [20].

Information on the prevalent *Leptospira* serovars among humans in Philippines dates back to the late 1960s and 1970s. Arambulo et al. [21] showed that *Leptospira*-agglutinating antibodies to serovars Pyrogenes, Bataviae, Pomona, Grippotyphosa, Manilae and Javanica were present among high risk workers such as abattoir employees, dog pound workers and fish inspectors in Manila.

Alora et al. [22] studied leptospirosis cases in St. Tomas University Hospital from 1967 to 1971. High fever, jaundice, oliguria, conjunctival suffusion and tenderness of the leg and thigh muscles were the clinical features mostly observed. Fifteen patients

were found to be positive in microscopic agglutination test (MAT). They were positive for serovars Manilae (5), Bataviae (3), Pyrogenes (3), Grippotyphosa (2) and Pomona (1).

In the early 1970s, Famatiga et al. [23,24] reported that serovars Pyrogenes, Manilae, Bataviae, Grippotyphosa, Hyos, Autumnalis, Australis, Pomona, Javanica, Icterohaemorrhagiae and Cynopteri were common among humans. Monkeys, on the other hand, were found to be positive for serovars Bataviae, Hyos, Australis, and Grippotyphosa. Santos-Ocampo et al. [25] tested 32 children aged 14 years old for leptospirosis in Philippine General Hospital (PGH). Serum samples from these patients were tested through MAT. Important clinical features included acute onset of fever, abdominal pain, jaundice, vomiting, anorexia, hepatomegaly, headache, muscle and/or joint pain, cough, oliguria or anuria, disorientation, convulsions and GIT hemorrhage. They also observed that the number of leptospirosis increased every year from 1960 to 1970 with most of the cases occurring during the rainy season (June to November). Pyrogenes, Manilae, Icterohaemorrhagiae, Pomona, Javanica and Grippotyphosa were the serovars detected in these children with the major serovars being Pyrogenes and Manilae.

Another study was done from 1971 to 1973 by Basaca-Sevilla et al. [26]. Eighty-two (21.0%) of a total of 390 human cases were found to be positive by isolation and serological tests. Among animals, serovars Pyrogenes, Grippotyphosa, Autumnalis, Pomona and Hyos have been isolated from rats; Autumnalis, Grippotyphosa from dogs and Pyrogenes and Pomona from pigs. Serological tests on animal samples have detected antibodies to serovars Pyrogenes, Pomona and Grippotyphosa among carabaos; Pomona, Pyrogenes and Cynopteri in cattle and Grippotyphosa, Pomona, Autumnalis, Cynopteri in pig's blood. It is therefore possible to relate exposure of humans to these animals as a possible source of infection.

Three rice-farming villages in Nueva Ecija and Bulacan were surveyed for leptospirosis by Padre et al. from October 1985 to March 1986 [27]. Results showed that 63 (43.5%, antibody titers ≥ 1:100) of 155 (humans aged 14 years old) sera tested were positive by MAT with a battery of 24 serovars. Antibodies were more frequent in men than women (48% vs. 31%, respectively), and exposure was found to be frequently occurring among rice farmers. However, they did not identify the prevalent serovars, although they detected serovars Shermani, Pyrogenes, Hardjo and Fort Bragg in humans. According to their study, leptospirosis is likely to be an under-diagnosed cause of both mild and severe febrile illness in Philippines.

Watt et al. [28] screened five patients with aseptic meningoencephalitis with ≥ 1:1600 set as a significant MAT titer. Their study showed that leptospirosis is an important but overlooked cause of aseptic meningoencephalitis in Philippines. They further stressed that *L. interrogans* infections remain a significant public health problem.

3.2. Outbreaks in jails

An outbreak of leptospirosis occurred in Sablayan Prison and penal farms in Mindoro (rural area of the Philippines) in 1976 [26]. There were 1539 prisoners and

280 employees and their families in this community. In September, 145 cases in Pusog, Yapang and Pasoque were reported and 60 patients were admitted to hospitals with five deaths reported. Blood samples were collected from 101 hospitalized patients and prisoners with clinical complaints. Results from the rapid macroscopic slide agglutination test that was performed on the blood samples showed that 35 sera (34.6%) were positive for one or more *Leptospira* antigen while 13 (37.4%) of the positive sera reacted to only one antigen. Pyrogenes and Autumnalis were observed to be the major infecting serovars. Among 517 prisoners and residents without complaints, only 10 (1.93%) tested positive but all people residing outside the penal farm were non-reactive to the serovars used.

An outbreak of leptospirosis occurred in Manila City Jail (MCJ) from September to October 1999 [29]. MCJ is located in an urban area of Philippines (Manila) and has 3532 inmates. Twenty-five cases of leptospirosis (0.7%, 24 males and 1 female) were identified during the outbreak.

As has been discussed in the preceding sections, Philippines was found to be endemic for leptospirosis with cases occurring in both urban and rural areas. Although the reason as to why the outbreak occurred in Sablayan Prison and penal farms in Mindoro in 1976 was not known, the background of the outbreak in MCJ was relatively clear [29]. A report from the Field Epidemiology Training Program (FETP)–Department of Health (DOH) Philippines stated that in the first week of August 1999, flooding occurred inside MCJ due to heavy rains. The incident was repeated on September 10. All cells were submerged underwater for 5 days. Inmates were forced to wade in floodwater in order to get their food from the kitchen and some of them slept standing up due to flooded floors. Once the flood water was pumped out using a motor pump and long hose, the flood subsided after a few days. One inmate started to develop clinical signs and symptoms of leptospirosis after 1 week followed by other cases after a few days. Health authorities inspected MCJ and found that the sewage canal of brigades 1–10 was filled with stagnant water and garbage. Furthermore, they also reported that the brigades were overcrowded and had poor ventilation and the toilets were also dirty.

4. Present status

In Philippines, the latest distribution of leptospirosis has not been well explained. In 2001, Masuzawa et al. [30] detected antibodies to prevalent *Leptospira* serovars in sera of 71 suspected leptospirosis patients from three major hospitals in Metro Manila by MAT and Western blot analysis. Since leptospirosis is endemic in Philippines, a titer of ≥1:400 was considered to be significant. This was used to determine the currently prevailing serovars causing the disease. Results from their study showed that sera of 53 subjects had antibodies against eight serovars, namely Poi, Tarassovi, Manilae, Pyrogenes, Australis, Grippotyphosa, Javanica and Autumnalis.

As Philippines, composed of about 7100 islands, is known to be a country where leptospirosis is endemic, preventive and control measures need to be established and

seroepidemiological studies would be a primary step in fulfilling these goals. From 1998 to 2001, the authors performed a seroepidemiological survey and isolated leptospires from humans and rats (house and field) in Metro Manila, nearby provinces, and Iloilo (Cabatuan). Furthermore, the background to the outbreaks of leptospirosis in the Philippines was also examined [Yanagihara Y, Villanueva SYAM, Masuzawa T, Kawabata H, et al. In Preparation and presented at 84th Kanto branch meeting of Japanese Society for Bacteriology, Yokohama, November 26–27, 2001].

More than 1200 samples from patients suspected of having leptospirosis from 10 major hospitals including PGH, San Lazaro Hospital (SLH) and others were tested from June 1998 to February 2001 and a patient distribution map in Metro Manila was prepared.

Rate of infection (morbidity): About 70% of the samples were positive in screening tests for leptospirosis, the Microcapsule Agglutination Test (MCAT) [31] and LeptoDipstick test [32]. The exact patient number in Metro Manila was difficult to obtain therefore the exact morbidity was not estimated. On the other hand, the morbidity in Cabatuan, Iloilo, where rural-type leptospirosis outbreaks occurred after flooding in 1999–2000, was 147 per 100,000 population. Of the suspected patients, 94.2% were seropositive and most of them were farmers and outdoor workers.

Socio-demographic data: Males, mostly involved in outdoor activities, were more at risk of developing leptospirosis than females. The average age of seropositive patients was 32 years and 87% of the patients were male (Fig. 1). Eighty-one percent of the working people were outdoor workers and 85.7% had contact with surface water (e.g., floodwater), although in Philippines there are many jobless people. In Cabatuan, 92.3% of the seropositive patients experienced contact with surface water and the average age was 25.4 years. The percentage was greater among males and people with occupation (81.5%). The average age of male patients was younger than that of females, 24.4 years vs. 29.7 years, respectively.

As mentioned above, most of the patients who tested positive were outdoor workers (72.1%). These patients were involved in construction, labor, stall keeping, agriculture, fisheries, driving and other occupations. Over 80% of them had prior contact with surface water such as flood or sanitary sewage.

Rainfall and season: The incidence of patients was closely related to rainfall. Outbreaks usually occurred during the rainy season (June to November) and just after the said season in flood-prone areas (Fig. 2).

Symptoms: The main symptoms of the patients were acute fever with headache, myalgia, conjunctival suffusion, oliguria and/or proteinuria, renal failure, jaundice, abdominal pain, arthralgia, etc.

Mortality and pathogenicity: Although patient follow-up in Philippines is usually difficult and thus data are inaccurate, mortality of patients was significantly high and was 12–14% in PGH and SLH. The major cause of death was renal failure. Isolated strains from patients were tested for virulence using golden hamsters. The animals injected peritoneally with 1×10^5 to 1×10^7 cells of the isolate died within 2 weeks. The organism, *Leptospira*, was then recovered from the blood and kidney of the dead hamsters.
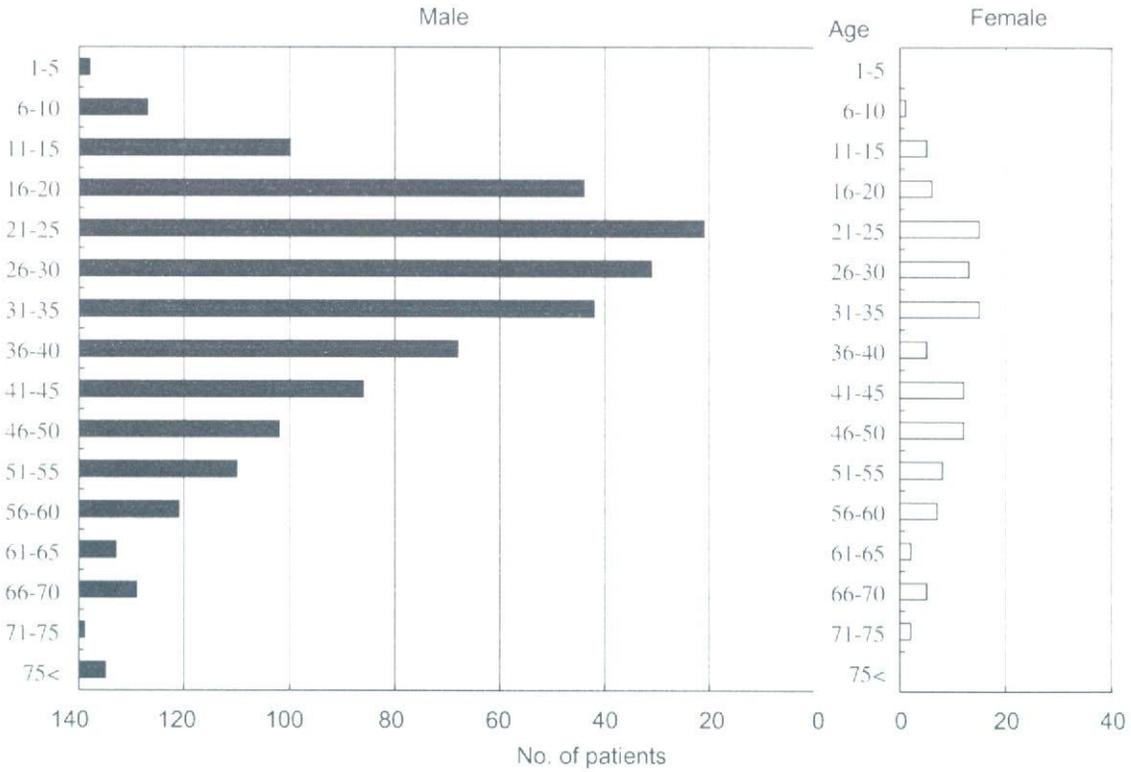
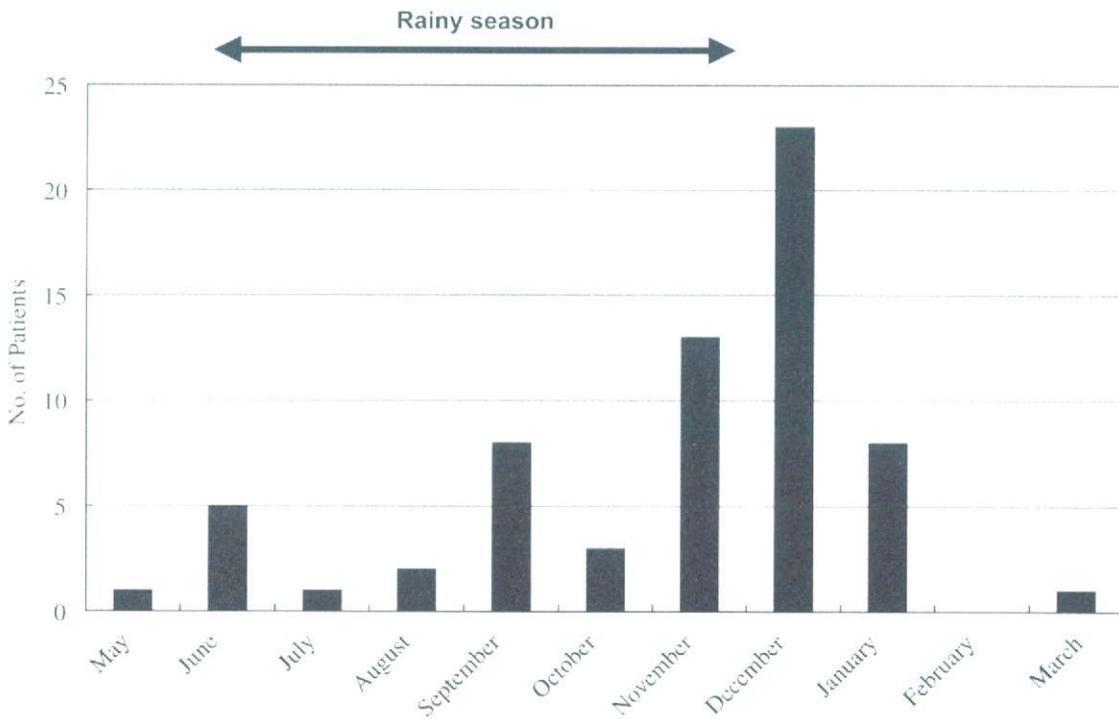Fig. 1. Distribution of leptospirosis cases in Philippines by age and sex.

Fig. 2. No. of patients reported by month in Cabatuan, Iloilo, Philippines (May 1999–March 2000).

Prevailing serovars: Based on MAT, the *Leptospira* serovars that prevail in Metro Manila and the vicinity are Manilae, Losbanos, Poi, Tarassovi and others.

Results of our study on leptospirosis in Philippines showed that this infectious disease is becoming a serious threat to the country. There are many reasons why leptospirosis has re-emerged in Philippines. One of the probable causes of the increase in cases is the rapid urbanization of the country. Many trees were cut down and forested areas were converted to either residential or business zones. The number of forested areas in the country has decreased over a period of 100 years (1900–1999). Data from the map entitled, "Decline of the Philippine Forest" in Environmental Science for Social Changes, Incorporated Metro Manila, Philippines 2000, showed that during the said period, the percentage of forested areas in the country has declined from 70% in 1900 to 18.3% in 1999 [33]. A very important period to note was from the 1960s until the present (about 30 years), when there was a 40% decrease in the forested areas. Trees from forests have a very important role in absorbing water, especially during the rainy season, thereby preventing the occurrence of floods. However, due to deforestation, a large part of Philippines, particularly the Metro Manila area and other low-lying areas, have been continually inundated during the rainy season.

Typhoons or cyclones directly hit the Philippines an average of 20 times per year. The average amount of rainfall from 1961 to 1990 in Quezon City (Science Garden), Pasay City (Ninoy Aquino International Airport) and Marikina City was 2258, 1834, and 3000 mm, respectively. In Manila City alone, the amount of rainfall from 1998 to August 2000 was 2096 and 2741 mm. Inundation of the streets of Metro Manila and other low-lying areas, especially during heavy rainfall, usually occurs because most of the drainage and the esteros or creeks in Philippines are clogged with garbage thus they are not functioning.

It was also observed that from the 1960s, the flooded areas in Philippines has been expanding [34]. At 50–100 mm of rainfall per month, cases of leptospirosis occurred. The problem is further aggravated by poor drainage systems, poor waste disposal system, rat infestation and the tropical temperature (around 30 °C annually). Such factors make it suitable for the growth and maintenance of leptospires. Data from the National Disaster Coordinating Council (NDCC) of the Department of National Defense of the Philippines showed that the frequently flooded areas in Metro Manila are those that are parallel to Pasig river, Marikina river, Taguig river, Paranaque river and Las Pinas river. Consequently, leptospirosis was found to have occurred most frequently among patients that live near these rivers particularly in Manila City, Pasig City, Caloocan City, Malabon, Valenzuela City, Rizal (Taguig), Pasay City, Makati City and the provinces of Cavite and Bulacan. Sta. Cruz, Sampaloc, Binondo and Paco in Manila City as well as Cabatuan in Iloilo and the provinces of Cavite and Bulacan were observed to be the endemic foci of leptospirosis.

In Philippines, Public Health officials and other persons concerned must consider not only the treatment of symptomatic patients but also the improvement of the environment such as drainage systems and control of rats, although eradication seem to be impossible. This must be done not only in urban but also in rural areas such as fishing and farming villages. Investigation of leptospirosis should also be carried out

not only among humans and rats but also among other animals, especially the domesticated ones, since it is a typical zoonotic infection.

Elucidation of the actual status of leptospirosis as well as the prevailing serovar(s) of *Leptospira* is an urgent issue that has to be addressed. These factors are very important in the development of a serovar-specific leptospiral vaccine. Vaccination is thought to be the most effective means of preventing leptospirosis. It is the most practical prophylaxis that can save Filipinos as well as domestic animals from leptospirosis.

5. Conclusion

In this article, we reviewed the current status of leptospirosis in Japan and Philippines, representative among Asian countries. In under-developed countries, leptospirosis is still a serious infectious disease and there is a need for early diagnosis and guidelines for treatment and prophylaxis. On the other hand, in developing countries, the main risk of infection may now be from animals imported from countries and regions where leptospirosis is endemic. To control infections, technical and infomercial support is needed. WHO collaborating centers and ILS are expected to play a role in the control and prevention of infections.

Acknowledgments

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