Original article:

Seroprevalence of Leptospirosis, Enteric fever and Dengue in patients with acute febrile illness in Tamil Nadu, India.

K.Mary Sushi[1], K.Sivasangeetha[1], A.Suresh Kumar[2], Pandi Shastri[3], A.Ganesan[1], D.Anitha[1], G.Thatchinamoorthy[1], S.Mini Jacob[1]

[1]Department of Experimental Medicine, TN Dr.M.G.R Medical University,Chennai, India.

[2]KumararaniMeenaMuthiah College of Arts & Science, Chennai, India.

[3] RVS college of Arts & Science, Sulur, Coimbatore ,India.

Corresponding Author: Dr.S.Mini Jacob , Professor and Head, Department of Experimental Medicine , The Tamil Nadu Dr.M.G.R Medical University, Guindy, Chennai -600032, Tamil Nadu, India.

ABSTRACT:

Introduction: In developing countries like India, alaria, Leptospirosis, Dengue and Typhoid presents as acute undiff-erentiated fever. Management of acute febrile illness differs and is mainly based on the causative infectious agents. In areas endemic for two or more infectious agents, co-infection is more common. The objective of the study was to estimate seroprevalence of Leptospirosis, Enteric Fever and Dengue in cases of acute febrile illness.

Methodology : Hundred patients with febrile illness who were referred for diagnosis of leptospirosis were included in the study. Their serum samples were tested for Leptospira using Microscopic Agglutination Test (MAT), *Salmonella typhi* using Widal rapid & tube agglutination test and Dengue virus by IgM ELISA. Demographic Characteristics were compared and analyzed.

Result: Out of 100 samples, 21 tested positive for Leptospirosis (21%), 17 for Typhoid (17%) and 8 for Dengue (8%). Four patients had Co-infections. Two of them tested positive for Leptospirosis & Typhoid, one for Leptospirosis & Dengue and 1 for Dengue & Typhoid.Forty eight percent (48 %) reported 1-5 days fever, 52% above 5 days, 86 % intermittent fever. Out of 100 patients, 46 tested positive for one or more of the three tests. 54 % were negative.

Conclusion: The laboratory diagnosis plays a very important role in determining the line of management in acute febrile illness. Possibility of co-infection with one or more causative organisms must also be borne in mind when treating patients with acute febrile illness.

Key words: Co-infection, Dengue, Leptospirosis

INTRODUCTION:

In developing countries, like India, Malaria, Leptospirosis, Dengue and Typhoid presents as acute undifferentiated fever [1]. Wide variety of causative agents and limited diagnostic tests to make prompt diagnosis pose major challenge in treating acute febrile illness in tropical countries [2]. The positivity rate of Leptospirosis among acute febrile illness is 25.6% in Southern India [3]. Leptospirosis is sometimes referred to as "The Great Mimicker" and it may be overlooked and under diagnosed due to its varied clinical presentations. In the early stages of the illness, leptospirosis is often indistinguishable from other common causes of acute febrile illnesses in the tropics such as dengue, malaria and typhoid (Enteric fever) [4]. Leptospirosis is a potentially serious but treatable disease [5]. They can cause considerable morbidity, mortality and economic burden to developing tropical nations [6]. Typhoid

and paratyphoid fever remain important public health problems globally and is a major cause of morbidity in the developing world. While both typhoid and paratyphoid fever share clinical features, paratyphoid fever tends to have a more benign course of illness. Without effective treatment, typhoid fever has a case-fatality rate of 10–30% [7]. Dengue has been rampant in parts of Tamil Nadu in the past two decades. The prevalence of dengue vector and silent circulation of dengue viruses have been detected in rural and urban Tamil Nadu, which is ever increasing [8].Management of acute febrile illness differs and is mainly based on the causative infectious agents. In areas endemic for two or more infectious agents, co-infection is more common.

With this background, the study was designed to estimate the seroprevalence of Leptospirosis, Enteric Fever and Dengue infection in cases of acute febrile illness.

MATERIALS& METHODS:

This study was done in Department of Experimental Medicine, the Tamil Nadu Dr.M.G.R Medical University, Chennai which functions as a Leptospira Diagnostic Center. Approximately, the department receives 200 serum samples per month for diagnosis of Leptospirosis referred by the physicians from areas in and around Chennai city. This prospective study was conducted for a period of 6 months from March 2011 to August 2011.Allpatients with febrile illness who were referred for diagnosis of Leptospirosis were given a proforma to fill containing age, gender, occupation and locality. Their clinical signs and symptoms and past history was obtained. Five ml of blood was collected from patient and serum was separated.

Every 12^{th} sample was included in the study by the method of systematic random sampling. Informed written consent was obtained. A total of 100 serum samples were tested for Leptospirosis, Enteric fever and Dengue fever and proforma of the 100 patients were analyzed.

Diagnosis of Leptospirosis:

All the samples were tested for presence of antibody to Leptospira by Microscopic Agglutination Test (MAT) as per standard procedure[9]. Standard Leptospiral strain serovars (*icterohaemorrhagiae, australis, autumnalis, canicola*) which causes Leptospirosis in Chennai and saphrophyticserovar (*Patoc*) *were* included in MAT. A titer of 1:80 and above was considered positive.

Diagnosis of Enteric Fever:

All the samples were tested for Typhoid using Widal Rapid Slide Screening test. All the positive samples by Widal Rapid test were confirmed by Widal Tube Agglutination test. The test included the serovars*Salmonella typhi, Salmonella paratyphi A* and *Salmonella paratyphi B* causing enteric fever.A titer of 1:80 and above was considered positive.

Diagnosis of Dengue:

All the samples were tested for Dengue virus using IgMMicrolisa**.** Tests were done as per the manufacturer's instructions.

RESULTS:

Among the 100 patients, 56% were males and 44% were females. With regard to age distribution, 28 patients were < 20 years, 46 were between the ages of 20 to 45years and 26 patients were > 45 years. Forty three percent (43%) of cases were from Chennai city and 57% from the surrounding areas. Twenty two percent (22%) were students, 18% were laborers, 18% were professionals and 4% were children and others (38%). Seventy four percent (74%) reported no history of fever in the family in recent times and 70% of patients reported the presence of mosquito in their locality. In

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

contrast, 79% reported good drainage system. Among 100 patients with fever symptoms, 48% reported 1-5 days of fever and 52% above 5 days. Eighty six percent (86%) of the patients reported intermittent pattern of fever. Frequency of other symptoms is shown in Table: 1. Past history of febrile illnesses of the 100 patients revealed 11% had typhoid, 2% suffered from Dengue, 2% from Leptospirosis & 7% had jaundice. One patient reported past history of Typhoid, Leptospirosis, jaundice and 2 reported for Typhoid and Jaundice. Of the 100 serum samples tested,21 were positive for Leptospirosis, 17 for Enteric fever and 8 for Dengue (Fig 1).

All the 21 positive patients for Leptospirosis reported high grade fever ranging from 3 days to 1 week and most of the patients had symptoms like headache, body pain, vomiting and abdominal pain. Out of 100 patients, 26 samples reported positive by Widal Rapid slide Agglutination test. On further confirmation, only 17 samples (36.9%) were positive by Widal Tube test. Thirteen patients (76.47%) had antibodies against *Salmonella typhi* followed by 3 patients for *Salmonella paratyphi A* (17.6%) and 1 patient for *Salmonella paratyphi B* (5.8%) (Fig 3). All the 17 positive patients reported high grade fever with headache, malaise,abdominal pain, joint pain and few patients had diarrhea. Eight patients (8%) were positive for dengue using the IgM ELISA. Dengue positive patients also reported high grade fever, head ache, vomiting, sore throat and some suffered from breathlessness.

Four patients were diagnosed to have one or more infection. Two patients were positive for Leptospirosis and Typhoid, one patient for Leptospirosis and Dengue and one patient positive for Typhoid and Dengue (Table 4).

DISCUSSION: All the 100 patients enrolled in this study reported high grade fever for 3 days to a week. They were suspected to have Leptospirosis by general physicians and were referred for testing. Twenty one patients (21%) were seropositive for leptospirosis among the 100 samples tested. D. Deodhar*et al* reported that 31% patients had serological evidence of leptospira among cases of acute febrile illness [3]. Smita B. Shekatkar*et al* from Pondicherry had reported 60% of seropositivity for leptospirosis by MAT [10].H Manocha*et al* from Uttar Pradesh had reported 7% of leptospirosis as a cause of acute febrile illness [11]. Clinically Leptospirosis presents as two forms: Anicteric leptospirosis is the most common form accounting for 85% and icteric leptospirosis or Weil's syndrome occurring in 5 to 10% of cases. Anicteric leptospirosis has 2 phases, septicemic phase and immune phase. Septicemic phase lasts for 3-7 days. Fever is high and remitting. Headache is intense, unremitting and possibly throbbing. Anorexia, nausea, vomiting and abdominal pain occur in most patients [12]. In the present study, all 21 patients who had antibodies to Leptospira also had high grade fever ranging from 3 days to 1 week and most of the patients showed symptoms like headache, body pain, vomiting and abdominal pain. Serological tests based on the detection of antibodies remain the most practical method of diagnosis of leptospirosis. Among the serological techniques, MAT remains the "gold standard" because of its unsurpassed diagnostic (serovar-/serogroup) specificity in comparison with other currently available tests [13].In the present study, out of 21 leptospira positive patients, 10 had antibodies against *L.australis* and 6 had antibodies against *L.canicola*, 4 against *L.icterohemorrhagiae* and one for *L.autumnalis*. S. Ratnam*et al* had reported that serogroup*L. autumnalis* was common in Chennai [14].Ganesan A *et al* had reported *L.australis* was most common serovar followed by

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

L.canicola in a study conducted in the same department previously [15]. Smitha*et al* from Puducherry had reported that serogroup L. *icterohemorrhagiae* was found in 27% of their patients followed by *L.pomona* and *L.pyrogenes* [10].Sharma.S*et al* from Andaman Islands had reported *L.grippotyphosa* as the commonest serotype followed by *L.australis*[16].In the present study, patients were positive for leptospirosis during summer months. Sumathi G *et al* from Chennai had reported in the year 2008 that leptospirosis occurs throughout the year and number of cases increases during monsoon months [17].

Dengue is an acute viral infection with potential fatal complications. It is transmitted mainly by *Aedesaegypti*mosquito and also by *Ae. albopictus.* All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe [18].Manifestations of dengue infection range from asymptomatic or mild febrile illness to circulatory failure and death from dengue hemorrhagic fever (DHF)[19].In the present study, 8 out of 100 patients (8%) were positive for Dengue by IgM ELISA. Megan E. Reller*et al* reported that Dengue accounted for 6.3% (54/859) of acute febrile illnesses in Srilanka [20].A study in Vietnam by H L Phuong reported that dengue is responsible for one third of fever cases presented to the public primary health services [21].PiyushTripathi*et al* in Uttar Pradesh reported 21% of their study patients had antibodies to Dengue [22]. Gunasekaran P et al from Chennai reported 15.5% of adult patients had antibodies to dengue for a period of three years from 2006 to 2008[23].In the present study, Dengue positive patients also reported high grade fever, head ache, vomiting, sore throat and some suffered from breathlessness. Dengue usually presents with symptoms of flu-like illness such as high-grade fever, generalized body ache, arthralgia, myalgia, nausea, and vomiting as well as maculopapular rashes. The symptoms of dengue may mimic other diseases such as leptospirosis and malaria which are also prevalent in areas where dengue is endemic [24].

In this study initially 26 samples reported positive by Widal Rapid slide Agglutination test. On further confirming, only 17 samples (36.9%) were positive by Widal Tube Agglutination test. Typhoid fever continues to be a global health problem with an estimated 16 million cases worldwide and 600,000 deaths each year. The disease is endemic in many developing countries [25].The signs and symptoms of uncomplicated typhoid fever are nonspecific and a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow. In areas where bacterial culture facilities are not available, Widal test remains the specific diagnostic test. The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever. Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart [26]. R. Shyamala*et al* in their study had reported 9% of seropositivity of Widal test among clinically suspected typhoid patients [27].In this study,17 cases of enteric fever was diagnosed from March to August. Of the 17 cases, 13 were due to *Salmonella typhi*. This could be due to onset of monsoon season in the month of July and August and lack of proper drainage leading to water contamination with fecal material. A study from Kolkata by Sur D

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

et al also showed a peak of the disease from July to September [28].

In the present study, 8 patients were positive by widal tube test in their first week of illness. Christopher M *et al* also reported that widal was positive in 90% of patients with blood culture-positive typhoid fever and admitted during the first week of their illness. This could be due to regular subclinical sensitization to Salmonella in endemic areas [26].In this study, 4 co-infections were noticed, 2 for Leptospirosis and typhoid, 1 for Leptospirosis and Dengue, 1 for dengue and typhoid. This could be explained by 2 ways. One by the fact that all the three infections are endemic in Chennai and the patients would have had infections with one or two causative agents.

On the other hand results of serology may be a false positive reaction caused by cross-reacting antibodies or non-specific polyclonal immune-oreactivity [28]. Moreover, IgM antibody against dengue infection stays in circulation for a period of two to three months.Levett P N *et al* from Barbados had also reported that 2 out of 25 leptospirosis patients had serological evidence of acute dengue infection [29].

L. S. Yong *et al* from Malaysia had also reported a case of co-infection with Dengue, Leptospirosis and malaria in their patient [4]. A study in Kerala by Anil Kumar *et al* reported 17 cases (1.3%) of co-infections due to both leptospirosis and dengue [30].Farid Ahmed *et al* from Bangladesh had reported a case co-infections of typhoid fever with Hepatitis A, Hepatitis E and dengue [31].

There are a few limitations to this study. The study was undertaken in a Diagnostic Testing Laboratory in the University rather than a hospital. In a hospital setup, the case history could have provided more information. The follow up of patients were not possible in case of extending the study in depth. Nearly half of the patient had fever for less than 5 days, absence of IgM antibody may not represent true Negatives. This could have been overcome by doing the serological test in paired serum samples. If paired serum samples were tested, which it would have demonstrated the increasing titers of antibodies. Financial constraints did not permit us to perform NS1 antigen in diagnosing Dengue fever. Blood culture was not done in the present study to confirm the diagnosis of typhoid and paratyphoid fevers. The present study was carried out in 100 samples. Entire workup for diagnosing the etiology of acute febrile illness could have been performed. In spite of malaria being the most frequent cause of acute febrile illness in Chennai, tests for malaria was not done.

CONCLUSION:

The laboratory diagnosis plays a very important role in determining the line of management in acute febrile illness. In resource limited setting, meticulous history taking, careful clinical examination and right choice of laboratory tests to detect the infections endemic in an area is crucial in diagnosing the cause of Acute Febrile Illness. Possibility of co-infections with one or more causative organisms must also be borne in mind when treating patients with acute febrile illness.

Table: 1 Presenting symptoms

Symptoms	No. of Patients
Headache	55
Rigor and chills	50
Cough	48
Joint pain	39
Abdominal pain	28
Muscle ache	29

Table: 2 Distribution of gender in Leptospirosis, Typhoid and Dengue Positive patients.

	MALE	FEMALE
Leptospirosis	11	10
Enteric Fever	9	8
Dengue	4	4
Total	24(52.15%)	22(47.8%)

Table: 3 Distribution of Age in Leptospirosis, Typhoid and Dengue Positive patients.

AGE	LEPTOSPIROSIS	Enteric Fever	DENGUE
<20 years	5	3	4
20-45 years	9	11	4
>45 years	7	3	0
Total	21	17	8

Table:4 Co-infections in Seropositive cases (n=4)

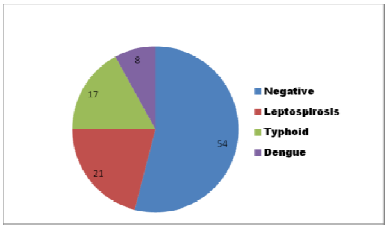
Co-infections	No. of cases
Leptospirosis and Typhoid	2
Leptospirosis and Dengue	1
Typhoid and Dengue	1
Total	4

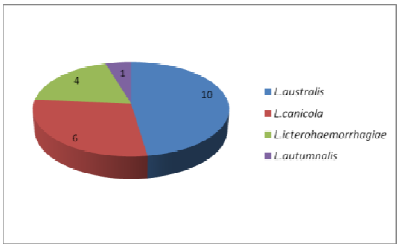
www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

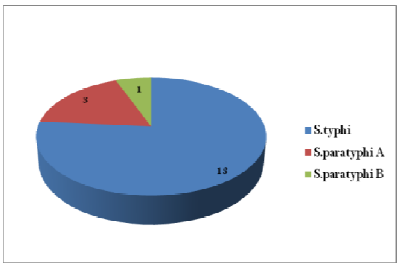
Fig: 1. DISTRIBUTION OF LEPTOSPIROSIS, TYPHOID AND DENGUE AMONG PATIENTS (n=100)

Fig. 2. DISTRIBUTION OF SEROVARS IN LEPTOSPIROSIS POSITIVE PATIENTS (n=21)

Fig. 3. DISTRIBUTION OF SEROTYPES IN ENTERIC FEVER PATIENTS (n=17)







REFERENCES:

1. Rajnish Joshi et al Nonmalarial Acute Undifferentiated Fever in a Rural Hospital in Central India: Diagnostic Uncertainty and Overtreatment with Antimalarial Agents, Am J Trop Med Hyg 2008; 78 (3): 393-39.
2. Kasper MR, Blair PJ, Touch S, Sokhal B, Yasuda CY, Williams M, Richards AL, Burgess TH, Wierzba TF, Putnam SD. Infectiousetiologies of acutefebrileillness among patientsseekinghealthcare in south-centralCambodia. Am J Trop Med Hyg2012;86(2):246-53.
3. D. Deodhar, M. John, Leptospirosis: Experience at a tertiary care hospital in northern India. Natl Med J India 2011;24:78–80.
4. Yong LS, Koh KC A case of mixed infections in a patient presenting with acute febrile illness in the tropics.Case Rep Infect Dis. 2013:562175. doi: 10.1155/2013/562175. Epub 2013; 2
5. HUMAN LEPTOSPIROSIS:GUIDANCE FOR DIAGNOSIS, SURVEILLANCE AND CONTROL WHO.International Leptospirosis Society.
6. Chrispal A, Boorugu H, Gopinath KG, Chandy S, Prakash JA, Thomas EM, Abraham AM, Abraham OC, Thomas K Acute undifferentiated febrile illness in adult hospitalized patients: the disease

spectrum and diagnostic predictors - an experience from a tertiary care hospital in South India.Trop Doct.2010 ;40(4):230-4.

7. Geoffrey C. Buckle, Christa L. Fischer Walker, Robert E. Black, Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010 Journal of Global Health 2012; 2(1): 010401
8. Tewari SC, Thenmozhi1 V, Katholi CR, Manavalan R, Munirathinam A, Gajanana A. Dengue vector prevalence and virus infection in a rural area in south India. Trop Med Int Health 2004; 9 : 499-507.
9. P.Vijayachari, Sameer Sharma,K.Natarajaseenivasan, Laboratory Diagnosis, In: Leptospirosis Laboratory Manual, Regional Medical Research Centre, Indian Council of medical Research port Blair. New concepts information systems Pvt.Ltd.,New Delhi 2007: 27-45
10. Smita B. Shekatkar, Belgode N. Harish, Godfred A. Menezes and Subhash C. Parija Clinical and serological evaluation of leptospirosis in Puducherry, India. J Infect DevCtries 2010; 4(3):139-143.
11. H Manocha, UjjalaGhoshal, SK Singh, J Kishore, ArchanaAyyagari Frequency of Leptospirosis in Patients of Acute Febrile Illness in Uttar Pradesh JAPI 2004;52: 623-5
12. Sambasiva RR, Naveen G, P B, Agarwal SK Leptospirosis in India and the rest of the world. Braz J Infect Dis.2003 ;7(3):178-93.
13. Velineni S, Asuthkar S, Umabala P, Lakshmi V, Sritharan M. Serological evaluation of leptospirosis in Hyderabad, Andhra Pradesh: A retrospective hospital-based study. Indian J Med Microbiol 2007;25:24-7
14. Ratnam S, SubramaniamS,MadanagopalanN,SundararajT,Jayanthi V; Isolation of Leptospires and demonstration of antibodies in human Leptospirosis in Madras, India. Trans R Soc Trop Med Hyg. 1983;77(4): 455-8
15. GanesanArumugam, S Mini Jacob, D Anitha, SivakumarMampakkamRajappa Occurrence of leptospirosis among suspected cases in Chennai, Tamil Nadu Indian Journal Pathology & Microbiology 2011 ; 54(1) :100-102
16. Sharma S, Vijayachari P. Sugunan AP et al. Seroprevalence of leptospirosis among high risk population of Andaman Islands, India. Am J Trop Med Hyg2006;74(2): 278-283.
17. Sumathi G, Narayanan R, Shivakumar S. Leptospirosis laboratory, Madras medical college: Review of our experience (2004-2006). Indian J Med Microbiol 2008;26:206-7
18. Nivedita Gupta, Sakshi Srivastava, Amita Jain and Umesh C. Chaturvedi Dengue in India Indian J Med Res. 2012 September; 136(3): 373–390.
19. GublerDJ. Dengue and Dengue Hemorrhagic Fever. ClinMicrobiol Rev. 1998; 11(3): 480–496.
20. Megan E. Reller, ChampikaBodinayake, AjithNagahawatte, VasanthaDevasiri, WasanthaKodikara-Arachichi, John J. Strouse, Anne Broadwater, TrulsØstbye, Aravinda de Silva, and Christopher W. Woods Unsuspected Dengue and Acute Febrile Illness in Rural and Semi-Urban Southern Sri Lanka. Emerging Infectious Diseases 2012; 18(2):256-263.
21. Phuong HL, de Vries PJ, Nga TT, Giao PT, Hung le Q, Binh TQ, Nam NV, Nagelkerke N, Kager PA. Dengue as a cause of acute undifferentiated fever in Vietnam.BMC Infect Dis. 2006; 25; 6:123.

22. Tripathi P, Kumar R, Tripathi S, Tambe JJ, Venkatesh V. Descriptive Epidemiology of Dengue Transmission in Uttar Pradesh, Indian Pediatr. 2008; 45(4):315-8.

23. P. Gunasekaran, K. Kaveri, S. Mohana, KavitaArunagiri, B.V. Suresh Babu, P. Padma Priya, R. Kiruba, V. Senthil Kumar & A. Khaleefathullah Sheriff Dengue disease status in Chennai (2006-2008): A retrospective analysis Indian J Med Res 2011;133: 322-32

24. T. P. Monath and T. F. Tsai, "Flaviviruses," in Clinical Virology, D. D. Richman, R. J. Whitley, and F. G. Hayden, Eds., pp. 1133–1186, Churchill Livingstone, New York, NY, USA, 1997.

25. Ivanoff B. Typhoid fever: global situation and WHO recommendations. Proceedings of the 2nd Asia-Pacific Symposium on Typhoid Fever and Other Salmonellosis. Bangkok: Infectious Disease Association of Thailand, 1994:39.

26. Christopher M. Parry, Nguyen ThiTuyetHoa, To Song Diep, John Wain, Nguyen Tran Chinh, Ha Vinh, Tran TinhHien, Nicholas J. White, Jeremy J. Farrar Value of a Single-Tube Widal Test in Diagnosis of Typhoid Fever in VietnamJ ClinMicrobiol. 1999; 37(9): 2882–2886.

27. R. Shyamala Prevalence of Widal positivity in a tertiary care hospital in South India, Der Pharmacia Lettre, 2012, 4 (5):1486-1489

28. Sur D, von Seidlein L, Manna B, Dutta S, Deb AK, Sarkar BL, Kanungo S, Deen JL, et al. (2006) The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community based study, Trans R Soc Trop Med Hyg 100(8): 725-33.

29. Levett PN, Branch SL, Edwards CN. Detection of dengue infection in patients investigated for leptospirosis in Barbados. Am J Trop Med Hyg2000;62(1):112-4.

30. Kumar A, Balachandran V, Dominic A, Dinesh KR, Karim S, Rao G. Serological evidence of leptospirosis and dengue coinfection in an endemic region in South India. Ann Trop Med Public Health 2012;5:286-90

31. Farid Ahmed, Kanta Chowdhury, Md. Jahangir Alam, ShamsulArefeen, M MorshedAlam Co-infection of typhoid fever with hepatitis A, hepatitis E and dengue fever: A challenge to the physicians. Dhaka Shishu Hospital Journal 2010; 26 (2) : 122-124

Date of submission: 15 January 2014 Date of Provisional acceptance: 05 February 2014
Date of Final acceptance: 27 February 2014 Date of Publication: 04 March 2014
Source of support: Nil; Conflict of Interest: Nil