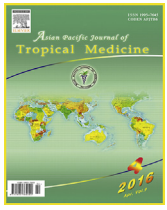
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Review http://dx.doi.org/10.1016/j.apjtm.2016.03.009

Etiological agents causing leptospirosis in Sri Lanka: A review

Chamidri Naotunna, Suneth Buddhika Agampodi*, Thilini Chanchala Agampodi

Department of Community Medicine, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, Saliyapura 50008, Sri Lanka

ARTICLE INFO

Article history:
Received 15 Jan 2016
Received in revised form 20 Feb 2016
Accepted 1 Mar 2016
Available online 9 Mar 2016

Keywords:
Leptospira
Leptospirosis
Sri Lanka
Serovar
Strain
Species

ABSTRACT

Objective: To systematically review the etiological agent causing human leptospirosis in Sri Lanka.

Methods: Published articles on leptospirosis and *Leptospira* in Sri Lanka were all reviewed to determine serovar, strain and species level identification of *Leptospira*. After screening process, 74 full text articles/reports were reviewed and among of them, 12 published papers describing isolation of *Leptospira* from Sri Lankan patients/animals, 5 molecular epidemiology papers on newer typing methods citing Sri Lanka isolates, with a descriptions of the isolates and 6 published papers reporting PCR based species level identification were identified.

Results: Published literature showed that more than 40 strains classified under at least 20 serovars and 10 serogroups have been isolated from Sri Lanka. These isolates belong to four species, namely, *Leptospira interrogans*, *Leptospira kirschneri*, *Leptospira borgpetersenii*, and *Leptospira santarosai*. In addition, recent studies on direct patient samples without culture and isolation showed *Leptospira* from *Leptospira weilli* is also circulating in Sri Lanka. Multi locus sequence typing showed 13 genotypes of *Leptospira* from Sri Lankan isolates.

Conclusions: This review shows the diversity of *Leptospira* in Sri Lanka, but culture isolation data has not been published in Sri Lanka during last 30 years.

1. Introduction

Leptospirosis is one of the most widely spread zoonotic disease in the world with an estimated 1.03 million annual cases and 58 999 deaths [1]. Control and prevention of leptospirosis are often limited due to knowledge gaps on disease agent, hosts and environment enabling the disease transmission in local settings. The disease is caused by spirochetes belonging to genus *Leptospira*, which has more than 230 serovars classified in to 31 serogroups based on the serology. Based on the DNA hybridization methods, 21 species has been identified to date [2–12]. Because of the diversity of *Leptospira*, management and control of leptospirosis is a challenge especially for countries in tropical setting where the facilities for isolation and typing are limited.

Sri Lanka is having one of the highest incidence of leptospirosis and considered as a leptospirosis high endemic country [13]. During the five year period from 2004 to 2008, the total number of cases reported to the Epidemiology Unit (National surveillance Centre) was around 4000 cases [14]. In 2008, Sri Lanka experienced the worst recent outbreak of leptospirosis with more than 7000 reported cases [15]. Cumulative annual incidence of leptospirosis during 2008–2014 period in Sri Lanka was more than 25 per 100 000 and during the last 6 years, an average of more than 5000 cases were reported annually to the Epidemiology unit [16–20]. Despite all control measures taken by the national control programme, leptospirosis continues to affect lives of people in Sri Lanka. This could be partly attributed to the knowledge gap in leptospirosis transmission. There were considerable numbers of research carried out since 2008, however investigations on disease causing agents are limited.

The present standard diagnostic method for leptospirosis is microscopic agglutination test (MAT) [21]. Several extensive studies showed that MAT could not be considered as the gold standard due to its very low sensitivity [22]. We experienced same in Sri Lanka in 2008 [15] and 2011 [23]. Though imperfect, MAT is still the standard test for leptospirosis

*Corresponding author: Suneth Buddhika Agampodi, Department of Community Medicine, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, Saliyapura 50008, Sri Lanka.
Tel: +94 777880096;
Fax: +94 252226252
E-mail: sunethagampodi@yahoo.com
Peer review under responsibility of Hainan Medical College.

diagnosis due to lack of other diagnostic facilities. Standard MAT panel should be based on the local knowledge on circulating serovars or it should be a broad MAT panel to cover all pathogenic serogroups. In high burden countries in South East Asia, including Sri Lanka, these standard MAT diagnostic facilities are still not available. As an example, till 2014 Sri Lankan diagnostic laboratory used genes specific Patoc serovar for MAT and still the MAT panel used in Sri Lankan reference center include only 11 serovars. This lack of diagnostic facilities are attributed due to lack of knowledge on circulating serovars or lack of resources to maintain labor intensive MAT diagnosis facilities. However, this may be partly due to lack of knowledge synthesis within the country. The purpose of this review is to identify the locally prevalent serovars of *Leptospira* in Sri Lanka based on published literature to fill this knowledge gap.

2. Materials and methods

We carried out a comprehensive search of literature to identify studies related to leptospirosis and *Leptospira* in Sri Lanka. Two main search platforms were used for internet based search: Pubmed and Google Scholar. The search strategies were as follows: Pubmed search string (56) ((("Leptospirosis"(Mesh) OR 'Weil Disease'(Mesh) OR "*Leptospira*"(Mesh) OR '*Leptospira interrogans* (*L. interrogans*) serovar Pomona'(Mesh) OR '*L. interrogans* serovar Icterohaemorrhagiae'(Mesh) OR '*L. interrogans* serovar Hebdomadis'(Mesh) OR '*L. interrogans* serovar Canicola'(Mesh) OR '*L. interrogans* serovar Autumnalis'(Mesh) OR '*L. interrogans* serovar Australis' (Mesh) OR '*L. interrogans*'(Mesh)))) OR ((('Leptospirosis' OR '*Leptospira*' OR 'Weils disease' OR 'Weil's syndrome'))) AND (('Sri Lanka' OR 'Ceylon')); Google Scholar search (2320) ("Leptospirosis" OR "*Leptospira*") AND ("Sri Lanka" OR "Ceylon").

In PubMed search, we used a specific search strategy. However, in Google Scholar, we manually screened all titles appeared after the initial search to include grey literature and articles that are not listed in PubMed.

We also carried out manual search, specially to retrieve data from non-indexed local publications. For this purpose we used three bibliographic references: a Bibliography of medical publications related to Sri Lanka 1811–1976 [24] and its supplement Bibliography on health in Sri Lanka, 1977–1980 [25] by Peiris and Uragoda and Bibliography of Medical Literature 1980–2005 compiled by Post Graduate Institute of Medicine (PGIM) Library, Colombo. In addition, we hand searched titles of theses and dissertations, and casebooks submitted to PGIM, all issues of Ceylon Medical Journals prior to 2008 and archived issues of Sri Lanka Journal of Medical Sciences and Kandy Medical Journal in four libraries (Sri Lanka Medical Association Library, PGIM library and Colombo and Peradeniya Medical School libraries). Further, we searched technical reports published by Medical Research Institute during 1960–1980 period and Quarterly Epidemiological Bulletins of Epidemiology Unit 1980–2014. Reference lists of relevant articles were also searched to identify any missing article. In addition, we searched for publications which included global collection of *Leptospira* isolates for typing to get information on Sri Lankan isolates.

The search was done in several steps. First we selected all Sri Lankan publications and publications on Sri Lankan patients or animals related to leptospirosis and *Leptospira*. This was done by three investigators. A title and abstract search was done to eliminate review articles or articles without primary data. Once the articles with original data were identified, we searched the full text for any article reporting culture isolation or inoculation of patient

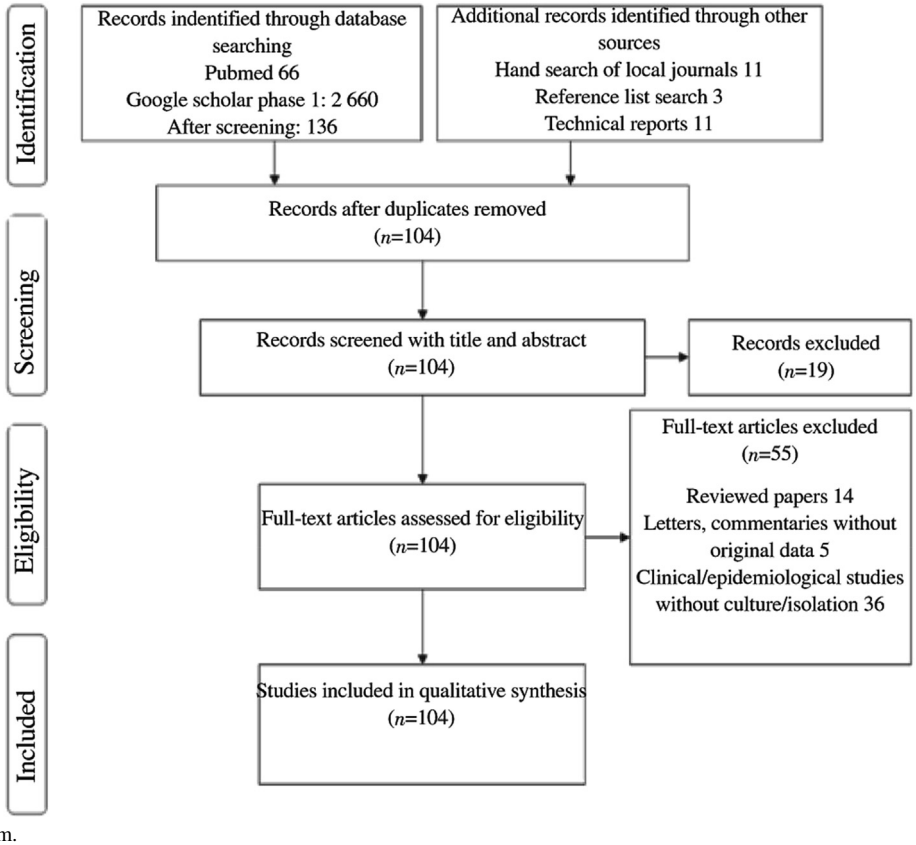


Figure 1. Flow diagram.

samples to animal models to isolate *Leptospira*. We did not exclude any article based on quality of culture isolation details, rather we cross checked the reported isolations specially strain information with online databases on *Leptospira* published by Pasture institute and data published in species classification studies using large collection of global isolates to validate our finding. We did not limit our search to languages and online translations were used to translate the title and abstracts of selected articles.

3. Results

The flow diagram shows in Figure 1.

Through full text review of selected literature, we identified 12 published papers [26–37] describing isolation of *Leptospira* from Sri Lankan patients/animals, 5 molecular epidemiology papers on newer typing methods citing Sri Lanka isolates, with a descriptions of the isolates [38–42] and 8 published papers [15,23,43–47] reporting PCR based species level identification.

First confirmed case of leptospirosis in Sri Lanka was reported by Rajasuriya *et al* [26] and the investigators have done guinea pig inoculation and demonstration of Leptospires, but isolation was not done. First report on isolation of *Leptospira* from Sri Lankan patient was by Nityananda in 1962 [27] and *L. interrogans* serovar *Icterohaemorrhagiae* was identified. Most of the early studies demonstrated *Leptospires* after guinea pig inoculation, but only few studies isolated and reported the serovars identified.

Early publications on isolation were based on serological classifications only. More than 20 serovars have been documented as isolated from Sri Lanka from four species; *L. interrogans, Leptospira kirschneri* (*L. kirschneri*), *Leptospira borgpetersenii* (*L. borgpetersenii*), and *Leptospira santarosai* (*L. santarosai*) (Table 1). However, the classification was not clear for some of the serovars and some of the recently isolated strains are listed as undetermined [40]. Of the strains isolated from Sri Lanka, eight strains are listed as the reference strains for the serovars.

Table 1

Summary of *Leptospira* serovars isolated from Sri Lanka[c].

Serovar	First isolated/reported	Note
L. interrogans serovar Icterohaemorragiae	1961	From a 46 years old male from Sri Lanka
L. borgpetersenii serovar Ceylonica[a]	1964	Strain Piyasena[b]. From a male baker from Colombo. First named as strain Dyananda and later renamed.
L. interrogans serovar Geyaweera[a]	1965	Strain Geyaweera[b]. From a patient from Sri Lanka. Published literature not retrievable. Reference is from KIT leptospirosis reference center
L. interrogans serovar Weerasinghe[a]	1965–66	Strain Weerasinghe[b]. Human case described in 1971.
L. kirschneri serovar Ratnapura[a]	1965–66	Strain Wumalasena[b]. From a patient from Ratnapura and reported in 1970
L. interrogans serovar Gem[a]	1966	Strain Simon[b]. From human case
L. santarosai serovar Alice[a]	1966	Strain Alice[b], From human case from Ratnapura
L. interrogans serovar Autumnalis	1967	From a Ratnapura, Sri Lanka (details not available)
L. interrogans serovar Canicola	1967	From Ratnapura, Sri Lanka (details not available)
L. interrogans serovar Hebdomadis	1967	From Ratnapura, Sri Lanka (details not available)
L. interrogans serovar Lanka	1967	Strain R 740[b]. Isolated from a patient from Ratnapura
L. kirschneri serovar Grippotyphosa	1967	From a human case from Ratnapura, Sri Lanka
L. borgpetersenii serovar Javanica	1968	From paddy field rats, sewer rats, bandicoots, shrew and dog
L. interrogans serovar Pomona	1968	From Dogs (details not available)
L. interrogans serovar Bangkinang	1971	Human case (details not available)
L. interrogans serovar Bulgarcia	1971	Human case (details not available)
L. interrogans serovar Ricardi	1971	Human case (details not available)
L. interrogans serovar Pyrogenes	1971–73	Human case from Ragama, Sri Lanka
L. interrogans serovar 52–73		Strain 457[b]. From a dog in Sri Lanka

[a] These serovars were first isolated from Sri Lankan patients. [b] These are the reference strains for the serovar. [c] Number of strains belongs to *L. interrogans* and *L. borgpetersenii* from 2006 to 7 period and isolated from Gampaha area are listed in MLST.net with undesignated serovars.

Table 2

Leptospira species identification based on molecular techniques without culture and isolation.

Year	Geographical location	Origin	Target gene	Deduced species	No. of positives
2008	Peradeniya	Human	*Leptospira flaB*	*L. interrogans*	1
				L. kirschneri	2
2009	Kandy district	Cattle	*Leptospira flaB*	*L. interrogans*	1
2009–10	Peradeniya	Human	*Leptospira flaB*	*L. interrogans*	7
				L. kirschneri	1
2008	Matale. Kandy, Kegalle	Human	16s	*L. interrogans*	30
				L. weilli	2
2010	Anuradhapura, Kurunagala, Puttalum	Cattle	*Leptospira flaB*	*L. interrogans*	3
				L. kirschneiri	7
				L. borgpetersenii	10
2011	Anuradhapura	Human	16s	*L. interrogans*	3
				L. kirschneri	20
				L. borgpetersenii	3
2013	Southern and Western provinces	Human	*Leptospira flaB*	*L. interrogans*	11
				L. kirschneri	1
				L. borgpetersenii	2

Table 3
Multi Locus Sequence based genotyping of Sri Lankan isolates using 7 loci published by Boonslip *et al* 2013.

Species	Serovar	Strain	Host	ST
L. interrogans	Gem	Simon	Human	38
L. interrogans	Weerasinghe	Weerasinghe	Human	43
L. interrogans	Geyaweera	Geyaweera	Human	44
L. interrogans	Pyrogenes	R122	Human	49
L. interrogans	Pyrogenes	R150	Human	49
L. interrogans	Pyrogenes	R168	Human	49
L. interrogans	Pyrogenes	R166	Human	49
L. interrogans	Pyrogenes	R358	Human	49
L. interrogans	Pyrogenes	R480	Human	49
L. interrogans	Pyrogenes	R163	Human	74
L. interrogans	Pyrogenes	R205	Human	75
L. interrogans	Pyrogenes	R493	Human	75
L. interrogans	Pyrogenes	R601	Human	75
L. interrogans	Undesignated	R444	Human	75
L. interrogans	Undesignated	R437	Human	76
L. interrogans	Undesignated	R457	Human	76
L. interrogans	Undesignated	R499	Human	80
L. kirschneri	Ratnapura	Wumalasena	Human	116
L. interrogans	Undesignated	R235	Human	140
L. borgpetersenii	Undesignated	R010	Human	144
L. borgpetersenii	Ceylonica	Piyasena	Human	144
L. borgpetersenii	Undesignated	R116	Human	157
L. santarosai	Alice	Alice	Human	178

In some of the published studies, only the serogroup was mentioned after the culture isolation but not the exact serovars. Combination of published serovars and serogroups showed that serovars belonging to at least 10 serogroups are circulating in Sri Lanka. The circulating serogroups of *Leptospira* include Icterohaemorragiae, Autumnalis, Sejroe, Grippotyphosa, Javanica, Louisiana, Canicola, Hebdomadis, Pomona and Pyrogenes.

Isolation of serovars was mainly from leptospirosis patients. However, several studies looked at the presence of *Leptospira* in mammals and rodents. The largest study on mammalian and rodent hosts were carried out in 1971 which included cattle, dog, swine, shrew, mongoose, hare, cat, bandicoots, sewer rats, rice field rats, Asian house shrew, Ceylon flat country house rat, Ceylon hill country house rat and rock squirrels [32]. Cattle, swine, mongoose and hare kidney tissue and samples did not revealed leptospires after culture. All other animals showed leptospires belonged to several serogroups.

Recent studies using molecular techniques without culture or isolation also confirmed the wide variation of species circulating in Sri Lanka (Table 2).

These recent studies also show regional variations of circulating serovars, which is common among human and animal species.

Multi Locus Sequence based genotyping of Sri Lankan isolates was reported using three different typing schemes [38,40–42]. The combined typing scheme published in 2013 using seven MLST loci using 23 Sri Lankan isolates (Table 3) shows that at least 13 genotypes are causing human leptospirosis in this country [41]. Two of these genotypes (ST 1 & ST 44) were demonstrated in direct clinical samples from confirmed cases of leptospirosis [48].

4. Discussion

Contrary to the popular belief among Sri Lankan researchers and practitioners, a large number of *Leptospira* serovars, stains and serotypes have been already identified in Sri Lanka, majority in 1960's and 70's. These include more than 40 strains classified in to more than 20 serovars from 10 serogroups representing five species. However, no published data available on culture and isolation of *Leptospira* in the recent past, where the leptospirosis has become hyper endemic in this country.

This review clearly shows the diversity of *Leptospira* in Sri Lanka, a small country with 20 million population in 74 000 square kilometers. This diversity is only for the disease causing agent, however the disease transmission is more complicated with large number of reservoir animals and a range of different ecological systems that facilitate disease transmission in animal–human interface. As shown in a recent publication [23], these complexities may lead to a completely different clinical presentation by different serovars through different reservoir hosts. This complicates the disease control activities even within a context of small country, where national level strategies may not be applicable in different geographical regions in the country.

Though we reported large number of serovars in this review, it is important to note that this is not providing data on prevalent serovars or serotypes circulating in Sri Lanka at present. With the climate change, change of ecological systems and different animal human interaction may completely change the circulating serovars and strain types which need to investigate using culture and isolation. Though the new robust methods of *Leptospira* classification based on MLST [41] is more scientific and provide more information on genetic relatedness of *Leptospira*, for MAT we need serovar-based identification with culture and isolation. This is a challenge in settings where the presentation is late due to initial treatment at out patient settings and also heavy use of antibiotics before coming to hospitals.

As we observed in Sri Lanka, knowledge synthesis of already available and published data will provide a strong platform for future research and baseline data for diagnosis and control activities of this globally important disease.

Conflict of interest statements

We declare that we have no conflict of interest.

References

[1] Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, et al. Global morbidity and mortality of leptospirosis: a systematic review. *PLoS Negl Trop Dis* 2015; http://dx.doi.org/10.1371/journal.pntd.0003898.
[2] Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS. Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira genomospecies*. *Int J Syst Bacteriol* 1999; **49**(Pt 2): 839-858.
[3] Levett PN, Morey RE, Galloway RL, Steigerwalt AG. *Leptospira broomii* sp. nov., isolated from humans with leptospirosis. *Int J Syst Evol Microbiol* 2006; **56**(Pt 3): 671-673.
[4] Levett PN, Morey RE, Galloway R, Steigerwalt AG, Ellis WA. Reclassification of *Leptospira parva* Hovind-Hougen et al. 1982 as *Turneriella parva* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2005; **55**(Pt 4): 1497-1499.
[5] Perolat P, Chappel RJ, Adler B, Baranton G, Bulach DM, Billinghurst ML, et al. *Leptospira fainei* sp. nov., isolated from pigs in Australia. *Int J Syst Bacteriol* 1998; **48**(Pt 3): 851-858.
[6] Ramadass P, Jarvis BD, Corner RJ, Penny D, Marshall RB. Genetic characterization of pathogenic *Leptospira* species by DNA hybridization. *Int J Syst Bacteriol* 1992; **42**(2): 215-219.
[7] Yasuda PH, Steigerwalt AG, Sulzer KR, Kaufmann AF, Rogers F, Brenner DJ. Deoxyribonucleic acid relatedness between serogroups

and serovars in the family *Leptospiraceae* with proposals for seven new *Leptospira* species. *Int J Syst Bacteriol* 1987; **37**(4): 407-415.
[8] Matthias MA, Ricaldi JN, Cespedes M, Diaz MM, Galloway RL, Saito M, et al. Human leptospirosis caused by a new, antigenically unique *Leptospira* associated with a rattus species reservoir in the Peruvian Amazon. *PLoS Negl Trop Dis* 2008; **2**(4): e213.
[9] Bourhy P, Collet L, Brisse S, Picardeau M. *Leptospira mayottensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from humans. *Int J Syst Evol Microbiol* 2014; **64**(Pt 12): 4061-4067.
[10] Slack AT, Kalambaheti T, Symonds ML, Dohnt MF, Galloway RL, Steigerwalt AG, et al. *Leptospira wolffii* sp. nov., isolated from a human with suspected leptospirosis in Thailand. *Int J Syst Evol Microbiol* 2008; **58**(Pt 10): 2305-2308.
[11] Slack AT, Khairani-Bejo S, Symonds ML, Dohnt MF, Galloway RL, Steigerwalt AG, et al. *Leptospira kmetyi* sp. nov., isolated from an environmental source in Malaysia. *Int J Syst Evol Microbiol* 2009; **59**(Pt 4): 705-708.
[12] Saito M, Villanueva SY, Kawamura Y, Iida K, Tomida J, Kanemaru T, et al. *Leptospira idonii* sp. nov., isolated from environmental water. *Int J Syst Evol Microbiol* 2013; **63**(Pt 7): 2457-2462.
[13] Agampodi S, Peacock SJ, Thevanesam V. The potential emergence of leptospirosis in Sri Lanka. *Lancet Infect Dis* 2009; **9**(9): 524-526.
[14] Gamage CD, Amarasekera J, Palihawadana P, Samaraweera S, Mendis D, Janakan N, et al. Analysis of hospital-based sentinel surveillance data on leptospirosis in Sri Lanka, 2005–2008. *Jpn J Infect Dis* 2012; **65**(2): 157-161.
[15] Agampodi SB, Peacock SJ, Thevanesam V, Nugegoda DB, Smythe L, Thaipadungpanit J, et al. Leptospirosis outbreak in Sri Lanka in 2008: lessons for assessing the global burden of disease. *Am J Trop Med Hyg* 2011; **85**(3): 471-478.
[16] Epidemiological Unit. Selected notifiable diseases reported by medical officers of health. *Wkly Epidemiol Rep* 2009; **36**(1): 4.
[17] Epidemiological Unit. Selected notifiable diseases reported by medical officers of health. *Wkly Epidemiol Rep* 2010; **37**(1): 4.
[18] Epidemiological Unit. Selected notifiable diseases reported by medical officers of health. *Wkly Epidemiol Rep* 2011; **38**(1): 4.
[19] Epidemiological Unit. Selected notifiable diseases reported by medical officers of health. *Wkly Epidemiol Rep* 2012; **39**(1): 4.
[20] Epidemiological Unit. Selected notifiable diseases reported by medical officers of health. *Wkly Epidemiol Rep* 2013; **40**(1): 4.
[21] World Health Organization. *Human leptospirosis – guidance for diagnosis, surveillance and control*. 2003 [Online] Available at: http://www.who.int/csr/don/en/WHO_CDS_CSR_EPH_2002.23.pdf.
[22] Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, Chierakul W, et al. Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clin Infect Dis* 2012; **55**(3): 322-331.
[23] Agampodi SB, Dahanayaka NJ, Bandaranayaka AK, Perera M, Priyankara S, Weerawansa P, et al. Regional differences of leptospirosis in Sri Lanka: observations from a flood-associated outbreak in 2011. *PLoS Negl Trop Dis* 2014; **8**(1): e2626.
[24] Pieris K, Uragoda CG. *A bibliography on health in Sri Lanka, 1977–1980*. Colombo: Ceylon College of Physicians; 1983.
[25] Pieris K, Uragoda CG. *A bibliography of medical publications relating to Sri Lanka, 1811–1976*. Colombo: National Science Council of Sri Lanka; 1980.
[26] Rajasuriya K, Somasunderam M, Nagaratnam N. Leptospirosis in Ceylon. *J Trop Med Hyg* 1959; **62**: 205-210.
[27] Nityananda K. Isolation of Leptospira in Ceylon. *Ceylon Med J* 1962; **17**(2): 95-96.
[28] Kokovin IL, Cheruukha YP. The etiological structure of the leptospirosis in man in Ceylon: new serotypes of leptospires. In: *Teheran, Iran: 8th International Congress on Tropical Medicine and Malaria*; 1968, p. 907-908.
[29] Nityananda K. In: *Leptospirosis in Ceylon-epidemiological and laboratory investigation*. Colombo: Medical Research Institute; 1969.
[30] Nityananda K, Sulzer CR. A new leptospiral serotype in the *Javanica* serogroup from Ceylon. *Trop Geogr Med* 1969; **21**(2): 207-209.
[31] Kokovin IL, Chernukha Iu G. Serological classification of leptospirae of the *Grippotyphosa* serogroup–new serological type ratnapura. *Zh Mikrobiol Epidemiol Immunobiol* 1970; **47**(10): 102-105.
[32] Nityananda K, Harvey T. Leptospirosis in Ceylon-epidemiological and laboratory studies. *Ceylon J Med Sci* 1971; **20**(1): 5-14.
[33] Nityananda K, Sulzer CR. A new serotype of *Leptospira* belonging to the autumnalis serogroup. *J Trop Med Hyg* 1971; **74**(8): 184-186.
[34] Nityananda K, Sulzer CR. A new leptospiral serotype in the *Icterohaemorrhagiae* serogroup from Ceylon. *Ceylon J Med Sci* 1972; **21**(1): 9-13.
[35] Ramachandran S, Rajapakse CN, Perera MV. Changing patterns in leptospirosis in Sri Lanka. *Ceylon Med J* 1974; **19**(3): 142-149.
[36] Cinco M, Nityananda K. Classification of 8 new strains of *Leptospira* isolated in Ceylon. *Zentralbl Bakteriol Orig A* 1975; **43**(2): 248-250.
[37] Ramachandran S, Rajapakse CN, Perera MV, Yoganathan M. Patterns of acute renal failure in leptospirosis. *J Trop Med Hyg* 1976; **79**(7): 158-160.
[38] Ahmed N, Devi SM, Valverde Mde L, Vijayachari P, Machang'u RS, Ellis WA, et al. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. *Ann Clin Microbiol Antimicrob* 2006; **5**: 28.
[39] Nalam K, Ahmed A, Devi SM, Francalacci P, Baig M, Sechi LA, et al. Genetic affinities within a large global collection of pathogenic *Leptospira*: implications for strain identification and molecular epidemiology. *PLoS One* 2010; **5**(8): e12637.
[40] Thaipadungpanit J, Wuthiekanun V, Chierakul W, Smythe LD, Petkanchanapong W, Limpaiboon R, et al. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. *PLoS Negl Trop Dis* 2007; **1**(1): e56.
[41] Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Bailey MS, Holden MT, et al. A single multilocus sequence typing (MLST) scheme for seven pathogenic *Leptospira* species. *PLoS Negl Trop Dis* 2013; **7**(1): e1954.
[42] Ahmed A, Thaipadungpanit J, Boonsilp S, Wuthiekanun V, Nalam K, Spratt BG, et al. Comparison of two multilocus sequence based genotyping schemes for *Leptospira* species. *PLoS Negl Trop Dis* 2011; **5**(11): e1374.
[43] Koizumi N, Gamage CD, Muto M, Kularatne SA, Budagoda BD, Rajapakse RP, et al. Serological and genetic analysis of leptospirosis in patients with acute febrile illness in Kandy, Sri Lanka. *Jpn J Infect Dis* 2009; **62**(6): 474-475.
[44] Gamage CD, Koizumi N, Muto M, Nwafor-Okoli C, Kurukurusuriya S, Rajapakse JR, et al. Prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents in Kandy, Sri Lanka. *Vector Borne Zoonotic Dis* 2011; **11**(8): 1041-1047.
[45] Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: association of level of leptospiremia and clinical manifestations in Sri Lanka. *Clin Infect Dis* 2012; **54**(9): 1249-1255.
[46] Gamage CD, Koizumi N, Perera AK, Muto M, Nwafor-Okoli C, Ranasinghe S, et al. Carrier status of leptospirosis among cattle in Sri Lanka: a zoonotic threat to public health. *Transbound Emerg Dis* 2014; **61**(1): 91-96.
[47] Bandara KK, Weerasekera M, Gunasekara CP, Ranasinghe N, Marasinghe C, Fernando N. Molecular characterisation and disease severity of leptospirosis in Sri Lanka. *Mem Inst Oswaldo Cruz* 2015; **110**(4): 485-491.
[48] Agampodi SB, Moreno AC, Vinetz JM, Matthias MA. Utility and limitations of direct multi-locus sequence typing on qPCR-positive blood to determine infecting Leptospira strain. *Am J Trop Med Hyg* 2013; **88**(1): 184-185.