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LEPTOSPIROSIS CASE FINDING FOR DEVELOPMENT OF LEPTOSPIROSIS SURVEILLANCE IN SEMARANG CITY, CENTRAL JAVA, INDONESIA

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PENEMUAN KASUS LEPTOSPIROSIS DALAM PENGEMBANGAN SURVEILANS LEPTOSPIROSIS DI KOTA SEMARANG, JAWA TENGAH, INDONESIA

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Abstract

Leptospirosis is endemic in some parts of Indonesia territory. This work aimed to find the leptospirosis cases in Semarang city, one of the endemic leptospirosis in Indonesia by both active and passive case findings. The leptospirosis was screened in the community base active case finding. In addition, the cases were passively found in primary health care center and hospitals using a WHO-SEARO criteria and laboratory confirmation test. There were 191 cases detected with WHO-SEARO criteria, and among those cases only 31 cases (3 from active finding and 28 from passive finding) confirmed by laboratory test, either by using Rapid Detection Test (RDT), Microscopic Agglutinations Test (MAT) or Polymerase chained reaction (PCR). Fever, headache and myalgia were the most common symptoms experienced by >90% patients. Based on an active case finding that no more than 21,4% (3/14) probable leptospirosis was detected in this study, meanwhile there was passively 15,8% (28/177) cases confirmed. It was concluded that leptospirosis case finding is still challenging in the endemic area, Semarang city. The application of WHO-SEARO criteria as diagnostic tool may be need to be further evaluated.

Keywords: *Leptospirosis, surveillance, case finding, passive, active*

Abstrak

Leptospirosis endemis di beberapa daerah di Indonesia. Penelitian ini bertujuan memperoleh kasus leptospirosis secara aktif dan pasif di Kota Semarang, Jawa Tengah yang merupakan salah satu daerah endemis leptospirosis di Indonesia. Kasus leptospirosis secara aktif banyak ditemukan di masyarakat sedangkan secara pasif banyak dilaporkan di Pusat Kesehatan Masyarakat dan Rumah Sakit dengan menggunakan Kriteria WHO-SEARO 2009 dan dikonfirmasi di laboratorium menggunakan RDT (Rapid Detection Test), MAT (Microscopic Agglutinations Test) dan PCR (Polymerase Chained Reaction). Penentuan kasus leptospirosis secara aktif dan pasif dilakukan oleh dokter dan tenaga medis Puskesmas dan Rumah Sakit (enumerator). Hasil penelitian menunjukkan bahwa 191 tersangka kasus leptospirosis tertapis dengan kriteri WHO-SEARO dan 31 kasus leptospirosis terkonfirmasi secara laboratorium (RDT, MAT dan PCR). Kasus leptospirosis tidak berhubungan dengan musim hujan. Demam, sakit kepala, dan myalgia merupakan gejala yang paling sering ditemukan

yaitu lebih dari 90% pasien. Hasil penemuan kasus leptospirosis secara aktif dan telah dikonfirmasi secara laboratorium adalah sekitar 21,4% (3/14 pasien), sedangkan penemuan kasus leptospirosis secara pasif yang telah terkonfirmasi secara laboratorium adalah 15,8% (28/177 pasien). Penemuan kasus leptospirosis secara aktif dan pasif di daerah endemis seperti Kota Semarang direkomendasikan untuk menurunkan angka kematian karena leptospirosis. Penerapan kriteria WHO-SEARO sebagai alat diagnostik mungkin perlu dievaluasi lebih lanjut.

Kata Kunci : *Leptospirosis, surveilans, penemuan kasus, daerah endemis, deteksi*

INTRODUCTION

Leptospirosis is a zoonosis acute generalized infectious disease characterized by extensive vasculitis, caused by spiral bacteria, *Leptospira sp.* In addition, the disease is primarily a disease of wild and domestic animals, and may be transmitted to humans through either direct or indirect contact with infected animal urine (WHO, 2003; Keenan *et al.*, 2009). The annual incidence of leptospirosis is estimated from 0.1–1 per 100,000 people in temperate climates to 10–100 per 100,000 people in the humid tropics. The incidence may be increased more than 100 per 100,000 people during outbreaks and in high-exposure risk groups. According to the World Health Organization (WHO), Leptospirosis is one of an emerging diseases of public health concern in South East Asian countries (WHO, 2009).

Leptospirosis is endemic in part of Indonesia territory (Gasem *et al.*, 2009). The incidence of leptospirosis in Semarang city, one of the endemic area in Indonesia in 2000 was 1.2 per 100,000 populations (WHO, 2009). However, there was a trend of increasing incidence of leptospirosis since 2002. Data from hospitals in Semarang showed that the morbidity was 4.14% annually with 16.92 % of mortality (Setyorini & Dangiran, 2017).

Diagnosis of leptospirosis remains controversial, which lead to under-diagnosis worldwide. Leptospirosis has clinical symptoms similar as other disease and many people showed mild clinical symptoms so it is difficult to diagnose and need confirmed laboratory test. However, WHO-SEARO (World Health Organization South-East Asia Region) criteria have been reported effective for leptospirosis case finding (Kumar, 2013). The criteria was introduced to overcome the limitation of confirmatory laboratory test for leptospirosis such as: microscopic agglutinations test (MAT), rapid diagnosis tests (RDT) and direct examination of molecular biology (WHO, 2003; Singh & Vijayachari, 2012; Chaudhry *et al.*, 2013). The result study of Andani (2014) in Kariyadi Hospital showed that WHO-SEARO criteria was facilitated leptospirosis diagnoses like as

clinical picture, exposure risk, and laboratory test on acute fever cases.

WHO (1999) recommend both active and passive leptospirosis case finding as part of the multi-diseases approach to surveillance. The aim of the study was found leptospirosis cases which active and passive performed in endemic area. Because leptospirosis is a largely neglected disease, it needs highly awareness of leptospirosis risk.

MATERIAL AND METHODS

Ethics Committee Approval

The Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Gadjah Mada University Dr. Sardjito General Hospital states that the leptospirosis surveillance protocol meets the ethical principle outlined in the Declaration of Helsinki 2008 and therefore can be carried out. The number of Ethics Committee Approval is KE/FK/108/EC

Active case finding:

Active case finding was performed in Semarang city. Active and passive leptospirosis case findings were performed on May 2014 to October 2015. Enumerators actively screened subjects in the community. Patient has fever in the last 2 days will be further screened by using WHO-SEARO criteria (Kumar, 2013). Subject matched with suspected leptospirosis will be asked to donate 10 ml blood for subsequent confirmatory laboratory tests for *Leptospira* infection. In this WHO-SEARO categories, the case definition has three categories: suspect (which consists of only clinical features), probable (which consists of clinical features + Rapid diagnostic tests) dan confirmed (which consists of clinical features + positive MAT/ PCR/Culture) (Kumar, 2013)

Passive case finding:

Passive case finding was performed in hospitals and primary health centers in Semarang city. Enumerator will screen patients fulfilled suspected leptospirosis criteria, and additional blood sample will be obtained for

subsequent confirmatory laboratory tests for leptospira infection. All procedures were received ethical approval from Medical and Health Research Ethics Committee Faculty of Medicine Universitas Gadjah Mada / Dr. Sardjito General Hospital.

DNA Extraction

Leptospira DNA was extracted from whole blood of patients by using standard method as described elsewhere. Leptospira DNA were extracted, purified and eluted using the invitrogen DNA extraction kit according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

DNA was subjected for PCR amplification by using Go Taq Green Master Mix PCR amplification was performed according to the manufacturer suggested protocol. Primers correspond to gen 16sRNA were employed. The primers sequences are as follow: Forward 5' GCAAGCATTACCGCTTGTGG 3' and reverse 5' TGTTGGGGAAATCATACGAAC 3'. The PCR produces 262 bp amplicon (Branger *et al.*, 2005).

Rapid Diagnostic Test (RDT)

Leptotek lateral flow (BioMerieux bv, Boxtel, NL) was employed to confirm the leptospirosis cases. Leptotek lateral flow 10 µl whole blood was spotted in the sample port of the device, running buffer was added and the test was read after 15 minutes. Leptotek lateral flow were

valid when the control band stained. Valid tests were scored positive when a test band was observed, negative when no band was observed and indeterminate when it was unclear whether a band was observed or not. Invalid tests were repeated.

Microscopic Agglutination Test (MAT)

MAT was performed to confirm the presence of antibody specific (IgM and IgG) against Leptospira in the whole blood of suspected leptospirosis patients. MAT was performed according to the suggested protocol of WHO (2007). The Leptospira panel which was used for MAT procedure was generously donated by Regional Medical Research Centre, Indian Council of Medical Research, India. Data was analyzed by univariate. Univariate Analysis saw the frequency distribution by active and passive leptospirosis cases based on WHO-SEARO criteria.

RESULTS

Fever, headache, and myalgia symptoms are the main symptoms that found in leptospirosis cases. Table 1 describes the clinical symptoms which were recorded in the index cases. Meningitis was the less frequent symptom found in the probable leptospirosis cases. The suspected leptospirosis cases were significantly higher in males than females, and more than leptospirosis cases are productive people (20-50 years old) and older people (>50 years old). (Table 2).

Table 1. Active and passive finding case of clinical symptoms of probable leptospirosis patients, 2014-2015

No	Clinical Symptoms	Total	Percentage
1.	Fever	191	100,0
2.	Headache	183	95,8
3.	Myalgia	176	92,4
4.	Calf pain	115	60,5
5.	Jaundice	55	28,6
6.	Conjunctival suffusion	48	25,2
7.	Meningitis	31	16,0

Table 2. Characteristics of patients leptospirosis based on the WHO-SEARO criteria, 2014-2015

No	Characteristics	Active case finding	Passive case finding
1	Sex		
	Male	11	111
	Female	3	66
2	Age		
	0-10	0	5
	11-20	4	17
	21-30	5	49
	31-40	3	67
	41-50	1	25
	>50	1	14

Only 31 out of 191 suspected leptospirosis cases were confirmed by using laboratory examination. This data showed that only 15,8 % (28/177) of the probable leptospirosis cases screened by passive case finding were confirmed as definite leptospirosis cases, and 21,4 % (3/14) in active case finding series (Table 3).

This data showed that finding the leptospirosis cases in the field by using modified WHO-SEARO criteria is tending to be over diagnosed compare to the gold standard. Our data which obtained from both primary health center (in the community) and tertiary referral hospital showed the same limitation of WHO-SEARO

Table 3. Laboratory confirmation of leptospirosis cases 2014-1015

Case Finding Methods	WHO Criteria Cases	Laboratory Confirmed Cases	Laboratory Confirmatory Methods		
			RDT*	MAT*	PCR*
Active	14	3	0	1	2
Passive	177	28	11	20	12
Total	191	31	11	21	14

*: RDT: *Rapid diagnostic test*; MAT: *Microscopic agglutination test*; PCR: *Polymerase Chain Reaction*.

DISCUSSION

The finding of leptospirosis was peaked on July 2014 is interesting, since leptospirosis traditionally reported high prevalence in rainy season. However this finding may highlight the important of risk factors, such as knowledge, perception, and behavior of individuals in the community and other environmental determinant than excessive water and flood that may occur in rainy season (Kamath *et al.*, 2014; Sumanta *et al.*, 2015). The result of study in Brazilia showed that there is the correlation between rainy season with leptospirosis cases and increased 0,55% of leptospirosis cases (Kupek *et al.*, 2000).

The results indicated that suspected leptospirosis cases was mostly found in the productive age population (20-50 years). The result was in agreement with the previous report in Brazil showing that leptospirosis was mostly occurred in productive age between 15-50 years old (Barcellos & Sabroza, 2001). This trend was also reported in Hawaii and India that most of the cases was found in productive people (Katsz *et al.*, 2011; Kamath *et al.*, 2014). It is a great challenges for the government to protect the productive ages to become ill because of the leptospira infection, otherwise will contribute to the social and economical burdens for the community.

The laboratory confirmed cases were found only 15,8% in passive case finding and 21,4% in active case finding. We used laboratorium examination like RDT (Rapid Detection Test), MAT (Microscopic Agglutinations Test) and PCR (Polymerase Chained Reaction) for confirmation of leptospirosis cases. Some of researches showed that the sensitivity of RDT was 65-93%, sensitivity of PCR was 52-63%, specificities of RDT was 83-98%, and specificities of PCR was 79-100% than gold standar (MAT) (Bhatia *et al.*, 2015); (Mullan & Panwala, 2016).

criteria compare to the gold standard. Similar result was also reported in India (Bhatia *et al.*, 2015). The three symptoms which were recorded, i.e: fever, headache, and maylgia in this series of patients are common symptoms of many infectious diseases. Furthermore, there is difficult to collecting leptospirosis data because scattered distribution of leptospirosis cases and limited location of laboratories for gold standard examination.

Active cases were found in Semarang and was identified 14 of 191 cases (7%) based on the WHO-SEARO criteria, and 3 of 14 cases (21.4%) for laboratory confirmation of leptospirosis infection. Considering very low additional cases could be identified using active case finding strategy, it seems that active cases finding is not recommended in the area where leptospirosis is endemic, such as Semarang city. However, active leptospirosis case finding may be useful to be applied when it is performed in a multi-diseases approach to make it more efficient and cost effective.

CONCLUTIONS AND RECOMMENDATION

Conclutions

It was conclude that leptospirosis case finding is still challenging in the endemic area, such as Semarang city. The active and passive finding of leptospirosis cases in the field using WHO-SEARO criteria tends to be over diagnosed compared to the gold standard.

Recommendation

Active and passive leptospirosis case finding using WHO-SEARO criteria can be applied at primary health center. It is necessary to examine leptospirosis surveillance indicators or variables for early warning systems.

AUTHOR CONTRIBUTIONS

R, main contributor, roles : conceptualization, data curation, methodology, writing - original draft, writing - review & editing. FDH, supporting contributor, roles: data analysis. AM, roles : laboratory examination, writing -editing. TW, SB, S, roles : editing draft article.

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