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The Presence of Pathogenic *Leptospira sp.* in Water Bodies in Klaten District

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ABSTRACT

Leptospirosis is a health problem in Indonesia. In the year 2016 cases of leptospirosis in Klaten reported death rate 15.38%. The presence of *Leptospira* bacteria in the body of water plays an important role in the transmission of leptospirosis. This study aims to determine the presence of *Leptospira* bacteria in water bodies in Klaten Regency. This research is descriptive research with survey method using cross sectional design. The sample was 100 water samples from wells, rivers and paddy fields in endemic and non endemic areas of Leptospirosis. Polymerase Chain Reaction examination there were 6 positive water samples of rpoB leptospira gene on well water (6.7%), irrigation channel (6.7%) and paddy field (5%). *Leptospira* is found at temperatures ranging from 24-31°C, pH 6.8-7.5 and dissolved oxygen 4-12ppm.

Keywords: *Leptospira sp., water bodies, irrigation channels, bacteria*

INTRODUCTION

Leptospirosis is one of the most widespread bacterial zoonoses in the world.[1] Leptospirosis, caused by pathogenic spirochetes belonging to the genus Leptospira, is a zoonosis that has important impacts on human and animal health worldwide.[2] Transmission to mammals occurs via direct contact with leptospira-infected urine or tissues or indirectly through contact with contaminated soil or water. Although infection may take place through unbroken skin after prolonged immersion, *Leptospira* sp. usually gain entry to the host via abrasions or cuts in the skin or through exposed mucosae (eyes, nose, *etc.*).[3] Soil,[4,5] mud,[6] and surface waters contaminated with urine from chronically-infected reservoir hosts remain important sources of human leptospirosis transmission worldwide.[7-9]

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Based on data from the Central Java Provincial Health Office, cases of leptospirosis in Central Java have increased cases from 2012 to 2014. In 2015 cases in Central Java declined from the previous year, but the Case Fatality Rate (CFR) stagnated.[10] Klaten District is one of the endemic areas of leptospirosis. Leptospirosis in Klaten District occurs every year for the last five years. In 2016 reported 39 patients with 6 deaths (CFR = 15.38%). The case spread sporadically, not clustered in one place. Leptospirosis in Klaten regency was dominated by patients with occupation as farmer and laborer (79.49%) and had wound history (87.18%).[11]

The high prevalence rate of Leptospirosis in tropical and subtropical climates can be attributed to adverse environmental conditions that allow the environment to be a suitable place to live and develop of *Leptospira* bacteria.[6,12] Outbreaks of leptospirosis have been associated with common water events such as rural and urban flooding, swimming, and other water sports as well as occupational exposure involved predominantly with farming and drinking contaminated water.[13,14] The presence of *Leptospira* sp. bacteria in the body of water plays an important role in the transmission of

leptospirosis. The purpose of this study was to determine the presence of *Leptospira* sp. bacteria in water bodies. Tested using PCR (Polymerase Chain Reaction) method to determine the presence of *Leptopsira* sp. bacteria in water bodies with high sensitivity reaches 90-100%.

METHOD

The design of this study is cross sectional conducted by survey method. Sample in this research is water well, irrigation channels and paddy field in case area and free of leptospirosis in Klaten District. The water sample is determined based on the proportion estimation formula from Lemeshow to obtain 100 water samples taken by puposive. Water samples taken from wells are commonly used by respodents, irrigation channels used by respondents to cleanse themselves and paddy fields where respondents work. Each water sample was taken with a volume of 150ml placed in a dark glass bottle. Water samples are stored at 4ºC and processed within 24 hours.[15]

Water samples taken measured temperature, pH and dissolved oxygen. Temperature measurements are carried out with a water thermometer that is by dipping the thermometer into water and allowing for 2-5 minutes until the thermometer shows a stable value. Then record the thermometer scale reading without lifting the thermometer in water.[16] The pH measurement is done by dipping the electrode into a water sample until the pH meter indicates a fixed reading. Then record the reading of the scale or number on the view of pH meter.[17] Dissolved Oxygen measurements using Dissolved Oxygen meter in accordance with WalkLAB Digital Dissolved Oxygen meter.[18]

Each water sample was filtered with one nitrocellulose membrane (Milipore) whose pore size was 0.22µm. Next the membrane is cut into small pieces and inserted into a microsentrifuge tube. The next stage is the DNA isolation of the niroselulose membrane used to filter the water sample. The DNA isolation was performed using the Genomic DNA Mini Kit isolation kit (Geneaid). The isolation stages are performed according to the procedures listed in the kit manual.

The Polymerase Chain Reaction (PCR) process performed on DNA samples was obtained using primers: rpoB-F-CCTCATGGGTTCACAATATCA and RpoB-R-CGCATCCTCRAAGTTGTAWCCTT using Go Taq Green Master Mix (Promega).[19] PCR Stages were as follows: predenaturation 94°C for 2 minute, followed by 40 amplification cycles consisting of denaturation at 94°C for 30s, annealing at 55°C for 1 min, extension at 72°C for 1 min followed by a final extension for 20 min at 72°C . Analysis of PCR results was performed with electrophoresis using agarose 1.5% at 100 volts for 15 min. Specific DNA visualizations were performed using UV transilluminators. Positive samples when electrophoresis results showed that the DNA bands were in the 600 bp position.

RESULTS AND DISCUSSIONS

One hundred water samples consist of well water, irrigation channels and paddy fields coming from sub-districts where leptospirosis cases occur every year, sub-districts almost every year occurs leptospirosis cases and sub-district without leptospirosis in Klaten District. Six positive water samples of rpoB gene were obtained from two wells, two rivers and two rice fields. This indicates that the water bodies are contaminated by the *Leptospira* sp. from infected animals. Prevalence of *Leptospira* sp. in water samples are tabulated in Table 1.

Table 1: Results of water sampling and Leptospira sp detection based on Leptospira rpoB gene detection. (n=100)

Distribution area	Water sources	Total sample	Positive sample	Percentage of positive sample (%)
Sub-district with cases occurs every years	wells	11	0	0.0
	irrigation channels	11	2	18.18
	paddy fields	15	1	6.67
Sub-district with cases occurs almost every years	wells	11	2	18.18
	irrigation channels	11	0	0.0
	paddy fields	15	1	6.67
Sub-district without cases	wells	8	0	0.0
	irrigation channels	8	0	0.0
	paddy fields	10	0	0.0

Our study revealed that *Leptospira* bacteria is found in sub-district with cases occurs every years and sub-district with cases occurs almost every years. In sub-districts with cases ocuurs every year, *Leptospira* bacteria found in irrigation channels (18.18%) and paddy fields (6.67%). While in sub-districts with cases occurs almost every year, *Leptospira* sp. are

found in wells (18.18%) and paddy fields (6.67%). The bacteria were found in irrigation channels, one of the positive irrigation channels found rats holes, water in the irrigation channels was relatively stagnant. The presence of rats around the river can contaminate the water in the irrigation channels. Leptopsires survive weeks or months in moist and warm soil, stagnant water at neutral or slightly pH.[20,21] And based on observation on two positive wells of *Leptopsira*, the floor around the well is made from soil, not segmented with cement. Wells are easily contaminated through floors which is not made with water resistant material.[22] Leptospira bacteria contamination in the paddy fields is caused, a paddy field is a place for the availability of food for rats. And the soil in the paddy fields is wet. It is suitable for *Leptospira* sp. to survive for long period of time. Besides that, there were water puddles in the rice field allows for *Leptospira* sp. spread the contaminated urine in the soil. Previous study revealed that *Leptospira* serovar Harjo has higher survival rate in moist soil at pH 6.9-7.4.[23]

Table.2: Results of water sampling and *Leptospira sp.* detection based on *Leptospira* rpoB gene detection (n = 100)

Distribution area		Sampling code	° C	pH	DO
Sub-district with cases occurs every years	Cawas irrigation channels 1	CB2	30	6,9	12
	Cawas irrigation channels 2	CB3	24	6,8	6,9
	Trucuk paddy fields	TC4	28	7,4	9,2
Sub-district with cases occurs almost every years	Kebonarum paddy fields	KBC3	31	6,8	8,7
	Klaten Selatan 1 well	KSA1	28	7,5	4
	Klaten Selatan 2 well	KSA2	28	7,2	5,7

Temperature was important factor for the survival of Letopsires in the nature.[21] Leptospires are obligate aerobes with an optimum growth temperature of 28 to 30°C.[24] In this study *Leptospira* sp. was found at a temperature of 24-31°C, pH 6.8-7.4 and DO 4-12ppm. These results show the ability of *Leptospira* sp. to survive in a variety of environmental conditions. Our results are consistent with the theory that the ability to survive *Leptospira* sp. in the environment is affected by variations in soil and water conditions in contaminated areas.[25] Well water and paddy fields in the leptospirosis case areas have more optimal temperatures compared to leptopsirosis-free areas. Temperature is influenced by external factors such as weather, wind and currents. In addition, water temperature variations are caused by natural processes such as biochemistry, through organisms that produce heat (endothermic and exothermic reactions) and microbiological processes (geothermal sources).[26] Optimal temperature makes *Leptospira* sp. survive last longer. The previous study found that The leptospires can survive for 10 months in adverse condition (4°C) and up to 20 months when stored at 30°C.[27] Length of life of Leptospira bacteria in water can be affected by water pH. Optimum growth of Leptospira bacteria occurred in the range of pH 7.2-7.6. [28] The result of examination found the existence of Leptospira bacteria at relatively acidic pH that is pH 6.8-6.9. This research is in line with research conducted by Benacer in Malaysia, that Leptospira bacteria found in water with acidic pH that is 5.77-6.63.[18] State of environmental conditions that are not suitable for breeding bacteria Leptospira can cause bacteria are inhibited growth even become die.[4] Based on research found Leptospira bacteria in DO 4-12ppm. The oxygen concentration is influenced by many factors including water temperature, photosynthesis rate, turbidity and water depth, degree of water turbulence or wave action, and the amount of oxygen used by respiration and decay of organic matter. The oxygen concentration is the limiting factor of growth.

CONCLUSIONS

Leptospira sp. can live at temperatures and pH beyond the optimal limit for its growth. From 100 water samples examined, six samples found positive rpoB gene from well water samples (6.7%), irrigation channels (6.7%) and paddy fields (5%).

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Conflict of Interest: The author reports no conflicts of interest in this work.

Ethical Clearance: Ethical clearance was obtained from Ethic Commission of Health Research, Faculty of Public Health UNDIP (123/EC/FKM/2017). All subjects signed informed consent to join the study.

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