Leptospirosis Transmission in Ponorogo District of East Java, Indonesia

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Abstract

This study aims to identify *Leptospira* bacterial infection in livestock owners and their livestock, as well as to examine potential risk factors correlated with the incidence. 50 participants were selected and their blood samples were collected. 50 urine samples were collected from livestock owned by the participants. Polymerase Chain Reaction (PCR) was used to identify the existence of the *Leptospira* bacteria. The questionnaire instrument was used to obtain information about individual characteristics and hygiene. The Chi-Squared test was adopted to examine the correlation between outcome and explanatory variables. The confirmation PCR test detected the bacterial DNA in 2 out of 50 blood samples examined (4%) and 3 out of 50 urine samples examined (6%). Human leptospirosis incidence is significantly correlated with occupation type (p=0.035), personal protective equipment (PPE) use (p=0.044), water puddle contact (p=0.044), cage sanitation (p=0.044) and *Leptospira* bacteria presence in livestock urine (p=0.007). Insignificant correlation was showed in owners' age variable. The presence of *Leptospira* bacteria both in livestock and the owners indicates the real threat of animal to human transmission. Further study with larger sample size and wider range variables and meticulous examination technique is required to comprehend the investigation.

Keywords: *Leptospirosis, livestock, sanitation, water contact, PPE.*

Introduction

Leptospirosis is an emerging disease with agent of *Spirochete* bacteria from *Leptospira* genus. This infectious disease is prevalent in tropical and sub-tropical region as the climate condition provides suitable environment for the proliferation of bacteria. *Leptospira* bacteria infect both animals and humans led to 58,900 mortalities and resulted on health burden for 2,9 million population annually[1,2]. WHO defines this zoonotic disease is transmitted from animal to humans[3] that causes symptoms of fever and jaundice for all patients, furthermore in a few patients it might lead to severe manifestation of serious icteric symptoms such as meningitis, respiratory distress, pulmonary hemorrhage and Weil's diseases[4].

Generally, all mammals can harbor *Leptospira* bacteria in their kidneys and become a source of infection to human and animals. Human acquires *Leptospira* infection by direct contact with the transmission source through the injured skin, mucous membranes of the eyes, and nose as port of entry. While, direct transmission in animals is through urine or close interaction with other infected animal[4,5]. *Leptospira* bacteria will survive for a long period in the body of an infected animal. They will contaminate the environment through water supplies, food, pastures, and soil when excreted through animal urine[1,6,7].

Even though leptospirosis transmission is dominated by rodents, it also comes from other sources such as

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livestock and pets such as pigs, cows, horses, dogs[8], and buffalo[9]. Previous study reported that *Leptospira* bacteria was found in 12 out of 188 (6.38%) samples screened among cattle[10]. Another research showed that 1 out of 20 (5%) animals in farming area are found to have positive infection of *ardjobovis Leptospira* and or *serovar pomona*[11]. While, the study about incidence of animal leptospirosis case among cattle in Girimulyo Sub-district of Kulon Progo reported was 3.3%[12].

There are many public health issues impacted by the existence of leptospirosis vector, rodents and infected animals. Farmers and workers who have close interaction with livestock are the most vulnerable to be infected by this infectious disease[1,11]. Current ongoing prevention intervention proposed by public health professionals focus on raising knowledge and awareness regarding the various risk factors including of contact with infected animals[13]. Even though most of cases can be diagnosed using real-time PCR before antimicrobial therapy is initiated, the intervention and control program are still facing some challenges due to nonspecific presentation of this disease, high complexity of laboratory confirmation, and multiple environmental factors involved[14].

During 2009–2013, there were 2,466 new notified cases of leptospirosis in Indonesia with case fatality rate (CFR) was 9.6%. Endemic areas such as East Java Province demonstrated an increasing pattern, from 210 cases with CFR 7.3 % in 2009 to 305 new cases with CFR 9.3% in 2013[15]. Ponorogo is one of districts in East Java Province that recorded 92 new cases between 2012 to 2015, which is 80% of those cases occurred in flood-free highlands. The local health authority stated that the cases were frequently found among farmers, breeders, and sand miners. The area with the highest incidence of leptospirosis in Ponororgo was Ngrayun sub-district where 90% of population own livestock behind their houses[16]. This study aimed to identify *Leptospira* bacterial infection in livestock and their owners and explore the risk factors from individual characteristics, personal hygiene and sanitation.

Methods

Study design

This research is an observational study with cross-sectional design. 50 participants were chosen by purposive random sampling based on admission record at outpatient department of Grayun Primary Health Center for the period between March 2017 and November 2018. The inclusion criteria for respondents were those having fever (>38 ^{o}C) accompanied by muscle pain, headache, conjunctivitis, and rash[3,17] and had livestock behind their houses.

Materials and Instruments

The tools used were isolation kit, absolute ethanol, PCR kit, primer, PCR tube, EDTA tube, gloves, micropipette of various sizes and tips, sterile 1.5 ml micro-tubes, centrifuges, water baths, and thermal cycler devices. Insulation was performed according to the procedure recommended in the Kit manual. PCR examination was conducted by the Technical Centre for Environmental Health and Disease Control, Indonesian MoH. PCR test was carried out using the Dream Taq Green Master mix. PCR products were electrophoretic in 1.5% agarose gel and 100 bp ladder were used as markers to analyze large PCR products[18]. The close ended questionnaire was prepared for obtaining the information about participants' characteristics, personal hygiene and sanitation.

Variables, Data Collection and Analysis

The outcome variable of interest was the presence of *Leptospira* bacterial infection in human blood sample. While the explanatory variables were including of *Leptospira* bacterial infection in livestock urine sample, demographic information and personal hygiene and sanitation. Blood serum samples were taken from selected participants and urine samples were taken from their livestock. Five millimeters of venous blood was taken from each participant by laboratory staff after obtaining informed consent. Ten milliliters of cattle or sheep urine was taken by the owners. demographic information, personal hygiene and sanitation were collected using an interviewer-administered questionnaire. The data were analyzed using the Chi-Square statistical test with SPSS statistical software with assessment of significance refers to p value <0.05.

Results and Discussion

Demographic Characteristics, Personal Hygiene and Sanitation

Leptospirosis cases confirmed as positive were found in 2 participants from different age groups of 45-55 years old group and above 55 years old group. Both of positive cases worked as farmer. Occupation, PPE use and water paddle contact variables were reported to have significant correlation with leptospirosis incidence with p value are ($p < 0.05$), ($p < 0.05$) and ($p < 0.05$) consecutively. While, insignificant correlation was found between age group and leptospirosis incidence ($p > 0.05$) (Table 1).

Table 1. Demographic and Characteristic Respondent with Leptospirosis Incident

Characteristics	Leptospirosis Incident		Total	p
	Positive	Negative		
Age (years)				
35-45	0 (0%)	2 (4%)	2 (4%)	0.950
45-55	1 (2%)	21 (42%)	22 (44%)	
>55	1 (2%)	25 (50%)	26 (52%)	
Total	2 (4%)	48 (96%)	50 (100%)	
Occupation				
Farmer	2 (4%)	14 (28%)	16 (32%)	0.035*
Others	0 (0%)	34 (68%)	34 (68%)	
Total	2 (4%)	48 (96%)	50 (100%)	
Personal Protective Equipment (PPE)				
Yes	0 (0%)	33 (66%)	33 (66%)	0.044*
No	2 (4%)	15 (30%)	17 (34%)	
Total	2 (4%)	48 (96%)	50 (100%)	
Water Puddle Contact				
Yes	2 (4%)	15(30%)	17 (34%)	0.044*
No	0 (0%)	33 (66%)	33 (66%)	
Total	2 (4%)	48 (96%)	50 (100%)	

*Significance level at < 0.05

Identification of *Leptospira* Bacteria in Livestock

Leptospiral DNA was detected in 3 out of 50 (6%) livestock and the environmental observation conducted by the researcher team found the 3 livestock cages have poor sanitation quality. 28 livestock cage out of 50 total cages observed (56%) were reported to have maintained good sanitation. There was a significant correlation between the presence of *Leptospira* bacteria in livestock with cages sanitation quality ($p < 0.05$) (Table 2).

Table 2. Identification of *Leptospira* Bacteria in Livestock

Sanitation of Cage	Leptospira in Livestock		Total	p
	Positive	Negative		
Good	0 (0%)	28 (56%)	28 (56%)	0.04*
Poor	3 (6%)	19 (38%)	22 (44%)	
Total	3 (6%)	47 (94%)	50 (100%)	

*Significance level at <0.05

Correlation of Human Leptospirosis and the Presence of *Leptospira* bacteria in Livestock

There was a significant correlation between human leptospirosis incidents with the presence of *Leptospira* bacteria in their livestock (p=0.005). Out of the two participants who had been diagnosed with Leptospirosis positive, one of them had their livestock been contaminated by *Leptospira* bacteria (Table 3).

Table 3. Correlation Leptospirosis with Existence of Leptospira in Livestock

Leptospira in Livestock	Leptospirosis Cases		Total	p
	Positive	Negative		
Positive	1 (2%)	2 (4%)	3 (6%)	0.007*
Negative	1 (2%)	46 (92%)	47 (94%)	
Total	2 (4%)	48 (96%)	50 (100%)	

*Significance level at <0.05

This study reveals that *Leptospiral* DNA presences both in human blood serum and animal urine samples around husbandry area in Ngrayun sub-district, Ponorogo, even though it was in small prevalence. Infection to human might occurs indirectly with livestock as maintenance host exacerbated by influencing factors such as climate, population density and contact intensity with the animals. Moreover, domestic and dairy cattle were recorded able to harbour number of serovars.

The age group of participants was not significantly correlated with the leptospirosis incident. It was showed that the participants who contracted leptospirosis were in the age group of 45-55 and >55 years old, which is in the productive age. This result is linear with a study in farming community in Brazil showed that the mean age was not significantly different between seropositive and negative subjects[19,20,21]. Nevertheless, age factor remains a risk factor for leptospirosis because farming is generally only done during the productive age. Previous study reported that patients who had positive leptospirosis in older age are at a higher risk for severe course and unfavorable outcomes. The group of men aged 50-59 years have a death risk 3.7 times in death greater than other age groups[22]. This finding is supported by study in Japan and Korea that found Leptospirosis cases among age group above 40 as the highest incidence Briefly, age and gender can be a specific risk factors in the incidence of fatality in patients with leptospirosis.

The PCR test showed that two farmers were positive with leptospirosis and there is significant correlation between occupation and leptospirosis incident ($p < 0.05$). This result magnifies that occupation and human activity as risk factors for the incidence of Leptospirosis case. The farmers most probably were exposed to the bacteria while working on farms. Furthermore, they have been continuously contacted with water and soil that has the potential to carry bacteria into their skin. The risk becomes even greater if the farmer has an open wound on the skin. The results of another study indicated that some occupations have a greater risk of being infected with *Leptospira* pathogen bacteria as their jobs require them to always be in contact with many animals such as scientists, laboratory staff, milking workers, veterinarians, and abattoir workers[9,23]. Agricultural workers are classified as at risk for Leptospirosis as well as other workers such as paddy field workers, fruit farmers and harvesters[24]. Until recently, Leptospirosis is prevalent among poor farmers[21] and agricultural workers or laborers[25]. Therefore, they must be given the understanding and awareness about the hazards of this infectious disease[26]. Aside from public education and health promotion, well-developed infrastructure of water treatment seems to be able to reduce the risk within rural area and agricultural setting like what have been done in Japan that make their Leptospirosis incidence was significantly lower than Korea.

In this study, PPE usage and water contact variables were significantly correlated with leptospirosis incident. As observed during this study, farmers who were found positive with leptospirosis mentioned that they did not use personal protective equipment (PPE) especially boots and gloves due to uncomforted and disturbed while working. This finding is similar to the study on fruit collectors in Malaysia, it was shown that the practice of using PPE that did not meet the standards led to the discovery of *Leptospira* bacteria in their bodies[27]. The researcher team made observation and noticed that participants who stated that every time they were in contact with water and feet buried in mud showed never used boots.

Conclusion

The leptospirosis infection presences in both humans and animals in Ngrayun sub-district is correlated with poor sanitation and inconsistent use of PPE. Public education regarding the hazard of the bacterial infection within domestic and husbandry setting is pivotal, therefore the local health authority needs to increase public awareness about personal hygiene and sanitation among the rural communities.

Conflict of Interest: The author declare that they have no conflict of interest.

Source of Funding : This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgements: Universitas Airlangga funded this study through the Annual Budget Activity Plan 2018. We would like to thank the participants who have voluntarily participated, the Ponorogo Government Regency that support this study and the enumerators who have collected data. We additionally thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study approved by the Ethics Committee from Faculty of Public Health, Universitas Airlangga (121-KEPK/2018).

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