

Spatial Analysis of *Leptospira* in Rats, Water and Soil in Bantul District Yogyakarta Indonesia

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Abstract

Leptospirosis is a potential threat to public health. An increasing number of people infected with *Leptospira* were reported in Bantul District, Yogyakarta special region with a case fatality rate (CFR) of 7.8%. Infected areas in the district have increased from 2 to 15 sub districts. Leptospirosis is caused by *Leptospira* bacteria and spread by direct contact with infected rodents and indirect contact through contaminated water or soil. *Leptospira* in rats, water and soil were detected using real-time quantitative polymerase chain reaction (qPCR). The sites of sampled materials were geocoded using Global Positioning System (GPS). Spatial analysis was used to predict the spread of *Spira*. This study aims to perform the mapping, clustering, and predicting the spread of *Leptospira* in Bantul Yogyakarta Indonesia. Data were collected from three sub-districts: Sedayu, Sewon and Bantul. The result showed that 38.04% from 368 samples were *Spira* positive. There were four significant clusters of infection spread source. *Spira* is predicted to spread in, and out from, Bantul District.

Keywords

Leptospirosis, *Leptospira*, Rats, Water, Soil, Spatial Analysis

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1. Introduction

Leptospirosis is a zoonoses infectious disease caused by the direct spiral shaped microorganisms called *Leptospira* [1]-[3]. Transmission of the disease can be through direct contact with urine and body fluids of infected animals, or through indirect contact with environmental water and soil contaminated with the bacteria *Leptospira* [4]-[6]. Michna and Faine, revealed that the urine of infected animals or carrier Spira healthy environment can contaminate the water, soil, grass, drinking water and food [7]-[9]. *Leptospira* lives in the kidneys and the urine is the source of infection when the reservoir is issue the urine in water or soil environments [10]-[12].

Leptospira can live up to several months in water and plays an important role as a source of transmission of leptospirosis. Spira needs an optimal environment to live and to reproduce such as a moist atmosphere, temperatures around 25°C, and pH near neutral (± 7). Dry weather conditions, strong sunlight, water and soil pH outside the range of 6.2 to 8.0 do not support the growth of *Leptospira* [4] [13] [14].

Rats are the reservoir with chronic leptospirosis infection. The infection is transmitted from rat to an other through direct contact at a young age or mature. According to Rejeki, *L. icterohaemorrhagiae* species were found in *Bandicota indica* and *Rattus diardii*, meanwhile *L. Ballum* species were found in *Mus musculus* [15]. Rats are suspected to have an important role in the incidence of leptospirosis are *R. norvegicus*, *R. diardii*, *Suncus murinus* and *R. exulans* [16].

Data from the Regional Health Office Yogyakarta showed that Leptospirosis cases rising during the last 5-year period (2009 - June 2013). In 2009, there were 10 cases in the 2 villages. An outbreak of leptospirosis occurred in 2010 with 116 cases in 43 villages. For the next year increased 154 cases and widespread in 51 villages. Cases declined in 2012 to 48 cases in 27 villages, but in the early semester of 2013 the case raised to 51 cases in 24 villages [17]. Syamsumin [18] reported that the outbreak occurred due to exposure to water irrigation like canal or river or pond, the presence of rats in the work environment and the presence of livestock manure in the house. Geographical conditions that favor outbreaks are agricultural lands such as rice fields, plantations and moor, and the presence of pools, ponds and swamps, and rainfall average of 11 mm days each month [18].

The spread of *Leptospira* in environment related to epidemiological aspects and geographical region. Spatial analysis can be used to determine the spread and clustering and to predict the incidence of leptospirosis in a region [19]. Spatial analysis with geographical information systems approach (GIS) has been widely applied in various studies in order to control leptospirosis. Mapping patterns of this disease is often done to determine the location of the source of infection. Tunissea, Sunaryo, Melani dan Fuadi, has conducted research on spatial analysis of patients with leptospirosis on the environment. These studies mapped the distribution patterns of patients with leptospirosis. Distribution of leptospirosis tends to follow the river flow pattern with high rainfall. Leptospirosis cases are found in slums and in area were rats are found [19]-[22].

Accordingly, this study examines whether there is a pattern of distribution and clustering of *Leptospira* bacteria, as well as to predict distribution patterns *Leptospira* bacteria in Sedayu, Bantul and Sewon Sub District. This study can be used as a guidance to control leptospirosis.

2. Methods

2.1. Study Site and Sample

This research was conducted in Sedayu, Bantul and Sewon Sub District which is highly endemic areas leptospirosis. Samples were determined based on the presence of patients with leptospirosis in Sedayu, Bantul and Sewon Sub District, from Hospital medical records and Health Office Bantul District in 2013. Samples in this study were rats, water and soil in the area where the patient lived. The number of rats in each district is calculated based on the sample formula according to Dohoo, were 30 rats [23]. Water and soil samples are taken from 1 - 3 points in around the site of rats catched. Determination of sampling sites was based on the presence of signs such as rat hole, trace of the rat, and rat droppings.

2.2. Data Collection and Analysis

Leptospirosis patient data obtained from District health offices and Hospitals Bantul regency in 2013, based on medical record data on the clinical and laboratory diagnosis by serological tests using dot leptotek leptotek dri or lateral flow. Catching mice using traps conducted around the patient's residence. Traps were installed in

places that there are signs of the rat both inside and outside the home or in a rice field for 3 nights to allow the rat entering the trap. One trap is stalled at any place or home with a spacious 0.015 to 0.035 ha, and each multiplication added one trap. While in paddy fields, the trap is installed at every 0.85 to 2.1 ha of paddy fields [24] [25]. Water and soil samples were taken around the location of the arrest the mouse with a radius of ± 50 meters around the house and ± 100 meters around the field. Criteria for water or soil samples to be taken where there were signs of the rat, such as the presence of a hole or nest of the rat, the presence of traces of the rat, and the presence of rat droppings. Water and soil sampling conducted in the morning at approximately 6:00 a.m. to 9:00 a.m. in the morning before the sun directly in to water or soil [24]. Determination of the presence of *Leptospira* bacteria in the samples was conducted by qPCR using appropriate primers. Forward primer is 5'¹⁷¹-CCCG-CGTCCGATTAGC-3'. Reverse primer is 5'²⁵⁸-TCCATTGTGGCCGR^{A/G}ACAC-3'. The probe is 5'²⁰⁵(FAM)-CTCACCAAGGCG ACGATCG GTAGC²²⁸-3' (TAMRA), FAM 6-carboxy-fluorescein, TAMRA 6-carboxy-tetramethyl-rhodamine [26]. Determination of coordinates using GPS performed on place of the rat was found, and water and soil samples were taken.

Data were analyzed qualitatively and quantitatively using computers, and were presented in the form of description and tables. Distribution patterns and clustering of bacteria *Leptospira* were analyzed by spatial analysis approach using ArcGIS software assistance, SaTScan and CrimeStat program.

2.3. Ethical Clearance

This study has obtained ethical permission (ethical clearance) for medical research from the Ethics Committee of Faculty of Medicine Gadjah Mada University.

3. Result

Three sub-districts of Bantul (includes Sedayu, Sewon, Bantul) purple were endemic leptospirosis areas that used for our research (Figure 1). Geographically, Bantul is located between 07°44'04" - 08°00'27" South Latitude and 110°12'34" - 110°31'08" East Longitude in the southern part of Yogyakarta. Bantul district has an area of 50.85 km² or 50.685 ha, which is composed of 17 districts and divided in to 75 villages and 933 hamlets.

Bantul district is an area that suitable for agriculture. But in this area there are many puddles and ditches with water that does not flow well. It is suitable for the development of *Leptospira* bacteria. Figure 2 shows the location of the data in the form of rice fields and ditches that there are many puddles. This location is also

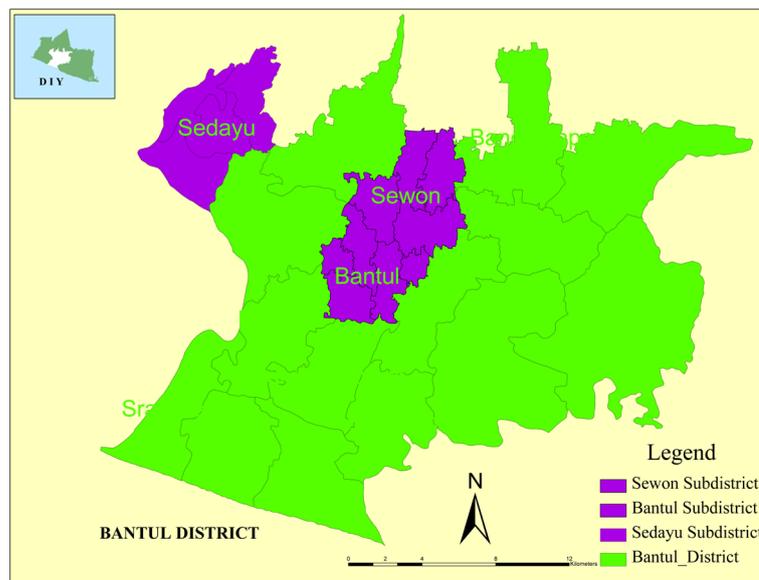


Figure 1. The study area at three sub districts in Bantul district. The purple color indicates the study area, Sub District of Bantul, Sewon and Sedayu. Green color, Bantul Regency excluding research area.



Figure 2. Images of the location, the source of exposure to *Leptospira* bacteria. (a) Rice field area. Farmers are vulnerable to *Leptospira* exposure. (b) Rice fields as a source of infection for the surrounding community. (c) Garbage in the rice field is a source of leptospirosis infection. (d) Puddles in the fields as a source of infection.

suitable for the habitat of the rat as a reservoir host of *Spira*. These conditions are potentially as place of *Leptospira* spread and transmission of leptospirosis in Bantul District.

Data collection was conducted from September to December 2013. There was found 18 patients with leptospirosis and 368 samples consisting of 99 samples of the rat, 119 water samples and 150 soil samples. Distribution of patients with leptospirosis in Sedayu, Bantul and Sewon Sub District has been shown in **Figure 3**.

The examination using qPCR to find bacteria *Leptospira* performed on 368 samples were successfully collected. The results obtained 140 positive samples pathogenic *Leptospira*, which consists of 25 (25.25%) the rat samples, 51 (42.86%) water samples and 64 (42.67%) of soil samples. Based on the location of samples collected, Bantul sub-district is an area with the highest positive *Leptospira* at 51.68% followed by Sewon Sub District and Sedayu respectively 32.32% and 25.83%. Detail of the number of *Leptospira* in each Sub District has been written as in **Table 1**.

Determination of coordinates of the samples was taken at the same time when samples had been taking using a GPS device. Distribution of samples obtained shown in **Figure 4**. Distribution of the sample includes the location where the rat was found, the water and soil were taken.

Buffering pattern identification based on rat *Leptospira* bacteria, water and soil is shown in **Figure 4**. Buffering pattern formed indicates areas that have affected the risk of becoming wide spread in accordance with the ability of the rat spread. It appears that this buffer are a past the administrative boundaries, not only in the Bantul district, but also extends to the districts of Sleman and Yogyakarta.

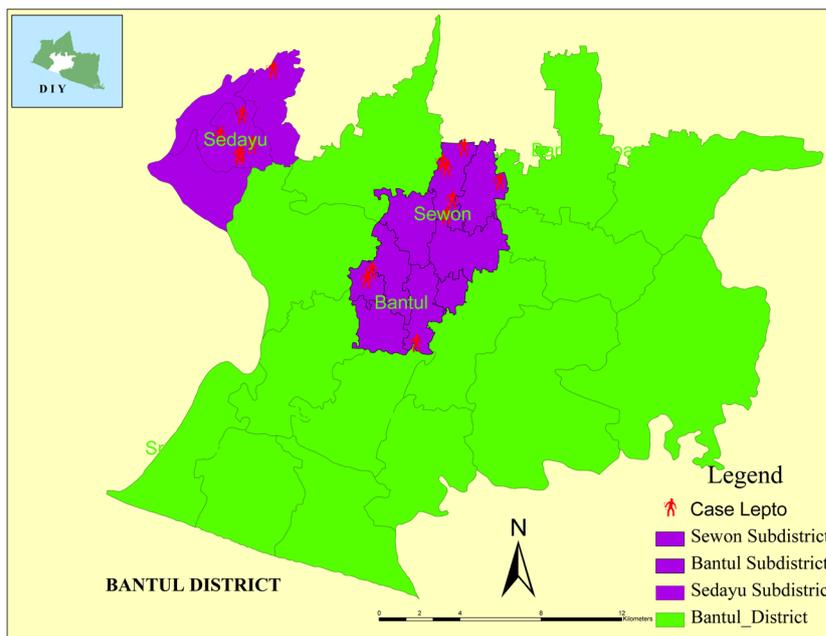


Figure 3. Distribution of patients leptospirosis in the research area of Bantul regency. The red color shows the distribution of patients with leptospirosis. The color purple, the study area in the sub district of Bantul, Sewon and Sedayu. The green color, the district of Bantul which does not include the area of research.

Table 1. The positive *Leptospira* of rats, water and soil samplesat Sedayu, Bantul and Sewon Sub District.

Sub District	Number of <i>Leptospira</i> /Total Sample			
	Rats	Water	Soil	Total
Sedayu	6/31 (19.35%)	13/32 (40.63%)	13/36 (36.11%)	32/99 (32.32%)
Bantul	10/36 (27.78%)	29/52 (55.75%)	38/61 (62.30%)	77/149 (51.68%)
Sewon	9/32 (28.13%)	9/35 (25.71%)	13/53 (24.53%)	31/120 (25.83%)
Total	25/99 (25.25%)	51/119 (42.86%)	64/150 (42.67%)	140/368 (38.04%)

SaTScan analysis shows that there are six clusters of infection source of leptospirosis as shown in **Figure 5**. The first cluster is located at the position of 7.793400 SL, and 110.276600 EL with a radius of 0:59 miles and there are 7 locations in Sedayu SubDistrict. This cluster is very significant with p value = 0.0000051. The second cluster is located at the position of 7.905350 SL, and 110.329617 EL with a radius of 0.58 km and there are six locations in Bantul Sub District. This cluster is also very significant with p value = 0.0018. The third cluster is located at the position of 7.840333 SL, and 110.351500 EL with a radius of 1.73 km, and there are ten locations in Sewon Sub District. This cluster is very significant with p value = 0.011. The fourth cluster is located at the position of 7.843200 SL, and 110.377300 EL with a radius of 0.31 km, and there are four locations in Sewon Sub District. This cluster is also very significant with p value = 0.014. The fifth cluster is located at position 7.888300 SL, and 110.361350 EL with a radius of 3.58 km and there are fifty locations in the district of Bantul. This cluster is not significant with p value = 0.062. The sixth cluster is located at the position of SL 7.886917, 110.318117 and EL with a radius of 0.40 km, and there are five locations in the district of Bantul. This cluster is not significant with p value = 0.258.

4. Discussion

Transmission of leptospirosis by *Leptospira* bacteria can be through direct contact with urine and body fluids of infected animals or indirect contact through contaminated water or soil by *Leptospira* bacteria [4] [6] [27]. Rice

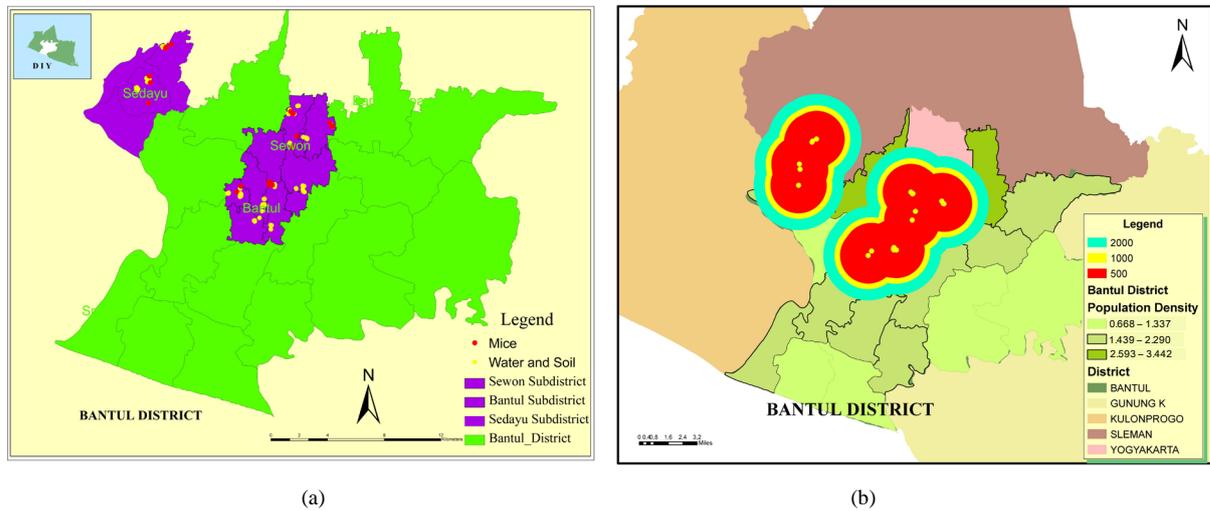


Figure 4. Distribution of positive samples (a) and positive sample buffer (b) in the three study areas Sedayu subdistrict, Bantul and Sewon, Bantul district. (a) The red color shows the distribution of samples positive mice *Spira* and yellow colors are water and soil samples were positive *Leptospira*. (b) Buffer *Leptospira* positive, blue is the buffer as far as 2 km, yellow as far as 1 km and red as far as 500 meters. The yellow color is a positive rat samples. The green color shows the population density gradation.

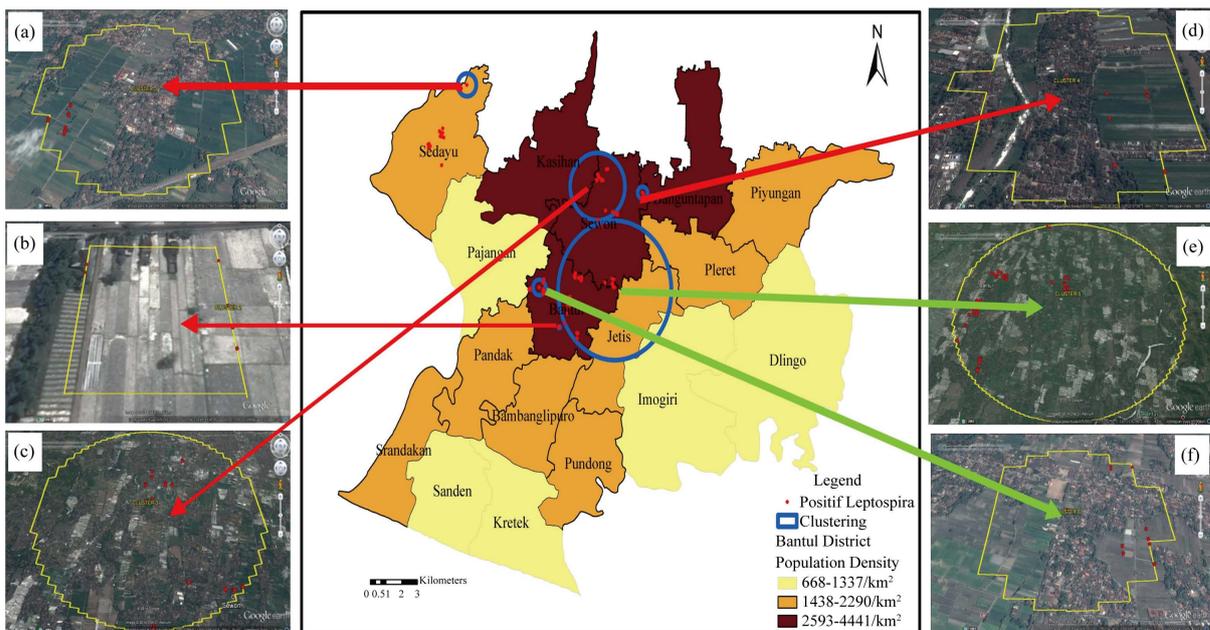


Figure 5. Overview the positive *Leptospira* clustered in three sub districts of research. (a) is located in District Sedayu. (c) and (d) is located in the sub district Sewon. (b), (e) and (f) located in the sub district of Bantul.

planting season in Bantul District done in conjunction with the rainy season, due to growth requires a lot of water. Puddle during rice grow this optimal conditions for the growth of *Leptospira* bacteria. According to [4] [13] [14] environment that is often waterlogged is a condition that easily contaminated with *Leptospira* [4]. Tunisia (2008) and Anies, (2009) stated that the puddle of water in the rice fields is a factor contributing to the occurrence of transmission of leptospirosis. Various factors that support the transmission of *Leptospira* which are found puddles, fields and bushes or wet grass and animals, especially rodents as a reservoir of *Leptospira* bacteria source [20] [28].

The presence of *Leptospira* bacteria in the environment caused by rats urine contamination of *Leptospira*

infected [10]-[12], because rats are there serovar and at the same time the main disseminators of *Leptospira* [29]. This bacteria is out along with the urine when rats explore the habitat to look for food, so the *Leptospira* bacteria can spread and contaminate water or soil and can infect other rats. Contaminated environment and rats with bacteria *Leptospira* would potentially be a source of infection and disease transmission sources of leptospirosis in humans [6]-[8]. *Leptospira* bacteria infect many farmers, farm workers and eel seekers who indulge in the fields [30] [31]. In general (Widarso, *et al.* 2005) mentions that leptospirosis usually attacks the farmers, plantation workers, mine or ditch workers, and abattoir workers. In Indonesia, the disease includes reemerging disease, so that it can appear sporadically at any time and has the potential to cause outbreaks [32]. Research conducted Simanjuntak *et al.* (1986) found the incidence of leptospirosis in the transmigration sites in Kuala Cinaku Riau Province have the chances of spread by rats [33].

Risk factors affecting the incidence of leptospirosis are the environmental risk factors of physical, biological environmental risk factors, behavioral factors, socioeconomic factors and health service factors [34]. Factors associated with the incidence Leptospirosis in DIY are work as a farmer, a habit of going to the paddy fields and the rats [30]. Indirect transmission occurs when *Leptospira* are excreted through the urine or excretion of careers animals to surface water environment, such as puddles, lakes, rivers, swamps, sewage, drainage, and mud puddles. The spread of the disease can spread to other are as due to flood waters contaminated rat urine containing *Leptospira* bacteria [4]-[6]. In the body of these animals, *Leptospira* lives in the renal tubules [35] and persist for many years as a chronic infection. This infection is transmitted from animals to another through direct contact. Typically, infection is acquired at a young age, and the prevalence of *Leptospira* exit through the urine increases with increasing the age of the animal [13]. Cases of leptospirosis associated with the location of land use. Leptospirosis cases are more common in residential land use locations, especially near bodies of water such as rivers, ponds, rice field sand puddles [36].

Leptospira can live up to several months in water and plays an important role as a source of transmission of leptospirosis. Optimal environment to live and reproduce Spira is in a moist atmosphere, temperatures around 25°C, and pH near neutral (± 7). Conditions of dry air, strong sunlight, and the pH is too low or high ($<6.2 - >8.0$) are not favorable atmosphere for the life and growth of *Leptospira* [4] [14] [37].

Bantul sub-district is an area that has a highest source of transmission of *Leptospira* (51.68%) in Bantul compared with two other sub-districts (Sedayu and Sewon), an area of research. Health Service data shows that Bantul sub-district not only have the area with the highest population density, but also have rice field sand locations conducive to rat habitat. Areas with dense population will impact on the high house hold waste be a source of food for rats. Like wise with many residential buildings around the fields and population behavior that less keeping the environment clean are another risk factor leading to high transmission of leptospirosis in Bantul sub-district. In **Figure 4**, it appears that the distribution of *Leptospira* in water and soil adjacent to the discovery of rats containing *Leptospira*. Distribution of *Leptospira* contaminated water and soil is dependent on home range rat sand high population density in a region.

Poor sanitation conditions such as the presence of midden and the presence of rats is a determinant variable leptospirosis cases. The presence of midden is used as indicators of the presence of rats [38]. This result is consistent with previous studies by Urmimala (2002) in Salvador Brazil stated that he poor shelter condition that is the midden is a risk factor for leptospirosis cases [39]. Barcellos (2001) who concluded that leptospirosis is influenced by the presence of litter, presence of rodents and bacteria *Leptospira* factors, it is possible there are other biotic factors associated with the incidence [38].

The result of the spatial analysis done by looking at the buffer of the distribution of these cases is an area at risk of the spread of bacteria *Leptospira*. *Leptospira* bacteria spread very dependent on rat home range and activities in its habitat. Home range and migration is a distance that commonly taken by rats or area passed rats for foraging activity [40]. Space home range for a mouse can be up to 0.0035 ha, where as field mouse can be up to 2.1 ha. Rats also have the ability to migrate as far as 1 - 2 km, if there is no food source around the home [40]. Buffer area of the source of infection can be determined based on the distance home range of mice. **Figure 5** shows that the location of the buffer is quite large, and has entered the area which is beyond the three sub-districts in Bantul area of research, and even extends to the districts of Sleman and Yogyakarta. This condition is of ten caused by *Leptospira* bacteria be came wide spread deployment. As we know that the areas around Bantul district is endemic of leptospirosis [17]. This study recommends that need to make a planning a cross borders to control the spread of the bacterium *Leptospira* Bantul and surrounding region.

Results of SaTScan analysis to see clustering of infection source of leptospirosis in three sub-districts of Bantul

District obtained 6 clustering distribution of the source of infection. The formations of a strong clustering and significant occur in clusters 1 to 4 for the distribution of sources of infection, where as clusters 5 and 6 were not statistically significant. The existence of a strong cluster grouping indicates that it is likely there has been a process of spreading the bacteria *Leptospira* in the group, so that the location of the cluster is the site as a potential source of infection leptospirosis. According to Sunaryo (2009), clusters distribution of leptospirosis cases occur under conditions of a polluted environment and land fills with the closest point distance between the case of 9 m (0.009 km) [21]. Tunisia (2008) showed that the body of water, temperature, light intensity, pH of water and soil, vegetation and the presence of rats associated with spread leptospirosis [20].

The study also predicts the occurrence of leptospirosis cases. Through the description of the distribution pattern of infection source of leptospirosis, it is known that the distribution of the source of infection can spread to non endemic areas such as parts of Yogyakarta regency and other Sub districts in Bantul. **Figure 6** displays the prediction of distribution direction of *Leptospira* bacteria. Overview the prediction of distribution extends to the southeast which will hit sub-districts in the southeast and the south of Bantul Sub district and partially Yogyakarta City. Location of this prediction of distribution is densely populated areas, so it will most likely be an outbreak Leptospirosis if no appropriate counter measures. This study is inline with research conducted by Nurbaeti in 2012 [38]. The study showed that the distribution of leptospirosis in the border does not spread through the flow of the river, but dealing with the flow of the river in the paddy field [41].

In the report of leptospirosis cases in 2009 showed that leptospirosis cases is most prevalent in Godean sub-district, Sleman regency. This case from year to year extends and spreads mainly to the east and the south to reach Yogyakarta and Bantul [41] [42]. Looking at the pattern distribution that occurs, it is possible the case will continue to spread, especially in line with the roaming rat as a reservoir host polluting water flow in the paddy fields.

5. Conclusion

Leptospira bacteria in the environment are spread through the rats urine infected Spira. It is found 4 clusters

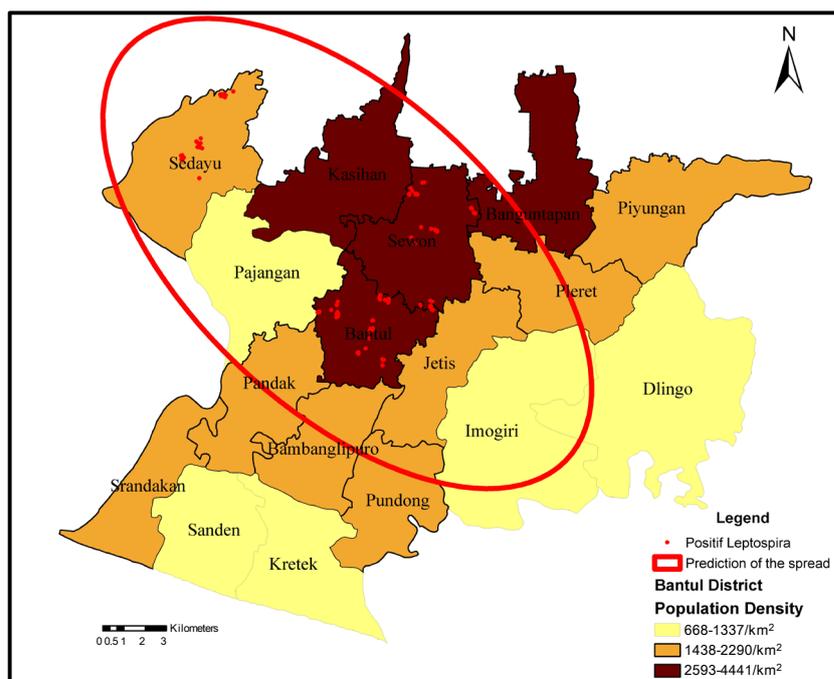


Figure 6. Prediction of the spread of *Leptospira* in the district of Bantul. Analysis using CrimeStat program. Almost all districts of Bantul, Kulon Progo and Sleman regency and Yogyakarta City is an area that can be a spread of *Leptospira*. Light brown until dark brown, showing population density in Bantul district. The red circle as a regional distribution prediction Spira. The red dots indicate positive samples Spira.

were formed and statistically significant with a p value < 0.001. This prediction of distribution extends to southwest (Sleman district) from source deployment in Sedayu sub-district. Other predictions of distributions are to the southeast which will hit sub-districts in the southeast and south of the Bantul sub-district and partially Yogyakarta City.

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