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## **Spatial Analysis of Pulmonary Tuberculosis Incidence in Kupang City in 2019-2021**

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### **Abstract**

#### **Introduction:**

Pulmonary tuberculosis has a fairly high number of cases in several regions of Indonesia, including Kupang City. In 2019, tuberculosis cases in Kupang City reached 667 cases, in 2020 it increased to 693 cases and in 2021 it decreased to 419 cases. This study aimed to analyze the factors that influence the incidence of pulmonary tuberculosis using a spatial analysis model.

**Methods :** This research is a quantitative research with an analytical observational design using an approach of Geographic Information Systems (GIS). The autocorrelation of population density, poor families, and healthy homes with the incidence of pulmonary tuberculosis were investigated. The data used in this research is secondary data taken from related agencies. The samples in this study were 11 community health centers in Kupang City, called Naioni Health Center, Alak Health Center, Manutapen Health Center, Sikumana Health Center, Penfui Health Center, Bakunase Health Center, Oebobo Health Center, Oepoi Health Center, Pasir Panjang Health Center, Kupang City Health Center, and Oesapa Community Health Center in 2019-2021. The QGIS and GeoDa applications were used. The GeoDa application was used through the LISA (Local Indicators Spatial Autocorrelation) tests.

**Results:** Based on the results of the LISA bivariate test, it showed that population density, poor families, and healthy housing coverage have no relationship with the incidence of TB in Kupang City ( $p$ -value  $> 0.05$ ) and show a random pattern of case distribution (Morans Index 'I' is smaller than  $E[I] = -0.1000$ ). However, in 2019-2020, the Morans I Index value covering healthy homes was greater than  $E[I] = -0.1000$ , which shows a clustered pattern of case distribution.

**Conclusion:** It was concluded that there was no positive spatial autocorrelation between population density, poor families, and healthy homes with the incidence of pulmonary tuberculosis in Kupang City in 2019-2021.

**Keywords:** Tuberculosis, spatial, autocorrelation, QGIS, GeoDa

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## Introduction

Spatial analysis is one of collecting data in environmental management efforts and is part of area-based disease management. Spatial analysis is generally used to record diseases geographically with regard to population, distribution, environment, behavior, social, economic, and interrelationships. The spatial approach in the health sector is a new approach that means problem-oriented health development and spatial priority of health (environmental) problems.

Spatial analysis using Geographic Information Systems (GIS) is an important method for surveillance and monitoring of public health. This is because the function of GIS in the health sector can produce a spatial picture of health events, analyzing the relationship between location, environment, and disease occurrence. Several software programs that can be used for spatial approaches include QGIS and Geoda. The use of GIS applications and spatial analysis methods makes it easier for mapping steps for the distribution pattern of tuberculosis to be adjusted to meet the specific needs of field teams as well as supervisors and program managers in assisting tuberculosis control programs and understanding the spatial distribution of a disease.<sup>1</sup>

One type of spatial analysis that is useful is autocorrelation, which is an analysis used to determine the correlation between variables in the area being observed. Apart from being used to determine the correlation between variables, this analysis also has the advantage that it can be used to identify areas that are more vulnerable compared to other areas. This analysis can also show whether the spatial distribution pattern of disease is clustered, scattered, or random.

Tuberculosis (TB) is currently a public health problem both in Indonesia and internationally, making it one of the Sustainable Health Development Goals (SDGs). Tuberculosis is an infectious disease caused by the bacteria *Mycobacterium tuberculosis*. TB germs usually attack the lung organs and can also be outside the lungs (extra pulmonary).

Transmission of TB disease increases if the community does not know about the

transmission of TB disease. Many things are risk factors for TB disease, including individual factors (age, gender, income level, education level, etc.), home environmental factors, and habits, contact history, and so on. The factors that cause the most frequent cases of TB are slums, crowded environments, and limited access to clean and healthy living habits.

Nearly a quarter of the world's population is infected with *Mycobacterium tuberculosis*; around 89% of TB is suffered by adults, and 11% is suffered by children. Tuberculosis is still the highest cause of death after HIV/AIDS and is one of the 20 leading causes of death worldwide. Indonesia is ranked 3rd with the highest number of TB sufferers in the world after India and China. Globally, it is estimated that 9.9 million people will suffer from TB in 2020. The number of deaths due to tuberculosis globally in 2020 was 1.3 million; this has increased compared to 2019, which was 1.2 million. According to the 2021 Global Tuberculosis Report, in 2020 the TB incidence rate in Indonesia was 301 per 100,000 population, a decrease compared to the TB incidence rate in 2019, which was 312 per 100,000 population. The TB death rate in 2019 and 2020 is still the same, namely 34 per 100,000 population.<sup>2</sup>

In 2017, the number of pulmonary TB cases in Indonesia was 420,994 cases. East Nusa Tenggara Province is in the 16th position out of 34 provinces, with a total of 2,842 pulmonary TB cases. In 2021, the number of tuberculosis cases found in Indonesia was 397,377 cases, an increase compared to all tuberculosis cases found in 2020, namely 351,936 cases.<sup>3</sup>

Based on Health Profile Data from East Nusa Tenggara Province, the number of pulmonary TB cases from 2015-2017 has increased, with the highest number of pulmonary TB cases in Kupang City at 13.98 per 100,000 population.<sup>4</sup> The highest incidence of pulmonary TB in Kupang City is in several sub-districts, including Oebobo District, Maulafa District, Kelapa Lima District, and Alak District.

The total number of TB cases in 2018 was 645, consisting of 374 men and 271 women. The increase in the number of new

cases occurred because the reporting system was better integrated with various government and health service units (TNI/Polri) and other private sectors reported to the Kupang City Health Service. The incidence rate of pulmonary TB cases increased in 2018 when compared to the previous year. The incidence rate of TB cases was 205 per 100,000 population in 2013, increasing in 2015 to 211 cases per 100,000 population, which then decreased to 152 cases per 100,000 population in 2018, with the success rate of treatment of smear-positive TB patients treated in 2016 amounting to 81%.<sup>4</sup>

The incidence of tuberculosis is a region-based disease, so it is necessary to analyze it using a spatial approach to determine its distribution pattern. One way to convey map-based information is the Geographic Information System (GIS), or web-based geographic information system. Web GIS and GeoDa can be used to provide information regarding the spread of tuberculosis in Kupang City. It can also be known what actions and policies need to be taken to reduce the number of cases of pulmonary TB, especially in areas that have a high tendency for smear-positive pulmonary TB cases. Apart from that, planning strategies for preventing and eradicating pulmonary TB more quickly and on target is very necessary in the city. In Kupang, one way is to look at the distribution pattern of smear-positive pulmonary TB sufferers in Kupang City for 3 consecutive years (2019-2021).

Until now, there has been no spatial analysis, especially spatial autocorrelation, to assist in formulating tuberculosis control policies in Kupang City. Based on the background of the problem of pulmonary tuberculosis and the benefits of spatial analysis with GIS, researchers feel it is necessary to conduct research on "Spatial Analysis of Pulmonary Tuberculosis Incidence in Kupang City in 2019-2021"

## **Methods**

The research design used is an analytical observational design using an approach of Geographic Information Systems (GIS). The population in this study was 11 health centers in Kupang City

in 2019-2021, called Naioni Health Center, Alak Health Center, Manutapen Health Center, Sikumana Health Center, Penfui Health Center, Bakunase Health Center, Oebobo Health Center, Oepoi Health Center, Pasir Panjang Health Center, Kupang City Health Center, and Oesapa Health Center.

The sampling technique used in this research is total sampling where the sample size is the same as the population. The sample in this study was 11 Community Health Centers in Kupang City. Data collection is carried out by visiting the relevant agencies and collecting the necessary data. The QGIS and GeoDa applications were used. QGIS produced a map of the distribution of pulmonary tuberculosis cases in 11 community health centers in Kupang City in 2019-2021. The GeoDa application was used to see TB pattern groups in Kupang City in 2019-2021 in general or globally. The Moran's I test and the LISA test were used. A p-value  $< 0.05$  indicates spatial autocorrelation. Meanwhile, a p-value  $> 0.05$  indicates there is no spatial autocorrelation. The I value is in the range  $0 < I \leq 1$  indicating positive spatial autocorrelation. Meanwhile, the I value is in the vulnerable range  $-1 \leq I < 0$ , indicating negative spatial autocorrelation. The value  $I > E[I]$  indicates a clustered distribution pattern. Meanwhile, the value  $I < E[I]$  indicates a random distribution pattern (no clusters). The data that has been analyzed will then be presented in the form of tables, maps and interpreted in narrative form.

## **Results**

### *Univariate Analysis*

Based on the map of the distribution of pulmonary tuberculosis incidents in Kupang City, it shows that in 2019 the community health center with the highest number of cases was the Bakunase Community Health Center with 125 cases. There is also a community health center with the lowest number of cases, namely the Kupang City Community Health Center with 10 cases. In 2020, the community health center with the highest number of cases was the Bakunase Community Health Center with 129 cases. This figure indicated an increased cases

from the previous year. There is also a community health center with the lowest number of cases, namely the Kupang City Community Health Center with 9 cases. In 2021, the community health center with the highest number of cases was the

Sikumana Community Health Center with 88 cases. There is also a community health center with the lowest number of cases, namely the Kupang City Community Health Center with 10 cases (Figure 1-3).

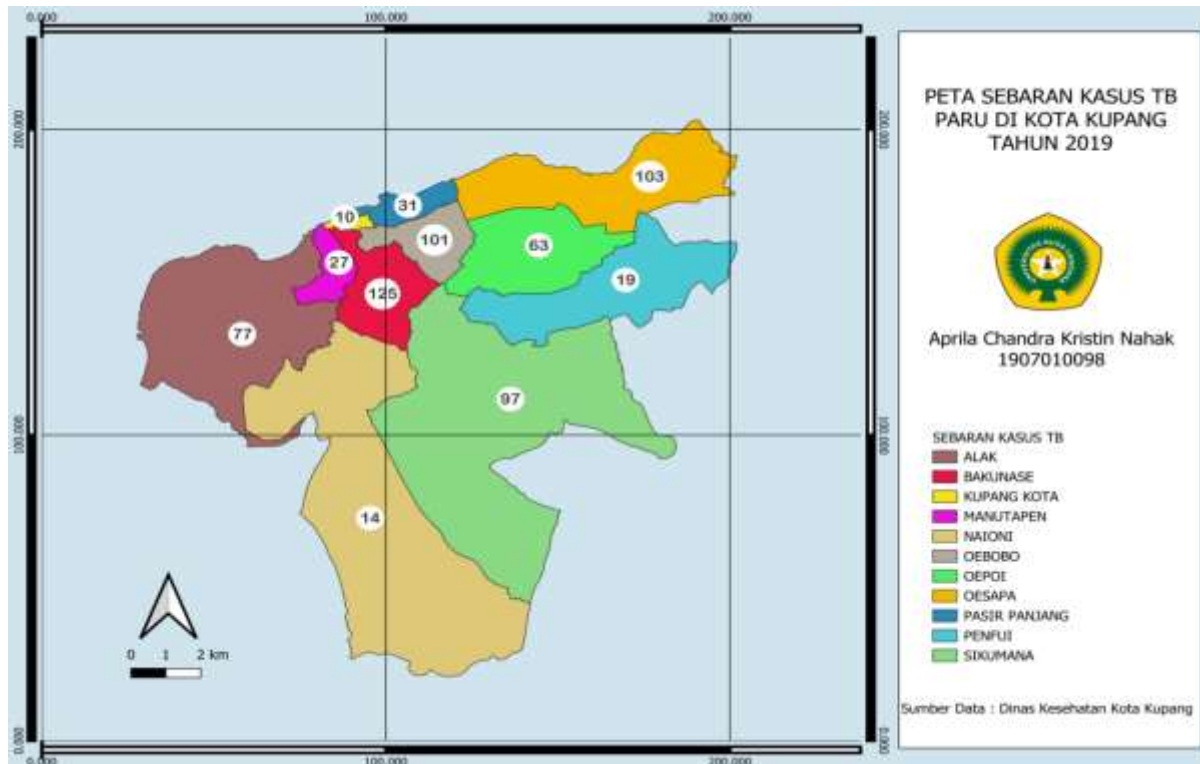


Figure 1. Distribution of Pulmonary TB Cases in Kupang City in 2019

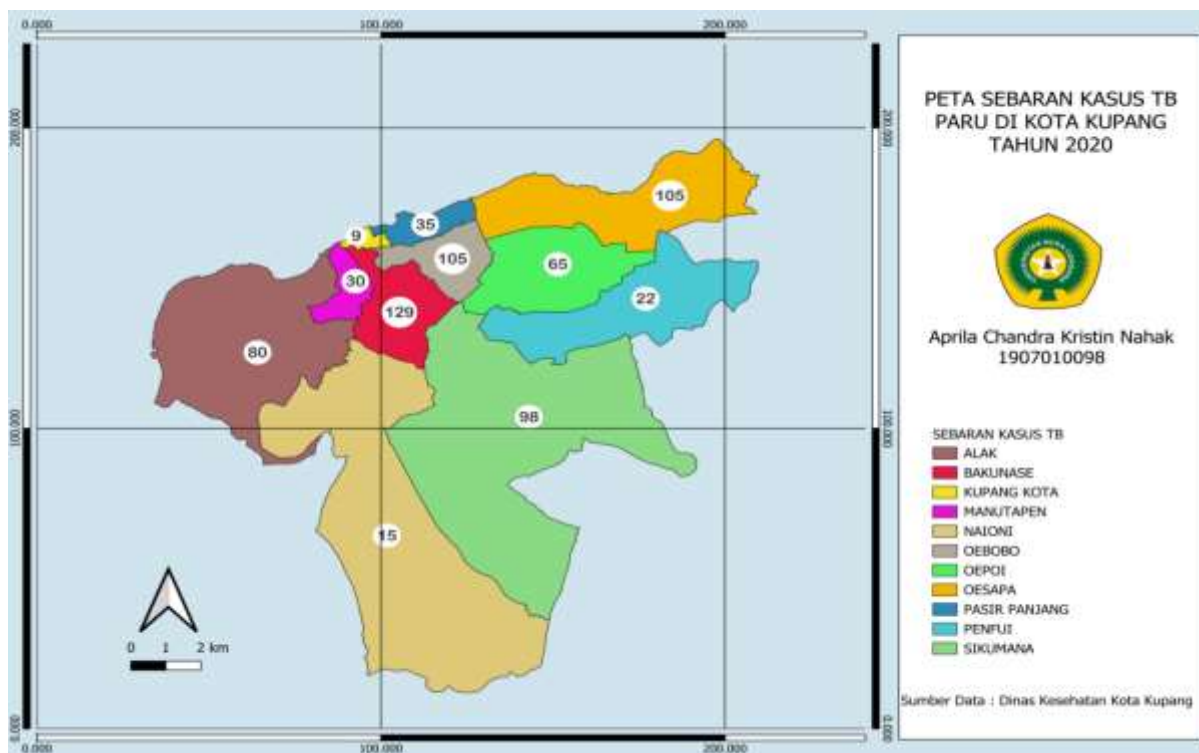


Figure 2. Distribution of Pulmonary TB Cases in Kupang City in 2020

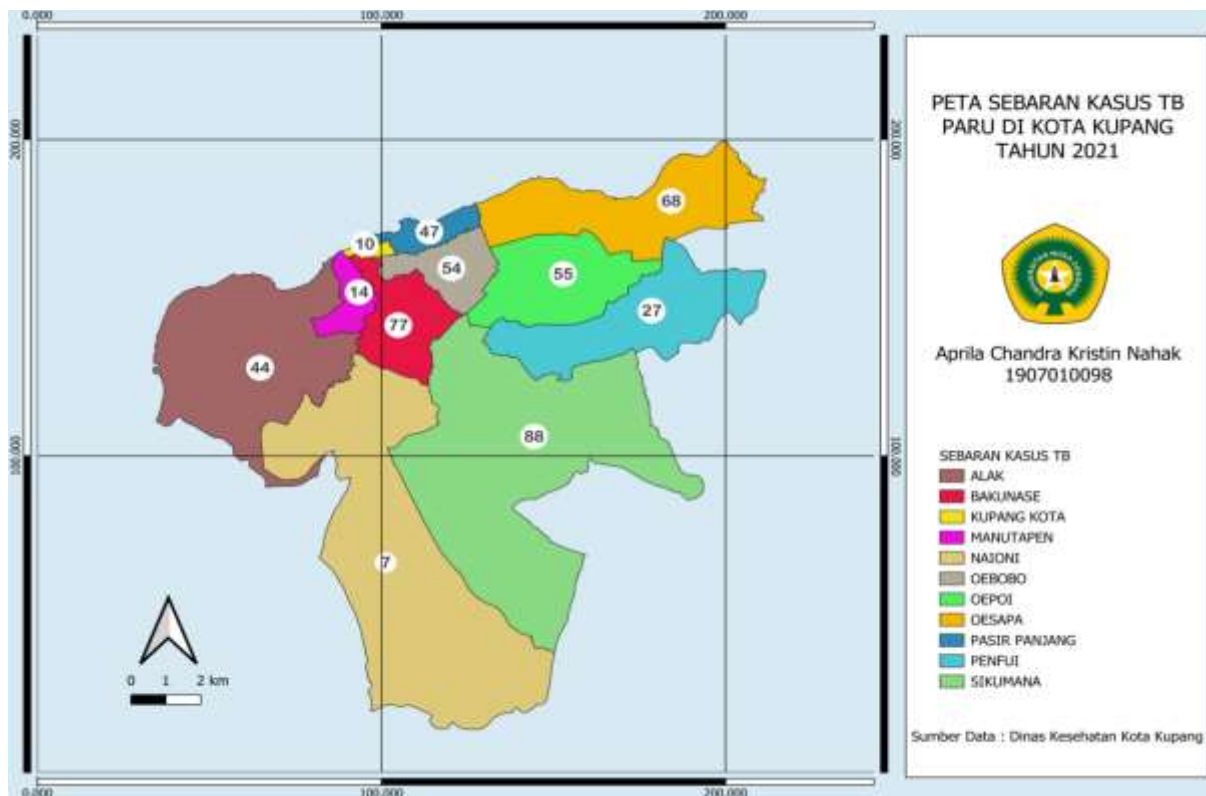
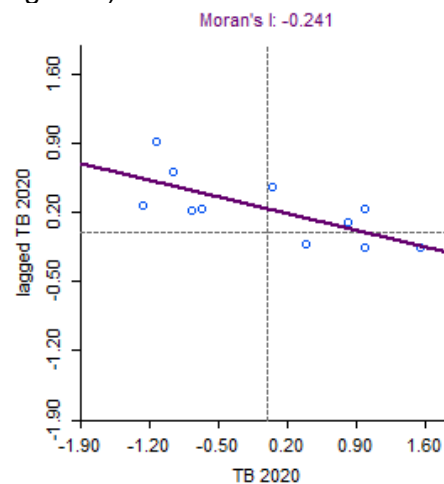
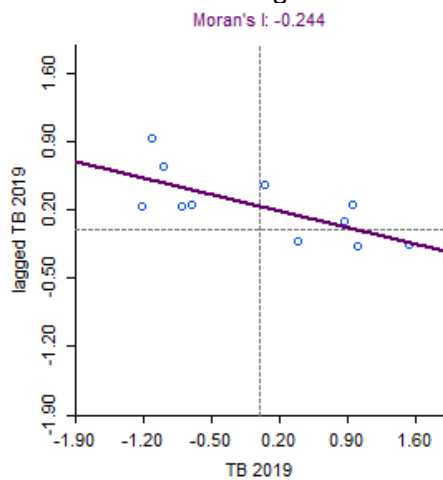


Figure 3. Distribution of Pulmonary TB Cases in Kupang City in 2021

Morans scatterplot of TB cases from 2019-2021 showed the pattern of relationship between the number of TB cases in a health center area and neighboring health center areas. The results of the Moran index throughout 2019-2021 showed a Moran negative value,

indicating that nearby locations had cases of pulmonary tuberculosis which tended to be random. Apart from that, the Moran's I value in pulmonary tuberculosis cases in 2019-2021 is smaller than  $E[I]$  which is  $-0.1000$ , which means that pulmonary tuberculosis cases are not clustered (Figure 4).



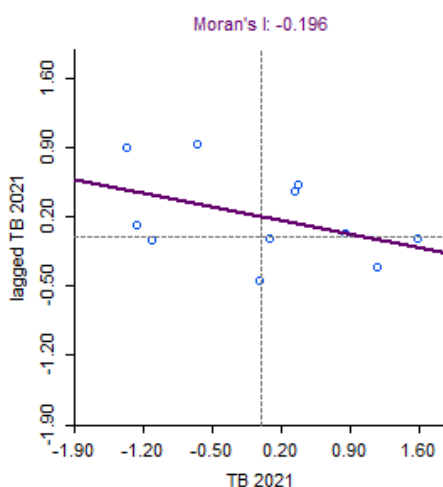


Figure 4. Moran's Scatterplot of Pulmonary TB Cases in Kupang City 2019-2021

**Bivariate Analysis**

Table 1. LISA Bivariate Analysis of Population Density and TB Cases in Kupang City 2019-2021

Tahun	Moran's I	E[I]	Mean	Standar Dev	z-value	p-value
2019	-0,1068	-0,1000	-0,0089	0,1245	-0,7866	0,224000
2020	-0,1076	-0,1000	-0,0081	0,1243	-0,8006	0,217000
2021	-0,1521	-0,1000	-0,0029	0,1249	-0,1945	0,114000

LISA bivariate test results (Local Indicators Spatial Autocorrelation) in the table 1 are obtained in the population density variable in 2019-2021 with *p-value* >0.05. This means that there is no spatial autocorrelation of population density with the incidence of pulmonary tuberculosis in Kupang City (Table 1). The Moran's I index value <0 which indicates negative spatial

autocorrelation. The test results above show that the Morans' I index value for population density in 2019-2021 is smaller than  $E[I] = -0.1000$ , which indicates that the pattern of relationship between population density and pulmonary tuberculosis in 2019-2021 between health center areas is random (Table 1).

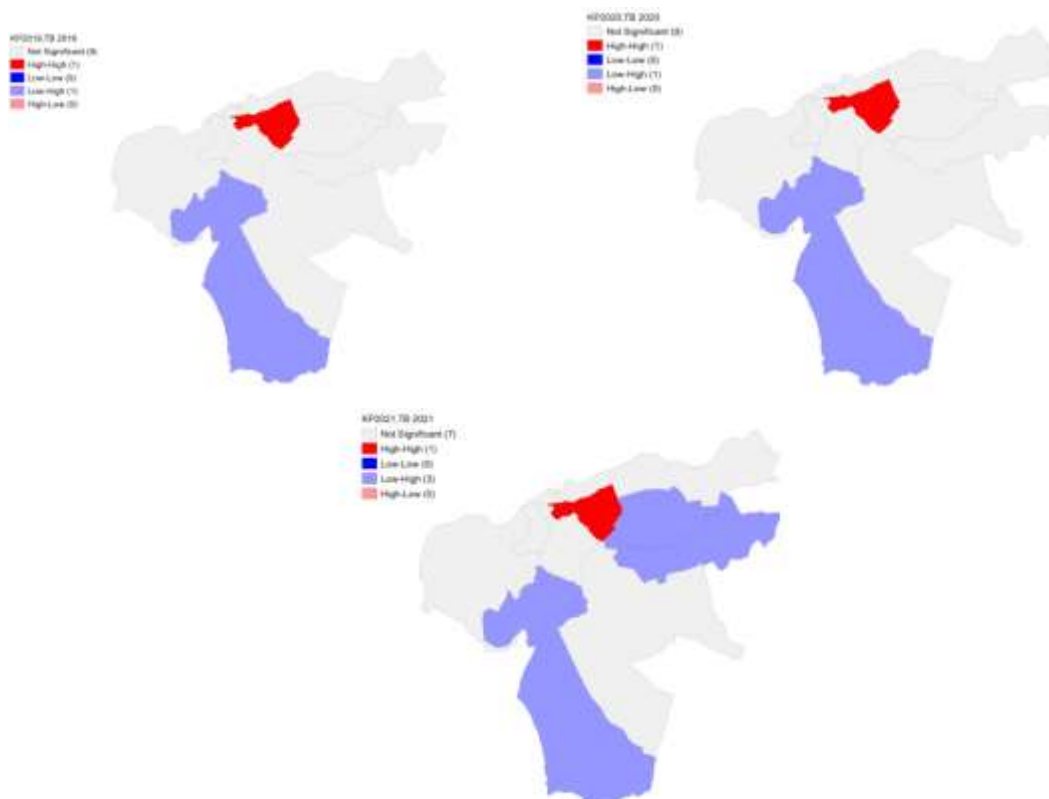


Figure 5. Map of Significant BiLISA Cluster Population Density with TB Cases in Kupang City 2019-2021

Bivariate Cluster Map Local Indicator Spatial Autoregression (LISA) in 2019-2020 showed the same results. There is 1 health center that has a spatial pattern in quadrant I (*High-High*) namely Oebobo Community Health Center and community health centers which have a spatial pattern in quadrant II (*Low-High*) namely the Naioni health center. In 2021

there will be 1 community health center with a spatial pattern in quadrant I (*High-High*) called Oebobo Community Health Center and there are 3 community health centers with a spatial pattern in quadrant II (*Low-High*) namely Oepoi Health Center, Penfui Health Center, and Naioni Health Center ( Figure 5).

Table 2. LISA Bivariate Analysis of Number of Poor Families with TB Cases in Kupang City 2019-2021

Tahun	Moran's I	E[I]	Mean	Standar Dev	z-value	p-value
2019	-0,2129	-0,1000	-0,0852	0,1671	-0,7645	0,237000
2020	-0,2068	-0,1000	-0,0843	0,1665	-0,7356	0,247000
2021	-0,2357	-0,1000	-0,0803	0,1602	-0,9703	0,164000

LISA bivariate test results (Local Indicators Spatial Autocorrelation) in the table 2 are obtained between in the poor family variable in 2019-2021 p-value >0.05. This means that there is no spatial autocorrelation between poor families and the incidence of pulmonary tuberculosis in Kupang City. The Moran's I index value <0 which indicates negative spatial

autocorrelation. The test results above show that the Morans' I index value for poor families in 2019-2021 is smaller than  $E[I] = -0.1000$ , which indicates that the relationship pattern between poor families and pulmonary tuberculosis in 2019-2021 between health center areas is random (Table 2).

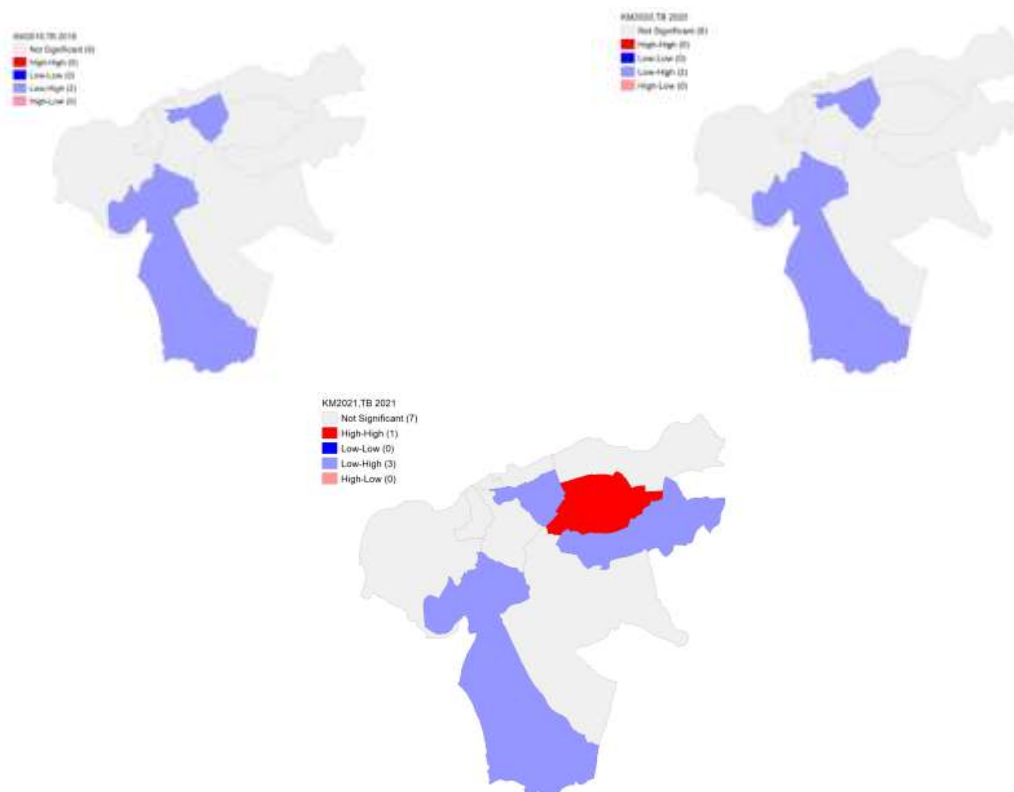


Figure 6. Map of Significant BiLISA Clusters of Poor Families with Pulmonary Tuberculosis Cases in Kupang City 2019-2021

Bivariate Cluster Map Local Indicator Spatial Autoregression (LISA) in 2019-2020 showed the same results. There are 2 health centers that have a spatial pattern in quadrant II (*Low-High*) namely Oebobo Community Health Center and Naioni Community Health Center. In 2021 there will be 1 community health center with a spatial pattern in quadrant I

(*High-High*) namely Oepoi Health Center and there are 3 health centers with a spatial pattern in quadrant II (*Low-High*) namely Penfui Health Center, Oebobo Health Center and Naioni Health Center (Figure 6).

Table 3. LISA Bivariate Analysis of Healthy Home Coverage with TB Cases in Kupang City 2019-2021

Tahun	Moran's I	E[I]	Mean	Standar Dev	z-value	p-value
2019	0,0428	-0,1000	0,0127	0,1219	0,2472	0,413000
2020	0,0266	-0,1000	0,0039	0,1230	0,1845	0,43700
2021	-0,0141	-0,1000	-0,0002	0,1218	-0,1138	0,432000

LISA bivariate test results (Local Indicators Spatial Autocorrelation) in the table 3 the values are obtained *p-value* in the 2019-2021 healthy home coverage variable >0.05. This means that there is no autocorrelation between healthy home coverage and the incidence of pulmonary tuberculosis in Kupang City. The test results above show that the Moran's I index value for healthy home coverage in 2019-

2020 is greater than  $E[I] = -0.1000$ , which indicates that the pattern of the relationship between healthy home coverage and pulmonary tuberculosis in 2019-2020 between health center areas is clustered. Meanwhile, in 2021, the Moran's I index value for healthy home coverage is smaller than  $E[I] = -0.1000$ , which indicates that the relationship pattern between healthy home coverage and pulmonary tuberculosis in



2021 between health center areas is random (Table 3).

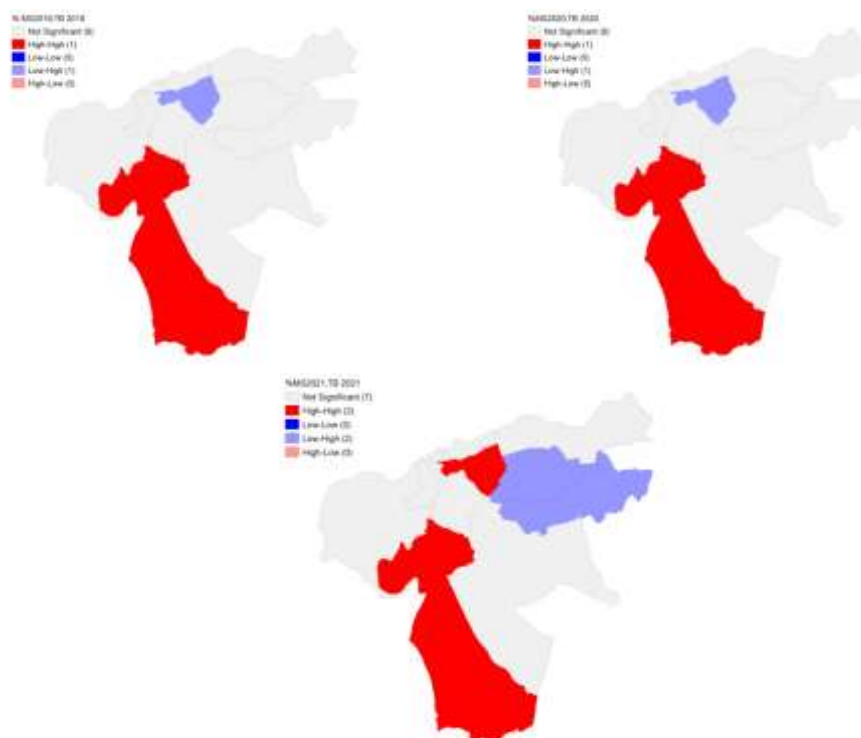


Figure 7. Map of Significant BiLISA Cluster Coverage of Healthy Homes with Pulmonary Tuberculosis Cases in Kupang City 2019-2021

Bivariate Cluster Map Local Indicator Spatial Autoregression (LISA) in 2019-2020 showed the same results. There is one community health center that has a spatial pattern in quadrant II (*Low-High*) namely Oebobo Community Health Center and in quadrant I (*High-High*) namely Naioni Health Center. In 2021 there will be two community health centers with spatial patterns in quadrant I (*High-High*) namely Naioni Health Center and Oebobo Health Center and there are two health centers with a spatial pattern in quadrant II (*Low-High*) namely Penfui Health Center and Oepoi Health Center (Figure 7).

### Discussion

#### *Spatial Autocorrelation of Population Density with TB Cases*

The number and distribution of population determines population density in an area. Apart from determining how quickly a disease can spread, population density also determines the number of sufferers if there are sudden changes such as extraordinary events and the size of adequate health service facilities.

According to (WHO, 2021) areas with high population density tend to have slum housing, poor hygiene and nutrition, so that if a resident is infected with TB it will increase the spread process.

The results of the bivariate test in this study showed that in 2019-2021, there was no spatial autocorrelation between population density and cases of pulmonary tuberculosis in Kupang City ( $p\text{-value} > 0.05$ ). In 2019-2021 there are community health centers that have low population density but have high TB cases, such as Sikumana Community Health Center, almost every year and it becomes a Community Health Center with pulmonary tuberculosis cases (88-98 cases) but has a low density (1647-1914 people/km<sup>2</sup>). On the other hand, there are also health center areas that have not too high pulmonary tuberculosis cases (9-10 cases) but high population density (10,118-10,914 people/km<sup>2</sup>) such as the Kota Lama Health Center.

Tuberculosis can spread through the air with a denser population can increase the risk of increased exposure to

people suffering from TB and will facilitate the spread of bacteria. Apart from population density conditions, the incidence of pulmonary tuberculosis is also influenced by other risk factors such as high poverty, low coverage of healthy homes and low PHBS so that the high number of cases of pulmonary tuberculosis in an area may be influenced by other variable characteristics. Apart from that, there are other factors that influence the incidence of pulmonary tuberculosis such as household contact, immunity, nutritional status, comorbidities, altitude and climate so that population density cannot be considered as the sole cause of tuberculosis cases. The city of Kupang is also synonymous with hot and dry because the air temperature is high so that TB germs die quickly in direct sunlight, but can survive in dark and damp places.

The Moran's index value shows negative spatial autocorrelation between population density and the incidence of pulmonary tuberculosis, meaning that adjacent areas are not similar or the distribution pattern is spatially diffuse. This is due to other factors that allow an explosion of cases in community health centers that have low population density. The health center that has a hotspot is the Oebobo Health Center, which means that the health center has a high population density followed by a high number of tuberculosis cases and is next door to a health center that has a high population density with also high tuberculosis cases.

This research is in line with research conducted by (Fitriyani & Sari, 2021) in West Java Province, in which there was no significant relationship between population density and positive smear pulmonary TB ( $p\text{-value} > 0.05$ ).<sup>5</sup> This research is also in line with research conducted by (Fikri Achmad, Syamsul Arifin, 2022) in Jambi City, in which there was no spatial autocorrelation so it can be said that there is no spatial relationship between BTA (+) TB cases and population density.<sup>6</sup> Another study conducted by (Wardani, 2015) in Bandar Lampung obtained a value of  $p = 0.97$ , greater than  $\alpha = 0.05$ , so there was no spatial relationship between population density and the prevalence of positive smear TB.<sup>7</sup>

This research is not in line with research conducted in Banda Aceh City which showed positive spatial autocorrelation or has a pattern that tends to cluster between population density and TB incidence  $p\text{-value} = 0.001$ .<sup>8</sup> Other research conducted by (Lestar, Makful, & Okfriani, 2021) in West Java Province showed that spatially the presence of population density has an effect on TB cases in West Java Province in 2019-2021. This showed that the pattern of distribution of tuberculosis incidents is in groups.<sup>9</sup>

#### *Spatial Autocorrelation of the Number of Poor Families with TB Cases*

The prevalence of TB disease is influenced by a person's income status, and has a large impact on economic productivity. TB disease is also the main disease that is most susceptible to affecting the world's poor and more than 90% of tuberculosis cases and deaths occur in developing countries. The prevalence of TB in economic groups has different variations and is inversely proportional to their economic level, even in the same country. Malnutrition is also an important factor in the high mortality and morbidity rates due to TB, in populations that are vulnerable to food shortages will increase the high prevalence of advanced TB which will make the situation worse.

The results of spatial analysis in this study showed that there was no spatial autocorrelation between areas with a high number of poor families and a low number of poor families on pulmonary tuberculosis cases. The results of statistical tests in this study also showed that there was no correlation between the number of poor families and the number of pulmonary TB cases in Kupang City in 2019-2021 with a  $p\text{-value} > 0.05$  which indicates there was no relationship, thus the hypothesis is rejected. The absence of significant spatial relationships and autocorrelation in this study could be due to other factors such as population movement and limited resources and health services, so that areas with good socio-economic conditions still have a high risk of transmission. Someone who has a high income may not necessarily be able to

maintain the cleanliness of the environment where they live, drink clean water and maintain the quality of their food so the possibility of contracting tuberculosis remains.

This research is in line with research conducted in Bandar Lampung City which showed that between the prevalence of TB and the proportion of prosperous families, a value of  $p = 0.23$  was obtained, greater than  $\alpha = 0.05$ , so there was no spatial relationship between the proportion of underprivileged families and the prevalence of TB. BTA positive (Wardani, 2015). Another study conducted in Kendari City by (Fitriyani & Sari, 2021) also showed statistical test results where there was no correlation between the number of poor families and the number of smear positive pulmonary TB cases in Kendari City in 2013-2015 with a  $p$ -value of  $r = 0.237$  which shows strength. weak relationship, with  $p$  value = 0.208 which indicates there is no significant relationship, thus the hypothesis is rejected.<sup>5</sup>

This research is not in line with research conducted by (Nariswari, 2022), namely that poverty has a positive and significant effect on the total number of tuberculosis cases in Indonesia. The addition of 1% of poor people will increase the total number of tuberculosis sufferers by 0.6744342% of people.<sup>10</sup> Poverty is one of the main causes of the increasing number of tuberculosis cases, especially in developing countries. The lower a person's income or the poorer a person is, the possibility of contracting tuberculosis is very high because they cannot meet adequate health requirements. People with poor conditions have limitations in accessing health services and healthy housing. Poverty also causes a low ability to consume nutritious food, thereby increasing vulnerability to the transmission of tuberculosis.

#### *Spatial Autocorrelation of Healthy Home Coverage with TB Cases*

One indicator to measure environmental conditions is a healthy house. A healthy house is a residence that meets health requirements consisting of house components, sanitation facilities

and other behaviors such as having a healthy latrine, clean water and waste disposal facilities, a rubbish dump, a healthy latrine, good ventilation, appropriate housing density and flooring. the house is not made of land. Unsanitary home conditions will become a reservoir for the entire environment. The better the coverage of healthy homes in an area, the lower the risk of people becoming sick. The presence of TB germs or tuberculosis sufferers in a house with inadequate conditions will increase the number of contacts with germs or sufferers, causing infection. On the other hand, the condition of the house meets the healthy requirements will reduce the potential for the development of disease-causing germs and the occurrence of disease transmission.

The bivariate test results in this study show that in 2019-2021, There is no spatial correlation between population density and pulmonary tuberculosis cases in the city. The value of the Moran's index in this study during 2019-2020 was more than the expected value of the Moran's index  $I[E]$ , thus it can be concluded that the distribution pattern is spatially clustered. Based on the results of the 2021 bivariate test, it shows negative spatial autocorrelation, which means that adjacent areas are not similar or the distribution pattern is spatially diffuse. This is due to other factors that allow an explosion of pulmonary tuberculosis cases in community health center areas that have high coverage of healthy homes. Naioni Health Center has high coverage of healthy homes and low TB cases compared to other health centers. In 2020-2021, the Kupang City Health Center had quite low coverage of healthy homes but also had low cases of tuberculosis. This research is in line with research conducted by (Fitriyani & Sari, 2021). which showed that there was no significant relationship found between healthy home coverage and positive BTA pulmonary TB ( $p > 0.05$ ).<sup>5</sup> Other research conducted in East Java Province also showed that the healthy home coverage variable had no significant effect on the number of tuberculosis cases ( $p=0.322239$ ).<sup>10</sup>

This can also be caused by the assessment of healthy houses in the field, carried out on a number of houses, so that the coverage of healthy houses is considered unable to describe the overall condition of healthy houses in the research location.

### **Conclusion**

In 2019-2021, there was no positive spatial autocorrelation between population density, poor families, and healthy housing coverage and the incidence of pulmonary tuberculosis in Kupang City. However, based on the 2019-2021 Moran's I on the population density variable, poor families have a random relationship pattern. Meanwhile, the healthy home coverage variable has a clustered relationship pattern.

The Kupang City Health Service is expected to improve the quality of published data, especially regarding case data by separating cases originating from within and outside the region and conducting regular screening of smear positive pulmonary TB sufferers so that handling/control efforts become more effective and efficient. People are also expected to check themselves at a health service facility when they have symptoms of tuberculosis and always keep their house and surrounding environment clean. It is hoped that future researchers can conduct research with smaller analysis units such as villages or sub-districts and ensure the validity of the data used so that the research results are more valid. A more in-depth study needs to be carried out regarding other risk factors that are at risk of increasing cases of pulmonary tuberculosis.

### **Ethics approval**

This research has received ethical feasibility approval from the Health Service, Health Service, Research Ethics Committee, Faculty of Public Health, Nusa Cendana University, with number: 2023184-KEPK

### **Availability of data and materials**

Available

### **Acknowledgment**

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### **Author Contribution**

Researchers collected and analyzed secondary data using the QGIS and GeoDa applications. The author has read and approved the final manuscript.

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