

Hantavirus Infection on Rats in the Leptospirosis Increased Case Area in Tangerang Regency

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Abstract—Hantavirus infection and leptospirosis are a serious zoonotic disease that spread across the globe. They have similar clinical manifestations, in which the rat is the main reservoir of them. The aim of the study was to identify the percentage of Hantavirus infection and co-infection of Hantavirus and *Leptospira* in the rats in an area where increasing cases of leptospirosis occurred. This study was conducted in Tangerang Regency, Banten Province on October to November in 2016. The identification of the trapped rats and the samples were examined by using an immunology test (Elisa) and a molecular test (PCR) to find out the result of *Leptospira* and Hantavirus infection. A total of 52 rats were caught in the study. They consisted of 2 species (*Rattus norvegicus* and *Rattus tanezumi*). Hantavirus infections were found in 8 of the 48 rats while co-infection (Hantavirus and *Leptospira*) were found in 6 of the 48 rats. 16.7% of the rats were positively infected by Hantavirus. Meanwhile, the total co-infections with both Hantaviruses and *Leptospira* were 12.5%. Cautions should be given if *R. norvegicus* and *R. tanezumi* were found in one residential area.

Keywords: *Hantavirus, leptospirosis, Tangerang*

I. INTRODUCTION

Hantavirus infection was a serious zoonosis diseases, in which the cases were reported to have an increase in the last 10 years. Hantavirus infection is caused by RNA viruses, a member of a *Hantavirus* genus of the bunyviridae family. There are two types of clinical manifestations of hantavirus infection. They are Haemorrhagic fever with renal syndrome (HFRS) and Hantavirus Pulmonary Syndrome (HPS). Haemorrhagic fever with renal syndrome (HFRS) has been a major epidemic and has been reported to occur in Asia and Europe. However, most of Hantavirus Pulmonary Syndrome (HPS) cases were only reported in America. Several genotypes of Hantaviruses caused HFRS, including Hantaan, Seoul, Puumala, and Dobrova virus. They have

similar clinical manifestations like fever, myalgia, headache, acute kidney failure, thrombocytopenia, conjunctiva suffusion, and bleeding with leptospirosis. Seoul virus infection caused liver dysfunction. [1].

Leptospirosis is a worldwide public health problem, in which Indonesia is one of endemic country in South East Asia. The Case Fatality Rate of leptospirosis in Indonesia is recorded as the third biggest cases in the world (after India and Brazil) [2]. The increased leptospirosis cases are reported annually in some sites in Indonesia, especially in Java Island. However, most of the suspected cases could not be confirmed as leptospirosis cases because the lack of specific diagnostic resources. In addition, leptospirosis symptoms demonstrated similar clinical features with other infection diseases like hantavirus infection. This can lead to a wrong diagnosis or negative diagnosis reports with bad consequences for public health actions.

Rats are the main reservoir host of hantavirus and leptospirosis infection [3–5]. The transmission of hantavirus infection and leptospirosis is related to one's exposure with the rats and its feces [6]. *Hantavirus* was transmitted as aerosol to human through air contaminated with saliva, urine, or feces of infected rats [7,8]. Leptospirosis also transmits to human through water or soil contaminated with *Leptospira*. Based on the same study, there are 7 kinds of rats as the main reservoir hosts of *Hantavirus* and leptospirosis in Indonesia i.e. *R. norvegicus*, *R. tanezumi*, *R. exulans*, *R. argentiventer*, *R. tiomanicus*, *Bandicota indica*, and *Maxomys surifer* [5,9]. As epidemiologist and clinical manifestation they have similar symptoms. A specific study is needed to know the percentage of hantavirus infection on trapped rats and co-infection among two pathogens in high risk sites with increased leptospirosis cases for prevention and control. efforts.

II. METHOD

The study was carried out on October to November 2015 in Kronjo Village, Tangerang Regency, Banten (Picture 1). In 2015, there was an increase in leptospirosis cases in the study site [10]. Rats were trapped by using 100 live traps in two consecutive days. The traps were installed in the evening, starting at 17:00 and was taken in the following morning, starting at 06:00. Two live traps would be used in each house, put them in places like kitchen or bedrooms. There were 25 houses in total where live traps were placed. 50 traps were also placed outdoors or in the fields. There was 1 trap in 10 m² area. The bait used to trap the rats was roasted coconut.

The identification of the rats was done by considering the morphology and morphometric characteristics. The morphology characteristics included the color and the kind of the fur, the color of the tails, as well as the scales and fur of the tails. The morphometric characteristics were weight, total length, tail's length, soles of back feet's length, ears' length and shapes, the size of the skulls, and the number of nipples for female rats. The measurement results and observation were matched to the rat identification key.

Before the blood sample was taken, the rats were anesthetized with ketamine and xylasin. The blood sample collection was done intracardially with a 3 ml syringe and a 22 g needle. The blood sample taken was stored in vacutainer tubes and was centrifuged in 3,000 rpm speed for 5 minutes to separate the serum. The collected serum was taken using Pasteur pipettes and was stored in the sterilized crytubes. The serum was kept in the fridge with the temperature of 4 to 8°C.

Elisa examination was used to detect the existence of rats' antibody toward *Hantavirus*. Xpress-Bio Elisa kits was used. The test was performed by following the instruction made by the kit producers. The first step of PCR test is DNA isolation from kidney of rats using Promega wizard kit reagent. The test procedure used in this study was from reagent maker factory. After isolation, the DNA amplification method with PCR using specific primer for 16s rRNA gene using the pairing of primer rrs1 & rrs2, in the following sequence: F(rrs1): 5' CATGCAAGTCAAGCGGAGTA 3' and R(rrs2) 5' AGTTGAGCCCGCAGTTTT 3'. The result of PCR was detected using electrophoresis for observing the matching gene amplification with the target and formed the bands at agarose gel 1%. Positive result of amplification showed 523bp on electrophoresis bands.



Picture 1. Geographical location of tangerang regency, banten province

III. RESULTS

A total of 52 rats were trapped, which were *R. norvegicus* and *R. tanezumi*. Only 48 of 52 rats were examined by using a *Hantavirus* test because some of the rats could not be taken their blood. The result showed that 16.7% of the rats had *Hantavirus* infection and 12.5 % of the rats had *Hantavirus* and *Leptospira* infection or co-infection (Table 1).

TABLE 1. TEST RESULT OF RATS WITH *HANTAVIRUS* AND *LEPTOSPIRA*

Rats Species	<i>Hanta virus</i>	%	<i>Lepto-spira</i>	%	<i>Hanta virus and Lepto-spira</i>	%
	(n/N)		(n/N)		(n/N)	
<i>Rattus norvegicus</i>	8/30	26.7	14/34	41.1	6/30	20
<i>Rattus tanezumi</i>	0/18	0	2/18	11.1	0/18	0
All	8/48	16.7	16/52	30.8	6/48	12.5

*n/N = no positive/no. tested

IV. DISCUSSION

The study showed that positive *Hantavirus* rats were only found in *R. norvegicus* (26.7%) and there was none (0%) in *R. tanezumi*. Positive *Hantavirus* found in *R. norvegicus* in the study site is higher than the number found in other studies performed in some countries in the world. The prevalence rate of hantavirus infection in *R. norvegicus* was reported in between 6.3% to 17.8% [11–13]. However, the result of study in Indonesia revealed various results. The percentage of positive hantavirus infection on *R. norvegicus* was between 8.2% to 44.7% [5,14,15]. Higher percentage of hantavirus infection on *R. norvegicus* in the study site showed that the site had a high risk for *Hantavirus* transmission. There is a positive correlation between prevalence reservoir and hantavirus infection cases on humans [16].

Rattus norvegicus is the natural host of Seoul virus (SEOV), which is one of strain viruses that spreads worldwide. SEOV infections have been reported in Asia, Europe, America, and

Africa. Recently, SEOV outbreak has also been found in the United Kingdom among rat breeders and pet sellers. In 2017 in the United States, SEOV infections outbreak occurred in 11 states with 17 positive SEOV infected cases [17]. SEOV was identified in Indonesia, too. A SEOV infections was found in a Germany tourist who came to Sulawesi in 2017 [18]. In 2018, SEOV infections also infected patients in Soetomo and Dr Cipto Mangunkusumo Jakarta Hospital [19]. SEOV was classified as the cause of moderate HFRS with a CFR of 1-2% in comparison to severe HFRS that was caused by DOBV and Hantaan virus (HTNV), with the CFR > 10% [20].

The study of Joharina et al in 2018 showed the percentage of positive *Leptospira* rats of 30.8% [10]. *Leptospira* infection in *R. norvegicus* was 41.1% and in *R. tanezumi* was 11.1%. Based on this result, the rate of *Leptospira* infection occurred in rats was more than 30% (very high) [21]. The difference of *Leptospira* prevalence in rats depends on the location [22]. The prevalence rate of *Leptospira* found in *R. norvegicus* in urban area was between 7% to 82%, whereas in *R. tanezumi* was between 7% to 34% [23,24]. They were the main reservoir of leptospirosis in urban slums area.

The study showed that the co-infection on *Hantavirus* and *Leptospira* found in *R. norvegicus* was 12.5%, and has been reported in Croatia, Brazil, and Maumere, Indonesia as co-infection cases [15,25,26]. It could be transmitted to human if at the same time, there were *Hantavirus* and *Leptospira* co-infection in *R. norvegicus* that caused acute clinical symptoms on humans [27]. Co-infection on humans was identified in Croatia and Sri Lanka, where the outbreak of leptospirosis showed co-infection on 7 of 31 patients with acute clinical symptoms [28].

The discovery of positive *Hantavirus* rats was higher in increased leptospirosis sites, which meant that serious attention was required. A preventive efforts and additional knowledge for the medical staff to detect the two diseases were highly needed. The high prevalence and restricted to co-infection at the leptospira sites caused a variety of nonspecific clinical symptoms that were similar to hantavirus. Specific medical examinations for suspected leptospirosis patients should be regarded as infected to either *Hantavirus* and/or co-infection. In Kandy, Sri Lanka, 8 of 105 suspected leptospirosis patients were reported to have positive results to Thailand virus, which was one of *Hantavirus* strains [29].

VI. CONCLUSION

16.7% of the rats were positive of *Hantavirus* while co-infections with both *Hantaviruses* and *Leptospira* were found at 12.5%. It should be given if *R. norvegicus* and *R. tanezumi* were found in one residential area. Both of diseases had similar clinical manifestations so we had to consider different diagnosis for leptospirosis patients with *Hantavirus* infection.

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