# Deteksi *Leptospira* Patogen secara Molekuler dan Histopatologi pada Tikus Liar di Kabupaten Bantul, Daerah Istimewa Yogyakarta

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## INTISARI

Leptospirosis yang merupakan salah satu penyakit zoonosis tersebar di seluruh dunia adalah penyakit yang disebabkan oleh bakteri *Leptospira*. Indonesia memiliki angka insidensi leptospirosis tinggi. Keberadaan tikus liar sebagai *reservoir* diduga berperan penting dalam penyebaran penyakit tersebut. Tujuan dari penelitian ini adalah mendeteksi *Leptospira* patogen pada tikus liar di Kabupaten Bantul Daerah Istimewa Yogyakarta (DIY) dengan uji molekuler secara *Polymerase Chain Reaction* (PCR) berdasarkan gen LipL32, mengetahui gambaran filogenetik spesies *Leptospira* yang patogen di Kabupaten Bantul berdasar sekuen gen LipL32, dan mengetahui histopatologis ginjal, hati, dan paru tikus liar yang terinfeksi alami *Leptospira* di Kabupaten Bantul. Pengambilan sampel tikus liar dilakukan di Kecamatan Sewon dan Pandak, Kabupaten Bantul. Tikus ditangkap dengan perangkap tikus (*single live trap*) selama 14 hari berturut-turut di masing-masing kecamatan. Tikus tertangkap kemudian dieutanasi dan dinekropsi. Ginjal, hati, dan paru tikus diambil untuk dilakukan uji PCR dan histopatologi menggunakan pewarnaan Hematoksilin-Eosin. Alkohol 70% digunakan untuk memfiksasi spesimen ginjal pada uji PCR, sementara *Neutral buffered formalin* (NBF) 10 % digunakan sebagai larutan fiksatif spesimen ginjal, hati, dan paru pada pemeriksaan histopatologi. LipL32 dengan 2 urutan basa yang berbeda digunakan sebagai primer pada uji PCR, dengan rincian yaitu Primer 1 menggunakan forward 5'-ATC TCC GTT GCA CTC TTT GC-3' dan reverse 5'-ACC ATC ATC ATC ATC GTC CA-3', menghasilkan produk 474 bp dan Primer 2 menggunakan forward 5'- GGA CGG TTT AGT CGA TGG AA -3' dan reverse 5'- GGG AAA AGC AGA CCA ACA GA -3', menghasilkan produk 498 bp. Tikus dan cecurut tertangkap dengan jumlah total 19 ekor, terdiri dari empat jenis yaitu tikus rumah (*Rattus tanezumi*) 9 ekor, tikus got (*Rattus norvegicus*) 7 ekor, tikus wirok (*Bandicota indica*) 2 ekor, dan cecurut (*Suncus murinus*) 1 ekor. *Leptospira* patogen ditemukan pada tikus liar di Kabupaten Bantul sebanyak 15,8% dari 19 ekor berdasarkan uji molekuler (PCR). Berdasar sekuen gen LipL32, *Leptospira* yang ditemukan pada tikus liar di Kabupaten Bantul, DIY adalah *Leptospira interrogans,* dan berkerabat dekat dengan *Leptospira interrogans* patogen asal Negara Amerika dan beberapa Negara di Asia. Infeksi alami *Leptospira interrogans* menyebabkan perubahan histopatologis berupa kongesti, perivaskulitis, nekrosis tubulus, dan nefritis interstitialis pada ginjal; kongesti, degenerasi vakuolar, nekrosis, dan hepatitis pada hati; serta kongesti, dan pneumonia interstitialis pada paru, namun tidak patognomonik.

**Kata Kunci** : *Leptospira* patogen, Tikus, *Polymerase Chain Reaction*, Histopatologi.

# Detection of Pathogenic *Leptospira* by Molecular and Histopathology Method in The Wild Rat in District Bantul Regency, Yogyakarta

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## ABSTRACT

Leptospirosis, one of the zoonotic diseases spread throughout the world, is a disease caused by *Leptospira*. Indonesia has a high incidence of leptospirosis. The presence of wild rats as a reservoir is thought to play a pivotal role in the spread of the disease. The purposes of this study were to detect pathogenic *Leptospira* in wild rats at Bantul Regency, Special Region ofnYogyakarta, using Polymerase Chain Reaction (PCR) molecular test based on LipL32 gene sequence, to find out phylogenetic feature of pathogenic *Leptospira* species at Bantul district based on LipL32 gene squence, and to observe histopathological alterations of kidney, liver, and lung of wild rats were naturally infected by *Leptospira* at Bantul Regency. Wild rat samples were taken in Sewon and Pandak Districts, Bantul Regency. Rats were caught in single live traps for 14 consecutive days in each district. The trapped rats were then euthanized and necropsied. Kidney, liver, and lung specimens of the rats were taken for two tests, PCR and histopathology using *Hematoxylin-Eosin* staining. Alcohol 70% was used for the fixation of renal specimens on the PCR test, whilst neutral buffered formalin (NBF) 10% was used for fixative reagent for histopathological method. LipL32 with 2 different base sequences was used for the primers on PCR test, with the details primer 1 using forward 5 '-ATC TCC GTT GCA CTC TTT GC-3' and reverse 5 '-ACC ATC ATC ATC ATC GTC CA-3', producing 474 bp products, and primer 2 using forward 5 '- GGA CGG TTT AGT CGA TGG AA -3' and reverse 5'- GGG AAA AGC AGA CCA ACA GA -3', producing 498 bp products. In this study, rats and shrews were caught with a total of 19 rats, consisting of four types which were 9 rats of home rats (*Rattus tanezumi*), 7 rats of sewer rats (*Rattus norvegicus*), 2 rats of wirok rats (*Bandicota indica*), and 1 rat of shrews (*Suncus murinus*). Pathogenic *Leptospira* were found in wild rats at Bantul district as much as 3 rats (15,8%) of 19 wild rats based on molecular tests (PCR). Based on the LipL32 gene sequence, *Leptospira* found in wild rats at Bantul Regency is *Leptospira interrogans* and is closely related to the pathogenic *Leptospira interrogans* from America and several countries in Asia. Natural infections of *Leptospira interrogans* caused histopathological changes such as congestion, perivasculitis, tubular necrosis, and interstitial nephritis on renal specimens; congestion, vacuolar degeneration, necrosis, and hepatitis on liver specimens; congestion and interstitial pneumonia on lung specimens, although all the found lesions were not pathognomonic.

**Keywords**: *Leptospira* pathogen, Rat, Polymerase Chain Reaction, Histopathology