Prevalence of Trypanosomiasis of Wild Rats (Rattus sp.) in Banjarnegara District and Potential Impact for Public Health

Prevalensi Tripanosomiasis Pada Tikus Liar (Rattus sp.) di Kabupaten Banjarnegara dan Pengaruhnya terhadap Kesehatan Masyarakat

Try Wijayanti*, Novia Tri Astuti1, Nuri Alfino Qur’ana1, Edi Surahman2, Jarohman Raharjo1, Bina Ikawati1, Didik Tulus Subekti2, Hari Ismanto1
1Balai Penelitian dan Pengembangan Kesehatan Banjarnegara Jalan Selamanik Nomor 16 A, Banjarnegara, Jawa Tengah, Indonesia
2Balai Besar Penelitian Veteriner, Kementerian Pertanian, Bogor Jalan R.E. Martadinata Haji Bin Ali Nomor 30, Kota Bogor, Jawa Barat, Indonesia
*E_mail: tri.wijayanti.76@gmail.com

Received date: 31-08-2021, Revised date: 12-05-2022, Accepted date: 27-06-2022

ABSTRACT
Trypanosomiasis is a zoonotic disease caused by Trypanosoma sp., a protozoan parasite that has a flagellum. It has the potential to cause emerging diseases. Generally, Trypanosoma infection is caused by T. evansi which causes Surra disease, and T. cruzi which causes Chagas disease. Trypanosoma lewisi has been considered a natural protozoan in mice, not pathogenic to humans but in recent years it has been reported in humans. This study aims to detect Trypanosoma in rats in Banjarneanga District and analyze the potential impact on public health. The research was observational with a descriptive approach, conducted in Banjarnegara from July-December 2020. Samples were taken by purposive sampling. Samples are rat’s blood that caught on wild rats survey in the main market of Banjarnegara District. Blood samples were made with a thin smear then th...
INTRODUCTION

Trypanosomiasis is a disease caused by *Trypanosoma* sp., a protozoan parasite that has a flagellum. The genus *Trypanosoma* is widespread, consisting of blood parasites that can infect all classes of vertebrates, causing several human and livestock diseases, especially in the tropics. There are two main groups of *Trypanosoma*, Salivaria and Stercoraria, according to their main mechanism. The most representative example for Stercoraria is *Trypanosoma cruzi* which completes its life cycle in the posterior section of insects. On the other hand, species from the Salivaria group complete their life cycle in the anterior section, which is the case for *T. brucei*.

The majority of zoonotic parasites are *T. brucei* ( subspecies *gambiense* and *T. brucei* subspecies *rhodesiense*) causing sleeping sickness in sub-Saharan Africa and *T. cruzi* causing Chagas disease in Latin America. The other *Trypanosoma* species, such as *T. lewisi*, *T. brucei* subspecies *brucei*, *T. congolense*, and *T. evansi*, can also cause disease in humans, although they are rare. The majority of atypical human infections are caused by pathogenic *T. lewisi* and *T. evansi* which can even cause death.

Research on trypanosomiasis in wild rats in several countries has been reported, for example in Brazil. *Trypanosoma lewisi* was observed in 21.7% of *Rattus norvegicus* (93 out of 429). The infection rate was different in both sexes, with 24.9% (68/273) being detected for males and 16% (25/156) for females. The monthly prevalence and infection rates of *T. lewisi* are related to seasonal periods. Rats presented significantly higher prevalences of infection in the warm, rainy season than in the winter, dry period. The highest values recorded in October (48.1%) and November (39.2%) coincide with the highest levels of infestation by *X. cheopis* flea in the municipality. A study in Abu Rawash, Giza, Egypt November 2016-October 2017 showed that 19 out of 117 (15.8%) *R. norvegicus* infected with *T. lewisi*. A survey in Malang, East Java Province, Indonesia from August to September 2020 showed that the *T. lewisi* infection rate in wild rats was 17.5%.

So far, there is no case fatality rate of human trypanosomiasis in Indonesia. Trypanosomiasis due to *T. evansi* (Surra disease) is endemic in Southeast Asia. It occurs on almost all major islands in Indonesia. *Trypanosoma evansi* can attack various types of livestock and wildlife, causing economic losses and farmers losing their jobs. Endemic areas for Trypanosomiasis in Indonesia are Bali, Sumbawa, Java (Pemalang, Banten), and Madura. An outbreak of Surra disease was reported in Banten in 2014, which caused several kinds of livestock to die. In Indonesia, cases of *T. evansi* in animals in recent years include those at Bali Cattle Superior Livestock Breeding Center in Jembrana, Bali. The prevalence of *T. evansi* in Bali cattle was 2% and in Bima in horses was 3.3%. Previously, the largest Surra cases occurred on Sumba Island during 2010-2012. Surra caused the death of around 2000 livestock (buffalo, cattle, and horses). The majority of them are horses. This condition can endanger the health of breeders. Examination of serum samples of 24 farmers in Southwest Sumba (22 men and 2 women) collected during the Surra outbreak in 2012 showed that 4 (16.7%) of them had antibodies to *T. evansi*. The potential for Surra and other species of *Trypanosoma* is quite high in Indonesia, while Thailand, Vietnam, and Malaysia have reported human trypanosomiasis. *Trypanosoma* vectors are fleas and flies. The mechanical vector of *T. cruzi* is *Ornithonyssus bacoti* (tick). The reservoir of *Trypanosoma* is a rat.

Research on trypanosomiasis in rats in Indonesia is still very limited. There isn’t any trypanosomiasis research in the Banjarnegara District. Therefore, it is necessary to know about trypanosomiasis in wild rats and its potential for public health in the Banjarnegara District.
Prevalence of Trypanosomiasis…(Wijayanti, et al)

METHODS
This study was an observational design with a descriptive approach. The research was conducted in the Banjarnegara District in July-November 2020. Samples were taken purposively. Data collection was carried out by rat survey in the main market of Banjarnegara District as many as four times. Each of the rat surveys was carried out for 4 days using 300 single-live traps with grilled coconut or salted fish bait. Rats were caught sedated with atropine and xylazine and then identified by morphology. Their blood was taken using a 3 mL syringe and stored in an EDTA tube. The blood samples were made into thin blood smears and then stained with Giemsa. All of the thin blood smears were examined microscopically at 1000x magnification. The data was analyzed by descriptive.

RESULTS
There were 157 rats caught during the study, consisting of 131 Rattus norvegicus (brown rats) and 26 R. tanezumi. This study not only examined rats as samples, but 12 Suncus murinus (shrews) were also examined (Figure 1).

There were 28 rats that were positive Trypanosoma sp., so the Trypanosoma infection rate in rats in the main market of Banjarnegara District is 16.57%. Trypanosomiasis in Rattus norvegicus was 18.3% and in R. tanezumi it was 15.38%. The Trypanosoma species could be identified as T. lewisi. It can be seen that the kinetoplast looks large, with the nucleus more towards the posterior. Extracellular hemoflagellates illustrated in Figure 2 have slender bodies with oval-shaped nuclei placed internally at the anterior-posterior junction of the body. There were no granular inclusions in the parasites. Meanwhile, none of the Suncus murinus caught was positive for Trypanosoma sp. However, molecular study was not done to establish the species of the trypanosome as the parasites were detected in its natural host.
DISCUSSIONS

*Trypanosoma lewisi* is the causative agent of murine trypanosomiasis in domestic rats throughout much of the world. Trypanosoma (Herpetosoma) species share common morphological features and hosts. These common characteristics are sufficient to distinguish species from different groups, although morphology itself is not adequate for differentiating species within groups. *Trypanosoma* (Herpetosoma) species that infect rodents of the Murinae subfamily (*T. grosi, T. lewisi,* and *T. musculi*) can be distinguished by their epimastigote phase, which occurs in the peripheral blood, whereas those that infect Microtinae rodents (*T. evotomys* and *T. microti*) can be distinguished by the amastigote phase, as they reproduce in the lymphoid tissue; amastigotes are never observed in the peripheral blood.

The host of *T. lewisi* is *Murinae* (rat), with the main site of reproduction in the kidney, a 3-7 day incubation period, and a 2-4 week duration of infection. Fleas species as vector of *T. lewisi* is *Nosopsyllus fasciatus* with rectum as site for development of *Trypanosoma*.1

There is a relationship between *T. lewisi* and the presence of *Xenopsylla cheopis* and *Polypax spinulosa* fleas as mechanical vectors of trypanosomiasis in Durban, South Africa, in areas with a poor environment, dirty and readily available food, which allows high transmission of fleas and trypanosomiasis, thus posing a threat to general public health. At least 10 species of trypanosomes are transmitted to rodents by fleas. Other rodent trypanosomes with confirmed flea transmission cycles include *T. musculi* (synonym: *T. duttoni*) of house mice, *T. rabinowitschi* of hamsters, *T. neotomae* of wood rats, and *T. grosi* of the European wood mouse (*Apodemus sylvaticus*).13

Wildlife, while generally immunotolerant to trypanosomes, however, develops clinical trypanosomiasis and shows varying levels of trypanotolerance among species. Trypanotolerance is influenced by multiple host intrinsic (age, sex, species, physiological state, state of nutrition) and extrinsic factors (temperature, humidity, nature of vegetation, nature of wildlife communities).15

This study showed the prevalence of *T. lewisi* in wild rats caught in the main market in Banjarnegara District is 16.57%. This prevalence is greater than previous studies in Banyuwangi (1.67%) and Surabaya (7.9%), but the type of rats is unknown.16 The rat samples in Banyuwangi were obtained from residential areas, markets, and rice fields. In this study, all wild rat samples were obtained from the main market in Banjarnegara District. The other research on trypanosomiasis showed infection of *T. lewisi* and *T. lewisi*-like trypanosomes were found in the blood smears of *Bandicota indica* (two rats), *Rattus argentiventer* (one rat), and *Rattus tiomanicus* (two rats).17

Sulawesi has a remarkable biodiversity and complex geology that has attracted biologists and geologists to study the area since the 19th century (e.g. Wallace, 1860).18
Prevalence of Trypanosomiasis…(Wijayanti, et al)

Sulawesi is a globally significant biodiversity hotspot for terrestrial vertebrates that is also likely to support substantial undocumented parasite diversity. Research on rodents was held on two (2) mountains (Mount Latimojong and Mount Bawakaraeng) in South Sulawesi in August–October 2016. *Trypanosoma* was detected in 151 of 441 (34.2%) rodents by PCR detection. *Trypanosoma theileri* was recovered from 117 individuals representing 8 host species and had a significantly higher mean prevalence of 26.5% compared to *T. lewisi*, which was detected in only 34 individuals from 6 species for a mean prevalence of 7.7%. The host species infected by *T. theileri* included 3 species of Bumomys (Bumomys coelestis, Bumomys penitus, and Bumomys torajae), 2 species of native Rattus (Rattus bontanus, Rattus facetus), Maxomys musschenbroekii, Paruromys dominator, and Paucidentomys vermidax. The host species infected by *T. lewisi* were similar but not identical and included 3 species of Bumomys (B. coelestis, B. penitus, and B. torajae), 2 species of native Rattus (R. bontanus and R. mollicomulus), and 1 species of introduced Rattus.

Several countries have reported trypanosomiasis in rats. In Europe, *T. lewisi* in rats was reported in Finland (*Rattus norvegicus, R. rattus*), Norway (*Clethrionomys glareolus, Microtus agrestis, Apodemus sylvaticus*), Poland (*Rattus norvegicus*), the United Kingdom (*R. norvegicus*), and Spain (*R. norvegicus, R. rattus*). A study in Abu Alnomros Center, Giza, from November to March 2016 showed trypanosomiasis prevalence among the different rodent species was (21 rats) 24.7%. All the infected rats belonged to *R. rattus* spp., where the prevalence of infection with *T. lewisi* among that species was very high, 51.2%. None of *R. norvegicus* were infected. All the positive rats were collected indoors (from houses), and all the rats that were captured from outdoors (farms) were negative for *T. lewisi*.

Research in Venezuela shows the prevalence of *T. lewisi* in *R. norvegicus* in Maracay slum areas was 6.3% and in *R. rattus* it was 31.1%, while the prevalence of *T. cruzi* in *R. norvegicus* was 10.5% and in *R. rattus* it was 24.6%. The prevalence of *T. lewisi* in the salivary glands of fleas was 76% and that of *T. cruzi* was 21.3%, indicating the large role of fleas as a vector of trypanosomiasis. Rats that play a role in the transmission of *T. cruzi* and *T. evansi* in Brazil are Clyomys laticeps, Thrichomys pachyurus, and Oecomys manorae.

*Trypanosoma* research in small mammals in three districts of Cotonou, Benin from the end of 2016 until 2017 found 369 small mammals (i.e., 251 *Rattus rattus*, 18 *R. norvegicus*, 56 *Mastomys natalensis*, 2 *Praomys derooi*, 5 *Cricetomys* sp., and 37 *Crocidura* sp.). Prevalences of *Trypanosoma*-carrying individuals using qPCR were found to be very high with 57.2% (211 animals). Identify *T. lewisi* using 16SDNA sequencing-based was found in 39.02% (144 animals). A survey of 1.298 commensal rodents from 20 cities and villages in Niger and Northern Nigeria showed the prevalence of *Trypanosoma* spp. was 14.6% qPCR positive. Presence of *T. lewisi* over all rat individuals tested is 27.4% and up to 68.8% locally.

This research found none of the *S. murinus* positive *Trypanosoma*. Previous research in Cambodia confirmed 27.6% (8 of 29) *Suncus murinus* (shrew) positivity for *T. lewisi*.

Surveys of rodents from May 2014 to February 2017 across New Orleans, USA at 98 trapping sites in 11 study area were got 1,428 animals (norway rat, roof rat, house mouse, and cotton rat). Molecular investigation showed the prevalence of *T. cruzi* in all host species was 11%, which included all positive rats from New Orleans but not in Baton Rouge.

A new public health problem arises from atypical human trypanosomiasis. Although humans have an innate protection against most *Trypanosoma* species, nineteen (19) cases of atypical human trypanosomiasis caused by the animal trypanosomes *T. b. brucei, T. vivax, T.
congolense, T. evansi, and T. lewisi have been recorded.\textsuperscript{21}

The presence of T. evansi in humans in Southeast Asia has been proven microscopically, serologically, and by PCR in Southern Vietnam. A case of trypanosomiasis occurred in a 38-year-old woman who came to a health care facility with a fever, headache, and joint pain. A survey of animals around the patient showed that 14 out of 30 (47\%) blood samples from cattle and buffalo were positive for T. evansi by PCR.\textsuperscript{2}

In theory, human infection with Trypanosoma in animals or livestock cannot occur because of the presence of trypanolytic apolipoprotein L1 in human serum. India is an endemic country for T. evansi in livestock or T. lewisi in mice. This country has reported several cases of T. evansi in immunocompetent adult humans in Nagpur Village since 2005. Trypanosoma evansi seropositivity in the village was reported to be 4.5\% by card agglutination test (CAT), although parasitemia was not found in the blood smear. The most recent case was reported in 2018. The patient is a 2-month-old baby boy who comes from a farming family with middle to lower socioeconomic conditions in Amreli, Gujarat. He has had a fever for 5 days with a history of 2 weeks before being bitten by insects or fleas (generally in livestock) on the axilla, causing local swelling. The baby becomes pale and hepatomegaly. Examination of blood smears and PCR shows the presence of T. lewisi.\textsuperscript{28} Although T. lewisi has been considered non-pathogenic to humans, during the period 2007-2012, there were 3 human cases of fever caused by T. lewisi in infants in Southwest India. Trypanosoma lewisi is also found in 2 out of 10 Rattus norvegicus in India.\textsuperscript{12}

There have been 9 cases of T. lewisi infection in humans worldwide. They were reported from Malaysia, India, Gambia, and Thailand. Patients are often babies (immunity is still low), live in areas with low hygiene conditions, and have close contact with infected rats around their house. Patients' clinical symptoms are generally mild.\textsuperscript{29} Doke and Far in 2011 reported that among the cases of atypical human trypanosomiasis, one patient died from a T. lewisi infection, sending out the message that this form of trypanosomiasis could also be fatal.\textsuperscript{30}

So far, there have been no reports of trypanosomiasis cases in humans in Indonesia. The research on human trypanosomiasis in Southwest Sumba District (Wewewa Barat, Kodi Bangedo, and Loura) showed 16.7\% positive antibody against T. evansi that supported the other research that found 38\% seropositive in livestock. This condition can indicate an active infection of T. evansi in humans and livestock in the region.\textsuperscript{10}

Patients with immunosuppression conditions or receiving organ transplants are susceptible to zoonotic infection.\textsuperscript{12} Patients with cancer and HIV/AIDS are humans with immunosuppression. The increasing number of people living with HIV/AIDS in Indonesia is something that needs to be considered. Central Java Province reported the highest AIDS cases in 2019 and in Januari–Maret 2021, with 1,613 cases and 307 cases, respectively.\textsuperscript{31,32} Cases of AIDS in Banjarnegeara District tended to increase from 2015–2019 with a number of new AIDS cases of 13, 11, 36, 64, and 70, respectively.\textsuperscript{33} On the other hand, the incidence of cancer in Indonesia (136.2/100,000 population) ranks 8\textsuperscript{th} in Southeast Asia, while in Asia it ranks 23\textsuperscript{th}.\textsuperscript{34}

The presence of rats as reservoirs for T. lewisi and fleas as vectors, human susceptibility to zoonotic infections, climate change, travel and migration of people and animals, and transportation of goods that are supported by poor sanitary hygiene conditions in Banjarnegeara District may be potential factors for trypanosomiasis transmission to humans.

CONCLUSION

Trypanosomiasis in wild rats in Banjarnegeara District’s main market is 16.57\%. Trypanosomiasis in R. norvegicus was 18.3\% and in R. tanezumi it was 15.38\%. The species of Trypanosoma is T. lewisi. It
needs awareness of trypanosomiasis transmission from rats to humans.

**RECOMMENDATION**

There is a need to increase awareness of trypanosomiasis transmission as a zoonotic disease. Some activities that can be done to realize it include restricting rats’ access to food in the market (one of them with waste management), traders and market visitors paying attention to personal hygiene, and periodically cleaning the water tunnel or drainage in the market area, which can be coordinated by the “Unit Pelaksana Teknis Daerah (UPTD) Pasar”.

**AUTHOR CONTRIBUTION**

The main contributors in this article are TW and DTS, who contribute as conceptors, data analysts, and article writers. The co-contributors are BI, NTA, NAQ, ES, and JR, who contributed to data collection and article correction.

**ACKNOWLEDGMENT**

The authors would like to thank the Head of Banjarnegarra Health Research and Development Unit for motivating us to write this article and the technicians who supported the implementation of the survey. We would also like to extend our sincere thanks to drh. Didik Tulus Subekti, M.Kes (Indonesian Research Center for Veterinary Science, Bogor), who was a guide in writing this article.

**REFERENCES**

1. Alharbi B. Arthropod-borne infections in the United Kingdom and Saudi Arabia. University of Salford Manchester; 2018.
2. Van Vinh Chau N, Buu Chau L, Desquesnes M, Herder S, Phu Huong Lan N, Campbell JI, et al. A clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosoma evansi* in Southeast Asia. Clin Infect Dis. 2016;62(8):1002–8. https://doi.org/10.1093/cid/ciw052.
3. Novita R. Kajian potensi tripanosomiasis sebagai penyakit zoonosis emerging di Indonesia. J Vektor Penyakit. 2019;13(1):21–32. doi:10.22435/vektorp.v13i1.934.
4. Linardi PM, Botelho JR. Prevalence of *Trypanosoma lewisi* in *Rattus norvegicus* from Belo Horizonte, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz, Rio Janeiro. 2002;97(3):411–4.
5. Mohammed ES, Kady AM El, Youseef AG, Hassan AA. Distribution pattern of *Trypanosoma lewisi* in (*Rattus norvegicus*) in Egypt. Biomed J Sci Tech Res. 2018;8(4):6636–9. doi:10.26717/BJSTR.2018.08.001688.
6. Yesica R, Holizah YN, Pratiwi H, Hardian AB, Kusumariini S, Wisesa IBGD. Data prevalensi, pemetaan spasial, analisis morfologi, dan morfometrik *Trypanosoma lewisi* pada tikus liiar di Malang. Acta Vet Indones. 2022;10(1):71–9. doi:10.30742/avi.v10i1.548.
7. Kurnianto A, Pratama JWA, Candrarisna M. Pengaruh infeksi *Trypanosoma evansi* terhadap kadar TNF-α dan perubahan histopathologi hepar pada tikus putih (*Rattus norvegicus*). J Ilm Kedokt Wijaya Kusuma. 2019;8(1):26–39. doi:10.37506/v10/i12/2019/ijphrd/192135.
8. Sari MD, Setyaningrum E, Rosa E, Sutyrso. Identifikasi ektoparasit pada tikus (*Rattus sp.*) sebagai vektor penyakit pes di Areal Pelabuhan Panjang Kota Bandar Lampung. J Med Malahayati. 2020;4(2):120–8.
12. Rayat CS, Vashista R. Wild rats as reservoir of *Trypanosoma Lewisi* in Northwest India. Austin J Pathol Lab Med. 2014;1(2):1–3.

13. Durden LA, Hinkle NC. Fleas (Siphonaptera). In: Medical and Veterinary Entomology. 2019. p. 145–69. doi:10.1016/B978-0-12-814043-7.00001-7.

14. Archer CE, Schoeman MC, Appleton CC, Mukaratirwa S, Hope KJ, Matthews GB. Predictors of *Trypanosoma lewisi* in *Rattus norvegicus* from Durban, South Africa. J Parasitol. 2018;104(3):187–95. doi:10.1645/17-92.

15. Kasozi KI, Zirintunda G, Sempijja F, Buyinza B, Alzahrani KJ, Matama K, et al. Epidemiology of trypanosomiasis in wildlife—implications for humans at the wildlife interface in Africa. Front Vet Sci. 2021;8(June):1–15. doi:10.3389/fvets.2021.621699.

16. Afifillia Z, Suwanti LT, Sudjarwo SA, Koeswanto S, Yunus M, Plumeriastuti H. Prevalence of trypanosomiasis of wild rats (*Rattus* sp.) in Banyuwangi. J Parasite Sci. 2017;1(2):39–42. doi:10.20473/jops.v1i2.16283.

17. Mafie E, Saito-Ito A, Kasai M, Hatta M, Rivera PT, Ma X-H, et al. Integrative taxonomic approach of trypanosomes in the blood of rodents and soricsids in Asian countries, with the description of three new species. Parasitol Res. 2019;118(1):97–109. doi:10.1007/s00436-018-6120-3.

18. Nugraha AMS, Hall R. Late Cenozoic palaeogeography of Sulawesi, Indonesia. Palaeogeogr Palaeoclimatol Palaeoecol. 2018;490(November 2017):191–209. doi:10.1016/j.palaeo.2017.10.033.

19. Winterhoff ML, Achmadi AS, Roycroft EJ, Handika H, Jaya Putra RT, Rowe KMC, et al. Native and introduced trypanosome parasites in endemic and introduced murine rodents of Sulawesi. J Parasitol. 2020;106(5):523–36. doi:10.1645/19-136.

20. Magri A, Galuppi R, Fioravanti M. Autochthonous *Trypanosoma* spp. in European mammals: A brief journey amongst the neglected trypanosomes. Pathogens. 2021;10(3). doi:10.3390/pathogens10030334.

21. Daheš SMA, Mikhail MW. Surveillance of *Trypanosoma* spp. of rodents and studies in their transmission probability by fleas in some rural egyptian areas. J Egypt Soc Parasitol. 2016;46(1):157–66. doi: 10.12816/0026161.

22. Garcia HA, Rangel CI, Ortiz PA, Calzadilla CO, Coronado RA, Silva AJ, et al. Zoonotic Trypanosomes in rats and fleas of Venezuelan slums. EcoHealth. 2019;16:523–33. doi: 10.1007/s10393-019-01440-4.

23. Wardhana AH. Surra: Trypanosomiasis pada ternak yang berpotensi sebagai penyakit zoonosis. Wartazoa. 2018;28(3):139–51.

24. Dobigny G, Gauthier P, Houéméou G, Dossou HJ, Badou S, Etoubéché J, et al. Spatio-temporal survey of small mammal-borne Trypanosoma lewisi in Cotonou, Benin, and the potential risk of human infection. In: 14th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID); 2018 Nov; Spain: Elsevier; 2018.

25. Tatard C, Garba M, Gauthier P, Himia K, Artige E, Dossou DKJH, et al. Rodent-borne *Trypanosoma* from cities and villages of Niger and Nigeria: A special role for the invasive genus *Rattus*?. Acta Trop. 2017;171:151–8. doi: 10.1016/j.actatropica.2017.03.027.

26. Pumhom P, Pognon D, Yangtara S, Thaprathorn N, Milocco C, Douangboupha B, et al. Molecular prevalence of *Trypanosoma spp.* in wild rodents of Southeast Asia: influence of human settlement habitat. Epidemiol Infect. 2014;142(6):1221–30. doi:10.1017/S0950268813002161.

27. Gheresi BM, Peterson AC, Gibson NL, Dash A, Elmayan A, Schwartzenburg H, et al. In the heart of the city: *Trypanosoma cruzi* infection prevalence in rodents across New Orleans. Parasites and Vectors. 2020;13(577):1–10. doi:10.1186/s13071-020-04446-y.

28. Bharodiya D, Singhal T, Kasodaria G, Banerjee P, Garg R. Trypanosomiasis in a young infant from Rural Gujarat, India. Indian Pediatr. 2018;55:69–70.

29. Cassan C, Diagne CA, Tatard C, Gauthier P, Dalecky A, Ba K, et al. *Leishmania major* and *Trypanosoma lewisi* infection in invasive and native rodents in Senegal. PLoS Negl Trop Dis. 2018;12(6):e0006615. doi:10.1371/journal.pntd.0006615.

30. Li SJ, Zhang X, Lukeš J, Li BQ, Wang JF, Qu LH, et al. Novel organization of mitochondrial
minicircles and guide RNAs in the zoonotic pathogen *Trypanosoma lewisi*. Nucleic Acids Res. 2020;48(17):9747–61. doi:10.1093/nar/gkaa700.

31. Pusat Data dan Informasi Kementerian Kesehatan. Infodatin [Internet]. Kementerian Kesehatan Republik Indonesia. Jakarta; 2020. Available from: https://pusdatin.kemkes.go.id/resources/download/pusdatin/infodatin/infodatin-2020-HIV.pdf.

32. Direktorat Jenderal P2P. Laporan Perkembangan HIV AIDS & penyakit infeksi menular seksual (PIMS) triwulan I Tahun 2021 [Internet]. [cited 2022 May 5]. Available from: https://hivaids-pimsindonesia.or.id/download.

33. Dinas Kesehatan Kabupaten Banjarnegara. Profil kesehatan Kabupaten Banjarnegara 2019. Banjarnegara: Dinas Kesehatan Kabupaten Banjarnegara; 2020.

34. Direktorat Jenderal Pencegahan dan Pengendalian Penyakit. Penyakit kanker di Indonesia berada pada urutan 8 di Asia Tenggara dan Urutan 23 di Asia [Internet]. [cited 202022 May 5]. Available from: http://p2p.kemkes.go.id/penyakit-kanker-di-indonesia-berada-pada-urutan-8-di-asia-tenggara-dan-urutan-23-di-asia/. 2019.
