





Molecular genotypes analysis of *Cryptosporidium* and *Hymenolepis* in rats on Lombok Island, Indonesia

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Abstract

Background and Aim: *Cryptosporidium parvum* and *Hymenolepis nana* are intestinal parasites that are commonly found in the unclean environment. Their presence in rats promotes the transmission of the cryptosporidiosis and hymenolepiasis to humans or animals nearby. This study aimed to determine the molecular characteristics of *C. parvum* and *H. nana* and their distribution in rats on Lombok Island.

Materials and Methods: *C. parvum* and *H. nana* were investigated in 50 rats from Lombok Island. The molecular-parasitological technique used was polymerase chain reaction and sequencing method.

Results: From 50 samples of rats' stool from 10 locations on Lombok Island, 8% (4/50) of *C. parvum* was detected molecularly with an *18S rRNA* gene and 2% (1/50) of *H. nana* with *COX 1* gene. Phylogenetic analysis indicated that *C. parvum* carrying rats on Lombok Island have a genetic relationship with *C. parvum* with *Obi7* isolates, Japan and *H. nana* has a genetic relationship with *Rodentolepis nana* identified with *Hn-VT* isolates, India.

Conclusion: The highest incidence of parasites was found in rats that were caught in the urban areas of Lombok Island, West Nusa Tenggara, Indonesia. Immediately, public health programs in these types of contaminated areas should receive priority attention to prevent further transmission of the parasites from animals to human beings.

Keywords: *Cryptosporidium*, *Hymenolepis*, intestinal parasite, *Rattus*.

Introduction

Diarrhea is a common global disease in both developed and developing countries. The cause of diarrhea generally occurs due to bacterial, fungal, viral, or parasitic infection. Diarrhea if not resolved properly, can cause death and become a significant burden on a country's economic development as well as medical resources. Parasites are one group of pathogens that contribute to diarrhea in developing countries and countries with low economic level. The digestive tract parasites are commonly found in third world countries, especially with tropical climates. The transmission can occur by any kind of animal, and rats are one common carrier [1,2].

Major changes in the natural habitat by humans cause human contact with animals to become more frequent. Diseases related to rats (rodent-borne diseases) began to increase in line with changes in the natural habitat. A rat

can act as a transmitter of infectious diseases due to their habitat and habits which are usually scavenging food in dirty places with garbage and consuming water contaminated with diseases so that the rats can cause transmission to not only humans but also animals [3].

Rats can carry various infectious diseases, both groups of viruses, bacteria, and parasites. Infectious diseases could be transmitted to humans through the saliva, urine, and stool or the ectoparasite bites. The dissemination of zoonotic parasites from rats to the human environment requires identification through an early investigation of the zoonotic parasite transmission source. The proximity of the rats with the human environment can be a risk factor for parasite transmission from rats to humans. Parasites can be transmitted through the digestive tract and the infected rats can then cause digestive tract disorders in humans. Parasites of the gastrointestinal tract from rats that have been reported to infect humans include the following, among others: *Hymenolepis nana*, *Giardia lamblia*, *Balantidium coli*, *Angiostrongylus cantonensis*, *Trichuris* spp., *Enterobius vermicularis*, and *Cryptosporidium* spp. [4-7].

Seventy-five million people of the world's population are estimated to be carriers of *H. nana*, and the highest incidence rates can reach up to 25% in some places. Research in Rio de Janeiro, Uttarakhand,

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North England, and Kuala Lumpur reported that *H. nana* is commonly found in unclean environments and where are many rats. *H. nana* commonly infects primary school-age children even with clean living habits [8-11].

H. nana infection was also found in 35% of the 17 rats in the plantation areas of Lampung with microscopic examination. Molecular identification used *ITS-1* gene in 35 adult cestode worms of rats in Lombok and identified two samples of *H. nana* and one of *H. diminuta* [12,13].

Cryptosporidium is an intestinal tract protozoan which can be transmitted through the stool of rats. Infections caused by *Cryptosporidium* can cause moderate to severe diarrhea and even death in children under 2 years old and people who are immunocompromised [14]. *Cryptosporidium* was found to have a high prevalence of 22.5% (14/62) in immunocompromised children, with microscopic and polymerase chain reaction (PCR) examination [15]. *Cryptosporidium* was reported in 12.7% (23/180) of *Rattus rattus* and *Rattus norvegicus* in Iran using the Ziehl-Neelsen staining [16]. Molecular examination using a small subunit *rRNA* gene could identify the presence of *Cryptosporidium parvum* and *Cryptosporidium muris* in rats in China. *C. parvum* subtype *11dA15G1* that infects humans was identified after gene sequencing using *gp 60* [17,18].

This study aimed to determine the molecular characteristics of *C. parvum* and *H. nana* and their distribution in rats on Lombok Island, Indonesia.

Materials and Methods

Ethical approval and informed consent

This study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Gadjah Mada University-Dr. Sardjito General Hospital, with approval number: KE/FK/1222/EC/2018, dated November 21, 2018. Informed consent was obtained from all individual participants included in the study.

Study period, area and sample collection

Fifty samples of rats were caught from five districts in Lombok Island, West Nusa Tenggara Province, from July to November 2019. Sampling was carried out purposively in a tourist-residential area. Each district provided 10 points for each sampling location of rats: Mataram, East Lombok, Central Lombok, West Lombok, and North Lombok. Samples of rat stool were obtained from rat cecum, and DNA of parasites was obtained by extraction used reagents and procedures QIAamp, Fast DNA Stool Mini Kit (Qiagen, Germany).

Parasitological examination technique

DNA extraction, 200 mg of the stool, was mixed with 1 mL InhibitEX Buffer divortex until homogeneous. The lysis process was done by incubating samples in a water bath at a temperature of 85°C for 5 min.

Samples were centrifugated at 10,000 rpm for 1 min to obtain a supernatant. Then, the supernatant as much as 600 µL was added with 25 µL of protein K, then combined with 600 µL of buffer AL and incubated at 70°C for 10 min. To get lysates, 600 µL of ethanol 96-100% was added. After transferring the 600 µL of lysate into the spin column, centrifugated at 10,000 rpm for 1 min, the filtrate was removed and placed in 2 mL tube. A back up of all lysate leftover was made and combined with 500 µL of buffer AW1 into the spin column with a new collection tube, centrifugated at 10,000 rpm for 1 min, the filtrate was removed. Next, 500 µL of buffer AW 2 was added in a new collection tube and centrifugated at 10,000 rpm for 3 min. After removing the spin column from the collection tube, it was installed in a new collection tube and centrifugated at 10,000 rpm for 3 min. Then, it was transferred from the spin column into 1.5 mL tube, then 100-200 µL of buffer ATE was added into the spin column and let stand 1 min to dissolve the DNA and finally centrifugated at 10,000 rpm for 1 min. After discarding the spin column, the 1.5 mL tube which contained DNA was stored at -20°C.

PCR amplification and detection, PCR as a determinant of DNA used Bioline mix with 0.2 mL PCR tube containing 2 mL DNA template DNA, 6.5 µL of ultrapure water, and 12.5 µL Master Mix in 2×2 µL. Primers used were *C. parvum 18S Ribosomal RNA* gene primer [19] F:5'-TAAACGGTAGGGTATTGGCCT-3'; R:5'-CAGACTTGCCCTCCAATTGATA-3'; *H. nana* gene *cytochrome oxidase subunit 1 (COX1)* [20,21], F:5'AGGTTGTAGTGTGTGGGCTC-3'; R:5'-CCAGTCACACCACCAAATCT-3'. PCR conditions used were 35 cycles, with initial activation temperature 95°C for 5 min, a temperature of 95°C for 30 s denaturation, annealing temperature of 59°C for 45 s, extension temperature 72°C for 3 min, and a final extension temperature of 72°C for 10 min. The final results were identified using electrophoresis on agarose 2% [22].

Sequencing, the analysis of the parasite's genetic relationship was done by phylogenetic test, with sample DNA amplification product followed by purification and sequencing. Sequencing used an Applied Biosystems 3500 Genetic Analyzer 2500 with BigDye Terminator kit. Edited DNA parasite sequencing used ClustalW Molecular Evolutionary Genetics Analysis (MEGA) software version 10, USA. The phylogeny tree was based on neighbor-joining [23-26].

Results

PCR examination of rats' stool samples on Lombok Island indicated the presence of *C. parvum* and *H. nana*. The results are shown in the following table, which shows four samples of *C. parvum* and one of *H. nana* were identified from the 50 samples of rats. Confirmation was done by molecular PCR examination followed by electrophoresis, namely, *C. parvum* at 240 bp and *H. nana* at 216 bp, in Table-1 and Figure-1.

C. parvum phylogenetic analysis used *18S rRNA* gene and *H. nana* used *COX1* to examine and determine the genetic relationship. The analysis of the parasites' genetic relationship used the "Neighbor-Joining" method. Each gene sequence was based on the access available in GenBank. The consensus of the bootstrap tree concluded 1000 replications, and evolution of parasites' distance was calculated based on the method of "Kimura 2." All calculations were performed by phylogenetic analysis using the free software of "MEGA X," USA [23-26].

C. parvum in rats caught on Lombok Island has a monophyletic kinship with *C. parvum* (FJ796268.1) in Japan and kinship synapomorphic with *C. suis* (AB694726.1 and AB449828) in Japan, as shown in Figure-2.

Table-1: The location of rats infected with *Cryptosporidium parvum* and *Hymenolepis nana*.

District/City	Parasite	
	<i>Cryptosporidium parvum</i>	<i>Hymenolepis nana</i>
Mataram	+	-
East Lombok	-	+
Central Lombok	+	-
Lombok Barat	-	-
North Lombok	+	-

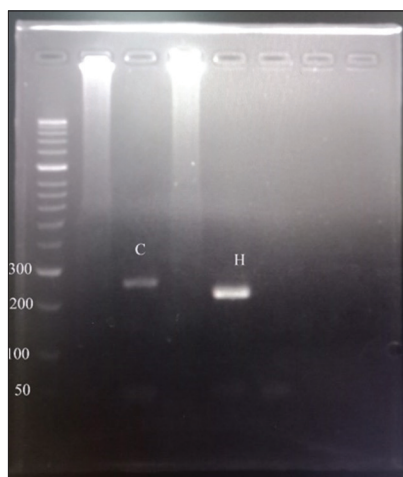


Figure-1: "C," the results of electrophoresis sample DNA *Cryptosporidium parvum* with the primer *18S Ribosomal RNA* gene and "H," the results of electrophoresis sample DNA *Hymenolepis nana* with the primer *COX1*.

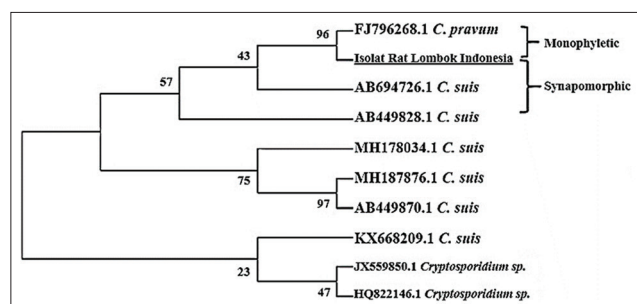


Figure-2: *Cryptosporidium parvum* phylogenetic trees based on gene sequences *18S Ribosomal RNA*, kinship analyzed using the method of "Neighbor-Joining."

H. nana in rats caught on Lombok Island, West Nusa Tenggara, has a monophyletic kinship with *Rodentolepis nana* (KU821727.1) in India and synapomorphic kinship with *H. nana* (AY121842.1) and *Taenia* spp. (AB905203.1) in Ethiopia, as shown in Figure-3.

The dissemination of *C. parvum* and *H. nana* in urban shown in Figure-4. The dissemination of *Cryptosporidium* parasite was more than *H. nana*. *C. parvum* was found in rats caught in Pemenang Village, North Lombok District, Ampenan and Mataram, Pujut Village in Center Lombok, while *H. nana* was found only in rats which were caught in Selong, East Lombok District, as shown in Figure-4.

Discussion

C. parvum was most identified in rats which were caught on the Lombok Island, involving 8% of carriers of *C. parvum* of the 50 rats caught on the Lombok Island. *Cryptosporidium* is a parasite in animals able to infect humans and can cause diarrhea. One of the worms contained in rats that could infect humans and cause diarrhea is *H. nana*. Severe infections can cause diarrhea in children and obstruct their development. According to genotyping, from the rats caught on the Lombok Island, there was only one sample with *H. nana*.

Cryptosporidiosis has been reported in a previous study in the farm areas of the Algerian population. Using microscopic methods with formyl-ether concentration and Ziehl-Neelsen staining, *Cryptosporidium* was found in moderately high incidence: 36.7% in cattle, sheep 15%, 8.9% of broiler chickens, and 2% in camels. The incidence of cryptosporidiosis was increased to 52.6% in calves with diarrhea [27]. *Cryptosporidium* was also reportedly found in wild rats caught in the area of Hainan, China. The results of PCR analysis using a small subunit of ribosomal DNA gene found 50% of rats infected by *Cryptosporidium* from 150 rats which were caught [18]. *C. parvum* was also reportedly found in two *Rattus* spp. among 134 rats that were used as laboratory rats, which was detected using PCR of *18S rRNA* gene [2]. *H. nana* is a tapeworm commonly found in rats, and human transmission is caused by unclean living habits, so the embryonated eggs or cysticercoids become swallowed. Several countries have reported the incident of hymenolepiasis, including the

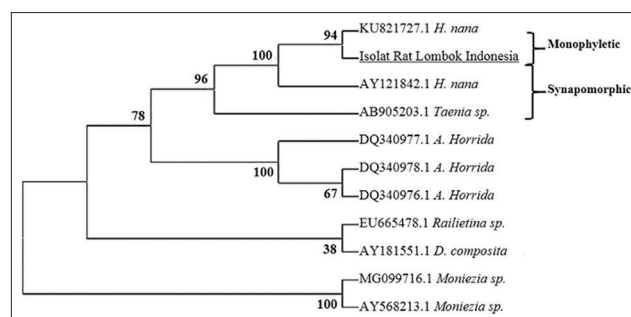


Figure-3: The phylogenetic tree of *Hymenolepis nana* based on gene sequences *cytochrome oxidase subunit 1*, kinship analyzed using the method of "Neighbor-Joining."

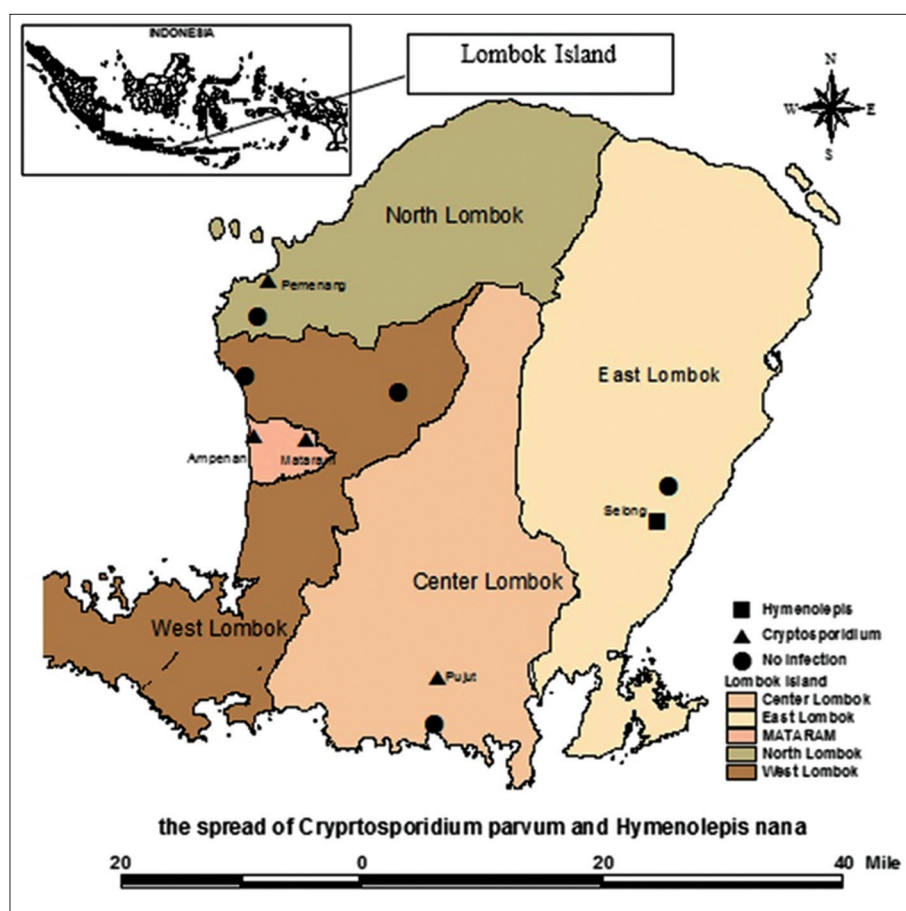


Figure-4: The location of the spread of *Cryptosporidium parvum* and *Hymenolepis nana* in rats on Lombok Island (Source: <https://tanahair.indonesia.go.id/portal-web>)

discovery of *H. nana* in Ethiopia that infected a pregnant women and severe infections with symptoms of abdominal pain, nausea, and diarrhea; the discovery of anemia in Bolivian children infected by parasites and identified as *H. nana* [28,29].

Gene molecular analysis used *COX I* against rats on Lombok Island, and there was one rat identified with hymenolepiasis out of the 50 rats which were caught. Molecular studies were used in Iraq against *R. rattus* and found 4% of rats infected with *H. nana* while in Iran, there was *H. nana* infection detection of 0.65% in the study population [30,31].

Cryptosporidium and *Hymenolepis* that were detected on Lombok Island tended to be widely identified in rats caught in urban areas. Parasites generally are found in a congested area with an unclean environment. *Cryptosporidium* and *Hymenolepis* which were found in rats can be transmitted to humans or animals nearby. One research report in Bolivia showed a high risk of anemia in children of school age who were infected by *H. nana*. Continued infection over long periods of time can diminish the performance index and degrade child development [28]. *Cryptosporidium* infections that cause diarrhea were found in as many as 37% of the 300 samples of diarrhea in Egypt. Patients suspected of cryptosporidiosis should be treated immediately since prolonged infection has been proven fatal in some patients who are immunocompromised. Research

recently has shown that the most serious impact is on children or people living with HIV/AIDS [32].

Conclusion

The highest incidence of parasites was found in rats which were caught in the urban areas of Lombok Island, West Nusa Tenggara. Immediately, public health programs in these types of contaminated areas should receive priority attention to prevent further transmission of the parasites.

Authors' Contributions

ER, WTA, and MAW designed the research study. ER, WTA, and FF gathered the data from the field. ER, WTA, and MAW interpreted the results and analyzed the data. ER, WTA, and MAW prepared the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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References

- Adam, E.A., Yoder, J.S., Gould, L.H., Hlavsa, M.C. and Gargano, J.W. (2016) Giardiasis outbreaks in the United States, 1971-2011. *Epidemiol. Infect.*, 144(13): 2790-2801.
- Ayinmode, A.B., Ogbonna, N.F. and Widmer, G. (2017) Detection and molecular identification of *Cryptosporidium* species in laboratory rats (*Rattus norvegicus*) in Ibadan, Nigeria. *Ann. Parasitol.*, 63(2): 105-109.
- Morand, S. (2015) Global parasite and *Rattus rodent* invasions: The consequences for rodent-borne diseases. *Integr. Zool.*, 10(5): 267-281.
- Zahedi, A., Paparini, A., Jian, F., Robertson, I. and Ryan, U. (2016) Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management. *Int. J. Parasitol. Parasites Wildl.*, 5(1): 88-109.
- Azzam, K.M. and El-Abd, N.M. (2016) Survey of endoparasites of different rodent species in Egypt. *Egypt. J. Biol. Pest Control*, 26(4): 815-820.
- Zahedi, A., Field, D. and Ryan, U. (2017) Molecular typing of *Giardia duodenalis* in humans in Queensland first report of assemblage e. *Parasitology*, 147(9): 1154-1161.
- Gordo, P. and Pomajbíková, J. (2017) *Balantidium coli*. In: Fayer, R. and Jakubowski, W. editors. Global Water Pathogen Project. Michigan State University, E Lansing, MI, UNESCO. Available from: <https://www.waterpathogens.org/sites/default/files/Balantidium%20coli1.pdf>. Retrieved on 15-08-2020.
- Crompton, D.W.T. (1999) How much human helminthiasis is there in the world? *J. Parasitol.*, 85(3): 397-403.
- Mirdha, B.R. and Samantray, J.C. (2002) *Hymenolepis nana*: A common cause of paediatric diarrhoea in urban slum dwellers in India. *J. Trop. Pediatr.*, 48(6): 331-334.
- Zain, S.N.M., Amdan, S.A.S., Braima, K.A., Abdul-Aziz, N.M., Wilson, J.J., Sithambaran, P. and Jeffery, J. (2015) Ectoparasites of murids in peninsular Malaysia and their associated diseases. *Parasit. Vector*, 8(1): 254.
- Simões, R.O., Luque, J.L., Gentile, R., Rosa, M.C.S., Costa-Neto, S. and Maldonado, A. (2016) Biotic and abiotic effects on the intestinal helminth community of the brown rat *Rattus norvegicus* from Rio de Janeiro, Brazil. *J. Helminthol.*, 90(1): 21-27.
- Dewi, K. and Purwaningsih, E. (2014) Helminth parasites on rats in rubber plantation in Gorejo Village, Gedongtataan Subdistrict, Pesawaran Regency, Lampung and their zoonotic review. *Zoo Indones.*, 22(2): 1-7.
- Tresnani, G., Suana, I.W. and Hadi, I. (2016) The detection of *Hymenolepis* sp. from house rats (*Rattus rattus diardii* Jentink, 1880) in Mataram through ITS-1 gene PCR analysis. *AIP Conf. Proc.*, 1744(1-4): 20023.
- Certad, G., Viscogliosi, E., Chabé, M. and Cacciò, S.M. (2017) Pathogenic mechanisms of *Cryptosporidium* and *Giardia*. *Trends Parasitol.*, 33(7): 561-576.
- Caner, A., Zorbozan, O., Tunali, V., Kantar, M., Aydoğdu, S., Aksoylar, S. and Turgay, N. (2019) Intestinal protozoan parasitic infections in immunocompromised child patients with diarrhea. *Jpn. J. Infect. Dis.*, 73(3): 187-192.
- Mirzaghavami, M., Sadraei, J. and Forouzandeh, M. (2016) Detection of *Cryptosporidium* spp. in free ranging animals of Tehran, Iran. *J. Parasit. Dis.*, 40(4): 1528-1531.
- Zhao, Z., Wang, R., Zhao, W., Qi, M., Zhao, J., Zhang, L., Li, J. and Liu, A. (2015) Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. *Parasitology*, 142(6): 800-806.
- Zhao, W., Zhou, H., Huang, Y., Xu, L., Rao, L., Wang, S., Wang, W., Yi, Y., Zhou, X., Wu, Y. and Ma, T. (2019) Parasites and wildlife *Cryptosporidium* spp. in wild rats (*Rattus* spp.) from the Hainan Province, China: Molecular detection, species/genotype identification and implications for public health. *Int. J. Parasitol.*, 9(1): 317-321.
- Zebardast, N., Yeganeh, F., Gharavi, M.J., Abadi, A., Tabaei, S.J.S. and Haghighi, A. (2016) Simultaneous detection and differentiation of *Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *Giardia lamblia* and *Cryptosporidium* spp. in human fecal samples using multiplex PCR and qPCR-MCA. *Acta Trop.*, 162(233-238): 233-238.
- Hailemariam, Z., Nakao, M., Menkir, S., Lavikainen, A., Yanagida, T., Okamoto, M. and Ito, A. (2012) Molecular identification of unilocular hydatid cysts from domestic ungulates in Ethiopia: Implications for human infections. *Parasitol. Int.*, 61(2): 375-377.
- Di Yang, W.Z., Zhang, Y. and Liu, A. (2017) Prevalence of *Hymenolepis nana* and *H. diminuta* from brown rats (*Rattus norvegicus*) in Heilongjiang Province, China. *Korean J. Parasitol.*, 55(3): 351.
- Nkouawa, A., Haukisalmi, V., Li, T., Nakao, M., Lavikainen, A., Chen, X., Henttonen, H. and Ito, A. (2016) Parasitology international cryptic diversity in hymenolepidid tapeworms infecting humans. *Parasitol. Int.*, 65(2): 83-86.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4(4): 406-425.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4): 783-791.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16(2): 111-120.
- Kumar, S., Stecher, G., Li, M., Nnyaz, C. and Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, 35(6): 1547-1549.
- Laatamna, A.K., Belkessa, S., Khalil, A., Afidi, A., Benmahdjouba, K., Belalmi, R., Benkrour, M., Ghazel, Z., Hakem, A. and Aissi, M. (2018) Prevalence of *Cryptosporidium* spp. in farmed animals from steppe and high plateau regions in Algeria. *Trop. Biomed.*, 35(3): 724-735.
- Spinicci, M., Macchioni, F., Gabrielli, S., Rojo, D., Gamboa, H., Villagrán, A.L., Vallejos, Y., Strohmeier, M., Roselli, M., Cancrini, G. and Olliaro, P. (2018) *Hymenolepis nana* an emerging intestinal parasite associated with anemia in school children from the Bolivian Chaco. *Am. J. Trop. Med. Hyg.*, 99(6): 1598-1601.
- Kandi, V., Koka, S.S. and Bhoomigari, M.R. (2019) *Hymenolepiasis* in a pregnant woman: A case report of *Hymenolepis nana* infection. *Cureus*, 11(1): e3810.
- Nabeel, M. and Al-Tameemi, M. (2019) Comparative study morphological and molecular for infected rodent with two cestoda *Hymenolepis nana* and *H. diminuta* in Al-Diwaniyah City, South of Iraq. *J. Phys. Conf. Ser.*, 124(6): 62058.
- Shahnazi, M., Mehri, M.Z., Alizadeh, S.A., Heydarian, P., Saraei, M., Alipour, M. and Hajjalilo, E. (2019) Molecular characterization of *Hymenolepis nana* based on nuclear rDNA ITS2 gene marker. *Afr. Health Sci.*, 19(1): 1346-1352.
- Gabr, N.S., Ahmed, A.K., Belal, U.S., Abd Rabou, R.A.M., Ahmed, R.F. and Abdel-Hafeez, E.H. (2019) Molecular characterization of *Cryptosporidium* isolates from humans by nested polymerase chain reaction-restriction fragment length polymorphism (nPCR-RFLP) analysis in Egypt. *Trop. Biomed.*, 36(1): 1-10.
