Parasitology International 69 (2019) 110–113

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short communication

Occurrence of zoonotic *Cryptosporidium* and *Giardia duodenalis* species/genotypes in urban rodents

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ARTICLE INFO

Keywords:
Cryptosporidium meleagridis
C. muris
C. parvum
Giardia
tpi
gp60
18S rRNA
Malaysia

ABSTRACT

This report describes the detection of zoonotic *Cryptosporidium muris*, *C. parvum* subgenotype IIa and *Giardia duodenalis* genotype B in urban rodents in Malaysia. A rare occurrence of *C. meleagridis* was also reported suggesting a role of rodents in mechanical transmission of this pathogen. Utilization of DNA sequencing and subtyping analysis confirmed the presence of zoonotic *C. parvum* subtypes IIaA17G2R1 and IIaA16G3R1 for the first time in rodents.

Cryptosporidium and *Giardia* are enteric protozoan parasites causing diarrheal diseases in humans and they have been responsible for at least 370 outbreaks worldwide [1]. To date, at least nine *Cryptosporidium* species (i.e., *Cryptosporidium andersoni*, *C. baileyi*, *C. canis*, *C. felis*, *C. hominis*, *C. meleagridis*, *C. muris*, *C. parvum*, *C. suis*, *C. ubiquitom*), and skunk genotype, and *Giardia duodenalis* genotypes A, B, C, D, E and F have been reported to infect humans and other animal hosts such as livestock and wild animals [2–7].

While the occurrences of *Cryptosporidium* and *Giardia* in humans and livestock in Malaysia have been extensively reported [8], the importance of both protozoa in rodents has been neglected, and the potential role of rodents as a reservoir or carrier to these pathogenic parasites remains unknown. Accordingly, the present study aimed to detect the presence of zoonotic *Cryptosporidium* and *Giardia* species/genotypes among urban rodents (i.e., rats) in a densely populated capital city of Malaysia, Kuala Lumpur.

The study protocol [Ethic Ref. No.: PAR/20/09/2011/J (R)] was reviewed and approved by Ethics Committee of Animal House, University of Malaya, Malaysia. A total of 134 urban rodents (e.g., *Rattus rattus diardii*, *R. norvegicus*, *R. argentiventer*, *R. tiomanicus*, and *R. exulans*) were captured from two human populated areas (Sentul and Chow Kit) in Kuala Lumpur using steel wire traps. A rectal faecal sample of each individual was collected, kept in stool container and subjected to genomic DNA extraction using NucleoSpin® Soil kit (Macherey-Nagel, Germany). The extracted DNA was subjected to polymerase chain reaction (PCR) using 1× ExPrime Taq™ Premix (GENET BIO, Korea), for amplifications of the 18S rRNA gene of *Cryptosporidium* spp. [9], glycoprotein 60 kDa (gp60) gene of *Cryptosporidium* [10], and triosephosphate isomerase (tpi) gene of *G. duodenalis* [11]. The nucleotide sequences were aligned with the available reference sequences deposited in the GenBank database, followed by maximum likelihood analysis using MEGA5 based on Kimura 2-parameter model with 1000 bootstrap values [12]. The microsatellite analysis of *Cryptosporidium* gp60 subtypes isolated from rodents was performed as described in Iqbal et al. [13].

According to the 18S rRNA gene fragment of *Cryptosporidium,* successful PCR amplification was found in 50 out of 134 individual rats (37.3% infection rate), and 40 PCR positive samples were successfully sequenced (Table 1). Maximum likelihood analysis (Fig. 1) revealed the occurrence of *Cryptosporidium* rat genotype II [accession numbers KY678457-KY678461] (13 of 134) and genotype III [accession number KY678463] (4 of 134), *C. muris* [accession number KY678448] (7 of

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https://doi.org/10.1016/j.parint.2018.12.007
Received 18 September 2018; Received in revised form 21 December 2018; Accepted 24 December 2018
Available online 24 December 2018
1383-5769/

Table 1
Cryptosporidium and *Giardia duodenalis* in urban rodents determined by DNA sequencing and maximum likelihood analysis according to type of genes used.

	PCR Positive	Successfully sequenced	Species/genotypes						
			Cryptosporidium spp.	*Cryptosporidium* rat genotype II	*Cryptosporidium* rat genotype III	*C. muris*	*C. meleagridis*	*C. parvum*	G. *duodenalis* assemblage B
Cryptosporidium									
18S rRNA	50 (37.3%)	40	15	13	4	7	1	–	–
gp60	23 (17.2%)	12	–	–	–	–	–	12	–
Giardia duodenalis									
tpi	4 (3.0%)	1	–	–	–	–	–	–	1

gp60: glycoprotein 60 kDa gene.
tpi: triosephosphate isomerase gene.

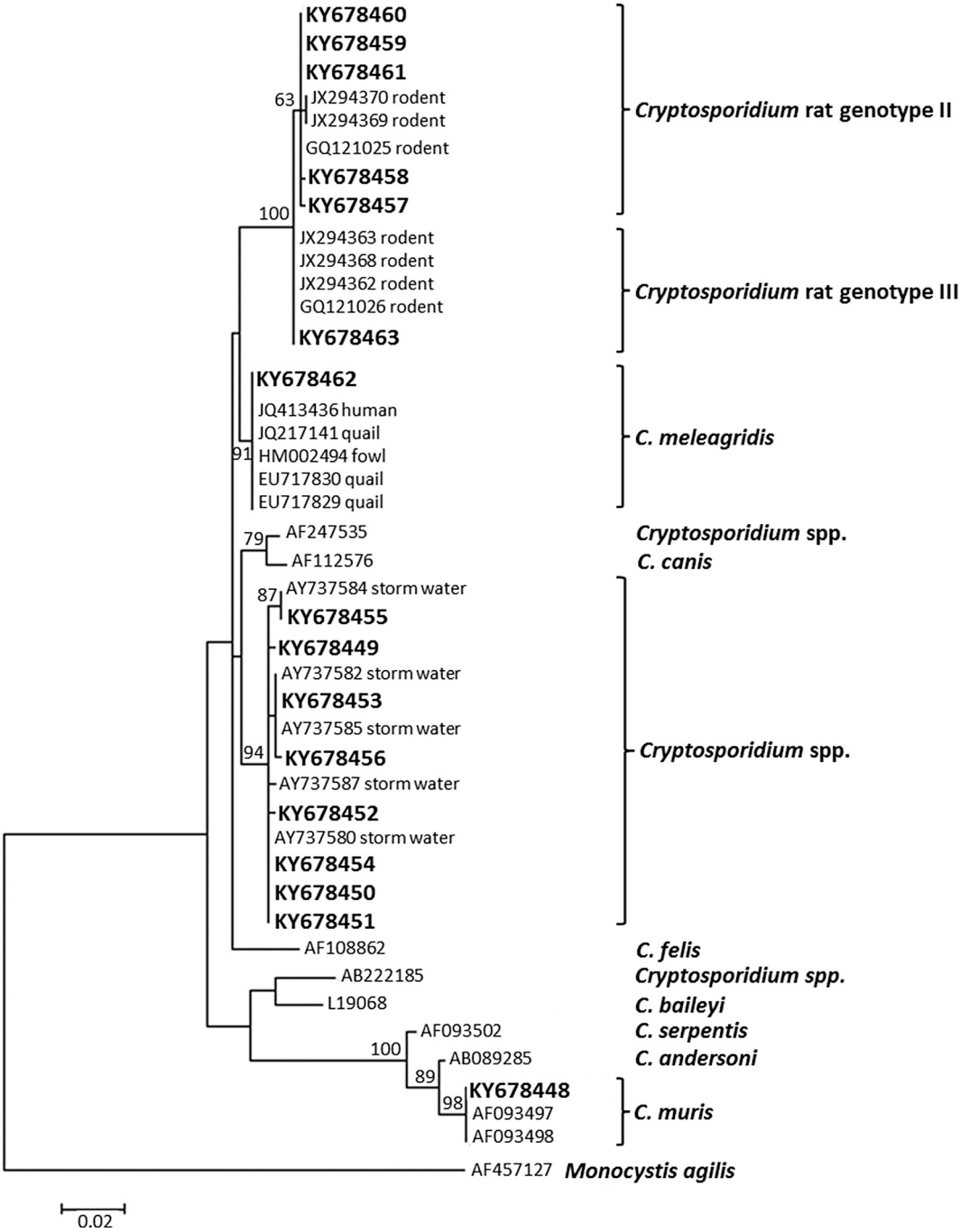

Fig. 1. The genetic relationships of *Cryptosporidium* spp. inferred from partial 18S rRNA sequence data following analysis using maximum likelihood. Sequences from the present study (bold type) as well as 27 reference sequences (acquired from the GenBank database) representing *Cryptosporidium* rat genotype II and III, *Cryptosporidium* spp., *C. meleagridis*, *C. muris*, *C. canis*, *C. felis*, *C. baileyi*, *C. serpentis*, *C. andersoni* and *Monocystis agilis* are indicated. Bootstrap values are indicated at all major nodes.

134), and *C. meleagridis* [accession number KY678462] (1 of 134) in the examined samples. Additionally, there were 15 isolates [accession numbers KY678449-KY678456] which could not be identified up to species level, but these sequences were clustered with some *Cryptosporidium* spp. reference sequences isolated from watersheds [accession numbers AY737580, AY737582, AY737584, AY737585, AY737587].

The occurrence of *C. meleagridis* is a rare phenomenon in rodents and it could be a mechanical transmission. Nevertheless, the prevalence of this species in humans has been globally reported, including Malaysia, where two of 122 (1.6%) HIV patients have been infected with *C. meleagridis* [8]. *Cryptosporidium muris* is not a major contributor to human cryptosporidiosis in Malaysia, but this species has been identified as the main cause to several symptomatic and asymptomatic cases in the adjacent countries of Malaysia, such as Thailand [14] and Indonesia [15].

Simultaneously, these rodent samples were analysed through amplification of the gp60 gene to determine the presence of *C. parvum* subtypes because this species is still the main zoonotic species for human cryptosporidiosis, especially in Malaysia. There were 23 samples tested positive to *Cryptosporidium* (17.2% of 134) and the species identity was determined in 12 PCR positive samples (Table 1). The obtained sequences [accession numbers KY696270-KY696274] were clustered with the *C. parvum* genotype IIa reference sequences (Fig. 2). Subtyping analysis on gp60 microsatellite region of *C. parvum* showed that IIaA15G2R1 [accession numbers KY696272-KY696274] was the commonest subtype in these urban rats (9 of 12). Likewise, this subtype has been documented in a wide range of hosts (e.g., ruminants, poultry and humans) [16], and has also been responsible for cryptosporidiosis outbreak, affecting an estimated 2780 persons in Oregon, United States [17]. In Malaysia, this subtype has been reported to be prevalent in HIV patients, compared to the IIaA13G1R1, IIaA13G2R, IIaA14G2R1, and IIdA15G1R1 subtypes [13].

This study also documented the presence of the IIaA17G2R1 [accession number KY696271] (2 of 12) and IIaA16G3R1 [accession number KY696270] (1 of 12) subtypes for the first time in rodents. As far as their potential zoonotic transmission is concerned, both subtypes have been incriminated as etiological agents for cryptosporidiosis outbreaks in many parts of the world [18–20].

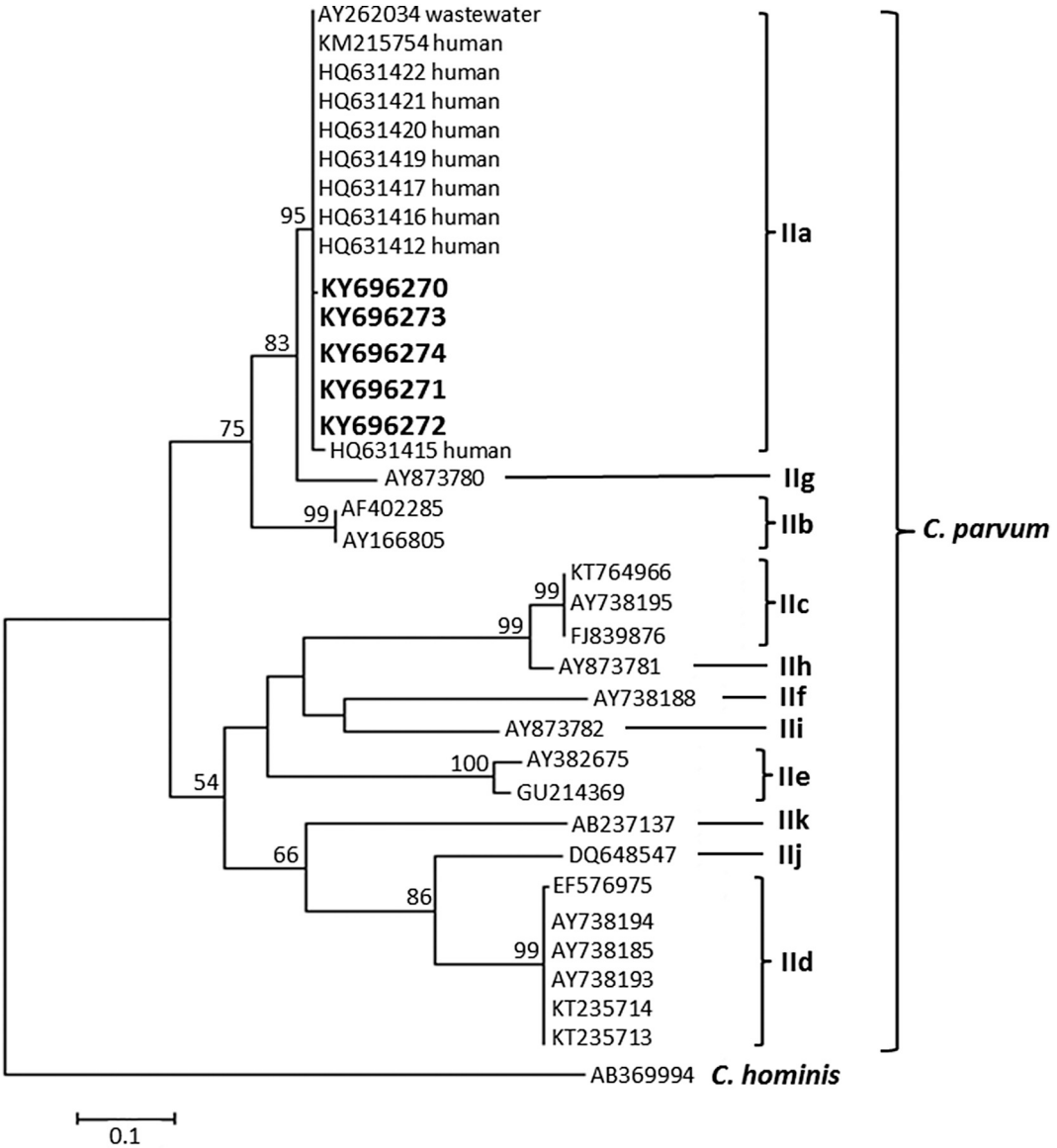

Fig. 2. The genetic relationships of *Cryptosporidium parvum* inferred from partial gp60 sequence data following analysis using maximum likelihood. Sequences from the present study (bold type) as well as 30 reference sequences (acquired from the GenBank database) representing subgenotype IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, Iii, IIj, IIk and *C. hominis* are indicated. Bootstrap values are indicated at all major nodes.

With regards to *G. duodenalis* detection, one of four PCR positive samples (3.0% infection rate) was successfully sequenced (Table 1). The obtained sequence [accession number KY709265] was identified as *G. duodenalis* genotype B [99–100% similarity to reference sequences AF069560 and AF069561] through maximum likelihood analysis (data not shown). The identified *G. duodenalis* positive sample was also co-infected with *Cryptosporidium* rat genotype II. In fact, occurrence of *G. duodenalis* genotype B in rodents has not been well-documented until recently, in that similar findings have been reported in other rodents, such as long-tailed chinchillas [21], bamboo rats [22], and voles [23]. In Malaysia, the *G. duodenalis* genotype B has been identified as a causative agent to human giardiasis in indigenous peoples [8].

In conclusion, the present study has detected zoonotic *Cryptosporidium* and *Giardia* species/genotypes, for the first time in urban rodents in Malaysia, pinpointing the highly possible role in zoonotic transmission because these rodents are living in close association with human populations. Indeed, the detected *C. muris*, *C. meleagridis*, *C. parvum* subtype IIaA15G2R1, and *G. duodenalis* genotype B in the present study have been shown to be prevalent in human populations in Malaysia [8]. In addition, the first discovery of the subtypes IIaA17G2R1 and IIaA16G3R1 in rodents provides insights into their adaptation to broad host ranges. The findings of this study have important implications for infectious disease control in the country, and further investigations are required to elucidate the role of rodent-borne transmission of *Cryptosporidium* and *Giardia* to humans.

Conflict of interests

The authors declare that there is no competing interests.

Acknowledgements

The study was supported by University of Malaya Research Fund (Grant No. E-000050-20001, H-20001-00-E000061, BK097-2016 and PG098-2016A).

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