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Understanding *Giardia* infections among rural communities using the one health approach

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ABSTRACT

The epidemiology of giardiasis in rural villages in Peninsular Malaysia was examined in the context of the One Health triad that encompasses humans, animals and environment (i.e. river water). A cross-sectional study was carried out among five rural communities in Malaysia to determine the prevalence of *Giardia duodenalis* in humans, animals and river water. Fecal samples collected from humans and animals were examined by light microscopy. Water was sampled from the rivers adjacent to the target communities and investigated for the occurrence of *Giardia* cysts. The isolated cysts were further genotyped targeting the glutamate dehydrogenase and triosephosphate isomerase genes. The overall prevalence of *G. duodenalis* was 6.7% (18/269) and 4.7% (8/169) among humans and animals, respectively. *Giardia* cysts (mean concentration range: 0.10–5.97 cysts/L) were also found in adjacent rivers at four out of the five villages examined. At Kemensah and Kuala Pangsun, *Giardia* cysts were isolated from humans [rate: 3.7% each (of 54 each)], animals [rates: 6.3% (of 62) and 11.3% (of 16), respectively] and river water [average concentration of 9 samples each: 0.83 ± 0.81 and 5.97 ± 7.00, respectively]. For both villages at Pos Piah and Paya Lebar, 12.2% (of 98) and 6.1% (of 33) of collected human samples were infected, respectively whilst none of the collected animals samples in these villages were found to be positive. The river water samples of these two villages were also contaminated (average concentration: 0.20 ± 0.35 (of 9) and 0.10 ± 0.19 (of 3), respectively). In conclusion, *Giardia* cysts were simultaneously observed in the human-animal-environment (i.e., river water) interfaces in at least two of five studied communities highlighting a vital need to improve understanding on the interplay of transmission dynamics, the role of infected humans and animals in contaminating the water sources and the role of water as a vehicle of disease transmission in these communities. Indeed, this study illustrates the One Health approach which is to recognize that the optimal health of humans are interconnected with the well-being of animals and their environment.

1. Introduction

Emerging parasitic diseases pose a major threat to human and animal populations globally. Disease outbreaks cause enormous and long-term damage to the national and global economy. The emergence of these parasitic diseases are caused by changes in natural and anthropogenic factors which include climate change, population growth, agricultural inflation, and human activities (e.g. hunting and intrusion into wildlife habitats) (Chromicz et al., 2016). To minimize the risk factors for these disease emergences, surveillance activities that require communication and co-operation efforts across inter-disciplines sectors (One Health approach) is crucial (Dixon et al., 2014). Generally, the One Health approach is to promote integrated multiple disciplines approach to clinicians, researchers, agencies and governments by working locally, nationally and globally for the benefits of human, animal and the environment (Gibbs, 2014). In a nutshell, the One health concept is an initiative that links the triad of human, animal and the environment.

Among the emerging pathogens, *Giardia duodenalis* is one of the waterborne protozoan parasites that has significant impact on humans, animals and the environment, especially in water. This zoonotic

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pathogen has accounted for a majority (40.6% of 315) of the global parasitic waterborne outbreaks (Baldursson and Karanis, 2011; Efstratiou et al., 2017; Karanis et al., 2007). In addition, giardiasis has been included in the 'Neglected Disease Initiative' of the World Health Organization from the year 2004 due to their impact on socioeconomic development, notably in developing countries (Lane and Lloyd, 2002; Savioli et al., 2006). Therefore, it is crucial to understand the transmission of *G. duodenalis* from the One Health perspective.

In Malaysia, *Giardia* has been found to be ubiquitous in human and non-human hosts (Lim et al., 2008). Surveillance studies in human reported variety of prevalence rates in indigenous communities (5.5–24.9%) (Al-Mekhlafi et al., 2005, 2013; Anuar et al., 2012; Choy et al., 2014; Kamel et al., 1994; Lim et al., 1997; Mahdy et al., 2007, 2008), children (0.9–28.3%) (Al-Delaimy et al., 2014; Al-Harazi et al., 2013; Al-Mekhlafi et al., 2013; Lai, 1992; Ludin et al., 1991; Mendez et al., 1988; Menon et al., 1999, 2001; Ng and Shekhar, 1993) and immunocompromised groups (3.2%–5.7%) (Lim et al., 2011; Asma et al., 2011). Meanwhile, the occurrence of *Giardia* in wild and domestic animals such as cattle, rodents and goats has also been recorded (Farizawati et al., 2005; Lim and Ahmad, 2001; Lim et al., 2013). As a main transmission agent for giardiasis, *Giardia* cysts have been observed at 16.4–66.7% of occurence rates in river systems in Malaysia (Azman et al., 2009; Farizawati et al., 2005, 2014a; Lim et al., 2004, 2008). Although there were numerous studies in humans, animals and the environment, all these previous studies were independent of each other and were not related. Hence, knowledge on the co-existence of *Giardia* cysts in humans, animals and their environment are limited.

In 2008, Mahdy et al. (2008) found that communities with high prevalence rates of giardiasis and low standards of personal hygiene were potentially at risk of acquiring the infection via consumption of contaminated post-treatment water and fresh vegetables with contaminated hands. However, assessment of the water quality did not recover any *Giardia* cysts during the study duration (Mahdy et al., 2008).

Since concrete evidence is still lacking, having a firm understanding of giardiasis epidemiological data in both humans and animals from the same communities coupled with *Giardia* contamination status in water system is crucial for the formulation of effective prevention and control measures for direct waterborne transmission. Hence, a study investigating the occurrence of *Giardia* in humans, animals and river within the same communities was initiated. In the present study, the human, animal and river water samples were collected at the same time. It is important to note that the information of *Giardia* in the river water samples has been published in Lee et al. (2014a). Nonetheless, certain aspects of the results are highlighted in this current analysis to enhance our understanding from the perspective of the dynamic interplay of *Giardia* transmission in humans, animals and the environment in these communities (relevant citations have been included where appropriate).

2. Materials and methods

2.1. Study area and population

A cross sectional study was conducted during December 2010–April 2012 at five rural indigenous villages in West Malaysia (covering the Selangor, Pahang and Perak states) as described in Lee et al. (2014b). Villagers at Kuala Pangsun, Bentong, Kemensah and Paya Lebar belong to the Proto-Malay tribe (Temuan subtribe) while those from Pos Piah belong to the Senoi tribe (Temiar subtribe). A total of 269 villagers gave consent to participate in this study. However, demographic data was only obtained from 255 (out of 269) villagers of which 132 are males and 123 are females (1–70 years of age). Although the villagers are provided with pour flush toilets, this facility is rarely used on a routine basis as they prefer to defecate at nearby bushes and/or in the river. Moreover, it is common for garbage to be burnt or thrown away in nearby bushes. Rearing animals like cats, dogs and chicken are also common practices and these rural communities live in close proximity with their companion animals.

2.2. Questionnaire and ethical considerations

Ethical considerations (i.e., MEC Ref. No. 824.11) by the Ethics Committee of the University Malaya Medical Centre (UMMC) Malaysia and approval by the Department of Indigenous Development (JAKOA) and Ministry of Health (MOH) were obtained prior to the commencement of the study. In brief, the objective of the study was explained to the participants before questionnaire and sample collections were performed. Written informed consent was obtained from each participant who has voluntarily agreed to be included. For participating individuals who are less than 12 years old, consent was acquired from their parents/guardians.

2.3. Fecal sample collection and parasitological analysis

Single screw-capped fecal containers were distributed to each participant and only filled containers were collected back the subsequent day. After seeking permission from animal owners, fecal samples from all types of animals were collected. The details of fecal storage and sedimentation method were as shown in Lee et al. (2014b). For the detection of *Giardia* cysts, the samples were examined using compound light microscope under 400× magnifications. Samples that were microscopically positive for *Giardia* cysts were further characterized using molecular techniques.

2.4. Water sample collection, concentration, purification and detection of Giardia cysts

A total of 10 L of river water samples were collected from 30 cm below the surface of rivers adjacent to the villages during each sampling exercise. Procedure of sample collection and detection method of *Giardia* cysts in water samples were performed as described in Lee et al. (2014a).

2.5. Molecular characterisation of Giardia

2.5.1. Genomic DNA extraction

Genomic DNA was extracted from the concentrated and purified *Giardia*-positive fecal samples using QIAamp DNA Stool Mini Kit (QIAGEN, Germany). Briefly, the pellet was re-suspended in 1.4 mL of Buffer ASL provided in the kit. DNA was then extracted, purified and stored at −20 °C until use. In addition, genomic DNA from water samples were subjected to freeze in liquid nitrogen and thaw in water bath at 56 °C for five cycles prior to purification using QIAamp DNA Mini Kit (QIAGEN, Germany) as instructed in the kit's manual.

2.5.2. DNA amplification by PCR using gdh gene

Molecular characterization of *Giardia* was conducted for humans, animals and water samples. Semi-nested PCR assay was used to amplify a region (432 bp) of the glutamate dehydrogenase (*gdh*) gene, according to Read et al. (2004). Primary PCR was carried out with primers GDHeF (5′-TCA ACG TYA AYC GYG GYT TCC GT-3′) and GDHiR (5′-GTT RTC CTT GCA CAT CTC C-3′) and the secondary PCR with primers GDHiF (5′-CAG TAC AAC TCY GCT CTC GG-3′) and GDHiR. Both primary and secondary PCRs were performed in a 50 μL reaction volume containing 0.5 μM of each primer (Bioneer Q-Oligos, Korea), 2.5 U of Taq polymerase (New England Biolabs, Ipswich, USA), 1 X PCR ThermoPol buffer (New England Biolabs, Ipswich, USA), 200 μM of each dNTP (Fermentas, Ontario, Canada), 1.5 mM $MgCl_2$ (Fermentas, Ontario, Canada) and 0.4 mg/ml BSA (New England Biolabs, Ipswich, USA). Two microliters of DNA template was used in both primary and secondary PCRs. In both amplifications, samples were incubated in the