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Prevalence of *Cryptosporidium* and *Giardia* in the water resources of the Kuang River catchment, Northern Thailand

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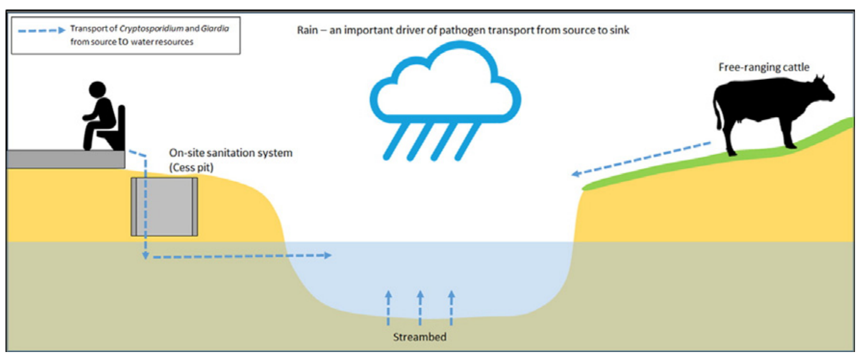
HIGHLIGHTS

- Cryptosporidium and Giardia are ubiquitous in the aquatic environment of N. Thailand.
- Rain is an important driver of pathogen transport.
- Streambed may be an important repository of Cryptosporidium and Giardia.
- Cattle farming management strategies may influence parasitic infection.
- Rural communities are especially at risk to giardiasis and cryptosporidiosis.

GRAPHICAL ABSTRACT



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ABSTRACT

A catchment-scale investigation of the prevalence of *Cryptosporidium* and *Giardia* in the Kuang River Basin was carried out during the dry and rainy seasons. Water samples were collected from the Kuang River and its tributaries as well as a major irrigation canal at the study site. We also investigated the prevalence of gastrointestinal parasitic infection among dairy and beef cattle hosts. *Cryptosporidium* and/or *Giardia* were detected in all the rivers considered for this study, reflecting their ubiquity within the Kuang River Basin. The high prevalence of *Cryptosporidium*/*Giardia* in the upper Kuang River and Lai River is of a particular concern as both drain into the Mae Kuang Reservoir, a vital source of drinking-water to many local towns and villages at the research area. We did not, however, detected neither *Cryptosporidium* nor *Giardia* were in the irrigation canal. The frequency of *Cryptosporidium*/*Giardia* detection nearly doubled during the rainy season compared to the dry season, highlighting the importance of water as an agent of transport. In addition to the overland transport of these protozoa from their land sources (e.g. cattle manure, cess pits), *Cryptosporidium*/*Giardia* may also be re-suspended from the streambeds (a potentially important repository) into the water column of rivers during storm events. Faecal samples from dairy and beef cattle showed high infection rates from various intestinal parasites — 97% and 94%, respectively. However, *Cryptosporidium* and *Giardia* were only detected in beef cattle. The difference in management style between beef (freeranging) and dairy cattle (confined) may account for this disparity. Finally, phylogenetic analyses revealed that the *Cryptosporidium*/*Giardia*-positive samples contained C. ryanae (non-zoonotic) as well as *Giardia intestinalis*

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assemblages B (zoonotic) and E (non-zoonotic). With only basic water treatment facilities afforded to them, the communities of the rural area relying on these water supplies are highly at risk to *Cryptosporidium*/*Giardia* infections.

1. Introduction

Diarrhoea claims more lives of children than AIDS, malaria and measles combined and is ranked as the second most common cause of death for children under five years of age worldwide after pneumonia (UNICEF and WHO, 2009). Nearly one in five children die each year from diarrhoea (UNICEF and WHO, 2009). Diarrhoea is caused by a wide range of pathogens including viruses, bacteria and protozoa. Of these, *Cryptosporidium* and *Giardia* are two of the most globally dominant and dangerous parasitic protozoa that infect not only humans, but also domestic animals and wildlife, (Caccio et al., 2005; Haque, 2007; Hunter and Thompson, 2005).

Cryptosporidium and *Giardia* are monoxenous: they complete their life-cycles within a single host, which excretes large numbers of infective stages (*Cryptosporidium* oocysts and *Giardia* cysts) in faeces. A gram of faeces from an infected host may contain as many as 1×10^7 and 2×10^6 *Cryptosporidium* and *Giardia* cysts, respectively (Smith et al., 2006). While there may be several modes of transmission, infection typically occurs following ingestion of water contaminated with *Cryptosporidium* and *Giardia* cysts — even in small doses. Infections in humans have been reported occur in doses as low as 9 and 10 cysts for cryptosporidiosis and giardiasis, respectively (Smith et al., 2006). The cysts are environmentally robust, allowing them to persist for long periods of time outside the host. Their small size allows them to penetrate the physical barriers of conventional water treatment systems. They are also insensitive or resistant to many disinfectants used in the water industry (e.g. chlorine). *Cryptosporidium* and *Giardia* therefore constitute a significant health hazard, even in developed countries.

Between World War I and 2003, a total of 325 recorded water-associated outbreaks of parasitic protozoan diseases occurred (Karanis et al., 2007). North America (Canada and the United States) and Europe (primarily the United Kingdom) accounted for 93% of the reported outbreak (Karanis et al., 2007) — most likely due to reporting bias (Baldursson and Karanis, 2011). Cryptosporidiosis and giardiasis make up nearly all of the reported cases: 51% and 41%, respectively. More recently, at least 199 outbreaks occurred between January 2004 and December 2010 (Baldursson and Karanis, 2011). Again, *Cryptosporidium* (60%) and *Giardia* (35%) were the main etiological agents of these waterborne parasitic outbreaks. Documented cases were mainly reported from North America and Europe, along with 'newcomers', Australia and New Zealand. Reports from these countries/continents make up approximately 96% of the documented outbreaks (Baldursson and Karanis, 2011).

Consideration of these two reviews discloses several important findings. Firstly, *Cryptosporidium* and *Giardia* are dominant causative agents of waterborne disease outbreaks, compared with other protozoan parasites. Secondly, even first world nations with reliable and modern water treatment systems and technology are susceptible to parasitic outbreaks. Thirdly, marked progress has been made in the detection and diagnostic methods, which in turn has resulted in the improvement in surveillance and reporting systems. Finally, there is a lack of research and monitoring in developing countries of Asia, Africa and Latin America. This latter issue is ironic, yet important, because the poorer communities from these regions without reliable water and sanitation facilities are likely more vulnerable to these diseases than those in the developed world where most cases are reported (Hotez et al., 2009; Prüss-Üstün et al., 2008; WHO, 2008).

Similarly, in the developing nations of Southeast Asia, *Cryptosporidium*- and *Giardia*-related studies are relatively rare compared with their first world counterparts. Wherever available, studies on *Cryptosporidium* and *Giardia* almost always pertain to their prevalence in hosts rather than the environment (Dib et al., 2008; Lim et al., 2010 and the references therein). In Thailand, for example, only five such studies have been published (Anceno et al., 2007; Diallo et al., 2008; Koompapong and Sukthana, 2012; Kumar et al., 2014; Srisuphanunt et al., 2010). These studies typically only investigated the occurrence of *Cryptosporidium* and *Giardia* in the aquatic environment and water resources. Investigations on the factors contributing to their distribution are rare.

Herein, this research void is addressed by investigating the prevalence of *Cryptosporidium* and *Giardia* in the surface water resources in a rural study area in northern Thailand. The role of seasonality (i.e. dry weather vs. wet weather) is also explored by investigating the association of hydroclimatological factors with the distribution of *Cryptosporidium* and *Giardia* in the environment. In addition, faecal samples from cattle, an important host for *Cryptosporidium* and *Giardia*, were also screened for both protozoa as well as other intestinal parasites. Finally, isolates of both organisms in faecal samples from cattle were molecularly characterised.

2. Study area

The area investigated for this study is centred on the Kuang River Basin, which is located on the eastern bank of the Ping River in Northern Thailand. The catchment area is 1661 km^2 and has a population of 291,000, of which, half are classified as rural (Ganjanapan and Lebel, 2014). The area spans across the districts of San Sai, San Kamphaeng, Mae On and Doi Saket within the Chiang Mai Province, as well as the districts of Ban Thi, Pa Sang and the capital district (*Amphoe Mueang*) of the Lamphun Province. Forests, mostly deciduous and dry dipterocarp, cover just over half of the drainage basin and are mostly restricted to higher elevations. Approximately one third of the area is devoted to agriculture and about 7–8% to residential use (Ganjanapan and Lebel, 2014).

The Kuang River is an important tributary to the Ping River, which drains into the Chao Phraya River in Central Thailand (Fig. 1). The Ping River basin is the largest (catchment area of over 35,000 km^2) in the Chao Phraya River basin. It is a vital source of water not only in the northern region, but also to the nation's capital, Bangkok, as well as many parts of Central Thailand for domestic, agricultural and industrial uses (Thomas, 2005).

The Lai River, Pong River and San River are major tributaries to the Kuang River. The former, together with the upper reach of the Kuang River, form the primary inflows to the Mae Kuang Reservoir. With a water storage capacity of approximately 260 million m^3 and a catchment area of over 550 km^2, the Mae Kuang Reservoir is a major source of irrigation, domestic and drinking-water supply for many locations in the provinces of Chiang Mai and Lamphun (Chansribut, 2002; Nutniyom, 2003).

A complex network of canals also distributes surface water across the study site. Importantly, the Mae Taeng-San Sai Canal, constructed and managed by the Royal Irrigation Department of Thailand, is a 40-km long, trapezoidal concrete canal that receives water from the Ping River, immediately downstream of the Mae Ngat Reservoir, and conveys water to the San Sai District from the Mae Taeng District, to the north. The water from this canal is typically used from irrigation purposes. The canal can also be used for recreational purposes where we observed villagers bathing in the waters particularly during the dry season.

Chiang Mai and Lamphun have a tropical wet and dry climate (Köppen *Aw*), typical of the northern region of Thailand. Annual rainfall