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Authors: R.C.A. Thompson, J.V. Conlan

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Emerging Issues and Parasite Zoonoses in the SE Asian and Australasian Region

R.C.A. Thompson*, JV Conlan

WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, Western Australia 6150, Australia.

*Corresponding Author: WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and the State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Western Australia 6150, Australia. Tel: 08 9360 2466. Email: a.thompson@murdoch.edu.au

Abstract

Parasite zoonoses are common in the SE Asian region. However, recent case reports and surveillance activities have revealed a number of emerging issues that threaten both biosecurity and ongoing control efforts. In all cases, the application of molecular tools has contributed to the identification of new foci of infection, novel aetiological agents and epidemiological investigations. These emerging issues are discussed with reference to trypanosomes, *Toxoplasma*, fish-borne flukes, cysticercosis and hookworm.

Keywords: Zoonoses, Emerging, SE Asia, Australia, *Trypanosoma*, *Leishmania*, *Toxoplasma* Fish-borne flukes, *Taenia*, cysticercosis, hookworm

Introduction

Parasite zoonoses continue to have a significant impact on public health throughout the world. This is particularly the case in developing countries and emerging economies where transmission is enhanced by poor hygiene, vectorial abundance and animal reservoirs. Global issues including climate change, migration, environmental changes and degradation, drug resistance and economic factors serve to exacerbate the problem. In addition, we are seeing the emergence of 'new' zoonotic problems associated with the recognition of novel parasite species/strains and new foci of infection. In many cases, these emerging issues have been identified with the application of molecular tools. In this review, we would like to highlight a few recent examples.

Trypanosomes

The trypanosomes provide an excellent example of why we should avoid complacency and challenge accepted dogma in trying to interpret new findings about diseases that were once considered well understood in terms of their distribution, host range and vectorial transmission.

Table 1 summarises chronologically the important reports and observations that have caused a reassessment of the geographical distribution, host range and vectorial specificity, and even the evolutionary biology, of *Leishmania* with respect to the SE Asian region. In most recent textbooks of tropical medicine and clinical parasitology that show maps of the global distribution of *Leishmania*, the parasite is markedly absent from SE Asia and Australia. The unconfirmed report of *Leishmania* in inhabitants of East Timor (Chevalier et al., 2000) gave rise to speculation on the possible vectors in the region (Thompson et al., 2003), and from an Australian perspective, heightened the significance of the discovery of a novel species of *Leishmania* in kangaroos, which has since been shown to infect several species of macropod marsupial (Rose et al., 2004; Dougall et al., 2009). In Thailand, the finding of novel and exotic species of *Leishmania* in humans raised questions about the nature of the vectors (Sukmee et al., 2008). Similar questions were raised by recent reports of *Leishmania* similar to the novel form in Thailand, in horses and cattle in Switzerland for which the vectors have yet to be identified (Muller et al., 2009; Lobsiger et al., 2010). It should be noted that in Europe there is much resistance at the present time to accepting proposals that non-sand fly vectors, such as fleas and ticks, may act as vectors of *Leishmania* (Ferreira et al., 2009; Dantas-Torres et al., 2010 ; Otranto and Dantas-Torres, 2010).

Two recent reports have served to emphasise that Chagas disease is now a global disease and raises questions about the possibility of vectorial transmission in areas not previously considered endemic for *Trypanosoma cruzi* (Gascon et al., 2010; Schmunis and Yadon, 2010). For example, in Australia, little attention has been given to the potential role of

triatomes as vectors of trypanosomes in native wildlife (Thompson et al., 2010). It is not known which species, if any, bite and feed on the blood of mammals although *Triatoma leopoldi* is thought to occur in northern Australia and is a vector of *T. cruzi* in South America (Monteith, 1974). If Australian triatomes can act as vectors of native species of *Trypanosoma*, they could presumably transmit *T. cruzi* from infected migrants from Chagas-disease endemic regions who are now living in Australia (Schmunis, 2007; Gascon et al., 2010; Schmunis and Yadon, 2010). Furthermore, the susceptibility of Australian native mammals to infection with *T. cruzi* is not known, although they are commonly infected with *Trypanosoma* species genetically similar to *T. cruzi* (Averis et al., 2009).

Toxoplasma

George Nelson concluded that although toxoplasmosis is one of the most common parasitic infections in the world, it is a rare disease (Nelson, 1979). This is true not only for humans but also for the majority of mammals *Toxoplasma* is known to infect. The low host specificity of *Toxoplasma* and the remarkable plasticity demonstrated by its life cycle in terms of routes of transmission and endodyogenous developmental phase have resulted in a virtually unlimited range of warm blooded intermediate hosts (Thompson et al., 2009). It is therefore surprising that for many years it was considered that the genetic diversity of *Toxoplasma* was relatively limited (Howe and Sibley 1995; Thompson et al., 2007).

The population genetic structure for protozoan parasites with both sexual and asexual modes of reproduction, such as *T. gondii,* is usually categorised as one of the following: **clonal**, where recombination is so infrequent that it does not disrupt genealogical relationships; **panmictic**, where recombination occurs regularly enough to create fully reticulate relationships; or **epidemic**, where an underlying panmictic structure is obscured by clonal expansion of a few genotypes (Smith et al., 1993). In Europe and North America, *T. gondii* has a remarkably clonal population structure, with just three predominant clonal lineages, referred to as Types I, II and III, accounting for >95% of strains isolated from humans and domestic animals (Howe and Sibley 1995). However, the results of laboratory crosses suggest that these so-called 'archetypal' strains emerged quite recently, probably within the last 10,000 years (Grigg et al., 2001; Su et al., 2003; Boyle et al., 2006). Recent studies in North and South America have revealed the existence of a diversity of biologically and genetically distinct isolates of *T. gondii* in wildlife assemblages (Ajzenberg et al., 2004; Miller et al., 2004; Lehmann et al., 2006). Although some of this diversity appears to be the result of recombination between the recognised Types (I-III), some strains appear to be novel, "atypical" or "exotic" strains. Thus, this diversity appears to be driven by regular cycles of sexual reproduction, with occasional expansion of clonal lineages, suggesting a panmictic population structure in South America and an epidemic expansion of three successful clones in parts of North America and Europe, where wildlife likely plays a less significant role in transmission of the parasite (Ajzenberg et al., 2004; Khan et al., 2007).

Until recently, almost nothing was known about the genetic diversity of *T. gondii* in Australia. Type II strains have been reported from a human patient and a domestic dog (Al-Qassab et al., 2009), but more recent studies have found that *T. gondii* is common and widely distributed among the native fauna, with the parasite isolated from over 25 species of native mammals and birds, with prevalences ranging from 40-100% (Parameswaran et al., 2010; Pan et al., submitted). Furthermore, a rich diversity of novel strains has been identified by multilocus PCR-DNA sequencing at polymorphic genes, with the majority (67%) of marsupial *T. gondii* infections found to be Type II-like or atypical strains (Parameswaran et al., 2010). Multiple infections with more than one strain have also been found in 80% of infected marsupials (Pan *et al.*, submitted). These results may suggest the possibility of a panmictic population structure for *T. gondii* infecting wildlife in Australia, although these findings have also raised questions about the transmission dynamics of *Toxoplasma* in Australian wildlife, particularly in arid areas, and the role of the definitive host (Thompson et al 2010).

These observations in wildlife challenge our current understanding of *T. gondii* transmission dynamics and the parasite's ability to rapidly evolve to new strains. They also support the need for studies on the population genetic structure of *Toxoplasma* in SE Asia, which could provide clues to the origin of *Toxoplasma* in Australia.

Fish borne Flukes

Opisthorchis viverrini and *Clonorchis sinensis* are the aetiological agents of hepatobiliary diseases and bile duct cancer in SE Asia (Sripa et al., 2010). Humans contract infection from metacercariae ingested with the flesh of fresh water fish. Thus, the distribution of human infections corresponds well with the availability of snail and fish intermediate hosts and the food consumption habits of local populations (Chai et al., 2005). However, a limiting factor in the accurate surveillance of human populations for infection with *Clonorchis* and *Opisthorchis* in endemic regions has been the lack of morphological differences between the eggs (Chai et al., 2005). It is important to differentiate between the two species because of differences in clinical presentation and prognosis in humans, as well as treatment regimes (Haswell-Elkins and Levri 2003; Keiser and Utzinger 2010; Sripa et al., 2010).

Direct characterisation from faecal samples is now possible using PCR-based tools which are now being applied in different geographical regions and have recently identified for the first time the presence of *C. sinensis* in Thailand (Traub et al., 2009), where previously only *Opisthorchis* was thought to occur. For many years it was considered that the only fluke endemic in Thailand was *O. viverrini* and that *C. sinensis* was restricted to Korea, China, Taiwan and northern Vietnam. The role of reservoir hosts such as dogs, cats and pigs in the maintenance and epidemiology of infections with *O. viverrini* and *C. sinensis* in different endemic regions is not well understood and opinions differ on their importance (Haswell-Elkins and Elkins 1998; Chai et al 2005). This is an issue that must be investigated since the presence of reservoir hosts will compromise efforts at controlling infection in humans through mass drug treatment (as with hookworm – see

158 below). The availability of molecular epidemiological tools will thus allow accurate
159 surveillance for *O. viverrini* and *C. sinensis* in human populations, and the determination
160 of which reservoir hosts are most important for each species in particular endemic foci.
161
162 ***Taenia***
163
164 Cysticercosis and taeniasis caused by the zoonotic cestode *Taenia solium* is an emerging
165 issue in SE Asia, with evidence that the parasite is extending its range (Thompson et al
166 2003; Willingham et al., 2010). Neurocysticercosis is the most severe manifestation of a
167 *T. solium* infection in humans and results in significant morbidity and may be fatal. Poor
168 hygiene, inadequate sanitation, poorly managed pig production systems and socio-
169 cultural factors enable transmission, and control efforts, which are principally based on
170 education, tend to have limited impact in endemic areas where education levels and
171 literacy rates are low. However, an important factor in the epidemiology of human
172 cysticercosis is the fact that, very often, we are dealing with more than one *Taenia*
173 species in a *T. solium* endemic area (Anantaphruti et al., 2007; Conlan et al., 2008;
174 Willingham et al., 2010, Conlan et al., in prep). This has been highlighted in rural areas
175 of Laos where conditions are suitable for *T. solium* hyperendemicity, but *T. solium* is only
176 one of four species of *Taenia* that may interact at various stages of the life cycle (Conlan
177 et al., 2009; Conlan et al., in prep). As such, Conlan et al. (2009) have demonstrated that
178 an ecological approach is essential to understand the epidemiology of cysticercosis in
179 regions where multiple *Taenia* species sympatrically co-exist and interact in the same
180 intermediate and/or definitive host.

Immune-mediated competitive interactions in the intermediate host may have a suppressive influence on one or more related *Taenia* species, and in the case of human cysticercosis in Laos, *T. hydatigena*, for which dogs are the definitive hosts, may well serve to suppress *T. solium* infections in pigs (Conlan et al., 2009). Ongoing research in Laos is thus giving support to the pioneering research of Michael Gemmell who developed such competitive theories over 20 years ago based on studies of ovine cysticercosis (Gemmell et al., 1987). Since dogs are the definitive host of *T. hydatigena* in Laos they may be acting to naturally moderate and suppress *T. solium* infection in pigs, and as such this raises questions about the control of enteric parasite infections in dogs. If dogs naturally infected with *T. hydatigena* are indirectly reducing the risk of human populations acquiring *T. solium* cysticercosis, then this tapeworm infection in the dog population should be maintained. Careful consideration will therefore need to be given on how to control the cohabiting *Ancylostoma ceylanicum* (see below) if therapeutic intervention is used.

Hookworm

Human hookworm infections continue to cause significant morbidity in developing countries, particularly among disadvantaged communities where sanitation, cultural factors and the lack of appropriate education support transmission (Thompson et al., 2001; Knopp et al., 2010), even though low cost effective chemotherapeutics are readily available (Awashi and Bundy 2007; Keiser and Utzinger 2010; Smith and Brooker 2010).

Most attention has focused on the human population and little attention has been given to the role of other hosts in the epidemiology of patent enteric hookworm infections in humans. This is despite the fact that, for many years, *A. ceylanicum* has been known to produce patent infections in dogs, cats and humans (Carroll and Grove 1986). Because of the inability to differentiate between different species on the basis of egg morphology and the associated difficulties and confusion regarding differentiation of the adult worms (Carroll and Grove 1987; Traub et al., 2007), it is likely that *A. ceylanicum* has been overlooked in the aetiology of enteric hookworm disease in humans, particularly in SE Asia. According to recent reports, this appears to be the case.

The recent development of PCR-based techniques for differentiating between hookworm species using DNA isolated from eggs in faeces, has provided a valuable epidemiological tool (Traub et al., 2008). Using this approach, *A. ceylanicum* has been identified in humans and dogs in endemic communities in Thailand, in dogs in Australia for the first time, and most recently in humans in Laos (Palmer et al., 2007; Traub et al., 2008; Sato et al., 2010). In particular, the latter report highlights the impact *A. ceylanicum* may have on control programmes since in rural areas of Laos, nearly 100% of dogs are infected with hookworm and up to half the human population (Conlan et al., in prep). Research is underway to determine the proportion of *A. ceylanicum* infections in the dog and human populations. *A. ceylanicum* is bound to impact on control since mass chemotherapy focusing on the human population alone is unlikely to be totally successful, and may even provide a unique ecological niche in which *A. ceylanicum* can thrive. The role of the dog in the transmission of hookworm infection to humans has to be considered and may

require better management and treatment of dogs. This will contribute to the cost of control, and care will have to be taken in the choice of anthelmintic used, given the value of maintaining *T. hydatigena* in the dog population of rural Laos (see above).

Conclusions

Here we discuss the emergence of issues critical to our understanding of zoonotic parasites of significant public health concern. Molecular technologies provide a tool to investigate emerging issues such as those described here, but these and other tools are no substitute for asking the right questions. Clearly we must not fall into the trap of unquestioningly accepting the established dogma as regards parasite ecology, vector competence and parasite interactions within an intermediate or definitive host. For example, dogs may truly be man's best friend in SE Asia if they are infected with *T. hydatigena* and are suppressing *T. solium* but may concurrently contribute to chronic disease in people if infected with *A. ceylanicum*. The established dogma would have us believe that *T. hydatigena* is a nuisance in pigs resulting in occasional liver condemnation at slaughter and that *A. ceylanicum* is inconsequential and does not cause chronic anaemia. The scientific and public health communities must take an unblinkered approach to investigations of zoonotic parasites if we are to have significant impact in reducing the burden of human disease.

Conflict of interest

The authors declare that there is no conflict of interest.

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475 **Table 1.** The emergence of *Leishmania* in SE Asia and Australia

Year	Observation	Reference
1996	First autochthonous case of visceral leishmaniasis reported in Thailand	Thisyakorn et al. (1999)
1999	46 cases of cutaneous leishmaniasis reported in indigenous population of East Timor (not confirmed)	Chevalier et al. (2000)
2000	Questions raised about the vectorial capacity of Australian phlebotomine sandflies	Thompson and Reid (2000)
2004	Novel species of *Leishmania* described in kangaroos in northern Australia	Rose et al. (2004)
2005-2006	Potential sandfly vectors identified in Thailand	Poleseela et al. (2007)
2007-2008	More autochthonous cases of visceral leishmaniasis reported in Thailand	Maharom et al. (2008)
2008	Novel aetiological agent of visceral leishmaniasis identified in Thailand	Sukmee et al. (2008)
2009	More reports of novel species of *Leishmania* in Australian macropods	Dougall et al., (2009)
2009-2010	*Leishmania* closely related to Thai species found in cutaneous lesions from horses and cattle in Switzerland	Muller et al. (2009) Lobsiger et al. (2010)
2010	Non-sandfly vectors of *Leishmania* identified in Australia	Dougall et al. (2010)*

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