*Tropical Biomedicine* 30(3): 355–366 (2013)

## Review Paper

# Human and animal invasive muscular sarcocystosis in Malaysia – recent cases, review and hypotheses

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Received 8 July 2013; received in revised form 31 July 2013; accepted 1 August 2013

**Abstract.** Sarcocystosis, an unusual parasitic zoonotic disease, is caused by coccidian/apicomplexan protozoa in humans and animals. The parasites usually develop in a heteroxenous predator-prey life-cycle involving final (carnivore) and intermediate (omnivore/herbivore) hosts. Besides the intestinal, non-invasive form of the disease in which humans and animals are the definitive hosts for certain *Sarcocystis* spp., the invasive form has come to recent attention. In the latter, humans and animals serve as intermediate host harbouring sarcocysts in their muscle tissue. Already in 1991 sarcocystosis was seen as a potential emerging food-borne zoonosis in Malaysia, and in 2011 and 2012 the largest cluster of symptomatic human muscular sarcocystosis world-wide was reported from Tioman Island, Pahang state. In this review, we focus on invasive sarcocystosis in humans and animals in Malaysia, review the recorded cases and epidemiology, and present hypotheses.

## INTRODUCTION

Sarcocystosis, a cosmopolitan zoonotic parasitic disease, is caused by small intracellular apicomplexan/coccidian protozoa of the genus *Sarcocystis* (Eucoccidiorida: Sarcocystidae). There are more than 120 recognized species in the genus, and the parasites usually develop in a heteroxenous predator-prey two-host life-cycle. The definitive host is often a predator and the intermediate host its respective prey. In the gut enterocytes of the definitive host, the parasite multiplies sexually by gamogony. Oocysts containing 2 sporocysts (diagnostic stages for the final host) with each sporocyst harbouring 4 sporozoites are eventually shed with the faeces into the environment. After ingestion of these infective forms (oocysts /sporocysts) by the intermediate host, asexual schizogony occurs in vascular endothelial cells. There, first and second generations of merozoites are released from schizonts, and finally invade muscle cells in order to form the typical tissue sarcocysts. With the exception of humans, the definitive host often does not show any symptoms, or only mild disease, of the non-invasive intestinal infection. In contrast, the intermediate host, including humans, usually shows pronounced symptoms of the invasive disease. In the intermediate host the sarcocysts are found most often in the heart, tongue, oesophagus, diaphragm, skeletal muscle, and rarely in the central nervous system and gut (Prakas & Butkausas, 2012). The main diagnostic criterium in the intermediate host is the presence, structure, size, and shape of the parasitic tissue cyst. This depends, however, on maturation of the cysts, type of host cell, and intermediate host species (Prakas & Butkausas, 2012). Electron microscopical studies revealed that the sarcocysts closely resemble the cysts of

*Toxoplasma gondii*, a related apicomplexan parasite (Matuschka, 1987). The shape of the cyst is elongated to oval (often 100-300 x 20-80 µm, sometimes much larger) with a wall thickness of 1-6 µm, and with or without internal villi or wall striations. Morphologically similar sarcocysts are found in muscles of taxonomically related intermediate host species (Prakas & Butkausas, 2012), and thus, the morphological features of the sarcocyst may be dependent on either the *Sarcocystis* species and/or the host. In the cyst, two stages are recognized, the peripheral metrocytes, and the central cystozoites or bradyzoites (Figure 1). The size and shape of the bradyzoites, the occurrence of septa or villi and a thick cyst wall are the characteristics to distinguish *Sarcocystis* cysts from *Toxoplasma* cysts. Moreover, metrocytes are not seen in *T. gondii* cysts, and multiplication in sarcocysts occurs by endodyogeny.

Sarcocystosis is one of the most prevalent parasitic diseases among wild and domestic animals in the world. Muscular cysts of various *Sarcocystis* spp. are found in a broad spectrum of intermediate hosts, such as mammals (74%), birds (14%) and reptiles (10%). Only 0.5% of the intermediate hosts are fish (Prakas & Butkausas, 2012). The specificity of the individual *Sarcocystis* sp. to the intermediate host is variable, and some *Sarcocystis* sp. can fully complete their life-cycle in one and the same host (dihomoxenous life-cycle). In contrast to what is known about intermediate hosts, 56% of the definitive hosts are unknown for *Sarcocystis* sp.. Of the known definitive hosts, mammals contribute 27%, reptiles 11%, and birds 6% (Prakas & Butkausas, 2012). The four most common combinations of intermediate/definitive hosts are mammals/ mammals, mammals/reptiles, reptiles/ reptiles, and mammals/birds (Prakas & Butkausas, 2012).

Humans serve as definitive host for only two *Sarcocystis* species: *S. hominis*, whose intermediate hosts are cattle and water buffaloes, and *Sarcocystis suihominis*, whose intermediate hosts are domesticated pigs and wild boars. Consumption of raw or undercooked meat infected with the intracellular cystic stage of the parasite leads to intestinal, non-invasive sarcocystosis with watery diarrhoea, nausea, and other gastrointestinal symptoms in humans (Prakas & Butkausas, 2012). Accidentally and rarely, human beings can also become intermediate hosts for unknown *Sarcocystis* sp. Initial human cases of such invasive sarcocystosis in Malaysia (1975-1992) were all incidental autopsy/biopsy findings in different ethnic groups of the Malaysian population (Pathmanathan & Kan, 1992). The

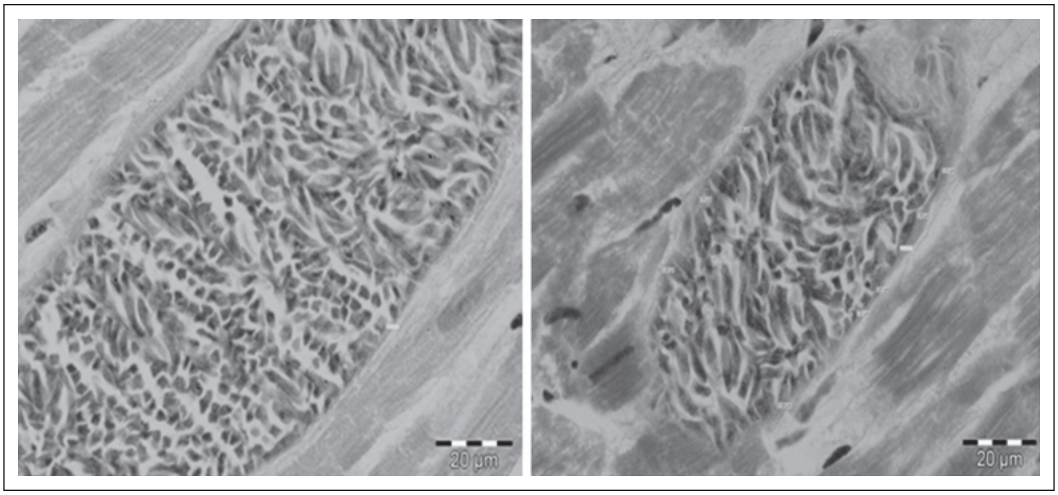


Figure 1. Typical sarcocysts found in muscle of bovine heart. The exact *Sarcocystis* species has not been determined. The sarcocysts are surrounded by a thin cyst wall and contain a multitude of bradyzoites. Haematoyxlin and eosin stain, original magnification x 100

first clinically symptomatic and documented cases in the country were those affecting a group of US servicemen working in rural peninsular Malaysia in 1993 (Arness *et al.*, 1999). Already in 1991, sarcocystosis was seen as a potential emerging food-borne zoonosis in Malaysia (Kan & Pathmanathan, 1991) because of high seroprevalence and results of autopsy cases. Nearly 50% of all globally recorded cases until 1992 were noted in Malaysia (Wong & Pathmanathan, 1992). In 2011 and 2012, the largest cluster of symptomatic human muscular sarcocystosis world-wide was reported from Tioman Island, Pahang state, affecting travellers (Esposito *et al.*, 2012; Von Sonneburg *et al.*, 2012; Tappe *et al.*, 2013). The prevalence of muscular sarcocystosis might be higher in South East Asia than in any other parts of the world (Wong & Pathmanathan, 1992).

In this article, we review human and animal cases of invasive sarcocystosis in Malaysia, discuss the epidemiology of this protozoan disease in the country, and present analyses and hypotheses concerning the current outbreak in travellers.

**Early Human Cases of Muscular Sarcocystosis in Malaysia**

The first human case of invasive muscular sarcocystosis in Malaysia was described in 1975 (Kannan Kutty & Dissanaike, 1975), followed by scattered singular reports until 1992 (Table 1). All were incidental biopsy findings and no clinical symptoms could be associated with the disease, except possibly for the first Malaysian case. In this case hoarseness of voice may have been caused by sarcocystosis of a fibromuscular nodule found during laryngoscopy. The sarcocysts reported in human muscle resembled

Table 1. Human cases of sarcocystosis in Malaysia

| Number of Cases | Tissue / Organ Involved | Signs & Symptoms | Reference |
|---|---|---|---|
| 1 | Laryngeal | Hoarseness of Voice / Maybe Incidental | Kutty *et al.*, 1975 |
| 1 | Oropharyngeal/Nasopharyngeal | Incidental Biopsy Finding | Kutty *et al.*, 1975 |
| 1 | Skeletal Muscle | Incidental Biopsy Finding | Prathap & Dissanaike, 1976 |
| 1 | Skeletal Muscle | Incidental Biopsy Finding | Prathap & Dissanaike, 1978 |
| 3 | Skeletal Muscles | Incidental Biopsy Finding | Kannan Kutty, Unpublished Data, 1978-1979 |
| 1 | Skeletal Muscle | Incidental Biopsy Finding | Pathmanathan & Kan, 1981 |
| 2 | Skeletal Muscles | Incidental Biopsy Finding | Pathmanathan & Kan, 1987 |
| 1 | Skeletal Muscle | Incidental Biopsy Finding | Pathmanathan *et al.*, 1988 |
| 3 | Skeletal Muscle | Incidental Biopsy Finding | Pathmanathan & Kan, 1992 |
| 21 | Tongue Muscles | Autopsy Study Findings | Wong & Pathmanathan, 1992 |
| 2 | Soft Tissue and Skeletal Muscles | Incidental Biopsy Finding | Shekhar *et al.*, 1998 |
| 7 | Systemic Illness; One Positive Muscle Biopsy | Fever, Myalgia, Lymphadenopathy, Rash, Subcutaneous Nodules, Bronchospasm | Arness *et al.*, 1999 |
| >100 (current cases) | Systemic Illness; Positive Muscle Biopsies (Investigations Ongoing) | Fever, Myalgia, Rash, Headache | Sonnenburg *et al.*, 2012; Esposito *et al.*, 2012; Tappe *et al.*, 2013; Slesak *et al.*, 2013 |

The cases and studies are listed according to the year they were published.

morphologically those seen in the moonrat, *Echinosorex gymnurus*, and the long-tailed macaque, *Macaca fascicularis* (Beaver *et al.*, 1979; Kan & Dissanaike, 1976; Kan *et al.*, 1979), however, the species infecting humans remained unknown. From human cases elsewhere in the world, a vascular host derived stroma around the parasite was described in histopathological investigations (Bonne & Soewandi, 1929). Atrophy of skeletal muscle fibres with degeneration and fibrosis and presence of varying number of lymphocytes and granulocytes in the connective tissue stroma was reported (Kean & Grocott, 1945; Gupta, 1973; Thomas, 1976). Even metaplastic osteochondroid foci in the parasitized muscles and vacuolation of muscle fibres have been recorded (Thomas, 1976). Investigators in Malaysia had not found any surrounding tissue reaction (Wong & Pathmanathan, 1992). Prompted by the incidental findings in Malaysian patients, a retrospective prevalence study was performed by serial examination of formalin-fixed and paraffin-embedded sections of tongue tissues procured from consecutive routine autopsies of subjects aged 12 years or above. Of a total of 100 tongues studied, 21% contained sarcocysts (Wong & Pathmanathan, 1992). The incidence of positive cases amongst Chinese, Indians and Malays was 62%, 33%, and 5%, respectively, in this study, reflecting in total a high positivity rate in the Malaysian population. Males (81%) were seemingly more often infected than female patients, but the patient cohort consisted of motorvehicle accident victims which may have biased gender ratio. The cysts had an average width of 77 µm and cyst walls were smooth with a thickness of < 1 µm. No surrounding inflammation was evident (Wong *et al.*, 1994). In these patients, also no symptoms could be attributed to the sarcocystosis findings, as no symptoms had been recorded prior to death. The first cases of clinically symptomatic muscular sarcocystosis in the country were not described until 1999, when 7 of 15 US servicemen who worked in a rural remote Malaysian village fell ill in 1993 (Arness *et al.*, 1999). In these patients, fever, myalgia, rash, eosinophilia and elevated levels of muscle enzymes were present (febrile eosinophilic myositis syndrome). Several sarcocysts were found in one patient by muscle biopsy, showing acute *Sarcocystis* myositis with eosinophil tissue infiltration. The sarcocyst measured 620 x 50 µm and had a thin cyst wall (< 0.5 µm).

A rather puzzling feature observed in the study of human sarcocystosis is the high prevalence of this parasitic infection noted in cancer patients (Shekhar *et al.*, 1998). Out of 36 cases reported hitherto 11 cases were associated with some form of malignancy. In one of our own cases, sarcocysts were incidentally detected in the nasopharynx and oropharynx of a patient with malignant brain melanoma during the search for the primary tumour (Kutty *et al.*, 1975). Arguably immunosuppression in cases of malignancy could predispose to opportunistic parasite invasion. In the cases reported so far there has not been any detectable evidence of tissue reaction at the site of the parasite invasion to provoke neoplastic alterations, and thus, sarcocystosis in cancer patients seems to be a coincidence in countries with high sarcocystosis prevalence.

While human intestinal sarcocystosis has hitherto not been reported in Malaysia, it can be presumed that such cases may not be infrequent in view of the occurrence of *Sarcocystis* cysts in meat animals, such as buffaloes, sheep, goat and cattle (Norlida *et al.*, 2012; Latif *et al.*, 2013). The overall seroprevalence of 19.8% reported among the main racial groups in Malaysia matched closely the autopsy positivity rate. Already in 1991 sarcocystosis (both the intestinal and the muscular forms) was seen as an emerging significant food-borne zoonotic infection in the country (Kan & Pathmanathan, 1991).

## Animal Muscular Sarcocystosis in Malaysia

In Malaysia, invasive sarcocystosis has been reported in several wild and domestic animals, including primates, water buffaloes, zoo animals, and a multitude of rodents (Table 2). Regarding the pathological effects of sarcocystosis, most infected animals are asymptomatic. The parasites are seen

Table 2. Sarcocystosis among wild and domestic animals in Malaysia

| Intermediate Animal Host | *Sarcocystis* sp. | Reference |
|---|---|---|
| Slow Loris (*Nycticebus coucang*) | Unknown *Sarcocystis* sp. | Zaman, 1970 |
| Long-Tailed Macaque (*Macaca fascicularis / irus*) | Unknown *Sarcocystis* sp. | Prathap, 1973; Kan *et al*., 1979 |
| Rat (*Rattus norvegicus*) | *S. orientalis* | Zaman & Colley, 1975 |
| Moon Rat (*Echinosorex gymnurus)* | *S. booliati* | Dissanaike & Poopalachelvam, 1975; Kan & Dissanaike, 1976 |
| House Rat (*Rattus rattus*) | Unknown *Sarcocystis* sp. | Kan & Dissanaike, 1977 |
| Various Rats (*R. annandalei*, *R. exulans*, *R. jolorensis*) | Unknown *Sarcocystis* sp | Lai, 1977 |
| Water Buffaloes (*Bubalus bubalus*) | *S. fusiformis*, *S. levinii*, and unknown *Sarcocystis* sp. | Dissanaike *et al*., 1977; Kan & Dissanaike, 1978; Dissanaike & Kan, 1978 |
| Bandicoot (*Bandicota indica*), Various Rats (*R. diardii*, *R. exulans*, *R. jalorensis*, *R. annandalei*) | Unknown *Sarcocystis* sp. | Kan, 1979 |
| Zoo Animals (Wallabies, Malaysian Sun Bear, Parrot, Owl, Hornbill) | Unknown *Sarcocystis* sp. | Latif *et al*., 2010 |
| Seven Undefined Rodent Species | Unknown *Sarcocystis* sp. | Ambu *et al*., 2011 |
| Sheep | Unknown *Sarcocystis* sp. | Norlida *et al*., 2012 |
| Cattle, Water Buffaloes | Unknown *Sarcocystis* sp. | Latif *et al*., 2013 |

The cases and studies are listed according to the year the were published.

Table 3. Morphology and size of *Sarcocystis* sp. cysts in tissues of wild and domestic animals

| Animal Host | Type of Sarcocyst | Shape of Sarcocyst | Size of Sarcocyst | Wall Thickness | Reference |
|---|---|---|---|---|---|
| Rats | Macroscopic | Elongate | 2 x 4.4 mm | 4.5 µm | Zaman & Colley, 1975 |
| Zoo Animals | Microscopic | Elongate | 24.5 x 254 µm | 2.5 µm | Latif *et al*., 2010 |
| Rodents | Microscopic | Elongate | 53.2–65.7 x 139.6–247.7 µm | Not Mentioned | Ambu *et al*., 2011 |
| Water Buffalo | Macroscopic Microscopic | Not Mentioned for Either Type | Macroscopic: Not Mentioned Microscopic: 0.1 x 0.9 mm | Not Mentioned for Either Type | Dissanaike & Kan, 1978 |
| Cattle & Water Buffaloes | Microscopic | Elongate/ Oval | 75.83 x 151 µm | 2.47 µm | Latif *et al*., 2013 |

mainly as an incidental finding at necropsy, similar to the recorded human cases. Tissue sarcocysts occur either as microscopically or as macroscopically visible structures in animal intermediate hosts (Dubey *et al*., 1989). Most studies on sarcocystosis in animals referred to the microscopic type and were based on histomorphological appearances using classical haematoxyline and eosin staining. An established method for detecting the infection rate in tissue is the muscle squash and digestion screening technique, followed by formalin-fixation and paraffin-embedding for microscopy (Latif *et al*., 1999). For several intermediate host animals, the morphological cyst characteristics have been reported (Table 3). However, based solely on these measurements, it is not possible to determine the exact species of *Sarcocystis* responsible for infection.

Recently, it was reported that infected zoo animals in Malaysia had died without previous clinical signs, but at necropsy there was atrophy of the sternal muscles with haemorrhage and oedema of the lungs (Latif *et al.*, 2010). In infected sheep, for example, there is degeneration of myocardial and skeletal muscle fibres with infiltration of inflammatory cells including eosinophils, neutrophils and lymphocytes (Norlida *et al.*, 2012). The pathological changes are due to the second stage schizonts which cause obstruction of capillaries of the infected organs, especially in the lungs, liver, and heart (Dubey *et al.*, 1989, 2000). In the recent zoo animal study it was shown microscopically after necropsy that of 20 captive wild mammals and 20 birds (12 animal species each), 15% of the mammals and 25% of the birds were infected with *Sarcocystis* sp. (Latif *et al.*, 2010). The place of infection of these captive zoo animals is unknown, but the sarcocystosis prevalence was strikingly lower than in animals living in the wild. Other studies showed a prevalence of *Sarcocystis* infection as high as 50% in wild and peri-urban rodents (Ambu *et al.*, 2011). Prevalences of 38% in sheep (Norlida *et al.*, 2012), and 35.3% in cattle and water buffaloes (Latif *et al.*, 2013) were also recorded. Except for a few *Sarcocystis* species the definitive hosts for the majority of the parasites found in animal tissue in Malaysia are unknown. For *Sarcocystis orientalis* and *Sarcocystis singaporensis* found in rodent tissue the reticulated python (*Python reticulatus*) is the definitive host (Zaman & Colley, 1975), and for *Sarcocystis fusiformis* and *Sarcocystis levinei* seen in water buffalo tissues, cats and dogs are the definitive hosts (Kan & Dissanaike, 1978). Studies in wild and domestic animals are valuable in unravelling the role of these animals as parasite reservoirs in the transmission of sarcocystosis, be it the invasive muscular form, or the non-invasive intestinal form. Identification of definitive hosts, and also identification of intermediate hosts (such as meat-producing animals), is therefore important for epidemiological studies and infection prevention. Most reports relied on histopathological findings without the identification of the *Sarcocystis* species. Molecular studies of the species infecting animal muscular tissue have not yet been performed. Such studies will follow in the future, with the aim of elucidating the life-cycles and the sources of transmission.

**Current Human Cases in Malaysia**

In the late summer of 2011, 5 German travellers who had spent a vacation in Malaysia had been seen with an acute illness upon returning home (Tappe *et al.*, 2013). Symptoms had consisted of fever, headache, malaise, and severe myalgia. In one of the patients, a typical sarcocyst had been found in a muscle biopsy. Laboratory parameters in all patients had shown eosinophilia and muscle enzyme elevation (creatine kinase [CK], lactate dehydrogenase [LDH], alanine-aminotransferase [ALT]). After serologies for toxoplasmosis, trichinellosis, dengue and chikungunya fever had been negative, the diagnosis of an invasive *Sarcocystis*-like infection with myositis had been made, based on results of the muscle biopsy. These patients had formed the first cluster, and soon, a total of 100 cases were seen successively in Europe and elsewhere in the following months and in the summer of 2012 (Esposito *et al.*, 2012; Von Sonnenburg *et al.*, 2012;). The outbreak appeared in two waves, with the first one comprising 35 patients in 2011, and the second one in 2012 consisting of 65 sick returned travellers. All patients had in common that they had stayed on Tioman Island (2º 48' 47" N, 104º 11' 17" E), Pahang state, 32 km off the east coast of peninsular Malaysia, before becoming symptomatic. They had mostly visited Tioman during July and August in either year (Esposito *et al.*, 2012). The majority had stayed in only a few villages, mostly on the northern tip of the island, where they were engaged in swimming, snorkeling and scuba diving in the sea. The estimated incubation period of 2 weeks (Slesak *et al.*, 2013) and the presence of a muscular sarcocyst as seen in the initial patient 8-9 weeks after leaving Tioman (Tappe *et al.*, 2013) matches animal records of experimental invasive *Sarcocystis* infection in cattle (Dubey *et al*., 1989). The symptoms and laboratory findings described in the initial German patients and the other returned travellers were also in line with what was known from the scarce human clinical cases of invasive sarcocystosis recorded before (Beaver *et al*., 1979; Arness *et al*., 1999; Fayer, 2004). A current analysis of 26 German cases from 2011 and 2012 after the stay on Tioman (3-15 days) revealed that the illness typically showed two phases: An initial phase of approximately 1 week with fever, headache, sweating and myalgia which was followed by an asymptomatic interval of approximately 2 weeks (Slesak *et al*., 2013). Finally, the second phase developed with protracted and severe myalgia, headache, recurring fever and sweating, characterized by the mentioned laboratory changes with eosinophilia, partial IgE elevation and hightened muscle enzyme serum levels. Such a biphasic course was also described in one patient from the outbreak involving US military personnel in peninsular Malaysia (Arness *et al*., 1999). Symptoms of the second phase were generally more severe than those of the first, and lasted much longer (>6 weeks) (Slesak *et al*., 2013). These symptoms and laboratory characteristics matched closely with the aforementioned data from the animal studies (Dubey *et al*., 1989). In the life-cycle of *Sarcocystis*, the first formation of schizonts and merozoites takes place in endothelial cells during the first phase, and the intermediate hosts develop fever. After the asymptomatic interval, in the second clinical phase, second generation schizonts and merozoites emerge, leading to fever and laboratory changes. Eventually the muscular sarcocysts develop after invasion of host myocytes (Dubey *et al*., 1989). Similar to the biopsy finding in the acute clinical case of the US serviceman in 1993, the German patient had also shown eosinophilic myositis. In contrast, the incidental biopsy and autopsy findings did not show eosinophil muscle infiltration, thus making eosinophil infiltration (and peripheral blood eosinophilia) a hallmark of acute, and not chronic disease. In the current patient cohort, severe pain intensity was associated with female gender, whereas longer pain duration was associated with older age (Günther Slesak, personal communication). Remarkably, the duration of illness was shorter in children than in adults. The wave-like pain mostly affected the proximal muscles of the limbs, was strongest when movement was initiated and eventually slowly subsided over weeks to months (Slesak *et al*., 2013). Cardiac involvement was seen in some patients (ECG changes, troponin and CK-MB elevations), but no dangerous courses were recorded. Steroids and albendazole were used for treatment and in some patients treatment was intensified by giving repeated courses leading to rapid improvement of symptoms (Slesak *et al*., 2013; Tappe *et al*., 2013). Apparently, asymptomatic courses exist, as one returned traveller has shown typical laboratory changes but no clinical symptoms (Slesak *et al*., 2013). Therefore, there maybe more patients who were infected but did not turn up for medical attention.

A local surveillance study for sarcocystosis conducted on Tioman in November 2011 could not determine the source of infection responsible for the case clusterings, possibly because the study was not performed during the period of time when travellers became infected (July – October) and the sample sizes were too small. The few local residents examined were asymptomatic, their stool was negative for sporocysts, as were animal faeces (cats, dogs, goats, cattle). However, the water supply pipes on the island were found to be leaking and *Escherichia coli* was detected as a fecal contaminant marker in the majority of water samples, but no *Sarcocystis* sporocysts were detected (Husna Maizura *et al*., 2012). Thus, the animal source of contamination and the kind of contaminated food or water is currently unknown.

## Unsolved Mysteries: The Source of Infection in Current Cases

The exact *Sarcocystis* species which infects humans as accidental intermediate host is unknown (Arness *et al*., 1999), also in the Malaysian cases (Pathmanathan & Kan, 1992). It is believed however, that human beings become infected by consuming food or water contaminated with faeces from a predator of non-human primates (Fayer, 2004). For example, *S. nesbitti*, a species which presumably uses snakes as final hosts, has been found in muscular tissues of macaques (Mandour, 1969; Yang *et al.*, 2005; Tian *et al.*, 2012). Already in 1991 it was speculated that human invasive sarcocystosis might be due to a species that naturally infects monkeys and rats, based on the structural similarities of the sarcocysts (Kan & Pathmanathan, 1991). In Malaysia, snakes, cats and dogs have been suggested to serve as final hosts for the early human cases (Kan & Pathmanathan, 1991). Phylogenetic analysis of *Sarcocystis* sp. infecting mammals and reptiles supported the concept of co-evolution of the parasites with their final hosts, and thus, such analyses can be useful tools in the search for an unknown final host (Dahlgreen *et al.*, 2008; Tian *et al.*, 2012). Reptiles have been shown to be intermediate and/or final hosts for *Sarcocystis* infection (Matuschka & Bannert, 1989). On Tioman, several species of snakes are found including the reticulated python, *P. reticulatus* (Lim & Lim, 1999), which has been demonstrated to be the final host for *S. orientalis* (Zaman & Colley, 1975) and *S. singaporensis* (Jäkel *et al.*, 1999), parasites closely related to *S. nesbitti*. *S. singaporensis* frequently occurs in rodents in Southeast Asia (Jäkel *et al.*, 1999). Besides this 'classical' rodent-snake cycle, there are lizard-snake cycles (and vice versa), and even lizard-lizard cycles (see below) for various *Sarcocystis* species. Thus, *S. nesbitti* might not be exclusively associated with snakes, but with reptiles in general, such as the growing population of water monitors (*Varanus salvator macromaculatus*) especially found in the north of Tioman, where all sick travellers had stayed. In contrast, snake sightings on Tioman are rare, and many travellers have independently reported on water monitors instead. They were often seen close to the beach, in restaurant areas and on river beds scavenging for food, and in small rivers, such as Sungai Salang (Figure 2). Water monitors spend much time in these small streams, where they also defecate, and the rivers flow into the open sea where people swim and prepare for snorkeling and scuba diving. It might thus be possible that *Sarcocystis* sporocysts shed with *Varanus* faeces are swallowed by humans whilst swimming in the sea close to the river mouths. Interestingly, in island-dwelling giant lizards (*Gallotia* sp.), some *Sarcocystis* species (*Sarcocystis simonyi*, *Sarcocystis gallotiae*, *Sarcocystis dugesii* and *Sarcocystis stehlinii*) may undergo sexual and asexual development in one and the same host or in different individuals of the same host species (dihomoxenous life-cycle), transmitted by cannibalism (Bannert, 1992) and thus abrogating the need of the parasite for a different intermediate host species. However, as invasive sarcocystosis is a food-borne zoonosis, any other food, drink, or water contaminated with oocysts or sporocysts of an unknown omnivorous or carnivorous final host could be responsible for human infection.

A striking feature is the time of the year when the travellers acquired the infection on Tioman (July-October), the last half of the dry season, in two successive years (2011 and 2012). This time period matches approximately with the breeding season of water monitors which begins in April and lasts until October, i.e. around the beginning of the wet season. Speculatively, increased local water monitor accumulations near tourist spots on the island during the mating and breeding season (e.g., more water monitors in rivers), altered reptile behaviour during such periods (e.g., biting and possible parasite dihomoxenous life-cycle), and the rise of newly susceptible water monitor generations (followed by intestinal *Sarcocystis* infection) might contribute to such a seemingly time-dependent characteristic of this outbreak. If there was any other omnivorous or carnivorous animal that showed such changes in population dynamics during this certain time of the year, it would be a good candidate as final host also. However, the period of infection mentioned might simply be a coincidence, for these months are usually the summer holiday months for foreign tourists and the best time to visit the island, or dependent on climate factors, such as low precipitation. The local

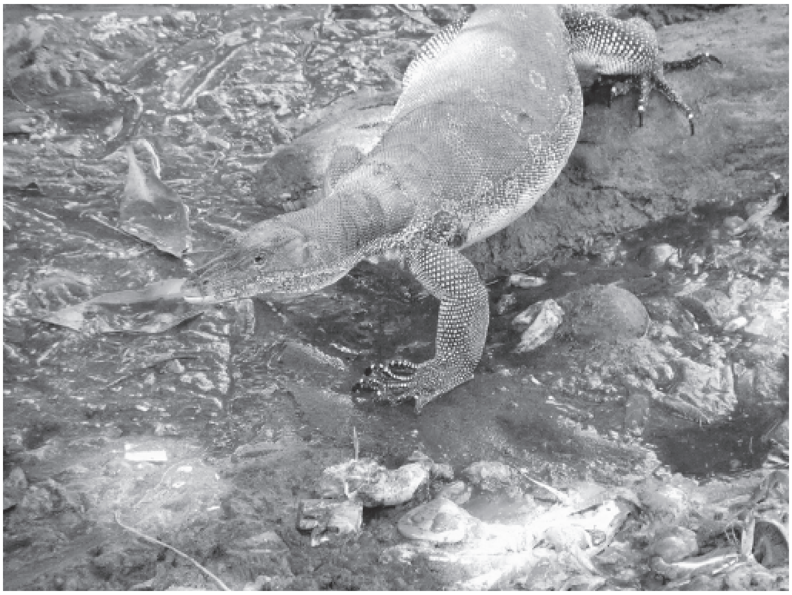

Figure 2. The water monitor, *Varanus salvator macromaculatus*, on Sungai Salang river bed. These animals have reportedly been noticed searching for food and swimming in streams in the north of Tioman by many travellers. The water monitors are known to eat fish, frogs, rodents, birds, crabs, and even snakes. They will also often eat carrion and discarded food. Besides other omnivorous and carnivorous animals (such as snakes), they could be a possible source of sarcocystsis in the current cases seen in Malaysia

study on Tioman (Husna Maizura *et al.*, 2012) was performed during the wet season and the authors deduced from their negative findings that rain might have diluted the source of infection in a way that it could not be detected. No possible reptile final hosts were screened either. Another unsolved mystery in this outbreak is the circumstance that apparently neither the local population nor the Malaysian tourists have come to medical attention. Whether this might be due to previous immunity to sarcocystosis (given the high seroprevalence and autopsy positivity rate in the country) would have to be elucidated. In conclusion, the search for an animal reservoir, faeces and tissue biopsies of water monitors, snakes and rodents living on the island could be screened parasitologically for oocysts/sporocysts and sarcocysts, respectively, and analyzed by molecular tools to the species level. Depending on these results, the most likely way of contamination of edibles and water can be determined, and further cases can be prevented. In addition, a serostudy involving the local population on the island seems favourable, in order to record the full extend of the outbreak, and to identify any asymptomatic cases.

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