

## ORIGINAL ARTICLE

# SURVEILLANCE FOR SARCOCYSTOSIS IN TIOMAN ISLAND, MALAYSIA

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## ABSTRACT

In October 2011, the National International Health Regulations (IHR) 2005 Focal Point for Malaysia received notification from the United States' Centers for Disease Control and Prevention (CDC) of a probable *Sarcocystis* outbreak amongst 23 travellers from six countries who had vacationed on Tioman Island between June and August 2011. The Ministry of Health, Malaysia (MOH) in collaboration with the Department of Veterinary Services, Malaysia (DVS) conducted a cross sectional study in November 2011 to determine the presence of *Sarcocystis* among humans, animals and in the environment in Tioman Island. Epidemiological investigations conducted involved a community health survey of 44 residents in Kampung Salang, Tioman and review of outpatient attendance cards for suspected or confirmed cases of *Sarcocystis*. Twenty-eight fresh stool samples were collected and sent to the National Public Health Laboratory (NPHL) for detection of *Sarcocystis* oocysts using fluorescence microscopy. Water samples taken from 27 water sampling points around the island were processed and analysed under the fluorescence microscope using ultraviolet (UV) light at the Institute for Medical Research (IMR) to detect the presence of *Sarcocystis* sporocyst. DVS collected 84 faecal samples from four types of domesticated animals and then analysed them at the Veterinary Services Centre in Tioman Island for *Sarcocystis* oocysts and other parasitic ova and cysts using qualitative Floatation Technique. The results showed that *Sarcocystis* was not present in humans, animals and in the environment in Tioman Island during the study period. Further surveillance among humans, wildlife and the environment is needed to determine *Sarcocystis* endemicity in Tioman Island.

**Key words:** Surveillance, *Sarcocystis*, *Sarcocystis*, Malaysia, epidemic reports, Tioman Island.

## INTRODUCTION

Sarcocystosis is a zoonosis caused by infection of the intracellular protozoan parasite with requisite two-host life cycle on the prey-predator host relationship, *Sarcocystis spp*<sup>1</sup>. Humans can present with two types of *Sarcocystis* infection, namely intestinal and muscular Sarcocystosis<sup>1</sup>. Intestinal Sarcocystosis are acquired when humans eat raw or undercooked meat and serve as the definitive hosts to the *Sarcocystis* parasite. In this instance, the infections are often asymptomatic and clear spontaneously. Muscular Sarcocystosis infections are acquired when humans act as an accidental host by ingesting sporocysts or infective oocysts in contaminated food or water<sup>1</sup>. In this case, humans are dead-end intermediate hosts and the infections resulted in formation of sarcocysts in tissues<sup>1</sup>.

The prevalence of Sarcocystosis in the world is low with fewer than 100 confirmed cases reported in the literature<sup>1</sup>. Most of the cases reported were incidental findings in asymptomatic persons<sup>1</sup>. Sarcocystis infection has been reported from tropical or sub-tropical countries, especially in Asia and Southeast Asia<sup>1</sup>. Cases have also been reported from Africa,

Europe, the United States, Central and South America<sup>1</sup>. In Malaysia, the prevalence rate of human intestinal Sarcocystosis is unknown<sup>2</sup>. However, muscular Sarcocystosis has been reported as an incidental finding from autopsied or biopsied materials from 11 local cases since 1975<sup>2</sup>. In 1978, a seroepidemiological survey found that 19.7% of 243 persons in West Malaysia had antibodies to *Sarcocystis*<sup>3</sup>. However, seropositivity for *Sarcocystis* antibodies could not differentiate between muscular or intestinal Sarcocystosis<sup>3</sup>. In 1999, an outbreak of acute eosinophilic myositis attributed to Sarcocystosis was reported involving seven out of 15 American military personnel who underwent an operation in rural West Malaysia<sup>4</sup>. Symptoms in five of the cases were mild to moderate and self-limited, while one case with laboratory abnormalities was asymptomatic<sup>4</sup>. Only the index case was reported with persistent symptoms for more than 5 years despite treatment<sup>4</sup>.

As the prevalence of infection is low with mostly asymptomatic or mild and self-limiting presentations, there is no specific ongoing surveillance programme for human Sarcocystosis in Malaysia. On 31 October 2011, multiple sites from the Geosentinel, the surveillance program

of the International Society of Travel Medicine and the United States Centers for Disease Control and Prevention (CDC), reported a cluster of approximately 23 cases of probable *Sarcocystis* infection in travellers from six countries who returned from vacation on Tioman Island between June and August 2011<sup>5</sup>. In a subsequent report, 32 symptomatic cases were detected but only two cases were confirmed as *Sarcocystis* from muscle biopsy<sup>6</sup>. All cases had fever, myalgia or musculoskeletal complaints, and eosinophilia, whilst some had diarrhoea initially and have improved either from a self-limiting disease or symptomatic treatment<sup>5</sup>.

## METHODOLOGY

Tioman Island lies some 56km off the east coast of Peninsular Malaysia in the South China Sea and is about 38km in length and 19km at its widest point<sup>7</sup>. The island is accessible by boat from jetties in Mersing, Johor and Tanjung Gemok, Pahang and by air from airports in Subang, Kuala Lumpur and Seletar, Singapore<sup>7</sup>. The island has a population of about 3,000 residents in nine kampungs or villages. The only available road at Tioman Island is from the main village, Kampung Tekek to Kampung Juara while the other villages are only accessible by boat. Tioman Island is a popular tourist destination with an average of 2,000 tourists staying on the island during peak seasons between March to July every year<sup>8</sup>. There are about 158 hotels, resorts or chalets and about 80 smaller food outlets that cater to tourists around the island<sup>8</sup>. The food premises in Tioman Island obtained their food supplies from mainland distributors in Mersing, Johor and Kuantan, Pahang<sup>8</sup>. Most of the residents of the island are fishermen or employees in the hospitality industry. During the Northeast monsoon season between November and February every year, there is limited activity on the island with most of the resorts being closed to tourists and the local residents travelling to the mainland for other activities<sup>8</sup>. Following notification by CDC, the Ministry of Health, Malaysia (MOH) collaborated with the Department of Veterinary Services, Malaysia (DVS) in November 2011 to carry out a cross sectional study to determine whether *Sarcocystis* is present among the human and animal population and the existence of the organism in the environment in Tioman Island. The study methods are as follows:

### Epidemiological investigations

#### Community health survey

Since most of the affected travellers in this

outbreak had stayed and eaten in Kampung Salang in Tioman Island, a community health survey was conducted in Kampung Salang from 9 to 18 November 2011<sup>8</sup>. The target population was all residents in Kampung Salang whilst households were sampled. During the period of the survey, there were 210 eligible persons including all persons residing in Kampung Salang, while children who attended boarding schools on the mainland were excluded<sup>8</sup>. A house to house survey was conducted and the residents were interviewed face to face using a structured questionnaire. Information gathered from the questionnaire included demographic data, medical history and history of food consumption<sup>8</sup>. Fresh stool samples were collected and sent to the National Public Health Laboratory (NPHL) in Sungai Buloh, Selangor for detection of *Sarcocystis* oocysts using fluorescence microscopy<sup>8</sup>.

#### Review of cases

Majority of the island population seek health and medical services from the Tekek health clinic while others sought treatment at the nearby district hospital located on the mainland (Mersing Hospital)<sup>8</sup>. All outpatient attendance cards from 1 January 2010 to 31 October 2011 at the health clinic and hospital were reviewed<sup>8</sup>. Case definitions were developed to classify cases as a suspect or confirmed case of *Sarcocystis* as follows: (a) suspect case - a person who presented with diarrhea or myalgia, or (b) confirmed case - a person who presented with diarrhea or myalgia and positive stool for *Sarcocystis* oocyst by fluorescence microscopy (intestinal *Sarcocystis*)<sup>8</sup>. Detailed information of patients who fit the case definitions was recorded in a format which included demographic data, onset of illness, symptoms and signs, and laboratory investigation<sup>8</sup>. Laboratory investigation for diarrhoea cases at the health facilities were reviewed for protozoa<sup>8</sup>.

#### Environmental water sampling

Environmental assessment was conducted in Tioman Island which included water supply storage, distribution and sanitary conditions. Site visits were made to identify the possible sources of water supply source contamination<sup>9</sup>. The main water supply system on the island is via Gravity Feed System (GFS) and tube wells managed by the local authority<sup>9</sup>. Some residents have direct well tubing from GFS or ground water to their homes<sup>9</sup>. Altogether, water samples were taken from 27 water sampling points source of water supply and reticulation systems around the island, namely from Kampung Tekek (3 sampling points), Kampung Juara (3 sampling points), Kampung Salang (6 sampling points), Kampung

Mukut (2 sampling points), Kampung Genting (3 sampling points), Kampung Paya (2 sampling points), Kampung Air Batang (2 sampling points) and from the main resort on the island (6 sampling points)<sup>9</sup>. In-situ physical examination of water samples, including turbidity and colilert test, was carried out<sup>9</sup>. For every sampling point, 50-100 litres of water were sampled and processed using the ultrafiltration machine for 2 hours<sup>9</sup>. The machine filtered water using a hemofilter which filters particles more than 50 kilodalton such as parasitic ova, trophozoites, cysts and oocysts<sup>9</sup>. This process was followed by backwashing method to produce 250 millilitres of processed water samples for analysis in the laboratory at the Institute for Medical Research (IMR) in Kuala Lumpur<sup>9</sup>.

At the IMR, 100 millilitres was taken from each sample to be processed and analysed microscopically while the remaining sample was kept for testing of other pathogens in the future<sup>9</sup>. The processed water samples were concentrated via the centrifuge method and divided into two parts (Sample A and B)<sup>9</sup>. Sample A was analysed using direct smear microscopy with iodine staining to detect any ova, trophozoites, cysts and oocysts<sup>9</sup>. Subsequently, the slide was viewed under the fluorescence microscope using ultraviolet (UV) light to detect *Sarcocystis* sporocyst. Further analysis was done using Trichrome and Modified Ziehl Neelsen staining to detect presence of coccidian protozoa especially *Cryptosporidium sp.* and *Isospora belli*. Sample B underwent concentration method through sedimentation to improve the probability of detection of parasites and then analysed using similar methods to sample A<sup>9</sup>.

**Animal surveillance**

DVS collected faecal samples from domesticated animals (cats, cattle, dogs and goats) from six villages in Tioman Island from 21 to 24 November 2011<sup>10</sup>. The animal faecal samples were then analysed for *Sarcocyst* oocysts and other parasitic ova and cysts using qualitative Floatation Technique at the Veterinary Services Centre in Tioman Island<sup>10</sup>.

**RESULTS**

**Epidemiological investigations**

**Community health survey**

Due to the monsoon season, only 15 households with 44 persons were available for interview while the rest have moved temporarily to the mainland. Majority of the residents were male

(77%) and 55% were between 21 to 40 years old (Table 1). Most of the residents worked in the hospitality industry as restaurant workers (46%), chalet operators or workers (14%) and cooks (11%)<sup>8</sup>. The main food consumed by the residents were chicken (91%), beef (80%) and mutton (23%) respectively<sup>8</sup>. All meat was well cooked before consumption<sup>8</sup>. The residents obtained their meat and chicken from food suppliers from mainland. The meat and poultry were ferried to Tioman Island in frozen form and distributed to food operators and household by local distributors<sup>8</sup>. There was no history of consumption of wildlife or exotic animals<sup>8</sup>. None of the 44 residents interviewed were symptomatic for Sarcocystosis from September to October 2011<sup>8</sup>. A total of 28 stool samples were collected and all were negative for presence of *Sarcocystis* oocysts by fluorescence microscopy<sup>8</sup>.

**Table 1. Demography of respondents in Kampung Salang**

| Variable           | n (%)   |
|--------------------|---------|
| Gender             |         |
| Male               | 34 (77) |
| Female             | 10 (23) |
| Age Groups (Years) |         |
| < 20               | 9 (20)  |
| 21-40              | 24 (55) |
| 41-60              | 11 (25) |

**Review of cases**

There were a total of 8,523 outpatient attendances at the Tekek health clinic in 2010<sup>8</sup>. Whilst from January to October 2011, a total of 7,934 attendees received outpatient treatment at the clinic<sup>8</sup>. Overall the attendance due to diarrhoea was low at the clinic comprising 205 (0.02%) and 89 (0.01%) cases respectively for 2010 and 2011<sup>8</sup>. Review of the outpatient attendance at Tekek health clinic till October 2011 found 116 myalgia cases<sup>8</sup>. Laboratory investigation was not conducted for the diarrhea and myalgia cases at the health clinic. Review of the cases from Tioman Island who presented at the nearby district hospital found only 11 cases of myalgia and diarrhea during the period of 2010 till October 2011<sup>8</sup>. Laboratory investigation was conducted for the diarrhoea cases at the hospital. None of the stool samples for the cases were positive for protozoa<sup>8</sup>.

### Environmental water sampling

The main source of water supply for the GFS in Tioman is from an uphill river that has been designated as a protected area (human residence or activity is not allowed upstream from the water source)<sup>9</sup>. The resort operators and residents received their water supply from GFS or individual tube wells<sup>9</sup>. Results of the interview with the residents found that only boiled water is used for consumption<sup>9</sup>. Observation during sampling found a footpath for jungle trekkers along the river and some leakages in the piping system at several locations<sup>9</sup>. All of the 27 water samples at source and distribution sites showed high turbidity and were found to be positive for total coliforms while 93% of the water samples were positive for *E. coli*<sup>9</sup>. Direct smear and

fluorescence microscopy analysis of all 27 water samples were negative for *Sarcocystis* oocysts<sup>9</sup>. However, ova and cysts of other parasites such as *Giardia spp*, *Ascaris lumbricoides*, unidentified cysts, ova and trophozoites were detected in small amounts (low concentration) in several samples<sup>9</sup>.

### Animal surveillance

A total of 84 animal faecal samples collected proportionate to the number of domesticated animals on the island (Table 2)<sup>10</sup>. All 84 animal faecal samples were negative for *Sarcocystis* oocysts by floatation technique<sup>10</sup>. However, several other parasites were detected from the samples (Table 3)<sup>10</sup>.

Table 2. Distribution of animal faecal samples by village in Tioman Island

| Villages   | Cat | Cattle | Goat | Dog |
|------------|-----|--------|------|-----|
| Tekek      | 48  | 6      | 1    | -   |
| Salang     | 9   | -      | -    | -   |
| Mukut      | 1   | 3      | 4    | -   |
| Paya       | 8   | 2      | -    | -   |
| Juara      | -   | 6      | -    | -   |
| Air Batang | -   | -      | -    | 2   |
| Total      | 66  | 11     | 5    | 2   |

Table 3. Results of floatation test for parasitology on animal faecal samples

| Animal (no. of faecal samples) | Ova and cysts detected   | n (%)   |
|--------------------------------|--------------------------|---------|
| Cat (n=66)                     | <i>Physaloptera</i>      | 3 (4)   |
|                                | <i>Ancylostoma</i>       | 57 (86) |
|                                | <i>Toxocara cati</i>     | 29 (44) |
|                                | <i>Toxoplasma gondii</i> | 3 (4)   |
| Cattle (n=11)                  | <i>Strongyle</i>         | 1 (9)   |
|                                | <i>Strongyloides</i>     | 1 (9)   |
| Goat (n=5)                     | <i>Strongyle</i>         | 2 (40)  |
|                                | <i>Strongyloides</i>     | 1 (20)  |
|                                | <i>Oocyst (Eimeria)</i>  | 1 (20)  |
| Dog (n=2)                      | Nil                      | Nil     |

## DISCUSSION

According to available literature, human *Sarcocystis* infection is low and mostly

asymptomatic<sup>1</sup>. In this study, none of the respondents were symptomatic or positive for *Sarcocystis* oocyst. A possible reason is because the sample size is too small to reveal any significant findings, whereby only 21% of the

residents were available for interview during that particular time. Other possible reasons include the criteria for case definition used in the study is not specific enough and of the different time frame involved, since the CDC cases were reported in June-August, whilst collected samples for this study were from October. Despite the low number of respondents, the study was able to collect 63% of stool samples<sup>8</sup>. The study also found that the local food culture did not include wildlife as part of the diet and all meat was well cooked before consumption<sup>8</sup>.

Based on the presenting symptoms of prominent muscle pain, mild diarrhoea and fever, the returning travellers could have had both intestinal and muscular Sarcocystosis infections. The confirmatory test for this study used stool samples for oocysts for detection of acute phase of intestinal Sarcocystosis infection. When human serve as dead-end host for non-human *Sarcocystis* spp, diagnosis is made by finding sarcocysts in tissue specimens (muscular Sarcocystosis). As such, the findings of this study cannot be associated with the cases reported by GeoSentinel, which was histologically diagnosed from muscle biopsy, which is muscular Sarcocystosis.

Microscopic analysis of all 27 water samples was negative for *Sarcocystis* oocyst<sup>9</sup>. This could mean that the oocysts were either not present or present in very low concentrations because of the sampling period during the monsoon season (dilution factor). However, the study found evidence of environmental and faecal contamination in the water reticulation system as shown by high total coliform and *E.coli* levels as well as several other parasitic ova and cysts<sup>9</sup>.

Results of faecal floatation test for 84 faecal animal samples (cats, cattle, dogs and goats) as conducted by DVS were negative for *Sarcocystis* oocyst<sup>10</sup>. The animal reservoir in Malaysia is unknown at this time, but a study by Kan in 1991 reported that the human muscular Sarcocystosis in Malaysia is a zoonotic infection acquired by contamination of food or drink with *Sarcocystis* shed by definitive hosts, such as domestic cat, dog or reticulated python<sup>2</sup>. Further study is recommended to detect *Sarcocystis* presence in wildlife in Tioman Island.

Sarcocystosis is a self-limiting disease with currently no vaccine or specific treatment. Health education is the most important aspect of prevention. Providing continuous health education to the local population and travellers to follow good personal, food and water hygiene is the standard and effective practice to prevent

most infectious diseases. Following the survey, several health education activities have been conducted in Tioman Island. The local authorities have taken further measures to ensure safe drinking water is available on the island.

## CONCLUSION

The surveillance studies in Tioman were not able to detect *Sarcocystis* infection in humans, animals or in the environment during the study period. The study was also unable to ascertain the possibility of endemicity among the population. Negative findings might be explained by the small sample size and different time periods of investigations. Further surveillance among humans, wildlife and the environment, preferably during the period of June to August, is needed to determine *Sarcocystis* endemicity in Tioman.

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