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First report on the seroprevalence of the Crimean-Congo haemorrhagic fever virus, a tick-borne virus, in Malaysia's Orang Asli population

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Abstract. – OBJECTIVE: The Crimean-Congo haemorrhagic fever virus (CCHFV), which is transmitted by the ticks of *Hyalomma spp*. in general and *H. marginatumin* particular, can cause severe disease in humans, with mortality rates of 3-30%. Other than from the bites of infected ticks, CCHFV can also be transmitted through contact with patients with the acute phase of infection or contact with blood or tissues from viraemic livestock. Outbreaks of human cases of haemorrhagic manifestations have been documented since 1945 and described in parts of Africa, Asia, Eastern Europe and the Middle East and most recently India in 2011. In addition, serological evidence of the disease has been reported in some countries where no human cases were reported. As regional neighbours China and India have been affected by this virus, this study was conducted to determine the seroprevalence of CCHFV among Orang Asli population of Malaysia as the most at risk people who residing in the deep forests.

PATIENTS AND METHODS: A total of 682 serum samples were collected from the Orang Asli population residing in eight states in peninsular Malaysia and analysed for the presence of anti-CCHFV immunoglobulin G (IgG) using a commercial enzyme-linked immunosorbent assay kit.

RESULTS: The study subjects comprised 277 (40.6%) men and 405 (59.4%) women. However, anti-CCHFV IgG was detected in only one female serum sample (0.1%). The presence of anti-CCHFV IgG could not be correlated to age or sex from these findings.

CONCLUSIONS: The results of this screening survey showed that the seroprevalence of the anti-CCHFV IgG among Malaysia's Orang Asli population is too low for detection or totally negative compared with that in neighbouring countries, such as India and China.

Key Words:
Tick-borne virus, Crimean-Congo hemorrhagic fever virus, Seroprevalence, Orang Asli, Malaysia.

Introduction

The Crimean-Congo haemorrhagic fever virus (CCHFV) was first characterised when severe haemorrhagic fever occurred in Crimea during 1944. In 1969, an antigenically identical virus was recognised after being isolated from a febrile patient in the Congo, from which the virus received the nomenclature by which it is known today. CCHFV is maintained in nature by the family of Ixodidae (hard ticks), in particular *Hyalomma spp*., which acts as both reservoir and vector. Ticks of other genera such as *Rhipicephalus*, *Boophilus* and *Dermacentor* have also been found to contribute to the ecological cycle of the virus, which is passed through trans-stadial and trans-ovarial transmission within the vector population.

A wide range of wild and domestic mammals are known to be the amplifying hosts, and the major concern is livestock that live closer proximity to human populations. In addition to vector-to-human transmission from infected tick bites and crushing the infected ticks, human-to-human transmission can occur through direct contact with infectious animal blood or body fluids and also by drinking unpasteurised milk from infected animals. Nosocomial outbreaks have mostly occurred through unprotected contact with highly infectious blood or body fluids from patients during the acute phase of infection as

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well as from the inadequate sterilisation of medical equipment, the re-use of syringe needles and the contamination of medical supplies; the fatality rates in hospitalised cases ranged from 9% to 50%[1-7].

CCHFV (*Nairovirus* of the *Bunyaviridae* family) was first described as a clinical entity in 1945 after the Nazi invasion of Crimea. In the twenty-first century, outbreaks have been reported in Pakistan, Iran, Senegal, Albania, Kosovo, Bulgaria, Turkey, Greece, Kenya, Mauritania and China, and most recently in India. Serological evidence of the virus has been found in Eygpt, Portugal, Hungary, France and Benin, but no human cases have been reported[8-11]. In summary, this virus is geographically distributed in different parts of Asia, Africa, the Middle East, eastern Europe and the Balkans[12].

A seroepidemiological study performed in Greece[13] showed that the seropositivity of the collected samples was significantly associated with older age, male sex, agricultural activities, contact with the livestock and prey, slaughtering, visits to forests and history of tick bites. Infections with the virus were also found to be mainly distributed among actively working rural inhabitants exposed to tick bites[14]. High-risk occupations include those of abattoir worker, medical personnel, animal herder, livestock worker, woodcutter and farmer. Travellers to endemic regions are also at high risk[3,5,6].

The pathogenesis of the infection has not been discussed in depth, but several mechanisms that explain the haemorrhagic diathesis in CCHF infection have been discovered[15]. Its haemorrhagic manifestations have caused substantial mortality rates in humans[16], and the World Health Organization has suggested the oral and intravenous administration of ribavirin because of its ensuing high survival rates, shorter recovery time and earlier return to normal levels of laboratory parameters[17,18]. The usual treatments for hospitalized infected patients include intensive and supportive care. To minimise the risk of infection in endemic areas, personal protective measures should be taken and grassy areas with high levels of vegetation should be avoided. Self-protection, such as through the use of repellents and wearing leather gloves and protective clothing, has been suggested for avoiding the occupational risk of infection[16].

No FDA-approved vaccine is available for CCHFV infection. Only the inactivated mouse brain vaccine and DNA vaccine have ever been used. The inactivated mouse brain vaccine, which was produced and available in Bulgaria, was tested on a small scale. High detectable antibody levels and a fourfold reduction in the number of reported cases in Bulgaria were observed over 21 years after initiation of the vaccination. However, it was not subjected to adequate trials and lacked commercial value due to the possibility of the occurrence of autoimmune responses. In contrast, the DNA vaccine, which contained the M segment of the CCHFV genome, induced neutralising antibodies in mice but no evaluation of its protective effects was performed. Antiviral therapy, such as human interferon (IFN), was given to six patients in the 1985 outbreak in South Africa, but no significant benefit was noted[19-21].

To the best of our knowledge, this was the first study to investigate the seroprevalence of CCHFV in Malaysia or even Southeast Asia. In addition, the target population was a specific group of forest dwellers who were chosen for their high probability of contact with ticks. As this virus is a constant threat for new regions, the impact of this study should be obvious, especially because we found one positive sample. Moreover, its expansion to other regions could be crucial.

The aim of this study is to determine the prevalence of the anti-CCHFV IgG among the Malaysian's Orang Asli population of the eight states in peninsular Malaysia.

Patients and Methods

Ethics Statement

The study received special permission from the Department of Orang Asli Development (JAKOA) and was approved by the Ethics Committee of the University Malaya Medical Centre (UMMC) (MEC Ref. 824.11). Participation in the study was purely voluntary. The participants were briefed about the project, allowed sufficient time for consideration, and written informed consent was then obtained. An additional assent form was completed by parents/guardian for participants below the legal age (18 years). The samples were handled with strict anonymously. All blood donors completed a prepared questionnaire to identify their history of tick bites, symptoms experienced, lifestyle and how frequently they visited a forest. All of the donors agreed, by written consent, to the anonymous use of the blood samples.

Population and Sample Collection

A total of 682 blood samples from the Orang Asli population residing in the states of Perak, Melaka, Pahang, Negeri Sembilan, Kedah, Kelantan, Selangor and Johor were successfully collected from September 2012 to February 2013 with the help of experienced medical assistants. Demographic data for each collected sample, including age and gender, were also recorded.

Anti-CCHFV Serology

Anti-CCHFV immunoglobulin G (IgG) antibodies were detected using the VectoCrimean-CHF-IgG enzyme-linked immunosorbent assay (ELISA) kit (VECTOR-BEST, Novosibirsk, Russia). This assay uses polystyrene strips coated with immobilised CCHFV antigen, and the tests were performed according to the manufacturer's instructions. The absorbance/cut-off ratios observed for all samples are shown in Figure 1.

Statistical Analysis

Categorical data were assessed in two-way contingency table analyses using Fisher's exact test. The correlation between age (by taking the median of the age range) and reactivity to the anti-CCHFV IgG ELISA (correlation coefficient, r value) test, and the *p* value, were determined by Spearman non-parametric correlation. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc.), with $p < 0.05$ considered to be significant.

Results

The general characteristics of the 682 samples studied are summarised in Table I and Figure 2 (according to state of residence) and Table I (according to age and gender). The study panel was composed of 277 men and 405 women aged 3-90 years with a median of 46.5 years. A total of 610 (89.4%) donors were aged 42 years or less, and 72 (10.6%) were older than 42 years.

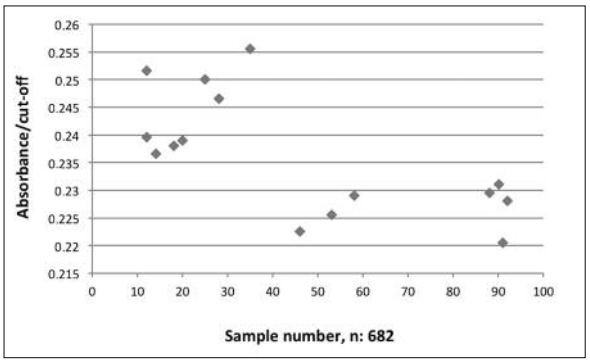

Figure 1. Absorbance/cut-off calculation.

Overall, anti-CCHFV IgG was found in one of 682 samples (0.1%). The seroprevalence was 0.0% (0/277) in men and 0.2% (1/405) in women ($p = 1.0000$); however, no association was association between antibody prevalence and sex, as depicted clearly in Figure 3. No variation with age for the reactivity to anti-CCHFV IgG ELISA could be evaluated because the only sample that was reactive was from an 8-year-old girl. As shown clearly in Figure 4 ($p = 0.1750$), no correlation between age and the reactivity of the serum samples to anti-CCHFV IgG ELISA was found.

Negeri Sembilan was the only state that contributed a positive sample, with the samples from the other states being non-reactive to the anti-CCHFV IgG ELISA test.

Discussion

We screened 682 serum samples from the Orang Asli population in eight states of peninsular Malaysia and found that the seropreva-

Table I. The number and percentage of reactive sample(s) among the sera collected by state.

Samples by state		Anti-CCHFV IgG reactive	Percentage of reactive sample(s)
Pahang	117	0	0.0%
Johor	60	0	0.0%
Melaka	30	0	0.0%
Kedah	63	0	0.0%
Perak	110	0	0.0%
Selangor	60	0	0.0 %
Kelantan	169	0	0.0%
Negeri Sembilan	73	1	1.4%
Total	682	1	0.1%

Figure 2. Distribution of reactive serum samples by state.

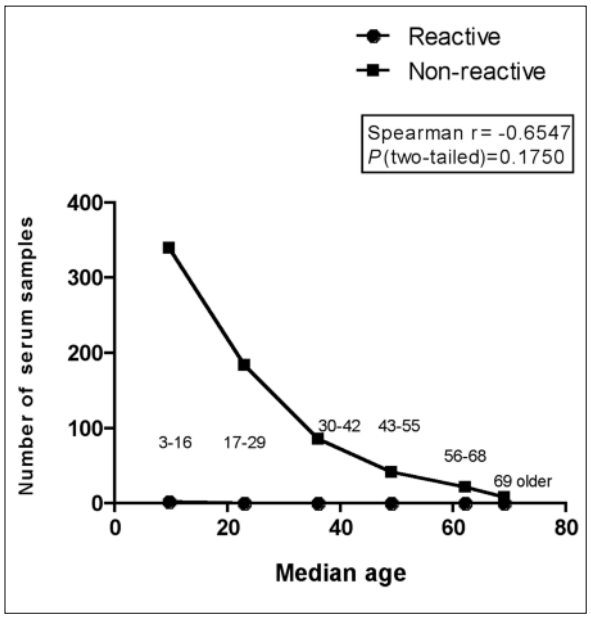

Figure 4. Age and sero-reactivity relationship. As it is shown in the figure there is no correlation between age and reactivity of the serum samples tow anti-CCHFV IgG ELISA.

lence of CCHFV was 0.1%. The only sample that was reactive to the anti-CCHFV IgG ELISA was collected from an 8-year-old girl from Negeri Sembilan. This was the first study of its kind to be conducted in Malaysia. The screening was conducted using the VectoCrimean-CHF-IgG ELISA kit (VECTOR-BEST, Novosibirsk, Russia), and a thorough literature review was carried out[4,5,22].

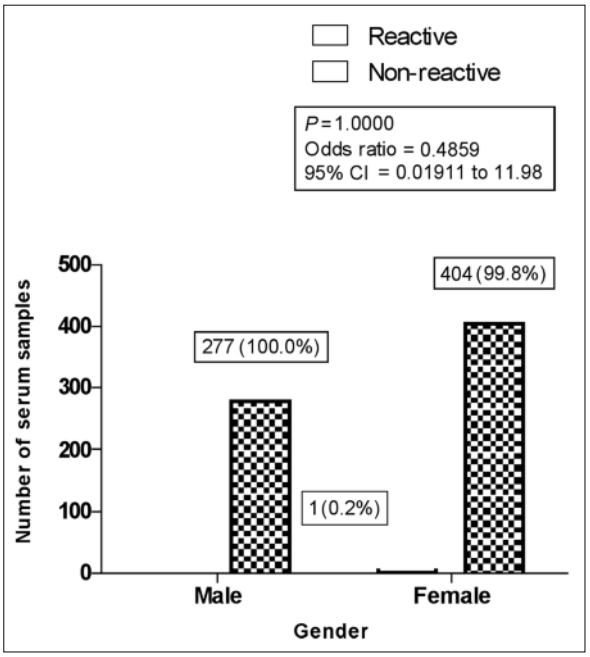

Figure 3. Gender and sero-reactivity relationship. As it is shown in the figure there is no association between gender and reactivity of the serum samples to anti-CCHFV IgG ELISA.

In nearby countries, CCHFV was first isolated in Xinjiang, China in 1966 from the blood of patients, organ autopsies and *H. Asiaticum* ticks. It is known as XHF or XHFV in the Chinese literature, based on the site of its isolation[23]. In one study, 3.4% of all samples from five counties in Yunnan, China, tested positive for CCHFV IgG and 14.3% tested RT-PCR positive, suggesting that these five counties were areas for potential outbreaks[24]. Moreover, an outbreak of CCHFV occurred in western India during 2011 and three medical professionals were among the four patients who died at a tertiary care hospital in Ahmadabad[4,25]. These studies and the high mortality rate of the disease drew our attention to this issue and led us to conduct this study due to trading of livestock and the lack of boundaries for travellers across the globe.

Because we were unable to determine the presence of CCHFV RNA in our investigation due to the absence of the wild virus in Malaysia, we could draw no conclusions about the presence of an active or past infection with CCHFV in the Orang Asli population. However, being con-

Table II. The number and percentage of samples reactive and non-reactive to the anti-CCHFV IgG ELISA test according to general profile.

General profile		All donors (n = 682)	Anti-CCHFV IgG non-reactive	Anti-CCHFV reactive
Sex	Male	277	277 (100.0%)	0 (0.0%)
	Female	405	404 (99.8%)	1 (0.2%)
Age	3-16	341	340 (99.7%)	1 (0.3%)
	17-29	184	184 (100.0%)	0 (0.0%)
	30-42	85	85 (100.0%)	0 (0.0%)
	43-55	42	42 (100.0%)	0 (0.0%)
	56-68	22	22 (100.0%)	0 (0.0%)
	69 and older	8	8 (100.0%)	0 (0.0%)

scious of the available epidemiology and being alarmed by this result, we are considering an expansion of our study of this virus as well as being prepared for any implications of its silent transmission within Malaysia.

In summary, we found that only one of 682 samples (0.1%) from the Orang Asli population of peninsular Malaysia was reactive in the anti-CCHFV IgG ELISA test in three independent experiments. Given the high mortality rate associated with the virus and the importance of the subject, a larger-scale study in different regions and populations is warranted. No borders can prevent infected individuals or vectors transmitting the infectious agent easily from endemic regions to non-endemic regions. However, it seems crucial to investigate the presence of CCHFV in ticks in Malaysia, especially those in Negeri Sembilan. Thus, as our study only provides preliminary data on CCHFV in Malaysia, the topic merits further exploration.

Conclusions

The findings of the present study indicate that the seroprevalence of anti-CCHFV IgG antibodies among Malaysia's Orang Asli population was too low for detection or totally negative compared to that in nearby countries such as India and China. Nevertheless, the situation should be reviewed further from other perspectives because it is crucial to raise the significant public health concerns related to this virus.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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