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Original article

An outbreak of scrub typhus in military personnel despite protocols for antibiotic prophylaxis: doxycycline resistance excluded by a quantitative PCR-based susceptibility assay

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Abstract

Scrub typhus is caused by the obligate intracellular bacterium *Orientia tsutsugamushi* and is endemic to many countries in the Asia–Pacific region, including tropical Australia. We describe a recent large outbreak amongst military personnel in north Queensland. A total of 45 clinical cases were identified (36% of all potentially exposed individuals). This occurred despite existing military protocols stipulating the provision of doxycycline prophylaxis. Doxycycline resistance in *O. tsutsugamushi* has been described in South-East Asia, but not Australia. In one case, *O. tsutsugamushi* was cultured from eschar tissue and blood. Using quantitative real-time PCR to determine susceptibility to doxycycline for the outbreak strain, a minimum inhibitory concentration (MIC) of ≤0.04 μg/mL was found, indicating susceptibility to this agent. It seems most probable that failure to adhere to adequate prophylaxis over the duration of the military exercise accounted for the large number of cases encountered rather than doxycycline resistance.

Keywords: Scrub typhus; *Orientia tsutsugamushi*; Doxycycline; Microbial sensitivity testing

1. Introduction

Scrub typhus is caused by the obligate intracellular bacterium *Orientia tsutsugamushi* and is transmitted by the bite of larval trombiculid mites, usually *Leptotrombidium* species (especially *Leptotrombidium deliense* in Australia). The disease remains endemic to many countries of the Asia–Pacific region, with a distribution described as approximating a triangular area of 13,000,000 km^2 including Eastern Russia, Korea, Japan, China, Taiwan, Thailand, southern Asia, tropical Australia and the islands of the south-west Pacific [9]. However, a disease resembling scrub typhus, caused by a bacterium related to *Orientia* has been described from Chile [1] and a new species of *Orientia* (*Orientia chuto*) has recently been isolated from a traveller returning from Dubai [7], some 500 km west of the previously recognised limit of epidemiological distribution. In Australia, the disease is geographically restricted to tropical regions and has historically been most commonly encountered in northern Queensland [12] and more recently the Torres Strait Islands [26,4]. Foci of endemicity are

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also recognised in the Northern Territory [18,2] and the Kimberly region of Western Australia [5,17]. Worldwide, it is predominantly a rural disease, with an estimated 1 million infections annually [19].

Small outbreaks were reported in Queensland, Australia, in 1996, 1997 [14], and 2005 [13], all occurring in soldiers performing military exercises in the Cowley Beach Training Area near Innisfail. Here we describe a fourth outbreak of scrub typhus in Australian army soldiers, which may represent the largest recorded outbreak in Australia for over 50 years.

1.1. Epidemiology of the outbreak

A full company of regular infantry soldiers and support elements deployed into the Cowley Beach training area in late April 2011. There is a requirement for all Australian Defence Force (ADF) members deploying into the Cowley Beach area to take chemoprophylaxis consisting of oral doxycycline 200 mg on entering the training area, 200 mg weekly thereafter, and 200 mg on exit from the area. This regimen is designed to prevent leptospirosis but has also been shown to be effective prophylaxis against scrub typhus [15]. In addition to this, dipping of uniforms in 25% permethrin (Perigen Defence; Bayer) is recommended to protect against mite bites, and soldiers are issued with personal insect repellent for the same purpose.

The first soldiers became unwell approximately two days after extracting from the training area, with the majority falling ill over the subsequent week. Because the soldiers were granted leave on conclusion of the exercise, suspect cases presented in other states within Australia, such as Victoria and New South Wales, in addition to Queensland, which delayed identification of the outbreak. More than 90% of the company were recalled and treated with doxycycline within 24 h, with the remainder captured in the following days. A subsequent dose of 200 mg seven days later was also recommended. A case met the clinical definition if the individual was deployed on the Cowley exercise and presented unwell between 23rd April and 5th May 2011 and displayed at least two of the following symptoms: headache, fever, maculopapular rash or arthralgia. A case met the laboratory definition if there was a ≥4-fold rise in IgG titres to *O. tsutsugamushi* between acute and convalescent serology, or a positive PCR on blood or tissue or a positive culture from clinical specimens.

1.2. Culture of O. tsutsugamushi *from an index case*

O. tsutsugamushi was cultured from one 19-year old soldier meeting the clinical and laboratory case definitions. Polymerase chain reaction (PCR) on whole blood and from a punch biopsy of eschar tissue from his trunk (see Fig. 1) detected *O. tsutsugamushi*–specific DNA [21]. These samples were also inoculated into Vero cell culture and severe combined immunodeficient mice. After culture, the presence of *O. tsutsugamushi* was confirmed by immunofluorescence of the Vero cells (see Fig. 2) and PCR of the mice spleens. He had been provided with doxycycline prophylaxis during the exercise, which he claimed to have taken as directed, and used clothing impregnated with insecticide. Given the development of scrub typhus despite the apparent provision of doxycycline prophylaxis, the possibility of doxycycline-resistant *O. tsutsugamushi* was considered, which has been described in Thailand [22,27] but never in Australia. Subsequent confirmation of many other cases from the same cohort of army personnel from the Cowley beach exercise further raised our concerns given the strong emphasis that the army places upon doxycycline provision. We therefore conducted further experiments to determine if the isolated strain demonstrated reduced susceptibility to doxycycline.

Fig. 1. Diffuse maculopapular rash and prominent eschar in scrub typhus.

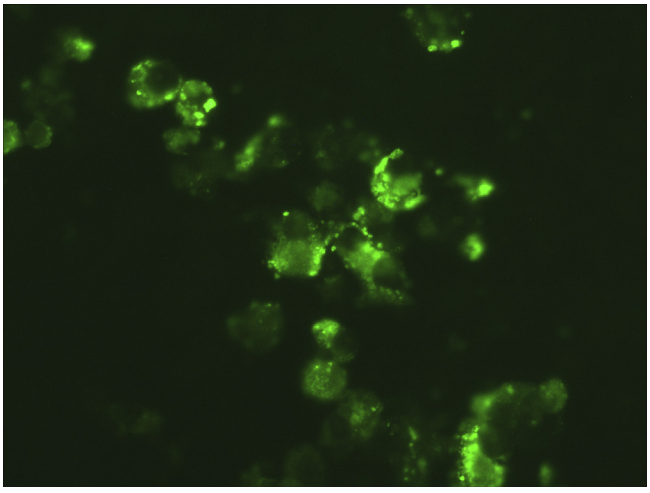

Fig. 2. *O. tsutsugamushi* detected by immunofluorescent (IF) assay following culture in Vero cells and using specific monoclonal antibody; original magnification ×1000. The Australian Rickettsial Reference Laboratory (ARRL) protocol was used for staining and fluorescence microscopy, as previously described [6].