Seroprevalence of antibodies to *Rickettsia typhi* in the Waikato region of New Zealand

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SUMMARY

The first reported New Zealand-acquired case of murine typhus occurred near Auckland in 1989. Since then, 72 locally acquired cases have been recorded from northern New Zealand. By 2008, on the basis of the timing and distribution of cases, it appeared that murine typhus was escalating and spreading southwards. To explore the presence of *Rickettsia typhi* in the Waikato region, we conducted a seroprevalence study, using indirect immunofluorescence, Western blot, and cross-adsorption assays of blood donor samples. Of 950 human sera from Waikato, 12 (1·3%) had *R. typhi* antibodies. The seroprevalence for *R. typhi* was slightly higher in northern Waikato (1·4%) compared to the south (1·2%; no significant difference, χ^2 $P = 0.768$ at $P < 0.05$). Our results extend the reported southern range of *R. typhi* by 140 km and indicate it is endemic in Waikato. Evidence of past *Rickettsia felis* infections was also detected in six sera. Globally, *R. felis* is an emerging disease of concern and this pathogen should also be considered when locally acquired rickettsiosis is suspected. If public health interventions are to be implemented to reduce the risk of rickettsioses as a significant public health problem, improvements in rickettsial diagnostics and surveillance will be necessary.

Key words: Emerging infectious diseases, murine typhus, *Rickettsia felis*, *Rickettsia typhi*.

INTRODUCTION

Murine typhus is a flea-borne rickettsial disease associated mainly with commensal rodents and is caused by the bacterium *Rickettsia typhi* [1]. Humans are incidental hosts, where the pathogen causes a non-specific febrile illness often with fever, headache, arthralgia and rash [2]. Murine typhus is one of the most widely distributed arthropod-borne infections throughout the world, yet it is frequently unrecognized or misdiagnosed due to its non-specific and usually mild clinical presentation [3]. Despite the fact that murine typhus can cause severe illness and death [4, 5], clinical signs and symptoms in untreated (undiagnosed) patients usually last for 7–14 days followed by spontaneous resolution of the illness [6, 7]. Hence, its incidence is likely to be under-reported.

Of the rickettsiae only *R. typhi* and *Rickettsia felis*, belonging to the typhus and transitional groups,

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respectively, have been reported to occur in New Zealand [1, 8]. *R. typhi* is primarily transmitted by the oriental rat flea, *Xenopsylla cheopis*, and humans become infected through the inhalation of *R. typhi*-containing flea faeces, or via contact through disrupted skin [3]. *R. typhi* is maintained by the classic rat–rat flea cycle, where *Rattus* rats are the main reservoir hosts that can be infected by the inoculation of infected flea faeces into the bite wound [9], or the regurgitation of rickettsiae by the infected flea during blood feeding [10]. Studies within the United States have reported antibodies against *R. typhi* in opossums and domestic cats and dogs [11] and a shift in the life-cycle of *R. typhi* from the classic rat–rat flea cycle to opossum–cat flea cycle [2, 12]. In comparison, the disease ecology of *R. felis* is less clear [1]. However, *R. felis* DNA is most highly reported in cat fleas, *Ctenocephalides felis,* worldwide [13].

Up to the end of 2014, 72 locally acquired murine typhus cases had been recorded in New Zealand ([14]; Institute of Environmental Science and Research, written personal communication). For *R. felis*, cat fleas hosted by cats and dogs have been found to carry the bacterium in New Zealand [15] and evidence of human *R. felis* infections have recently been reported [14]. It is important to note that because *R. felis* rickettsiosis shares a similar clinical profile to murine typhus (and potentially similar vectors and reservoirs), infection can be mistaken for a suspected case of *R. typhi* [13].

The first reported New Zealand-acquired murine typhus case occurred in greater Auckland in 1989 [16], where it has continued to occur at low-incidence levels. Since then cases have also been reported up to 130 km away to the south in the Waikato region (Fig. 1) [6, 17]. A marked increase in cases reported in this region from 2006 to 2008 raised concerns with health authorities that the disease may be continuing to both increase in prevalence and spread further south, i.e. is an emerging disease. In response, we conducted the first seroprevalence study in New Zealand for *R. typhi* and *R. felis*, using indirect immunofluorescence, Western blot, and cross-adsorption assays of samples from blood donors. Our goal was to explore the presence of *R. typhi* in the Waikato region, covering beyond the southernmost known distribution.

METHODS

Clinical suspicion of rickettsial infections is most widely confirmed by serological tests with the indirect immunofluorescence assay (IFA) being the gold standard [18, 19]. However, members of the *Rickettsia* genus are notorious for cross-reactivity in serological tests [3]. For example, antibodies against *R. felis* in human sera have been reported to cross-react with *R. typhi* in IFA [20]. For this reason, coupled with the fact that both *R. typhi* and *R. felis* rickettsioses share similar clinical manifestations, we established, in New Zealand, a specific laboratory methodology using Western blot (WB) and cross-adsorption assays to distinguish between *R. typhi* and *R. felis* infections [14]. These assays, in combination with IFA, have been shown to be reliable in serological differentiation among several rickettsioses [20, 21].

From 2009 to 2010, serum samples were obtained from 989 blood donors visiting the New Zealand Blood Service in Hamilton City (Fig. 1). Of these, 950 were from the Waikato District Health Board region with 435 residing to the north and 515 to the south of the city limits. All donors visiting the collection centre, except those residing within Hamilton city, were invited to participate and were enrolled sequentially during the study period. Participants were provided with a project information sheet, gave written, informed consent, and completed a standardized questionnaire. The questionnaire covered risk factors such as overseas travel, animal contact, and place of residence/employment. No participants withdrew from the study. The Northern Y Regional Ethics Committee of New Zealand approved this study (no. NTY/08/09/085).

Although samples from Waikato were the focus of our study, all sera were tested to identify any range expansions in the known distribution of *R. typhi*. Samples were first screened for seropositivity by IFA using slides acetone-fixed with *R. typhi* antigen [Australian Rickettsial Reference Laboratory (ARRL), Geelong, VIC, Australia; henceforth referred to as ARRL slides]. Each serum, including positive and negative control sera (ARRL, Australia), was diluted in 2% skim milk in phosphate-buffered saline before being applied to the slides and incubated at 37 °C for 30 min in a humidified container. Slides were washed three times, incubated with fluorescein-conjugated anti-human IgG, IgM and IgA (ARRL, Australia) at 37 °C for 30 min, washed again and examined using fluorescence microscopy. The cut-off titre for seropositivity was 1:128 as recommended by the manufacturer. All samples that were positive at this dilution were then tested using a commercially available group-specific, but not species-specific, IFA
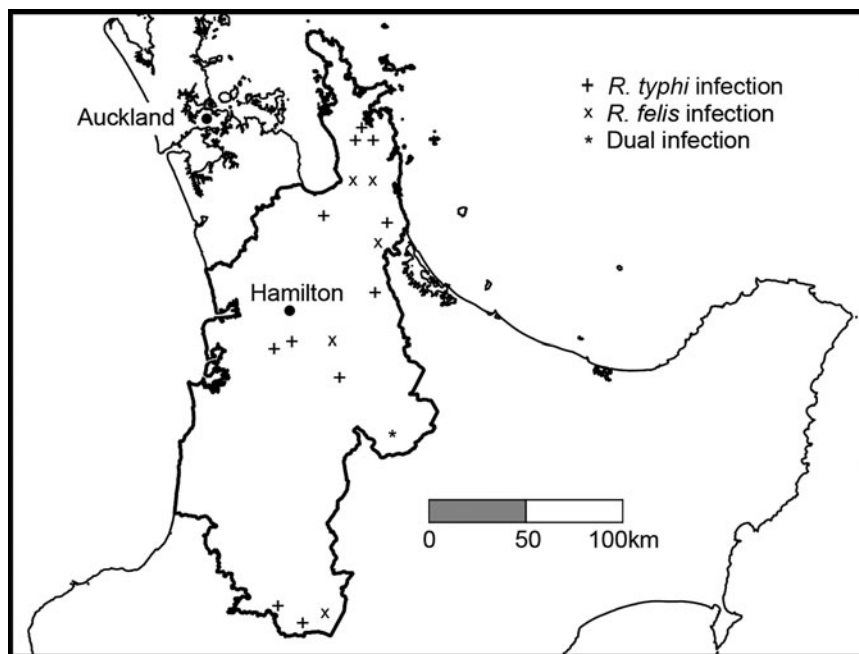
Fig. 1. Northern part of North Island, New Zealand, highlighting the Waikato District Health Board study region and the location of the 12 *Rickettsia typhi* and six *Rickettsia felis* infections (including one dual infection).

kit (henceforth referred to as Focus slides) for IgG against Typhus group (TG) *R. typhi* and Spotted Fever group (SFG) *R. rickettsii* (Focus Diagnostics, USA). The Focus slides include members of the transitional group within the SFG for interpretation of testing results.

Endpoint titres were determined for all samples tested on the Focus slides. According to kit instructions, endpoint titres ⩾1:64 and <1:256 indicate either past infection or early response to a recent infection, and titres ⩾1:256 are considered presumptive evidence of recent or current infection. Hence, the cut-off titre for seropositivity on the Focus slides was 1:64. Because only *R. typhi* and *R. felis* have been reported as occurring in New Zealand, study sera that are TG-positive and SFG negative, and those that are SFG-positive and TG negative, are likely to represent *R. typhi,* and *R. felis* infections, respectively. Since *R. typhi* can cross-react with SFG rickettsiae [22], and *R. felis* can cross-react both with other SFG rickettsiae and *R. typhi* [20], those sera that are both TG- and SFG-positive may be due to either rickettsia. Positive reactivity may also represent overseas-acquired rickettsioses, or infections of other cross-reactive pathogens [23]. Hence, WB and cross-adsorption assays using *R. typhi* (Wilmington) and *R. felis* (URRWXCal2) antigens (Unité des Rickettsies, France) were performed as described in

Lim *et al.* [14] to confirm and differentiate between *R. typhi* and *R. felis* infections [18, 21].

RESULTS

Of the 989 sera, 106 (10·7%) tested positive on the ARRL slides. Of these 106 samples, 37 (3·7%, 37/989) showed seropositivity on the Focus slides to either the TG or SFG antigen, or to antigens of both groups (Fig. 2). Seven (0·7%, 7/989) were TG-positive and SFG-negative, nine (0·9%, 9/989) were SFG-positive and TG-negative, and 21 (2·1%, 21/989) cross-reacted with both TG and SFG antigens.

WB was performed for all 37 samples that tested positive on the Focus slides. Five samples showed no specific WB reaction against either rickettsia (Fig. 2). Four of the five participants had not travelled overseas in the past 4 years, and thus could be infected with other IFA cross-reactive bacteria found in New Zealand. The participant with overseas travel could have been infected with rickettsiae other than *R. typhi* and *R. felis*, or other IFA cross-reactive bacteria. A rickettsial antigen was considered to represent the agent of infection when sera reacted only with the specific proteins of this antigen in the WB assay [20]. Using this criterion, three were considered to be *R. typhi* infections, all of which were TG-positive and SFG-negative on the Focus slides (Fig. 2). Three
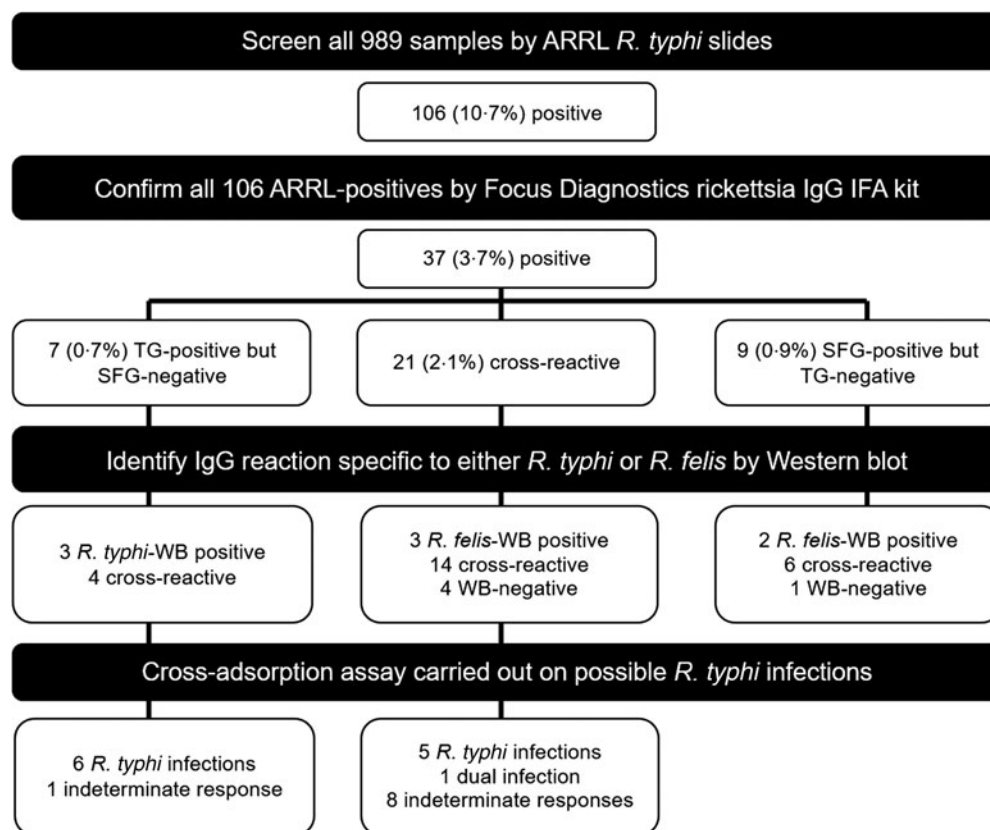
Fig. 2. Laboratory methodology for the identification and confirmation of *Rickettsia typhi* infections. Our combined approach of using indirect immunofluorescence assay (IFA), Western blot (WB) and cross-adsorption assay identified a total of 12 *R. typhi* infections, including one dual infection of both *R. typhi* and *Rickettsia felis*. ARRL, Australian Rickettsial Reference Laboratory; SFG, Spotted Fever group; TG, Typhus group.

TG- and SFG-positive samples and two SFG-positive but TG-negative samples reacted only against the *R. felis* WB, and thus were considered to be *R. felis* infections.

In identifying the final number of *R. typhi* infections, results from the IFA and WB assays eliminated the need for cross-adsorption assays to be performed on the five *R. felis* WB-positive samples, the five WB-negative samples, or the six WB cross-reactive SFG-positive samples (Fig. 2). Of the seven TG-positive but SFG-negative serum samples, six were confirmed to be *R. typhi* infections by the cross-adsorption assay. One participant was classified as having an indeterminate response and could have been infected with other bacteria that were known to cross-react with rickettsiae (such as *Bartonella hensalae* and *Legionella bosmanii*). Five other *R. typhi* infections were identified in samples that were cross-reactive on the Focus slides. One of the samples that showed cross-reactivity on the Focus slides was confirmed by the cross-adsorption assay to be a case of concomitant or consecutive infection by *R.*

typhi and *R. felis*. The remaining eight samples produced indeterminate responses. When an *R. typhi*-infected serum sample was adsorbed with *R. typhi*, all homologous and heterologous antibodies were removed. After adsorption with *R. felis*, only homologous antibodies were removed, indicating that antibodies were specific for *R. typhi*. In contrast, when adsorption with *R. typhi* was performed on an *R. felis*-infected serum sample, antibodies against *R. typhi* were removed while antibodies specific to *R. felis* remained. Adsorption with *R. felis* resulted in the disappearance of all the antibodies, indicating *R. felis* rickettsiosis. In the case of dual infection, when the serum sample was adsorbed separately by *R. typhi* and *R. felis*, all homologous antibodies were removed in both adsorptions while antibodies against *R. felis* and *R. typhi* remained, respectively.

In summary, we identified 12 *R. typhi* and six *R. felis* infections (including one dual infection; Fig. 2). Endpoint titres, overseas travel, and animal contact for the 12 *R. typhi* infections are detailed in Table 1.

Table 1. *Serological data and risk factors of participants that were confirmed to have* Rickettsia typhi *infection*

| Participant no. | Focus kit* IFA serological titres | | IgG reaction against *R. typhi* or *R. felis* in WB | Agent of infection identified after cross-adsorption assay | Risk factors | |
	TG *R. typhi*	SFG *R. rickettsii*			Flea or animal contact at home/work environment	Travelled overseas in the past 4 years
1	64	Neg.	Cross-reaction	*R. typhi*	Rat (seen), cat, dog	No
2	256	Neg.	Cross-reaction	*R. typhi*	Flea, rat, cat, dog	Yes
3	64	Neg.	*R. typhi*	*R. typhi*	Flea, rat (seen), cat, dog	No
4	256	Neg.	Cross-reaction	*R. typhi*	Flea, rat, cat, dog, possum	No
5	64	Neg.	*R. typhi*	*R. typhi*	Rat (seen), cat, dog	Yes
6	64	Neg.	*R. typhi*	*R. typhi*	Rat (seen), cat, dog	No
7	64	64	Cross-reaction	*R. typhi* + *R. felis*	Rat (seen), dog	Yes
8	64	64	Cross-reaction	*R. typhi*	Flea, rat (seen), cat, dog	No
9	64	64	Cross-reaction	*R. typhi*	Rat (seen), cat, dog	No
10	64	64	Cross-reaction	*R. typhi*	Flea, rat, cat, dog, possum	Yes
11	512	128	Cross-reaction	*R. typhi*	Rat (seen), cat, dog	Yes
12	256	64	Cross-reaction	*R. typhi*	Rat (seen), cat, dog, possum	Yes

IFA, Indirect immunofluorescence assay; TG, Typhus group; SFG, Spotted Fever group; WB, Western blot; Neg, negative.
* According to kit instructions, endpoint titres ⩾1:64 and <1:256 indicate either past infection or early response to a recent infection, and ⩾1:256 are considered presumptive evidence of recent or current infection.

Six of the 12 participants had no overseas travel in the past 4 years, with all participants listing contact with a range of risk-contributing animals [11]. All the *R. typhi* and *R. felis* infections were found in blood donors from the Waikato region (Fig. 1), giving a seroprevalence for *R. typhi* antibodies of 1·3% (12/950). Of these 12 *R. typhi* infections, six were in participants living north of Hamilton city (up to 100 km away) and six were found south of the city (up to 150 km away), indicating a seroprevalence for *R. typhi* antibodies of 1·4% (6/435) and 1·2% (6/515), respectively (no significant difference, χ^2 $P = 0.768$ at $P < 0.05$).

DISCUSSION

Although IgG titres decline over time, detectable levels can remain for up to 3–4 years and thus exposure to *R. typhi* may have occurred at any time during this period [24]. Since 6/12 *R. typhi*-infected participants, including the one with dual infection by *R. typhi* and *R. felis*, had travelled overseas in the past 4 years, it is possible that exposure could have occurred overseas. Other risk factors for murine typhus, that were common to all 12 participants, included animal contact such as rats, cats or dogs, or the presence of these animals in the home/work environment [11]. In the case of possums in New Zealand, there is as yet no clear link with the disease, unlike the

involvement of opossums in the dynamics of murine typhus in the United States [2, 12]. Previous New Zealand studies have also identified rural lifestyle as a risk factor [6, 17, 25]. We cannot confirm this in our study as we did not enrol urban participants from Hamilton city against which to compare the rural samples.

While *R. typhi* seroprevalence was slightly higher to the north (1·4%, 6/435) than to the south (1·2%, 6/515) of Hamilton city, which lies near the centre of the study region (Fig. 1), it did not reach statistical significance. Since seroprevalence is similar in the north and south, it is likely that *R. typhi* is already established in southern Waikato, and endemic in the study region. Two participants with evidence of past *R. typhi* infections lived ~150 km due south of Hamilton city. These and two other participants south of Hamilton extend the southernmost range previously reported of human infections from *R. typhi* by up to 140 km [17]. The two southernmost participants have lived in the same area for more than 7 years and have not travelled overseas in the past 4 years; hence the infection is likely to have been acquired locally after 2005 (given the limited length of time for detectable IgG levels).

Unlike many overseas countries, no large-scale seroprevalence studies have previously been conducted in New Zealand for rickettsioses. Compared to our study, investigations from Brazil and Greece have revealed similar seroprevalence levels for

antibodies against *R. typhi* of 1·1% and 2%, respectively [26, 27]; while studies from Colombia and Bosnia-Herzegovina have found seroprevalences as high as 25·2% and 37·7%, respectively [28, 29]. However, caution should be exercised when comparing seroprevalence studies as factors such as study populations, serological methods, or diagnostic criteria for seropositivity may differ [19].

In our study in New Zealand, we trialled the use of WB and cross-adsorption for confirming IFA results and differentiating between *R. typhi* and *R. felis* infections. Although IFA is regarded as the gold standard for serological diagnosis of rickettsioses, cross-reacting antibodies within and among the rickettsial groups are not unusual [30]. WB is considered to be the most specific serological assay for determining rickettsial infections and is especially useful for distinguishing true-positive from false-positive results obtained from IFA [18]. If we study the results based on the ARRL and Focus kit IFAs, we would conclude a higher prevalence for rickettsioses, i.e. being up to 10·7% and 3·7%, respectively (Fig. 2). However, using WB we were able to rule out five of the 37 Focus IFA-positive samples, as these five WB-negative samples were neither specific for *R. typhi* nor *R. felis.* The WB assay also eliminated the need for cross-adsorption to be performed on five identified *R. felis* WB-positive samples.

Cross-adsorption assays were used to identify the cross-reacting bacterium and the bacterium responsible for the infection when results from WB were not definitive. Our subsequent cross-adsorption assays allowed for species-specific diagnosis, resulting in nine further *R. typhi* infections being identified. However, we believe this technique is of limited applicability in New Zealand due to the large amount of antigen required and the relatively time-consuming methods, both resulting in a high cost per sample. Hence, we do not suggest the routine use of cross-adsorption assays to assist in confirmatory tests but WB may be useful for confirming IFA results and differentiating between true and false positives.

Our study also provided evidence of past *R. felis* infection in individuals living in New Zealand. All of the six *R. felis*-infected participants recorded risk factors associated with *R. felis*, having had contact with cats and dogs, the host reservoirs on which *C. felis* fleas have been found to carry *R. felis* in New Zealand [15]. However, five out of these six participants also recorded overseas travel in the past 4 years, and since *R. felis* has a worldwide distribution [13], there

remains the possibility that these infections were acquired overseas. It is interesting to note that *R. felis* infections were found within 5–25 km of those for *R. typhi* (Fig. 1). The identification of *R. felis* infections is of public health concern because of the high prevalence of *R. felis* in *C. felis* fleas in New Zealand [15] as well as the high rate of ownership of cats and dogs in the nation. Moreover, *R. felis* infections may be considerably under-reported in our study due to the ARRL slides being pre-treated with *R. typhi* antigen only, as was desired in answering our original study question. Globally, *R. felis* is an emerging disease of concern [13] and where *R. typhi* and *R. felis* have been found co-circulating, *R. felis* can be more prevalent in fleas and hosts [12, 31, 32].

The overall findings from our study have important public health implications. We were able to demonstrate that *R. typhi* has a wider geographical endemicity than originally thought. As murine typhus is frequently misdiagnosed overseas and therefore substantially under-reported [3], it is likely the disease is also under-diagnosed in New Zealand. In addition, while *R. felis* is not yet acknowledged as a public health issue in New Zealand, it may pose a significant risk. This issue mirrors the situation found overseas where *R. felis* is recognized as an emerging disease potentially overshadowing *R. typhi* in some regions [12, 13, 31, 32]. Infection from *R. felis* should be routinely considered when locally acquired murine typhus is suspected. Given that many of the routine diagnostic tests for rickettsioses are not species-specific, alternative laboratory methods that already exist may need to be utilized by public health laboratories. Furthermore, as the animal vectors and exposure route for *R. typhi* and *R. felis* can differ (predominantly rat fleas, *X. cheopis*; compared to cat and dog fleas, *C. felis*, respectively), targeted public health interventions (e.g. vector control) may need to be modified to match the associated disease. Thus, for public health interventions to be more effectively targeted in time and place, and thereby decrease the risk of rickettsioses emerging as a significant public health problem, it is recommended that whenever possible the rickettsial species involved in human infection should be identified.

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DECLARATION OF INTEREST

None.

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