Rickettsial Infections, New Zealand

To the Editor: Members of the genus Rickettsia have garnered much attention worldwide in recent years with the emergence of newly recognized rickettsioses. In New Zealand, only Rickettsia typhi and R. felis, belonging to the typhus and spotted fever groups, respectively, have so far been found (1). R. typhi, primarily transmitted by the oriental rat flea (Xenopsylla cheopis), has a worldwide distribution and causes murine typhus in humans (2). At the end of 2009, a total of 47 cases of murine typhus had been recorded in New Zealand. In contrast, although the cat flea (Ctenocephalides felis) can carry R. felis in New Zealand (3), no human infections have been reported. However, because R. felis shares a similar clinical profile to murine typhus, infection can be mistaken for a suspected case of R. typhi (4).

Clinical suspicion of rickettsial infection is widely confirmed by serologic tests with the indirect immunofluorescence assay (IFA) being the standard test. However, antibodies against R. felis in human sera are known to cross-react with R. typhi in IFA (5). Western blot (WB) and cross-adsorption assays, in combination with IFA, can differentiate between several rickettsioses (5,6). We report on the trial in New Zealand of WB and cross-adsorption assays for differentiating retrospectively between past R. typhi and R. felis infections and evidence of R. felis infection in persons living in the country.

Serum samples were obtained from 24 volunteers from the Institute of Environmental Science and Research Limited, Porirua, New Zealand. Samples were tested using R. typhi IFA slides (Australian Rickettsial Reference Laboratory [ARRL], Geelong, Victoria, Australia). After incubation (37°C for 30 min), slides were washed 3 times, incubated with fluorescein-conjugated antihuman IgG, IgM, and IgA (ARRL), and washed again before examination. All samples were then tested by using an IgG IFA kit (Focus Diagnostics, Cypress, CA, USA) against typhus group (TG) R. typhi and spotted fever group (SFG) R. rickettsii.

TG-positive and SFG-negative serum samples may represent R. typhi infections, and SFG-positive and TG-negative serum samples may represent R. felis infections. Because R. typhi can cross-react with SFG rickettsiae (7), and R. felis with R. typhi (5), results that are TG positive and SFG positive may cause by either rickettsiae. Positive reactivity may also represent overseas-acquired rickettsioses. Thus, WB and cross-adsorption assays using R. typhi (Wilmington) and R. felis (URRXWCal2) antigens (Unité des Rickettsies, Marseilles, France) were used to confirm any R. typhi or R. felis infections (6).

Antigens (2 mg/mL) were solubilized (100°C for 10 min) in 2× Laemmli buffer (6) and subjected to electrophoresis (20 μg/well; 20 mA, 2.5 h) through polyacrylamide gels (12.5% resolving; 4% stacking) (BioRad, Hercules, CA, USA). Resolved antigens were electroblotted (100 V for 1 h) onto 0.45-μm polyvinylidene difluoride membranes, which were blocked by using 5% milk–Tris-buffered saline membranes, which were blocked by using 5% milk–Tris-buffered saline with 0.1% Tween 20. Each antigen lane was divided into 2 strips before incubation (room temperature for 1 h) with serum (diluted 1:200). After three 10-min washes with Tris-buffered saline with 0.1% Tween 20, strips were incubated (room temperature, 1 h) with horseradish peroxidase–conjugated antihuman IgG (1:150,000; SouthernBiotech, Birmingham, AL, USA) and washed again. Enhanced chemiluminescent detection of bound horseradish peroxidase (ECL Plus; GE Healthcare, Buckinghamshire,

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UK) enabled identification of reactive band sizes with Precision Plus standards (BioRad). Cross-adsorption was carried out by incubating serum diluted 1:30 in boiled antigen (37°C for 5.5 h, then 4°C overnight) before centrifugation (10,000 × g for 10 min) (6). Supernatants were applied to WB strips and results compared with R. typhi and R. felis antisera.

Of the 24 serum samples, 3 (12.5%) were positive on the ARRL kit, and 11 (45.8%) showed IgG reactivity on Focus slides (Table). Of these 11 serum samples, 8 (33.3%) were SFG positive and TG negative, and 3 (12.5%) were SFG positive and TG negative. Of the 24 serum samples, 3 (12.5%) were positive on the ARRL kit, and 11 (45.8%) showed IgG reactivity on Focus slides (Table). Of these 11 serum samples, 8 (33.3%) were SFG positive and TG negative, and 3 (12.5%) were SFG positive and TG negative. Of the 12 serum samples that still reacted with R. typhi and R. felis after cross-adsorption were considered as indeterminate responses.

Serum samples that showed no specific reactions to R. typhi and R. felis in the WB assay were classified as negative for R. typhi and R. felis.

Table. Serologic data and risk factors of volunteers that showed positive reactivity in rickettsial IFA, New Zealand*

| Volunteer no. | ARRL kit† | Focus kit‡ | WB and cross-adsorption results | Risk factors in the past 4 years |
|---------------|------------|------------|---------------------------------|-------------------------------|
| 4             | Neg        | 64         | 64                              | Indeterminate                  |
|               |            |            |                                 | Flea or animal contact         |
| 5             | Neg        | 64         | 64                              | Cat, dog                      |
|               |            |            |                                 | Traveled overseas              |
| 8             | Neg        | 64         | 128                             | Indeterminate                  |
|               |            |            |                                 |                                    |
| 9             | Neg        | 64         | 64                              | Indeterminate                  |
|               |            |            |                                 |                                    |
| 16            | Neg        | 64         | 128                             | Indeterminate                  |
|               |            |            |                                 |                                    |
| 17            | Neg        | 64         | 128                             | R. felis                      |
|               |            |            |                                 |                                    |
| 18            | 128        | 64         | 64                              | R. felis                      |
|               |            |            |                                 |                                    |
| 19            | 128        | 64         | 128                             | R. felis                      |
|               |            |            |                                 |                                    |
| 21            | Neg        | 64         | 256                             | R. felis                      |
|               |            |            |                                 |                                    |
| 22            | Neg        | 64         | 64                              | Cat, dog                      |
|               |            |            |                                 |                                    |
| 23            | Neg        | 64         | 64                              | Flea, cat, dog, possum        |
|               |            |            |                                 |                                    |
| 25            | Neg        | 64         | 128                             | Indeterminate                  |
|               |            |            |                                 |                                    |

*Serum samples that showed no specific reactions to R. typhi and R. felis in the WB assay were classified as negative for R. typhi and R. felis.
†The cutoff titer for seropositivity was 128 as recommended by the manufacturer.
‡According to kit instructions, endpoint titers ≥64 and <256 indicate either past infection or early response to a recent infection, and ≥256 are considered presumptive evidence of recent or current infection.
§Serum samples that still reacted with R. typhi and R. felis after cross-adsorption were considered as indeterminate responses.
¶Serum samples that showed no specific reactions to R. typhi and R. felis in the WB assay were classified as negative for R. typhi and R. felis.

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Identifying Risk Factors for Shiga Toxin–producing Escherichia coli by Payment Information

To the Editor: During May and June 2011, a large outbreak of hemolytic uremic syndrome (HUS) and diarrhea caused by Shiga toxin–producing *Escherichia coli* (STEC) occurred, centered on northern Germany (1,2). Early on, salads and raw vegetables were suspected to be food vehicles (3). Also in May, the staff department of a local company informed the Health Protection Authority in Frankfurt in southwestern Germany about the rapidly increasing number of patients with bloody diarrhea and HUS among employees at 2 company office sites. Both sites were served by cafeterias run by the same caterer. Main dishes were prepared in the cafeterias’ kitchens and differed between the 2 sites. However, in both cafeterias various fresh foods from a salad bar and fruits, desserts, and daily asparagus dishes originated from the caterer’s main kitchen. The salad bar included 30 items. Suspecting that this outbreak was linked to the one in northern Germany, we conducted an outbreak investigation to confirm the epidemiologic link to focus epidemiologic and traceback investigations.

A face-to-face survey among hospitalized employees and by email among all other employees was conducted, which included personal details, symptoms, and information about general food eaten at the cafeterias. We defined outbreak cases as infections in employees of the company at 1 of the 2 sites who by May 23, 2011, were either hospitalized with bloody diarrhea or HUS or who self-reported onset of bloody diarrhea from May 8 through May 23. A total of 320 persons responded to the survey, and 285 (89%) of 320 responders stated they used the caterer’s main kitchen. The salad bar included 30 items. Suspecting that this outbreak was linked to the one in northern Germany, we conducted an outbreak investigation to confirm the epidemiologic link to focus epidemiologic and traceback investigations.

A nested case–control study design was chosen, limited to a fraction of the cohort to obtain rapid risk estimates. Exposures included were purchases of any fruit, salad bar item, dessert, or asparagus dish in either cafeteria from May 2 through May 13. On the basis of customer identification numbers, the caterer provided billing information for persons with early cases (n = 23). Controls were randomly chosen persons from the caterer’s database whose disease status was checked against the survey information (n = 30) and who did not report symptoms of diarrhea (nonbloody), vomiting, or nausea during the same period. Univariable logistic regression was performed.

In univariable analysis, salad bar purchases were highly associated with illness (odds ratio 5.19; 95% CI 1.28–21.03), and desserts, fruit, and asparagus dishes were not (Table). Three (9%) of the case-patients remained unexposed to salad bar items according to the payment system data. The analysis of main courses

| Risk factor       | No. case-patients exposed/total no. (%) | No. controls exposed/total no. (%) | Univariable analysis* |
|-------------------|----------------------------------------|-----------------------------------|-----------------------|
| Salad bar         | 20/23 (87)                             | 16/30 (53)                        | 5.83 (1.42–23.88)     | 0.014 |
| Dessert           | 16/23 (70)                             | 18/30 (60)                        | 1.52 (0.48–4.81)      | 0.473 |
| Fruits            | 5/23 (22)                              | 10/30 (33)                        | 0.53 (0.15–1.81)      | 0.312 |
| Asparagus dish    | 7/23 (30)                              | 11/30 (37)                        | 0.76 (0.24–2.41)      | 0.635 |
| Female sex        | 16/23 (70)                             | 15/30 (50)                        | 2.28 (0.73–7.15)      | 0.155 |
| Age, y            |                                        |                                   |                       |       |
| <30               | 12/23 (52)                             | 6/30 (20)                         | 2.80 (0.62–12.66)     | 0.181 |
| 30–<40            | 5/23 (22)                              | 7/30 (23)                         | Reference             |       |
| 40–<50            | 4/23 (17)                              | 13/30 (43)                        | 0.43 (0.09–2.14)      | 0.303 |
| >50               | 2/23 (9)                               | 4/30 (13)                         | 0.70 (0.09–5.43)      | 0.733 |

* Estimates in a multivariable model remained virtually unchanged.