Serological cross reaction between legionella and campylobacter in the rapid microagglutination test

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Abstract
Aims—To investigate the serological cross reaction between legionella and campylobacter using the rapid microagglutination test (RMAT).
Methods—Serum samples from 49 patients with campylobacter infection were tested for legionella antibodies using the indirect fluorescent antibody test (IFAT) and the RMAT. Serum samples that had positive RMAT titres were retested in the presence of a campylobacter immunosorbent. The specificity of the immunosorbent was evaluated with serum from patients with genuine legionella infection (legionella culture or antigen positive, or both).
Results—Fourteen (28%) patients with campylobacter infection had positive IFAT titres (≥16) and 16 (32%) patients had positive RMAT titres (≥8) in one or more serum samples. In addition, serum samples from 11 of 17 patients with campylobacter infection, previously shown to have positive legionella IFAT titres, were also RMAT positive. Sixteen patients had RMAT titres of ≥32, including seven with titres of ≥128. RMAT titres from all but one patient were significantly reduced after campylobacter absorption, but serum samples from 48 patients with legionella infection were unaffected.
Conclusions—Serological cross reaction between campylobacter and legionella can occur in the legionella RMAT, as well as the IFAT. This cross reaction can be eliminated in most cases by incorporating a campylobacter immunosorbent in the RMAT.

Keywords: legionella, campylobacter, serological cross reaction, rapid microagglutination test.

The serological diagnosis of Legionnaires’ disease has been complicated recently by the observation that patients with campylobacter gastroenteritis can develop false positive results in the legionella indirect fluorescent antibody test (IFAT). This assay was previously thought to be highly specific for the serological diagnosis of Legionnaires’ disease. Approximately 30% of patients with campylobacter gastroenteritis can have IFAT reactions sufficiently high to cause diagnostic confusion. This serological cross reaction can be inhibited using campylobacter blocking fluid (CBF), prepared from campylobacter Penner reference strain 11, as an immunosorbent in the IFAT. Over 90% of false positive reactions are inhibited using this method, but titres from patients with genuine legionella infection are unaffected. A significant number of unexpected false positive results were revealed when this immunosorbent was incorporated into routine diagnostic serology tests.

Preliminary results showed that this serological cross reaction could also occur in the legionella rapid microagglutination test (RMAT). The RMAT is commonly used by laboratories in England and Wales as an alternative to the IFAT. This aim of this study was to assess the full extent of the cross reaction in the RMAT and to evaluate whether a simple absorption step could be successfully incorporated into this test.

Methods
Serum samples from 49 patients with campylobacter gastroenteritis, confirmed by culture, were examined for legionella antibodies by the IFAT and the RMAT. Serum samples from a further 17 similar patients, known to contain cross reacting legionella antibodies by IFAT, were tested by the RMAT. Serum samples were stored at −20°C pending analysis. The IFAT was performed as described previously, using formalised yolk sac antigen (FYS) of Legionella pneumophila serogroup 1, supplied by the Laboratory of Microbiological Reagents (LMR), Central Public Health Laboratory, Colindale, London. The RMAT was performed as described previously, using antigen supplied by the LMR. IFAT and RMAT antibody titres are expressed as the reciprocal of the highest dilution of serum giving a positive result.

Serum samples were considered to be positive in the RMAT if they had a titre of ≥8. The RMAT was repeated on these samples with and without initial dilution of the serum in CBF, prepared from campylobacter Penner 11 reference strain. Briefly, a dense suspension of bacteria in phosphate buffered saline (PBS) was heated at 100°C for 60 minutes. After centrifugation the supernatant was decanted and stored at −20°C, with the addition of sodium azide (0.08%). Positive serum samples were retested as follows. A 1 in 4 dilution of serum (15.45 μl) was made separately in both PBS and CBF. After incubating overnight at 4°C, 25 μl from each dilution was transferred to wells in a microtitre plate and doubling dilutions from 1 in 4 to 1 in 512 were made using PBS for both titrations. The RMAT was performed with PBS and CBF titrations in parallel. Serum
samples that showed a fourfold or greater decrease in titre in CBF compared with PBS were considered to be significantly absorbed.

To determine whether absorption could reduce the RMAT titres of serum samples from patients with genuine legionella infection, serum from patients with confirmed Legionnaires’ disease was examined. Serum samples were available from 23 patients with a positive legionella urinary antigen test and from 25 patients in whom *L. pneumophila* had been isolated from a clinical specimen. Table 1 shows the serogroups and subtypes of *L. pneumophila* isolated from these patients. In total 62 serum samples from these patients had a RMAT titre of ≥8 and were retested after dilution in PBS and CBF as above.

**Results**

Table 2 shows the legionella IFAT and RMAT results from 49 patients with campylobacter gastroenteritis. Seventeen (34%) patients had a positive IFAT or RMAT, or both, titre in at least one sample. Of the 17 patients previously shown to have cross reacting antibody in the IFAT, 11 also had a positive RMAT titre. Figure 1 shows the comparison of IFAT and RMAT antibody titres in the 34 patients who were positive in one or both tests.

Serum samples from 27 patients infected with campylobacter with positive RMAT titres were retested with and without campylobacter absorption. Table 3 shows the RMAT titres obtained in PBS and CBF. Campylobacter absorption produced a fourfold or greater reduction in RMAT titres in all but one patient.

When campylobacter serum samples were diluted in CBF the usual appearance in the RMAT was a button of stained bacteria that streaked down the side of the V-bottomed microtitre well on inclination (negative result). The corresponding dilutions in PBS produced a tight button with no streaking. In seven patients, however, CBF inhibited the formation of a tight button during centrifugation, giving a diffuse appearance similar to that seen with some genuine high titre legionella serum samples. Absence of button formation in CBF, compared with PBS, was interpreted as a negative result.

Initial dilution of serum samples in CBF had no significant effect on the RMAT titres of 62 serum samples from 48 patients with confirmed legionella infection. Fifty nine serum samples had the same titre in PBS and CBF. Two serum samples showed a twofold reduction from 32 to 16 and one sample a twofold reduction from 8 to 4. In one of these patients a second serum sample showed a titre of 16 in both PBS and CBF. The absence of button formation in CBF compared with PBS was not observed in any sample.

**Discussion**

The results shown in table 2 and fig 1 demonstrate that the serological cross reaction between legionella and campylobacter occurs to a similar degree with both the legionella IFAT and RMAT. The cross reacting antibody in campylobacter positive serum samples is predominantly IgM and this is the predominant antibody detected by rapid microagglutination. Thus, the cross reaction was likely to occur in the RMAT even though different strains of *L. pneumophila* serogroup 1 are used to produce the IFAT and RMAT antigens (Harrison TG, personal communication). RMAT titres in campylobacter positive serum samples were generally two to fourfold lower than the corresponding IFAT titres (a more
sensitive assay), and in seven samples the RMAT titre was <8 when the IFAT was positive (≥16). However, three patients had a positive RMAT result (titres of 8 or 16) with a negative IFAT result. Sixteen patients had RMAT titres of ≥32, including seven with titres ≥128. As a RMAT titre of ≥32 in a single serum sample from a patient with a relevant clinical history is regarded as evidence of a presumptive case of legionella infection,7 the cross reaction has the potential for significant diagnostic confusion for laboratories using the RMAT.

The campylobacter immunosorbent was suitable for distinguishing between genuine and cross reacting serum samples in the RMAT. There was no significant reduction in RMAT titres in any of serum samples from the 48 patients with genuine legionella infection, whereas RMAT titres from 26 of 27 patients with campylobacter infection were significantly reduced (table 3). No alteration in the RMAT assay performance was seen when the immunosorbent was used with genuine legionella serum samples.

Absence of button formation in the RMAT occurred when some of the campylobacter positive serum samples were diluted in the immunosorbent. This could be because of large complexes of campylobacter antigen—antibody—legionella antigen, preventing the formation of a tight button of legionella antigen on centrifugation. Absence of button formation in CBF, but not PBS, could identify cross reacting serum samples as this pattern was not seen with genuine legionella serum samples.

Serum from a patient infected with campylobacter had a high RMAT titre which was not reduced by absorption. The failure of Penner 11 CBF to absorb this cross reaction in the IFAT has been reported before.7 Further work has shown that the IFAT and RMAT titres can be reduced in a CBF prepared from Campylobacter lari NCTC 11352. Further evaluation of this alternative CBF is planned.

In conclusion, laboratories using the legionella RMAT need to be aware of this important cross reaction. Incorporation of a campylobacter immunosorbent is technically simple and easily distinguishes genuine from cross reacting serum samples.

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