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Cross-Reactions in *Legionella* Antisera with *Bordetella pertussis* Strains

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While preparing slide agglutination test antisera and immunofluorescence conjugates for the identification of *Legionella* species and serogroups, we found that several of the reagents cross-reacted with *Bordetella pertussis* strains. To determine the extent of this problem and to estimate the specificity of *Legionella* reagents, we tested slide agglutination test antisera against 22 species and 35 serogroups with 92 bacterial strains representing 19 genera. The only cross-reactions observed were with *Legionella pneumophila* serogroup 10, *L. maceachernii*, *L. gormanii*, and *L. feeleii* serogroup 1 antisera and 4 of 10 *B. pertussis* strains. Nineteen conjugates, previously available from the Centers for Disease Control but no longer distributed as reference reagents, were tested with the four cross-reactive *B. pertussis* strains. Two conjugates, *L. micdadei* and *L. wadsworthii*, stained three of the *B. pertussis* strains at a fluorescence intensity of ≥3+. All cross-reactions were removed from the antisera and conjugates by absorption with the cross-reacting strain without diminishing the homologous reaction. Special emphasis should be placed on the identification and removal of cross-reactions in *Legionella* reagents with strains that have similar morphologic and growth characteristics.

We have previously described intrageneric cross-reactions in slide agglutination test (SAT) antisera prepared against 22 *Legionella* species and 33 serogroups (8). Most of the antisera were made specific by absorption with cross-reactive strains, and the SAT was selected as the reference test for *Legionella* isolates. Advantages of SAT over the direct immunofluorescence assay (DFA) included less complicated reagent preparation, test performance, and equipment and the fact that only the isolates that are *Legionella*-like in growth, morphology, and Gram-stain reaction are tested. However, a test such as DFA is still required for the examination of primary specimens when cultures cannot be obtained. For this purpose, we recommended a polyvalent conjugate to circumvent the need to produce and use 33 individual conjugates per specimen.

While developing the above *Legionella* reagents, we found that some *Legionella* antisera and conjugates cross-reacted with *Bordetella pertussis* strains. Previous studies of potential cross-reactivity between *Legionella* DFA conjugates and non-*Legionella* bacteria included *Legionella pneumophila* serogroups 1 to 4 and *L. micdadei* (2, 3, 6, 10), but we are not aware of such evaluations with antisera to additional serogroups or species.

The purpose of this study was to test *Legionella* antisera against a variety of heterologous bacterial antigens and to determine if they could be made specific by absorption with any strains found to be cross-reactive. We also tested all DFA conjugates previously distributed by the Centers for Disease Control against *B. pertussis* antigens to determine the extent of cross-reactivity against this respiratory pathogen.

MATERIALS AND METHODS

Strains. The *Legionella* species which were used to prepare SAT antisera and antigens as well as DFA antigens were *L. anisa*, *L. bozemanii* serogroups 1 and 2, *L. cherrii*, *L. dumoffii*, *L. erythra*, *L. feeleii* serogroups 1 and 2, *L. gormanii*, *L. hackeliae* serogroups 1 and 2, *L. jamestowniensis*, *L. jordanis*, *L. longbeachae* serogroups 1 and 2, *L. maceachernii*, *L. micdadei*, *L. oakridgensis*, *L. parisiensis*, *L. pneumophila* serogroups 1 to 10, *L. rubrilucens*, *L. sainthelensi*, *L. santicrucis*, *L. spiritensis*, *L. steigerwaltii*, and *L. wadsworthii*. This included 35 serogroup reference strains previously described (4, 8, 11). Non-*Legionella* strains used to screen SAT antisera for cross-reactions are shown in Table 1. All the strains were obtained from the stock culture collection that had been stored in rabbit blood at −70°C at the Immunology Laboratory.

Antisera. SAT antisera were prepared with whole-cell vaccines of the strains listed above, as previously described (9). DFA conjugates were prepared as described by Cherry et al. (2). For absorption, *B. pertussis* cells that had been grown on buffered charcoal-yeast extract (BCYE) agar plates for 72 h at 35°C were harvested in 1% formalin in 0.01 M phosphate-buffered saline (pH 7.6), centrifuged, and then washed twice in phosphate-buffered saline by centrifugation. Absorption was carried out at a 1:20 ratio (volume of cells to volume of serum) at 37°C for 2 h and then at 4°C overnight.

SAT. SAT antigens were prepared as described previously (9). Briefly, strains were grown on BCYE agar for the *Legionella* and *Bordetella* species and on heart infusion or Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar, supplemented with 5% rabbit blood, for the remaining bacteria. The growth from each agar slant was suspended in 2 to 3 ml of 10% (vol/vol) buffered Formalin, pH 7.0, and heated in a boiling-water bath for 15 min. The SAT was performed by mixing 1 drop (approximately 0.025 ml) of antigen with 1 drop of antiserum on a glass slide for 30 to 60 s. The reaction was scored on a scale of 1+ (barely visible) to 4+ (strong agglutination).

DFA. *Legionella* antigens were prepared as described by Cherry et al. (2), except that the strains were grown on BCYE agar and suspended in phosphate-buffered saline containing 0.5% normal chicken yolk sac. *Bordetella* antigens were prepared from strains grown and harvested as described above and then adjusted to a turbidity approximating a McFarland no. 1 standard. The DFA procedure was

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TABLE 1. SAT with *Legionella* antisera and non-*Legionella* bacteria

Organism (no. of species if >1)	No. of strains	
	Tested	Positive[a]
Acinetobacter lwoffii	1	0
Alcaligenes faecalis	1	0
Bordetella spp. (3)	10	4
Capnocytophaga sp.	1	0
Enterobacter aerogenes	4	0
Escherichia spp. (3)	3	0
Francisella tularensis	1	0
Haemophilus influenzae serotypes A to F	8	0
Klebsiella spp. (2)	6	0
Kluyvera ascorbata	1	0
Neisseria meningitidis serogroups A, B, C, L, W135, X, Y, and Z	9	0
Providencia spp. (2)	2	0
Pseudomonas spp. (2)	10	0
Serratia marcescens	4	0
Shigella spp. (2)	2	0
Staphylococcus spp. (2)	5	0
Streptococcus groups A, B, C, D, and G	9	0
Streptococcus pneumoniae types 1, 4, 6A, 6B, 9N, 14, 18C, and 23F	13	0
Yersinia spp. (2)	2	0

[a] Positive, ≥3+ agglutination.

performed as previously described (1) with *B. pertussis* fluorescein isothiocyanate-labeled chicken globulin and with the following *Legionella* conjugates: *L. pneumophila* serogroups 1 to 8, *L. dumoffii*, *L. gormanii*, *L. micdadei*, *L. longbeachae* serogroups 1 and 2, *L. jordanis*, *L. oakridgensis*, *L. bozemanii* serogroups 1 and 2, *L. feeleii*, and *L. wadsworthii*.

RESULTS

SAT antiserum pools reacted with only 1 of 19 non-*Legionella* bacterial genera (Table 1). Four *B. pertussis* strains reacted with three of the pools and, subsequently, with four individual antisera: *L. pneumophila* serogroup 10, *L. maceachernii*, *L. gormanii*, and *L. feeleii* serogroup 1. Reaction strength varied from 1+ to 3+. Preimmune sera from rabbits subsequently injected with *L. maceachernii* and *L. feeleii* serogroup 1 vaccines were nonreactive with the four *B. pertussis* strains. Preimmune sera for *L. gormanii* and *L. pneumophila* serogroup 10 were unavailable. The cross-reacting antibodies were easily removed by absorption with *B. pertussis* cells without affecting homologous titers.

To determine the extent of cross-reactivity between DFA reagents and *B. pertussis* antigens, we tested 19 *Legionella* conjugates. The *L. micdadei* and *L. wadsworthii* conjugates reacted with several strains of *B. pertussis* at a fluorescence intensity of ≥3+. All strains were negative with a fluorescein isothiocyanate-labeled normal rabbit control serum. Cross-reacting antibodies were removed from the conjugates by absorption with *B. pertussis* cells without affecting homologous titers. In contrast, the *B. pertussis* conjugate was nonreactive with *Legionella* strains representing 22 species and 35 serogroups.

DISCUSSION

Initial studies of cross-reactions between *L. pneumophila* serogroup 1 conjugates by Cherry et al. (2) and Cherry and McKinney (1) revealed only one strain of *Pseudomonas fluorescens* which cross-reacted. Additional studies by Thomason et al. (10) with a polyvalent *L. pneumophila* serogroup 1 to 4 conjugate showed cross-reactions with 10 strains of *B. pertussis*. This cross-reaction was due to preexisting antibodies in the *L. pneumophila* serogroup 4 conjugate and was blocked by the use of rhodamine-labeled normal rabbit serum as a counterstain. Orrison et al. (6) isolated six organisms that cross-reacted with three *Legionella* conjugates. These organisms, one of which was isolated from a human tracheal swab, were similar morphologically to *Legionella* cells and, therefore, could cause false-positive results when primary clinical specimens are tested with *Legionella* conjugates. After isolation, however, all of these organisms could be easily discriminated from *Legionella* cells by their growth on blood agar or BCYE agar without cysteine. Similarly, the nonspecific staining of *Bacteroides fragilis* strains with *Legionella* conjugates could give false-positive results unless isolation of the organism is attempted.

In our study, four strains of *B. pertussis* were tested with 19 *Legionella* conjugates. Three of these four strains cross-reacted with two conjugates, *L. micdadei* and *L. wadsworthii*. This staining could not be explained on the basis of preexisting antibodies since the conjugates contained normal rabbit serum as a diluent and since the *Bordetella* strains were nonreactive with fluorescein isothiocyanate-labeled normal rabbit globulin. Absorption removed the cross-reactive antibodies, but DFA results should probably be considered only presumptive unless the organism is subsequently isolated and retested. There is always the possibility of cross-reactions between conjugates and strains not yet tested.

Isolation of the organism with subsequent testing in the SAT provides a higher degree of confidence since only organisms that meet the phenotypic characteristics of the *Legionella* genus are tested with antiserum pools. Our results showed that four *B. pertussis* strains agglutinated with *L. pneumophila* serogroup 10, *L. maceachernii*, *L. gormanii*, and *L. feeleii* serogroup 1 antisera. Like *Legionella* spp., *B. pertussis* strains grow poorly or not at all on blood agar, and most of them require cysteine for growth (7). Two additional organisms, submitted to our laboratory as possible *Legionella* species, but subsequently identified as *Francisella tularensis* and a *Capnocytophaga* species, failed to grow on blood agar and on BCYE without cysteine. They were nonreactive with all available SAT antisera and, therefore, might have been considered a new *Legionella* species or serogroup without gas-liquid chromatographic data (5). Strains such as these should be used to evaluate *Legionella* antisera used for diagnostic testing, and all observed cross-reactions should be removed by absorption with the appropriate strains.

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