Serum sensitivity and lipopolysaccharide characteristics in *Bordetella bronchiseptica*, *B. pertussis* and *B. parapertussis*

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Summary. The viability of four strains of *Bordetella bronchiseptica*, two strains of *B. pertussis* and one strain of *B. parapertussis* exposed to hyperimmune and pre-colostrum porcine serum was examined. Viable cell numbers (cfu/ml) of the *B. pertussis* strains and a rough strain of *B. bronchiseptica* (CSU-P-1) decreased by 99% and 99.99%, respectively, after exposure for 1 h to porcine hyperimmune serum. In contrast, smooth *B. bronchiseptica* strains and the *B. parapertussis* strain showed no significant decrease in viable cell numbers after the same treatment. *B. bronchiseptica* strain CSU-P-1 also showed a 99% decrease in viable cell numbers after exposure to pre-colostrum porcine serum for 1 h whereas the other strains tested showed no decrease in viable numbers under the same conditions. Heating the hyperimmune and pre-colostrum serum at 56°C for 30 min resulted in the loss of bactericidal activity suggesting the involvement of complement in both systems. Analysis of silver-stained SDS-PAGE profiles of lipopolysaccharide (LPS) extracted from the bacterial cells indicated that the smooth strains of *B. bronchiseptica* and the *B. parapertussis* strain possessed high mol. wt O-side chain-like material, whereas the *B. pertussis* strains and *B. bronchiseptica* strain CSU-P-1 did not. Gel filtration of acid-hydrolysed LPS samples indicated two distinct carbohydrate peaks for the strains with high mol. wt O-side chain-like material, whereas the other strains each yielded one distinct peak. Western-blot analysis indicated a positive reaction for anti-*B. bronchiseptica* antibodies to the high mol. wt O-side chain-like material of all serum-resistant strains used in this study. The serum resistance of smooth *B. bronchiseptica* strains may be an important factor in their association with wound infections.

Introduction

Members of the genus *Bordetella* are aerobic, gram-negative, non-spore-forming, pleomorphic coccobacilli that typically infect the ciliated respiratory epithelium. *B. pertussis* is a highly communicable bacterium that causes whooping cough in man;¹ *B. parapertussis* causes a milder form of whooping cough which may go undetected.² *B. bronchiseptica* is associated with respiratory disease in laboratory animals, kennel cough in dogs and atrophic rhinitis and bronchopneumonia in pigs.³ *B. bronchiseptica* rarely causes clinical respiratory disease in man, but has been isolated sporadically from wound infections in man⁴ and dogs (H. P. Veit and R. M. Roop, unpublished observations). Unlike *B. bronchiseptica*, neither *B. pertussis* nor *B. parapertussis* has been reported to infect beyond the respiratory tract.¹

Complement-dependent serum killing is an important host defence mechanism against bacterial infections. In response, some pathogenic bacteria have

evolved means of avoiding this defence. One proposed way in which gram-negative bacteria may avoid complement-dependent serum killing is through the presence of high mol. wt O-side chain material on the lipopolysaccharide (LPS).^{5–8} The high mol. wt O-side chains apparently block access of the activated terminal complement complex C5b-9 to the outer cell membrane, thus preventing bacterial lysis.^{5,7}

Because *B. bronchiseptica* has been isolated from wound infections, this organism apparently can produce an invasive type of infection under certain conditions.⁴ This suggests that *B. bronchiseptica* can sometimes evade complement-dependent killing in serum, whereas *B. pertussis* appears more sensitive to killing by serum. This report presents the results of an investigation of the serum resistance of *B. bronchiseptica*, *B. pertussis* and *B. parapertussis* strains and its relation to their LPS characteristics.

Materials and methods

Bacterial strains

The origin and colonial morphology^{9,10} of the strains used in this study are listed in the table. Stock

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Table. Origin and colonial morphology on BGS of the *Bordetella* strains used in this study

Strain	Origin	Colonial morphology*
<i>B. pertussis</i> Bp-338†	Human: nasopharyngeal	Dom + Scs + Hly +
<i>B. pertussis</i> ATCC 9797‡	Human: nasopharyngeal	Dom + Scs + Hly +
<i>B. parapertussis</i> ATCC 15311§	Human: nasopharyngeal	Dom + Scs + Hly +
<i>B. bronchiseptica</i> CSU-P-1	Porcine: nasal	Dom - Scs - Hly -
<i>B. bronchiseptica</i> B205BT	Porcine: nasal	Dom + Scs + Hly +
<i>B. bronchiseptica</i> 64-C-0406	Canine: nasal	Dom + Scs + Hly +
<i>B. bronchiseptica</i> 1120-A-83-013	Porcine: lung	Dom + Scs + Hly +

* Nomenclature of Peppler and Schrupf,⁹ designed for describing the colonial morphology of *B. bronchiseptica* strains on BGS agar. Dom + designates the domed colony type, Scs + designates a smooth colony surface, and Hly + indicates production of a zone of haemolysis around the colony.

† Obtained from Dr E. L. Hewlett, University of Virginia School of Medicine, Charlottesville, VA, USA.

‡ *B. pertussis* type strain obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA.

§ *B. parapertussis* type strain obtained from ATCC.

|| These strains have been described previously.¹⁰

cultures were lyophilised in skimmed milk and stored at -70°C . Working cultures were grown on Bordet Gengou agar supplemented with defibrinated sheep blood 15% (BGS) at 37°C for 48 h (*B. bronchiseptica* and *B. parapertussis*) or 72 h (*B. pertussis*) and stored at 4°C with bi-weekly transfer. Working cultures were transferred a maximum of four times; new working cultures were then prepared from stock cultures.

Preparation and testing of serum

Hyperimmune serum was obtained from a sow that had been immunised four times during an 8-week period with a commercial *Bordetella-Pasteurella* vaccine (Rhinobac, Norden Laboratories, Omaha, NE, USA). Blood was collected 2 weeks after the last vaccination. The sow was bled from an ear vein by means of a butterfly infusion set (Abbott Hospitals, Inc., North Chicago, IL, USA) and a 5-ml syringe. Blood was allowed to clot overnight at 4°C in a 10-ml blood collection tube (Vacutainer, Becton Dickinson, Rutherford, NJ, USA) without additives. Serum was removed and stored at -70°C until needed. Precolostrum serum was taken from a newborn piglet which had not received colostrum and thus should not have had any specific antibodies to *Bordetella* spp. or other antigens.¹¹ Blood was obtained by intravenous puncture, and the serum was removed and stored as described above.

An enzyme-linked immunosorbent assay (ELISA) based on previously described procedures^{12,13} was used to test the serum samples for antibodies against *B. bronchiseptica*. The antigen for the ELISA was prepared as follows. *B. bronchiseptica* strain B205BT was grown for 48 h at 37°C on BGS. Cells were harvested in 0.01 M Tris-HCl, pH 7.8, and adjusted to an optical density of 1.0 at 660 nm. The cells were lysed by sonication (Fisher Sonic Dismembrator, Pittsburgh, PA) with short pulses at 60% power for 30

min while being cooled in an ice bath. The lysate was centrifuged at 800 *g* for 20 min at $0-4^{\circ}\text{C}$ and the pellet was resuspended in 0.05 M Na_2CO_3 - NaHCO_3 , pH 9.6 (coating buffer). Protein concentration was determined by the method of Lowry¹⁴ and adjusted to 50 $\mu\text{g}/\text{ml}$. Ninety-six-well microelisa plates (Nunc, Immuno-plate II, Vanguard International, Inc., Neptune, NJ, USA) with 50 μl of cell lysate per well were incubated overnight at 4°C . Plates were washed five times with phosphate-buffered saline (0.15 M NaCl, 0.003 M KCl, 0.006 M Na_2HPO_4 , 0.0015 M KH_2PO_4 , pH 7.4) supplemented with Tween 80 0.05% v/v (PBS-T). PBS-T (100 μl) supplemented with bovine serum albumin (BSA) 1% w/v was added to each well and incubated at 37°C for 30 min. Plates were washed five times as described above and 50 μl of test or control serum diluted from 1 in 10 to 1 in 25 600 in acetate-buffered saline (0.07 M $\text{NaC}_2\text{H}_3\text{O}_2$, 0.15 M NaCl, pH 5.0) supplemented with Tween-40 0.2% v/v (ABS-T) was added to the appropriate wells and incubated for 1 h at 37°C . Serum from the hyperimmunised sow was used as the positive control serum. Serum from the newborn piglet was used as the negative control serum. Wells were washed five times with ABS-T, and 50 μl of peroxidase-conjugated rabbit anti-swine IgG (heavy and light chains; Cooper Biomedical, Malvern, PA, USA), diluted 1 in 800 in ABS-T, was added to each well; the plates were incubated for 30 min at 37°C . Plates were washed five times with ABS-T, and 50 μl of freshly-prepared substrate solution (10 mg of *o*-phenylenediamine dissolved in 1 ml of methanol, in 100 ml of distilled H_2O and 100 μl of H_2O_2) was added to each well and incubated for 5 min in the dark. The reaction was terminated by the addition of 10 μl of 8 N H_2SO_4 to each well. The absorbance at 492 nm was determined with an automated microelisa reader (Titertek Multiskan, Flow Laboratories, McLean, VA, USA). A positive reaction at dilutions less than 1 in 20 was considered to represent the background.

Positive reactions at dilutions greater than 1 in 20 were considered to be *Bordetella* specific.

Serum sensitivity assay

Growth from a 48-h culture of *B. bronchiseptica* or *B. parapertussis* or a 72-h culture of *B. pertussis* grown on BGS at 37°C was used to inoculate a 16 × 125 mm screw-capped glass test tube containing 5 ml of Stainer-Scholte Broth (SSB).¹⁵ The *B. bronchiseptica* cultures were incubated for 8 h, and the *B. parapertussis* and *B. pertussis* strains were incubated for 24 h at 37°C in a shaking water bath at 120 rpm. The contents of the tube were transferred to a 250-ml screw-capped glass Erlenmeyer flask containing 50 ml of SSB, and incubated at 37°C in a water bath with shaking at 120 rpm until logarithmic growth occurred (c. 5 h for the *B. bronchiseptica* strains and c. 12 h for the *B. parapertussis* and *B. pertussis* strains). The cultures were then centrifuged at 10 000 *g* for 10 min, and the cells were washed once with sterile physiological saline (0.15 M NaCl) and resuspended in sterile saline to an optical density of 0.2 at 525 nm. A 10-fold serum dilution was prepared by adding 0.2 ml of the appropriate serum to 0.2 ml of the bacterial culture to be tested and 1.6 ml of Hanks's Balanced Salts Solution (Gibco Laboratories, Chagrin Falls, OH, USA) containing 0.15 mM CaCl₂ and 1.0 mM MgCl₂ (HBSS + +), buffered with 10 mM HEPES (Sigma) and NaH₂CO₃ 0.075% w/v.⁵⁻⁸ Two control media were used in the assay. One consisted of 0.2 ml of the appropriate culture with 1.6 ml of buffered HBSS + + plus 0.2 ml of serum that had been heated at 56°C for 30 min to inactivate complement. The second consisted of 0.2 ml of the appropriate culture plus 1.8 ml of buffered HBSS + + with no serum. All samples were incubated in 16 × 125 mm sterile screw-capped glass tubes at 37°C in a shaking water bath at 120 rpm. At 0, 20, 40, and 60 min, 100-μl samples were taken from the tubes. Ten-fold dilutions in sterile saline were made of each sample. Four 10-μl replicates were plated on to BGS agar for each dilution. Plates were incubated for 48–72 h at 37°C and the number of cfu/ml was determined.

LPS extraction

Bacterial strains were grown on BGS for 72 h at 37°C. Cells were harvested in distilled water and centrifuged at 10 000 *g* for 10 min, then washed twice in distilled water. LPS was extracted by the hot phenol-water method of Westphal and Jann.¹⁶ The aqueous and phenolic phases were dialysed in tubing with a mol. wt cut-off of 3.5 Kda (Spectrapor membrane tubing, Baxter, McGaw Park, IL, USA) against distilled water for 3 days at 4°C with fresh water added twice daily. After dialysis, the samples were lyophilised. LPS was recovered almost entirely from the aqueous phase lyophil, which was stored in air-tight containers at 4°C until used.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

LPS preparations were subjected to Laemmli discontinuous SDS-PAGE¹⁷⁻²¹ with the modifications of Tsai and Frasch.²² A 4% stacking and a 15% resolving gel were used. Each LPS sample was prepared at 2 mg/ml in 10 mM Tris buffer, pH 8.0. To 50 μl of each sample, 25 μl of DNAase (Sigma) stock solution was added and the preparations were incubated at 37°C for 1 h. Stock solutions of DNAase were prepared at 1.0 mg/ml in 10 mM Tris, pH 8.0. A stock solution (20 mg/ml in distilled water) of proteinase K (Sigma) was diluted 1 in 20 with 10 mM Tris, pH 8.0, and 20 μl of the diluted proteinase K solution was added to 100 μl of each sample and incubated for 1 h at 60°C. Sample buffer (120 μl) was added to each sample, the mixture was boiled for 5 min and 12 μl was applied to each lane on the gel. Gels were run at 20 mA constant current until the dye front reached the bottom of the gel (c. 5 h). Gels used for Western blotting were prepared as described above, except that 36 μl of the enzymatically-digested solution was added to each lane on the gel. The polyacrylamide gels were stained with silver as described by Tsai and Frasch.²²

Western blotting

A modification of the electrophoretic transfer procedure described by Towbin *et al.*²³ was used to transfer LPS from polyacrylamide gels to nitrocellulose paper. Briefly, LPS was transferred to nitrocellulose paper (Micron Separations Inc., Westboro, MA, USA) in a transfer apparatus at a constant voltage of 25 V for 16 h. All incubations after this step were performed on a table-top shaker at room temperature. The nitrocellulose paper was removed from the apparatus and incubated for 1 h in Tris-buffered saline (TBS) (0.02 M Tris-base, 0.5 M NaCl, pH 7.5) supplemented with BSA 2% w/v (TBS-B). The paper was then washed three times with TBS supplemented with Tween-20 0.3% v/v at 10 min/wash. Anti-*B. bronchiseptica* hyperimmune porcine serum diluted 1 in 100 with TBS-B was added to the paper and incubated for 2 h. The nitrocellulose paper was washed as previously described. Peroxidase-conjugated rabbit anti-swine IgG (heavy and light chains; Cappel, Durham, NC, USA) diluted 1 in 1000 with TBS-B was then added to the paper and incubated for 2 h. After washing, the paper was developed by adding 60 ml of freshly prepared substrate solution (30 mg of 4-chloro-1-naphthol dissolved in 10 ml of methanol, in 50 ml of TBS with 30 μl of H₂O₂) and incubated until colour developed. The nitrocellulose paper was washed with distilled water, dried, photographed and stored in the dark.

Acid hydrolysis and gel filtration

LPS samples (1 mg/ml) were acid-hydrolysed by boiling for 2 h in screw-capped glass tubes containing

acetic acid 1% v/v. After hydrolysis, samples were centrifuged at 10 000 *g* for 10 min. The supernate, which contained the oligosaccharide fraction of the LPS, was lyophilised. The pellet, which contained the lipid-A fraction, was discarded. The lyophilised oligosaccharides were resuspended in distilled water to a concentration of 10 mg/ml. Each sample (100- μ l) was placed on a 30 $\times$ 1 cm Sephadex G-75 (Pharmacia Inc., Piscataway, NJ, USA) column which had been equilibrated with distilled water. Samples (1 ml) were collected at a rate of 4.0 ml/h, and fractions containing carbohydrate were identified by the phenol-sulphuric acid assay.²⁴

Results

ELISA titres

The hyperimmune porcine serum had a titre of 12 800 against the *B. bronchiseptica* antigen. Pre-colostrum porcine serum had a titre of <10 which indicated no measurable levels of specific antibodies to *Bordetella* spp.

Serum sensitivity

B. bronchiseptica strains B205BT, 64-C-0406 and 1120-A-83-013 and *B. parapertussis* strain ATCC 15311 maintained nearly constant viability after exposure to unheated porcine hyperimmune serum for 1 h. In contrast, *B. pertussis* strain ATCC 9797 showed a 98% decrease in cfu/ml and *B. pertussis* strain Bp-338

showed a 99.5% decrease during the same period (fig. 1a); the rate of loss of viability was almost linear during the entire incubation period. *B. bronchiseptica* strain CSU-P-1 showed a 99.99% decrease in viable cell numbers after exposure to the unheated hyperimmune serum for 1 h (fig. 1a), with the largest decrease in viable cell numbers occurring during the first 20 min of incubation. *B. bronchiseptica* strain CSU-P-1 was the only strain tested that was sensitive to unheated pre-colostrum porcine serum (fig. 1b). This organism showed a 99.5% decrease in cell viability (cfu/ml) after 1 h, with the decrease being almost linear. When exposed to hyperimmune or pre-colostrum porcine serum that had been heated at 56°C for 30 min to inactivate the complement, none of the seven *Bordetella* strains showed a measurable decrease in viable cell numbers (data not shown).

LPS profiles

LPS preparations from *B. bronchiseptica* strains B205BT, 64-C-0406 and 1120-A-83-013 and *B. parapertussis* showed similar smears with high mol. wt bands on silver-stained SDS-PAGE gels (fig. 2a, identified as "a"). These bands were thought to represent LPS fragments which contained high mol. wt O-side chains on core structures. Similar high mol. wt material was not present in *B. pertussis* strains nor in *B. bronchiseptica* strain CSU-P-1, although unique intermediate mol. wt bands appeared for both *B. pertussis* strains (fig. 2a, bottom of "a" zone); these bands may represent intermediate mol. wt O-side

Fig. 1. Sensitivity of *Bordetella* strains to porcine serum: *B. pertussis* Bp-338 (○—○); *B. pertussis* ATCC 9797 (▲—▲); *B. parapertussis* ATCC 15311 (■—■); *B. bronchiseptica* CSU-P-1 (○---○); *B. bronchiseptica* B205BT (□—□); *B. bronchiseptica* 64-C-0406 (△—△); *B. bronchiseptica* 1120-A-83-013 (●—●). (A) Unheated hyperimmune serum; (B) unheated pre-colostrum serum (NB. From 40 min to 60 min, *B. pertussis* ATCC 9797 and *B. parapertussis* ATCC 15311 have similar values, and are represented as a single line).

Fig. 2. (a) Silver-stained SDS-PAGE profiles of proteinase K-treated LPS preparations from *Bordetella* strains. (b) Western blot analysis of LPS preparations from *Bordetella* strains with porcine hyperimmune serum. Lane A, *B. pertussis* Bp-338; B, *B. pertussis* ATCC 9797; C, *B. parapertussis* ATCC 15311; D, *B. bronchiseptica* CSU-P-1; E, *B. bronchiseptica* B205BT; F, *B. bronchiseptica* 64-C-0406; G, *B. bronchiseptica* 1120-A-83-013. Arrow a points to zone of high mol. wt bands interpreted to contain O-side chain-like materials. Arrows b, b' point to zones of lower mol. wt bands, interpreted as core structures.

chains on core structure fragments. *B. bronchiseptica* strains B205BT, 64-C-0406 and 1120-A-83-013 and both *B. pertussis* strains had bands which occupied similar mol. wt ranges on silver-stained SDS-PAGE gels (fig. 2a identified as b and b'), but such bands were absent from *B. parapertussis* and *B. bronchiseptica* strain CSU-P-1. These lower mol. wt bands were thought to represent primarily core structures with small to non-existent O-side chains.

Western blot analysis

The high mol. wt region (fig. 2a, identified as "a") of *B. bronchiseptica* strains B205BT, 64-C-0406 and 1120-A-83-013 and *B. parapertussis* strain ATCC 15311 developed a positive reaction on nitrocellulose paper when exposed to porcine hyperimmune serum. The lower mol. wt bands of all *Bordetella* strains tested, except for *B. bronchiseptica* strain CSU-P-1, gave a positive reaction when treated with porcine hyperimmune serum. Smooth *B. bronchiseptica* strains developed stronger reactions on nitrocellulose paper than did the *B. parapertussis* or *B. pertussis* strains (fig. 2b).

Chromatographic analysis

Gel filtration of oligosaccharide samples from *B. bronchiseptica* strains B205BT, 64-C-0406 and 1120-A-83-013 and *B. parapertussis* strain ATCC 15311 on Sephadex G-75 yielded two distinct peaks of carbohydrate, with K_{av} values of 0.2 and 0.8, where K_{av} is the distribution coefficient from the formula:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

where V_e is the volume at which the peak elutes, V_o the void volume of the column, and V_t the total volume of the column. Gel filtration of oligosaccharides from *B. bronchiseptica* strain CSU-P-1 and *B. pertussis* strains ATCC 9797 and Bp-338 resolved into one distinct carbohydrate peak with a K_{av} of 0.8.

Discussion

The smooth strains of *B. bronchiseptica*, B205BT, 1120-A-83-013 and 64-C-0406, and the *B. parapertussis* strain ATCC 15311 used in this study had LPS profiles with similar high mol. wt bands which were interpreted as core structure fragments with variable length O-side chain-like material. Similar LPS profiles on SDS-PAGE for *B. bronchiseptica* and *B. parapertussis* strains have been reported by others.^{20,25} The smooth strains were also found to remain viable in the presence of heated or unheated pre-colostrum or hyperimmune porcine serum. Similar survival responses have been observed with strains of *Escherichia coli*,⁶ *Salmonella minnesota*,²⁶ *S. montevideo*,⁵ *Klebsiella pneumoniae*,⁸ *Pseudomonas aeruginosa*⁷ and *Neisseria* spp.²¹ Protection against serum killing by complement-dependent mechanisms for these bacteria has been postulated to be a function of the size of the O-side chain.²⁶ In some of these studies^{5,7} it has been found that serum-resistant strains activate the complement cascade and cause the formation of the membrane attack complex (MAC). However, it is believed that the MAC can be sterically hindered from attacking the outer membrane of bacteria by the presence of high mol. wt O-side chains. This view is supported by the observation that

variants having smaller mol. wt O-side chains lose viability in the presence of serum containing complement.^{5,21} The positive correlations observed in this study between the presence of high mol. wt bands, reactivity of anti-*B. bronchiseptica* antibodies to the high mol. wt bands and maintenance of viability in specific antiserum suggest that a similar mechanism for serum resistance may operate for *Bordetella* spp.

In contrast to the resistant smooth *B. bronchiseptica* strains and the *B. parapertussis* strain, *B. pertussis* strains Bp-338 and ATCC 9797 lost viability in unheated hyperimmune porcine serum, but not in unheated pre-colostrum porcine serum. SDS-PAGE profiles indicated that the LPS of the *B. pertussis* strains lacked high mol. wt bands, suggesting a lack of long O-side chains. Their serum resistance and LPS profiles are thus similar to those of *Neisseria*^{21,27,28} and *Haemophilus*²⁹ spp. It was noted that the two *B. pertussis* strains had a similar unique band of intermediate mol. wt (fig. 2a). This band may represent core protein fragments with intermediate length O-side chains. Others have shown that LPS profiles of *B. pertussis* strains contain intermediate to small mol. wt bands typical of O-side chain material.^{2,20,28} This latter type of LPS has been referred to by some as a "lipo-oligosaccharide" or LOS.^{21,28} Specific antibodies, i.e., those directed against certain outer-membrane proteins, allow the MAC to form at sites on the outer membrane of such bacteria where it can effectively induce cell lysis.²⁶ Gram-negative bacteria with this type of LPS are usually susceptible to loss of viability only in the presence of specific antibodies.²⁶ Serum sensitivity of the *B. pertussis* strains used in this study showed this relationship.

B. bronchiseptica strain CSU-P-1 lost viability in both unheated pre-colostrum and hyperimmune porcine serum, suggesting that neither specific antibody nor immunoglobulins in general are necessary for the loss. This strain has a rough colonial morphology on BGS,¹⁰ and LPS profiles on SDS-PAGE indicate that it has a low mol. wt core structure with no apparent O-side chain-like material present, similar to the LPS of deep rough mutants described for *Salmonella* spp.³⁰ It has been shown that the lipid-A of some bacterial species is capable of binding complement components and activating the complement pathway in the absence of specific antibodies.³¹ Possibly, the lipid-A of CSU-P-1 is sufficiently exposed to activate the complement pathway.

The results outlined in this report show that *B. bronchiseptica*, *B. pertussis* and *B. parapertussis* have LPS characteristics and serum sensitivity similar to those observed for other gram-negative bacteria. *Bordetella* spp. are primarily respiratory pathogens which have a predilection for attachment to the cilia of the respiratory tract epithelium;^{3,32} serum resistance apparently does not play a major role as a virulence determinant in this type of infection. However, *B. bronchiseptica* is occasionally isolated from wound infections in man⁴ and animals (H. P. Veit and R. M. Roop, unpublished observations). *B. bronchiseptica* has also been isolated from tissues of pigs showing signs of sepsis which were experimentally infected via nasal inoculation or aerosol exposure^{33,34} (H. P. Veit, unpublished observations). Thus, for *B. bronchiseptica* to colonise outside the ciliated respiratory epithelium, serum resistance is likely to be necessary.

The serum resistance of *Bordetella* spp. *in vitro* noted in this study correlated well with clinical observations of invasiveness *in vivo* for all strains examined except one, *B. parapertussis* strain ATCC 15311. This strain had high mol. wt O-side chain-like material and serum resistance, but has not been reported to cause infection outside the respiratory tract.¹ It is possible that this strain would have shown serum sensitivity if *in vitro* periods longer than 60 min had been used. There was an observable trend (not significant, $p < 0.05$) for reduced viability of this strain between 20 and 60 min. Longer exposure to serum *in vitro* would have more closely mimicked *in vivo* conditions. Also, *B. parapertussis* strain ATCC 15311 had lower mol. wt core structures, compared to the core structures of the smooth *B. bronchiseptica* strains which are known to be invasive *in vivo*. Possibly, the core structures are important for long term (at least, greater than 60 min) serum resistance, and viability *in vivo*.

We concluded that the *Bordetella* strains that developed high mol. wt bands (interpreted as long O-side chain-like material) and higher mol. wt core structures (fig. 2a, identified as "b") are resistant to antibody and complement-mediated reduction in viability *in vitro*, and that such resistance closely correlates with the ability of such strains to infect outside the respiratory epithelium *in vivo*.

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