THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE ROLE OF FIMBRIAE IN *BORDETELLA* COLONIZATION

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Spring 2010

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Microbiology
with honors in Microbiology

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Abstract

Bordetella bronchiseptica is a respiratory tract pathogen of many mammalian species that is closely related to *B. pertussis* and *B. parapertussis,* the causative agents of whooping cough. Fimbriae are little-studied adhesins of the *Bordetella* and are a component of the *B. pertussis* acellular vaccine. In this study, we explored the role of fimbriae in murine *B. bronchiseptica* infection and began constructing fimbrial mutants of *B. pertussis* and *B. parapertussis* for future investigations of these human pathogens. We show that there is no statistically significant difference in colonization of the mouse respiratory tract between a wild type *B. bronchiseptica* strain and a fimbrial mutant following a high-dose, high-volume inoculation, but that fimbriae may be an important factor in dissemination of infection throughout the respiratory tract when a low-dose, low-volume inoculation method is used. Furthermore, there was a statistically significant higher IgG3 antibody titer observed in C57Bl/6 mice infected with the fimbrial mutant compared to the parental strain. We also show that the fimbrial mutant is substantially less likely to cause lethal bordetellosis in mice deficient in TNF-α than wild type *B. bronchiseptica*. Plasmids were constructed for the creation of fimbrial mutants of *B. parapertussis* and are being developed for *B. pertussis* knockouts. Together, our data indicate that fimbriae may play a role in dissemination of bacteria throughout the respiratory tract and suggest fimbriae are involved in immune system regulation.

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Acknowledgements

I would like to thank Laura Weyrich for all her support and advice in the laboratory and on this thesis. I also thank Sara Hester, Anne Buboltz, and Elizabeth Goebel for their infinite patience and tutoring and Eric Harvill for his guidance and mentorship.

Introduction

The three classical *Bordetella* species are Gram-negative coccobacilli that cause diseases of the respiratory tract. *Bordetella bronchiseptica* does not typically cause disease in humans but infects a wide range of mammalian species causing a variety of disease severities ranging from asymptomatic persistent infection in the nasal cavity to fatal pneumonia (19, 33, 39). *B. bronchiseptica* is the causative agent of kennel cough in canines, snuffles in rabbits and atrophic rhinitis in swine (1, 15, 30). The closely related species *B. pertussis* and *B. parapertussis* are the causative agents of whooping cough in humans, a disease that has been classified as re-emerging by the Centers for Disease Control (3, 8, 24, 33). *B. pertussis* and *B. parapertussis* are thought to have evolved separately from a *B. bronchiseptica*-like progenitor, primarily by genome degradation (14, 41, 42). These human-adapted species, along with *B. bronchiseptica*, share several highly conserved virulence factors (33, 41).

B. bronchiseptica, *B. pertussis*, and *B. parapertussis* each have a highly conserved, two component signal transduction system encoded by the *bvgAS* locus that is responsible for regulating the expression of many virulence factors (12, 33). BvgS is a transmembrane sensor protein that causes the activation of BvgA by phosphorylation, which then promotes the transcription of a wide range of genes (10, 12, 40). The stage in which these genes are expressed is known as the Bvg+ phase. When BvgA is not active, bacteria enter the Bvg^- phase. In this phase, which is thought to be important for survival outside of the host, flagellar genes and genes for nutrient acquisition are expressed (10, 11, 40). This phase can be observed when bacteria are grown at 26°C or under modulating conditions in the presence of nicotinic acid or $MgSO_4$ (35).

Among the virulence factors regulated by BvgAS are the five components of the *B. pertussis* acellular vaccine, including filamentous hemagglutinin (Fha), pertactin (Prn), pertussis

toxin (Ptx), and two serotypes of fimbriae (Fim) (12, 33). Fha is a highly immunogenic hairpin-shaped adhesin found on the bacterial cell surface that has been shown to contribute to ciliated epithelium and macrophage attachment by *B. bronchiseptica* (9, 43). In *B. pertussis, in vitro* studies have shown that Fha plays a role in the inhibition of T-cell proliferation and suppression of IL-12 (6, 34). Another adhesin, Prn, belongs to a group of proteins which direct their own transport to the outer membrane and contains motifs commonly present in proteins involved in eukaryotic cell binding via protein-protein interactions (16, 26). The one acellular vaccine component found only in *B. pertussis* is Ptx, a secreted ADP ribosylating toxin (33). Ptx is thought to contribute to disease morbidity and has a variety of effects on the host immune system, including causing leukocytosis, as well as inhibition of chemotaxis, oxidative responses, and release of lysosomal enzymes by neutrophils and macrophages (4, 12, 33, 37, 38, 46).

The remaining acellular vaccine components are two serotypes of fimbriae, Fim2 and Fim3, which are the only antigens in the vaccine shown to convey a significant degree of cross protection from *B. parapertussis* (28, 49). These are immunogenic filamentous polymeric cell surface proteins composed of a minor subunit, which makes up the fimbrial tip, and a major subunit, which distinguishes the protein serotype (45). The operon *fimBCD* contains the genes responsible for assembly and secretion of fimbriae as well as the minor fimbrial subunit gene. Disruption of the *fimBCD* locus results in a complete lack of fimbriae on the cell surface (51, 52). Based on predicted amino acid similarity to *Escherichia coli* fimbrial proteins, PapD and PapC, FimB is proposed to serve as a chaperon for fimbrial assembly and FimC as an usher protein (29, 52). FimD is the minor fimbrial subunit and has been shown to mediate monocyte binding via the integrin VLA-5 during *B. pertussis* infection (22, 23, 50). The major fimbrial subunits, Fim2 and Fim3, are found in all three classical *Bordetella* and are thought to mediate

binding to the ciliated epithelium (17, 36). Little is known about several additional Fim genes. Additional major subunit genes, *fimN* and *fimA*, are found in both *B. bronchiseptica* and *B. parapertussis* but are deleted and truncated respectively in *B. pertussis* (5, 27, 42). The fimbrial gene, *fimX*, is found in all three classical strains; however this gene contains a frameshift mutation in *B. parapertussis* (13, 44). Therefore, each of the classical *Bordetella* express a unique subset of fimbrial genes.

Regulation of fimbrial gene expression is under the control of the BvgAS system, but expression of the genes encoding the major subunits also undergoes phase variation. This is mediated by ~15 cytosine residues located upstream of the -10 element of the promoters of *fim2, fim3, fimN,* and *fimX*. Slip-strand mis-pairing during replication can cause insertions and deletions in the poly-C region altering the distance between the -10 and -35 elements and therefore affecting transcription initiation and causing varied levels of expression of *fim2, fim3, fimN,* and *fimX* (53). Expression of the major fimbrial subunits, *fim2* and *fim3*, in *B. bronchiseptica* usually remains relatively stable when grown *in vitro*, but has been shown to change more rapidly during the course of rabbit infection, likely due to immunological pressure (53).

While a significant amount of research has been done to elucidate the roles of the other protective antigens incorporated into acellular pertussis vaccines, little work has been done to determine the role of the fimbriae during infection or their effect on the immune response. An infection study of *B. bronchiseptica* showed that, compared to the wild-type, a fimbrial mutant strain lacking *fimBCD* was defective in the colonization of rat tracheae *in vivo* (32). In the same study, fimbriae were also found to be important for the long-term bacterial persistence in the trachea (32). Furthermore, a study of *B. pertussis* in BALB/c/Rivm mice showed that a strain

lacking *fimB* colonized throughout the respiratory tract at lower levels than the wild-type; the defect was most pronounced in the trachea (18).

Given the comparatively lower degree of understanding of the role fimbriae play in infection and their inclusion as a major component of acellular *B. pertussis* vaccines, we proposed to compare a previously constructed fimbriae mutant strain of *B. bronchiseptica* to a wild type *B. bronchiseptica* strain in our well-developed murine model of infection. We also performed enzyme-linked immunosorbant assays (ELISA) to measure the antibody titers from infected mice and studied the effect of fimbriae on antibody generation. Lastly, we undertook the creation of knockout constructs of the fimbrial genes, *fimA*, *fimBCD, fimX, fim2,* and *fim3* in *B. pertussis* and *fimBCD, fimN, fim2,* and *fim3* in *B. parapertussis.* This will effectively allow us to investigate the role of fimbriae in respiratory tract infection and immunity in these human-adapted species, both as a whole and as a function of individual fimbriae genes. Together, this data allows us to determine the importance of fimbriae during colonization of the respiratory tract and helps to clarify the role these highly conserved proteins play in infection.

Materials and Methods

Strains and Bacterial Growth

B. bronchiseptica strains RB50 and RB50 Δ*fimBCD* (RB63), in which the genes for the assembly and secretion of fimbriae have been deleted, have been previously described (11, 32). *B. parapertussis* strain 12822, the gentamicin-resistant derivative 12822G, and *B.pertussis* 536, a streptomycin resistant derivative of *B. pertussis* Tohama I, have been characterized previously (21, 25, 54). All *Bordetella* strains were maintained on Bordet-Gengou (BG) agar (Difco, Sparks, MD) containing 10% sheep's blood (Hema Resources, Aurora, OH) with streptomycin (RB50, RB63, 536), gentamicin (12822G), or no antibiotics (12822) and were grown at 37°C. Liquid cultures were grown overnight at 37°C in Stainer-Scholte (SS) broth (47) to mid-logarithmic phase. *E. coli* strains were grown on LB agar or in LB broth containing kanamycin under the same conditions listed above.

Animal Experiments

C57BL/6 and TNF-$\alpha^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME) and were bred in our specific pathogen and *Bordetella*-free breeding rooms at The Pennsylvania State University (University Park, PA). For inoculation, bacteria were grown overnight to mid-exponential phase, and bacterial cell concentration was estimated by measuring the optical density at 600 nm using the equation 1 OD_{600nm} = 1 x 10^9 CFU/ ml. Bacteria were then diluted to the appropriate concentration in 1% phosphate buffered saline (PBS). Bacterial concentration of the inocula was determined by plating on BG agar and counting colonies after two days growth at 37°C. Groups of four- to six-week-old mice were lightly anesthetized with 5% isofluorane

(IsoFlo; Abbot Laboratories) in oxygen and 50 µl of inoculum containing 5 x 10^5 CFU of either RB50 or RB63 (survival curves and high-dose, high-volume time courses) or 5 µL of inoculum containing 50 CFU of bacteria (low-dose, low-volume time courses) was gently pipetted onto the external nares. For survival curves, groups of four mice were inoculated, and survival was monitored over a 28-day period. Mice showing signs of lethal bordetellosis, including ruffled fur, difficulty breathing, and diminished responsiveness, were euthanized to eliminate unnecessary suffering (20, 31). For time course experiments, groups of four mice were sacrificed and dissected on days 0, 3, 7, 14, and 28 post-inoculation (high-dose, high-volume) or days 0, 3, 5, 7, 14, and 28 post-inoculation (low-dose, low-volume). The lungs, trachea, and nasal cavity of each mouse were harvested and homogenized in 1mL PBS, diluted to appropriate concentrations, and plated on BG agar for the determination of CFU . Colonies were counted after 2 days incubation at 37°C. Statistical significance between infection groups was determined using a Student's *t*-test. All animal experiments were performed in accordance with institutional guidelines and protocols were approved by the university's Institutional Animal Care and Use Committee.

ELISAs

Enzyme-linked immunosorbant assays (ELISA) were performed as previously described (54, 55). Briefly cultures of *B. bronchiseptica* strains RB50 and RB63 were heat-killed at 65°C for 30 minutes. The heat-killed bacteria were diluted to 7 x 10^6 CFU/ml in a 1:1 mixture of 0.2 M sodium carbonate and 0.2 M sodium bicarbonate buffers. 100 µl of this mixture was added to each well of 96-well plates and incubated at 37°C for 4 hours. The wells were then washed three times in PBS containing Tween 20 (PBS-T) and filled with 200 µl blocking buffer consisting of

1% bovine serum albumin (BSA) in PBS, and plates were stored in a sealed container at 4°C until use. Before use, plates were again washed in PBS-T, and wells were filled with 100 μL blocking buffer. To the first column, 96 μL blocking buffer and 4 μL mouse serum were added to each well to achieve a 1:50 dilution of serum. The serum was then serially diluted down the plates to achieve dilutions of 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800, 1:25600, 1:51200, and 1: 102400, and plates were incubated for 2 hours at 37°C. The plates were then washed, and goat antimouse horseradish peroxidase-conjugated antibody diluted in blocking buffer was added to the plates. The following dilutions of conjugated antibody in blocking buffer were used: polyvalent Ig – 1:4,000, IgG – 1:4,000, IgG_1 – 1:2,000, IgG_{2A} – 1:2,000, IgG_{2B} – 1:20,000, and IgG_3 – 1:2,000. After incubating for 1 hour, the plates were again washed, and 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) and hydrogen peroxide in a phosocitrate buffer was added to the wells. Plates were incubated in the dark at room temperature for 20-30 minutes. Absorbance was then measured at 405 nm. Anitbody titers were determined using an endpoint method by comparing wells containing either RB50 or RB63 serum to wells treated with naïve serum. Statistical significance between groups was determined using a Student's *t*-test.

Primer Design and PCR

DNA sequences for *B. pertussis* strain Tohama I and *B. parapertussis* strain 12822 are available through NCBI under the accession numbers NC_002929 and NC_002928 respectively. Primers for the construction/creation/ of knockout constructs for the genes *fimBCD, fimN, fim2,* and *fim3* in *B. parapertussis* and genes *fimBCD, fimX, fim2* and *fim3* in *B. pertussis* were designed with the aid of the OligoAnalyzer (Integrated DNA Technologies, www.idtdna.com)

and are described in Table 1. Restriction sites were added to the 5' ends of primers to assist with DNA manipulation. PCR was performed under the conditions of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, the annealing temperature listed in table for 30 s, and 72°C for an appropriate elongation time according to fragment size; and then a finishing step of 72°C for 5 min.

Knockout Construct Construction

Vectors for the creation of gene knockouts were constructed as previously described. (7) Briefly, genomic DNA was extracted from *B. parapertussis* strain 12822 and *B. pertussis* strain 536 using a Qiagen kit (Qiagen, Valencia, CA) and the manufacturer's protocol. PCR was performed using the set of primers described for both the 5' and 3' flanking regions of each gene at an appropriate annealing temperature based on the melting temperatures listed in Table 1. The PCR product was run on a 1% agarose gel, and the band containing polynucleotides of the correct size was extracted and gel purified using a Qiagen gel purification kit (Qiagen, Valencia, CA). Fragments were then digested at 37°C for 2 hours with the appropriate restriction enzyme listed in Table 1. Restriction enzymes were inactivated via Qiagen PCR purification kit, and the 3' and 5' flanking regions of the same gene were ligated overnight at ~4°C. The ligated product was PCR amplified using the 5'F and 3'R primers for each indicated gene. PCR products were again run on a 1% agarose gel, and the bands of the appropriate size were gel extracted and ligated into TOPO sequencing vectors (Invitrogen, Carlsbad, CA) as per supplier's instructions. The vectors were then transformed into Mach1 *E.coli* DH5-α cells and screened for the uptake of the vector on LB agar containing 100 mg/L kanamycin. Resulting colonies were screened for the presence of the insert in the plasmid by performing a plasmid extraction, digesting with the

appropriate restriction enzyme, and running on a 1% agarose gel to detect a band of the anticipated size. Plasmids containing inserts of the expected size were sent for sequencing. After sequencing confirmation, the inserts were digested from the vectors, purified, and ligated into *Bordetella* specific allelic exchange vector pSS4245 (S. Stibitz, unpublished data) cut with the same enzyme. The vector was transformed into DH5-α cells and plated on LB agar with kanamycin to screen for vector uptake. Resulting colonies were screened for insert as described above. Colonies confirmed to have the desired sequence were grown overnight in LB broth, and freezer stocks were made by adding 20% glycerol to the culture media. Freezer stocks were stored at -80°C.

Results

Infection with high-dose, high-volume inoculum of *B. bronchiseptica* RB50 and RB63

To investigate to role of fimbriae in colonization of the respiratory tract of mice, C57BL/6 mice were intranasally inoculated with a high-dose, high-volume inoculum of either wild-type RB50 or fimbrial mutant RB63. Groups of mice were sacrificed and dissected on days 0, 3, 7, 14 and 28 post-infection, and the CFU of bacteria in the lungs, trachea, and nasal cavity was determined. In the nasal cavity and trachea, CFU numbers increased until they peaked at day 3 and then decreased gradually through day 28 (Figure 1). In the lungs, CFU numbers increased until day 7 post-inoculation and then decreased until day 28. In all three organs, there was no statistically significant difference in colonization between strains at any timepoint studied. This indicates that fimbriae are not required for the colonization of the murine respiratory tract under high-dose, high-volume inoculation conditions.

Infection with low-dose,low-volume inoculum of *B. bronchiseptica* RB50 and RB63

To determine if fimbriae are required for colonization and dissemination throughout the murine respiratory tract when subjects were only inoculated with a small number of bacteria, we utilized a low dose infection model where 50 CFU of RB50 or RB63 in 5 µl of PBS were intranasally inoculated. This procedure better replicates the course of a natural infection as the innoculum is not washed into the lungs, requiring bacteria to first colonize the nasal cavity before disseminating to the lower respiratory tract. Mice receiving a low-dose. low-volume inoculum were sacrificed and dissected on days 0, 3, 5, 7, 14, and 28, and the CFU of bacteria present in each organ was determined. Bacterial numbers in the nasal cavity increased from 10 CFU on day 0 post inoculation to 690,000 CFU (RB50) and 450,000 CFU (RB63) on day 7 and

then decreased gradually (Figure 2). There was no statistically significant difference at any timepoint during infection in the nasal cavity, indicating that fimbriae are not important for colonization of the nasal cavity. In trachea, a statistically significant difference in colonization was observed on day 14 with RB63 being present at about 100 times greater CFU than RB50; by day 28, this had decreased to a 10-fold difference. In the lungs, numbers of RB50 peaked on day 7, while the highest levels of RB63 CFU were seen on day 14. Throughout the course of infection, RB50 showed a trend toward higher colonization in the lungs, but at no point was the observed difference in colonization statistically significant. As may be expected in low-dose experiments, a large variation in CFU numbers was found between individuals leading to large error. As some individuals did not have any detectable level of *B. bronchiseptica* in their lungs, a comparison was made between the number of mice in each group that did have detectable CFU levels and those that did not. A higher number of individuals infected with RB50 (42% or 8/19 mice at all timepoints combined) were found to have pulmonary infections compared to those infected with RB63 (28% or 5/18 mice at all timepoints combined) (Table 2). The greatest difference was observed on day 7 post inoculation where bacteria were detected in the lungs of three out of four mice infected with RB50 and one out of four mice infected with RB63. On day 28, one out of four of mice in both groups were found to have detectable levels of *B. bronchiseptica* in their lungs. The trend toward a higher percentage of pulmonary infection in mice inoculated with RB50 sugges that fimbriae may play a role in allowing infection to progress to the lungs.

Infection of TNF-$\alpha^{-/-}$ mice with RB50 and RB63

The early innate immune response elicited by TNF-α is required to efficiently control a

B.bronchiseptica infection in mice (31). Additional infection experiments were performed in a lethal model of infection using TNF-$\alpha^{-/-}$ mice. In this model, TNF-$\alpha^{-/-}$ mice inoculated with 5 x 10^5 CFU RB50 succumb to infection by day 3 post-inoculation. We observed 66% survival (8/12 mice) by day 30 in mice infected with RB63 as opposed to 6.2% survival (1/16 mice) in mice infected with RB50 (Figure 3). Wild-type mice do not succumb to infection with *B. bronchiseptica* when receiving this dosage of bacteria. These data indicated that fimbriae contribute to lethality in TNF$\alpha^{-/-}$ mice.

Serum antibody titers

Fimbriae are highly immunogenic and could affect overall antibody titers, to determine if variation in serum antibody levels could be detected in animals infected with RB63 as opposed to RB50, ELISAs were performed with serum collected from C56/BL6 mice infected with both high and low doses of RB50 and RB63 on day 28 post-inoculation. Results showed no statistically significant difference in titer of polyvalent antibodies recognizing heat killed RB50 and RB63 (Figure 4). ELISAs to detect IgG and IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 were performed using sera from mice inoculated with high doses of each strain. Statistically significant higher titers of IgG3 antibodies were found in mice infected with RB63 compared to those infected with RB50. No statistically significant differences were seen in titers of IgG, IgG1, IgG2a, or IgG2b antibodies; however there was a non-statistically significant trend toward higher IgG and IgG1 levels in serum from individuals infected with RB63. These data indicate that while most serotypes of antibodies are not affected by the presence of fimbriae, higher levels of IgG3 antibodies are produced in response to infection with a strain of *B. bronchiseptica* lacking fimbriae.

Creation of knock-out constructs

In addition to experiments with *B. bronchiseptica*, we undertook the construction of fimbrial knock-out strains in *B. pertussis* and *B. parapertussis.* The individual *fimBCD* loci, as well as *fimA, fimX, fim2,* and *fim3* in *B. pertussis* and *fimN, fim2,* and *fim3* in *B. parapertussis* were targeted. DNA was manipulated using PCR, restriction digest, and ligation. *B. pertussis* knock-out constructs remain at various points of completion, while knock-out constructs for all *B. parapertussis* fimbrial genes were completed and inserted into the *Bordetella*-specific allelic exchange vector pSS4245. Wild type gene sizes are 6474 bp, 1864 bp, 1924 bp, and 1771 bp and predicted sizes of knockout constructs were 1569 bp, 1297 bp, 1162 bp, and 1240 bp for *fimBCD, fimN, fim2, and fim3,* respectively. PCR was used to confirm that the knock-out construct of *fimBCD* was of the expected size, the wild type locus was not easily amplified due to its large size (Figure 5a). PCR confirmed that wild type gene and knockout constructs of *fimN, fim2,* and *fim3* were of the expected sizes (Figures 5b, 5c, and 5d) Successful insertion of the desired sequences was confirmed by DNA sequencing at the Nucleic Acid Facility at The Pennsylvania State University. Sequencing of each knockout construct reveals a stretch of six nucleotides that deviate from the predicted sequence due to the insertion of a restriction site (Figures 6a, 6b, 6c, and 6d). Sequencing coverage of the *fimBCD* and *fim3* knockout constructs did not extend to the extreme 5' and 3' ends of the expected inserts (Figures 6a and 6d). Additionally there is a point mutation at position 1457 in the expected *fimBCD* sequence and two point mutations at nucleotide numbers 890 and 961 of the expected *fim3* sequence. All point mutations are located outside of the coding regions of these genes and are not expected to interfere with our ability to knockout the genes. The creation of plasmids containing fimbrial

gene knockout constructs will allow us to create fimbrial mutants of *B. pertussis* and *B. parapertussis*, allowing for a future in depth-study of the importance of fimbriae in these human pathogens.

Discussion

Previous studies have shown that fimbriae play a role in colonization of rat tracheae during *B. bronchiseptica* infection (32). To determine if fimbriae were required for colonization of the murine respiratory tract, mice were infected with either wild type *B. bronchiseptica* or a strain lacking fimbriae. Results indicated no statistically significant difference between the wild type and the mutant at any of the timepoints analyzed, indicating fimbriae are not required for *B. bronchiseptica* colonization of the murine respiratory tract using a high-dose, high-volume model. It was unexpected that a highly conserved, highly immunogenic virulence factor that is energetically demanding to produce would have no clear effect on colonization. While fimbriae have been thought to be important in adherence, other major adhesins present on the bacterial cell surface, such as Fha and Prn, may be sufficient for adherence during infection using this model. The high-dose, high-volume inoculation method is useful for studying infection because it leads to a reproducible, highly uniform course of infection, but because large numbers of bacteria are washed into the animals, this method masks the importance of factors required early in infection for the dissemination of bacteria throughout the respiratory tract.

It has been observed that *B. bronchiseptica* fimbriae are important for causing ciliostasis of the ciliated respiratory epithelium *in vitro* (2). Based on this and the earlier negative result using a high-dose, high-volume model, we hypothesized that fimbriae-induced ciliostasis in the trachea may be important in allowing bacteria to spread to the lower respiratory tract and colonize the lungs. One way to observe this is by utilizing a low-dose, low-volume model of infection that requires *B. bronchiseptica* to spread throughout the upper respiratory tract before disseminating to the lungs. The results of murine infection with a low-dose, low-volume inoculum of bacteria yielded fairly uniform colonization of the nasal cavity among groups of

mice infected with the wild type or the strain lacking fimbriae. However, highly variable numbers of bacteria were observed in the lungs and tracheae of individual mice infected with both strains. This is not unexpected, because many variables might affect dissemination throughout the respiratory tract. When inoculating with lower bacterial quantites, host microflora, immune sensitivity, and small volume variability all play a role in colonization variation. Due in part to the large error values caused by this variation, the only point at which a statistically significant difference in colonization was observed was on day 14 in the trachea, at which time RB63 colonized approximately 100 times more than wild type. This finding was opposite of what would have been expected based on previous studies in both *B. bronchiseptica* and *B. pertussis* which indicate that strains lacking fimbriae are deficient in tracheal colonization (32, 18). This could suggest that there are few fimbriae-specific receptors found in the respiratory tract of mice. Additionally, the results could indicate that adhesion is not the main role played by the fimbriae during infection. The lack of fimbriae could even potentially allow other adhesins, such as Fha and Prn, to be expressed in higher numbers on the cell surface or to bind to cells of the respiratory epithelium of mice more efficiently.

Because the observed CFU numbers in the lungs varied considerably between individuals and timepoints and because there were a number of individuals with no detectable infection in the lungs at each timepoint, we decided to examine the presence or absence of bacteria in the lungs of individual mice. A comparison of percentages of mice with detectable *Bordetella* infection in their lungs at each timepoint revealed that mice infected with *B. bronchiseptica* lacking fimbriae were generally less likely to have lung colonization than mice infected with wild type bacteria. These results suggest that fimbria are important for lung colonization during *B. bronchiseptica* infection, a result congruous with the observation that fimbriae are important

for ciliostasis of the respiratory epithelium (2), and that the ability to induce ciliostasis is important in facilitating the dissemination of bacteria into the lungs.

An early TNF-α response is required for the induction or augmentation of an early innate immune response critical for survival of RB50 infection (31). In a lethal model of infection in TNF-$\alpha^{-/-}$ mice, animals infected with RB63 were more than 10 times more likely to survive infection than those infected with RB50, suggesting fimbriae are a contributing factor for RB50 lethality in this model and that TNF-α is less essential for controlling RB63 infection. A potential explanation is that RB63 is unable to colonize the respiratory tract of TNF-α deficient mice at the high numbers seen during lethal RB50 infection (31). Alternatively RB63 may not be capable of inducing the extensive inflammation caused by RB50 in this model.

As fimbriae are a major component of acellular *B. pertussis* vaccines, we sought to determine whether infection with a strain of *B. bronchiseptica* lacking fimbriae would result in a serum antibody profile different from that of the wild type. When using titer ELISAs to analyze the antibody response in a low-dose, low-volume model, we found no statistically significant difference in antibody titer between infection with RB50 and RB63 (Figure 4). Lower titers of antibodies were produced in mice inoculated with low doses of bacteria compared to the response in a high-dose, high-volume model . This is as expected and was most likely caused by the lower levels of colonization seen in these experiments. Similarly, no statistically significant difference in antibody titer was seen in the high-dose, high-volume model of infection. The lack of a difference in overall antibody titer was not unexpected because even though fimbriae have been deleted, there are a large number of other highly immunogenic proteins that remain on the cell surface. Although antibody titers in mice infected with a low-dose, low-volume of *B. bronchiseptica* were too low to analyze further, ELISAs were used to determine titer of IgG and

IgG subclass antibodies in sera during high-dose, high-volume infections. This revealed no significant differences with the exception that higher IgG3 production was observed in RB63 infected mice. This result, as well as the trend toward higher IgG, IgG1, and total antibody production in RB63 infection, can be explained by higher surface expression of other highly antigenic proteins due to a lack of fimbriae. Alternatively, the lack of fimbriae could cause a reduced steric hindrance allowing an increased exposure of other epitopes on the surface of *B. bronchiseptica* for antibody recognition. Our ELISA results indicate that it is possible fimbriae mask other antigens of *B. bronchiseptica* which, coupled with the ability to change fimbrial serotype, could contribute to evasion of a secondary immune response upon re-infection. Future studies will examine whether there is a difference in the degree of protection against a secondary infection in mice initially infected with either RB50 or RB63.

Our work indicates that fimbriae are not required for *B. bronchiseptica* murine respiratory tract colonization in a high-dose, high-volume model, but analysis in a low-dose, low-volume model indicates they may play a role in the dissemination of bacteria throughout the respiratory tract during natural infection. The fimbriae may also be involved in modulating the immune response to *B. bronchiesptica.* The creation of allelic exchange vectors containing fimbrial knock-out constructs in *B. pertussis* and *B. parapertussis* will allow the creation of mutants lacking fimbriae or specific fimbrial serotypes in these two human pathogens. This will allow us to examine the role of fimbriae and individual fimbrial proteins during infection. We will also be able to establish the importance of an antibody response to fimbriae in preventing infection and re-infection, and providing cross-protective immunity. This information will be important for future vaccine design, because the current acellular vaccines do not effectively confer protection against *B. parapertussis*.

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Figures

Table 1: Table showing sequences of primers used to make knockout constructs for *B. pertussis* and *B. parapertussis* and their respective melting temperatures and restriction sites.

B.pertussis knock-out primers

Gene	primer	Primer sequence	Tm	Restriction Site
fimA	5' forward	ATTGAATTCGTGAAGCCCGAAACCACCAAG	62.7°C	EcoRI
	5' reverse	GACAAGCTTTGAGAACCCTGCGAATCC	61.6°C	HindIII
	3' forward	CAGAAGCTTGTGAAAGAGGGGTCACCC	62.8°C	HindIII
	3' reverse	GAGGAATTCTGCCGTCATCGATCCATG	60.9°C	EcoRI
fimBCD	5' forward	TAGGAATTCATGGTCAGCAAGTGGGATTGTTCT	62.3°C	EcoRI
	5' reverse	ACTCTCGAGCTTTACCGTAAATGCAGGCGT	64.3°C	XhoI
	3' forward	ACTCTCGAGCAGACCTCCACTTCAACC	63.2°C	XhoI
	3' reverse	GGCGAATTCATCAGCCATCCCTTGATG	61.7°C	EcoRI
fimX	5' forward	GTGGAATTCGTTCCATATACCGCGCCA	62.6°C	EcoRI
	5' reverse	CAACTCGAGACAGGCTGGAGTAAAGAAGGGATC	63.6°C	XhoI
	3' forward	GACCTCGAGTACCCCTGAAACGGCAAC	64.2°C	XhoI
	3' reverse	CAGGAATTCGAGCTGGTGCTGAAGATC	61.1°C	EcoRI
fim2	5' forward	GCTGAATTCGTGAGTGCCCATGGAGAC	63.1°C	EcoRI
	5' reverse	ACGCTCGAGTATGTGGGTTTTTCCGTG	62.6°C	XhoI
	3' forward	AATCTCGAGTAACACGGGCGTCTTGGC	64.0°C	XhoI
	3' reverse	GCCGAATTCCTGTCGCATTCCAACTTC	61.9°C	EcoRI
fim3	5' forward	TAGGATCCATGCGTCTATTCGATACATAACGC	60.6°C	BamHI
	5' reverse	ATCTCGAGGGTGATTCTGATACTGAGGGGTG	63.2°C	XhoI
	3' forward	ATACTCGAGAAACCGGCAAGCCTTGCG	64.5°C	XhoI
	3' reverse	ATTGGATCCTACATCACGCACAACCAGATGCTG	64.3°C	BamHI

B.parapertussis knockout primers

fimBCD	5' forward	AATGGATCCCAATTGCAGATCGAGATA	58.0°C	BamHI
	5' reverse	TAGAAGCTTTATGACTGACGCAACGAA	58.2°C	HindIII
	3' forward	CGCAAGCTTTGTATTCATGATTCAAGA	56.3°C	HindIII
	3' reverse	GACGGATCCTGAATATTCTTAGGCATC	56.8 °C	BamHI
fimN	5' forward	AATGAATTCTCTCCACTGGCCTTCTTC	58.7°C	EcoRI
	5' reverse	GAGAAGCTTAGCGCGATAACCACTTAG	58.9 °C	HindIII
	3' forward	CGTAAGCTTCTTCATGCTGGTATTGGC	59.8°C	HindIII
	3' reverse	GATGAATTCTCTCCAAGAGCGCATTGA	59.0°C	EcoRI
fim2	5' forward	GTCGAATTCCTGCTGTCGCATTCCAAC	61.8°C	EcoRI
	5' reverse	TATGGATCCTTGCATGGGTAACACGGG	62.1°C	BamHI
	3' forward	ATAGGATCCATCTATCCGTAACCCCGA	60.0°C	BamHI
	3' reverse	GACGAATTCTGCGCTAGATGCCGATGC	63.3°C	EcoRI
fim3	5' forward	GTCGAGCTCCCGAGATCTACATCACGC	59.3°C	SacI
	5' reverse	GGAAAGCTTGTCTATCCCTGAGTTCTC	58.5°C	HindIII
	3' forward	CAGAAGCTTGGACATGGTGATTCTGAG	59.3°C	HindIII
	3' reverse	TACGAGCTCAAGGGTTGTTTCATTTGG	59.3°C	SacI

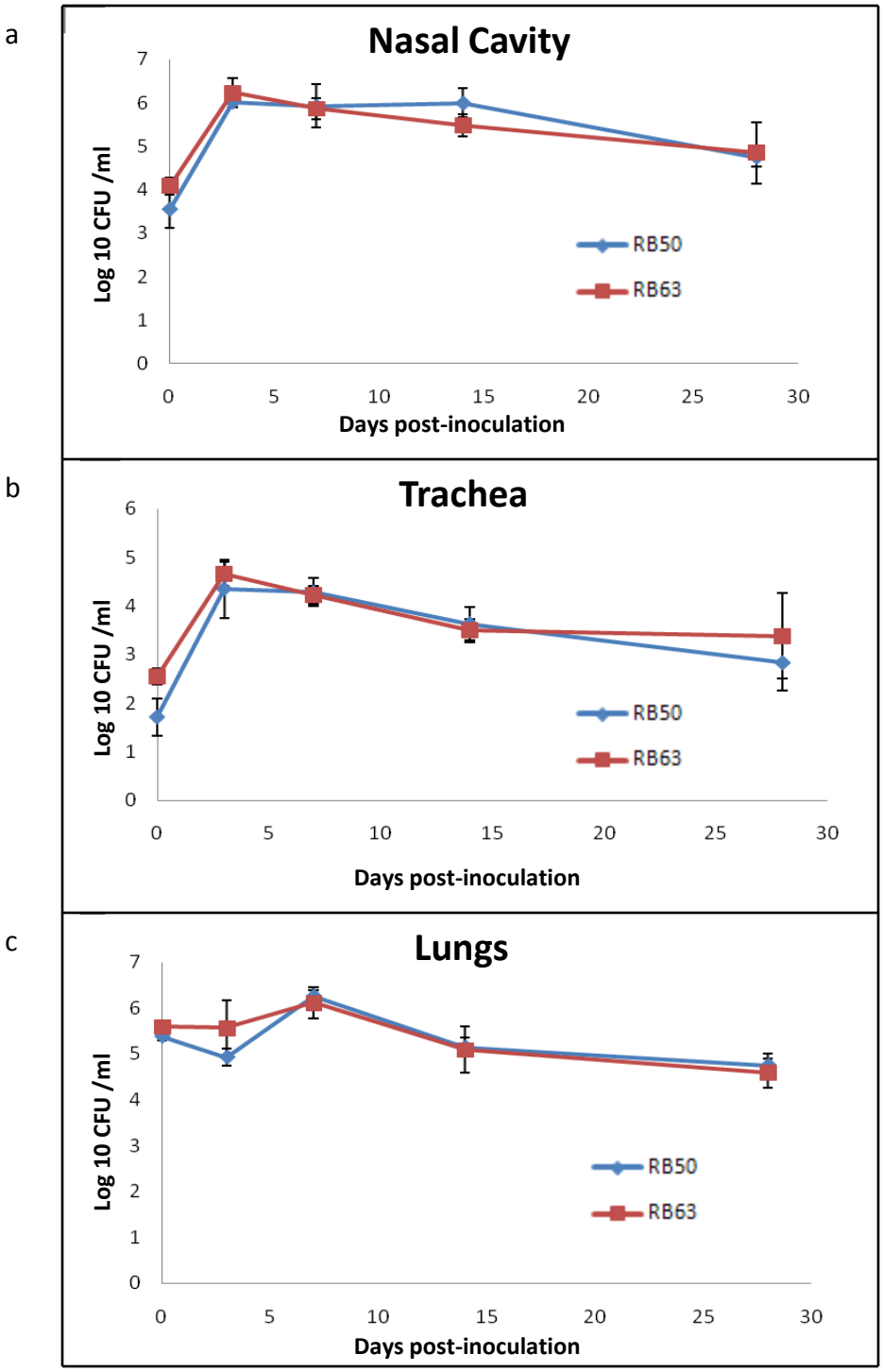
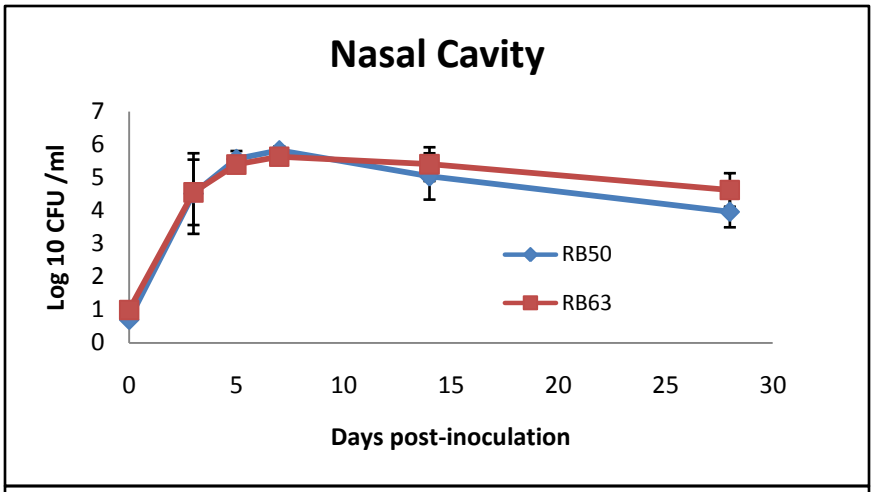


Figure 1: Comparison of colonization of the (a) nasal cavity, (b) trachea, and (c) lungs of groups of four C57BL/6 mice by RB50 ( ) and RB63 ( ) on days 0,3,7,14, and 28 post-inoculation with 5 x 10^5 CFU bacteria in 50 µl PBS.

a



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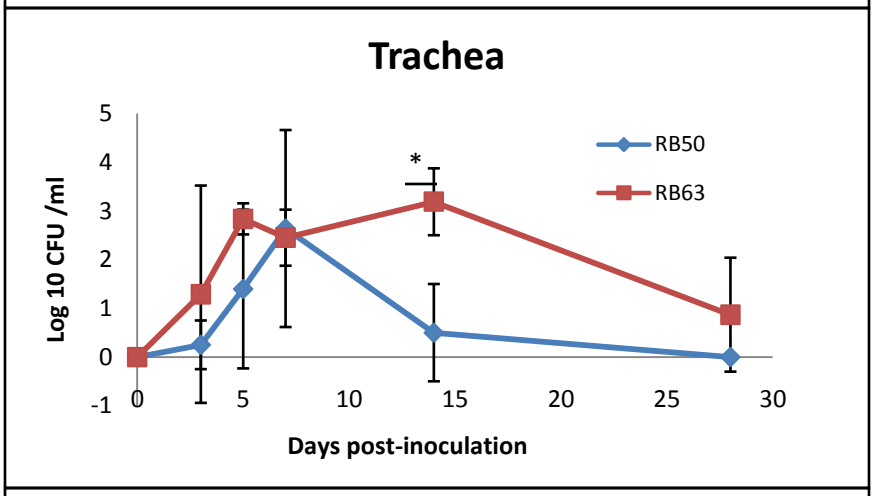

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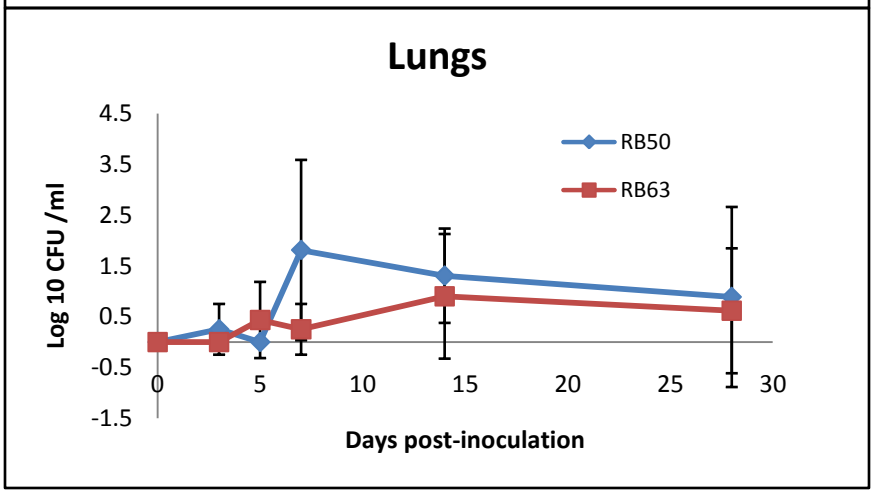


Figure 2: Colonization of the (a) nasal cavity, (b) trachea, and (c) lungs of C57BL/6 mice infected with RB50 ( ) or RB63 ( ) on days 0,3,7,14, and 28 post-inoculation with 50 CFU bacteria in 5 µl PBS. * denotes a statistically significant difference from wild type (P value ≤ 0.05)

Percentage of mice with pulmonary infection

	RB50	RB63
Day 3	25	0
Day 5	0	33.3
Day 7	75	25
Day 14	75	50
Day 28	25	25

Table 2: Chart showing the percentage of mice inoculated with 50 CFU of RB50 or RB63 in 5 µl PBS with detectable levels of *Bordetella* in the lungs on the days indicated.

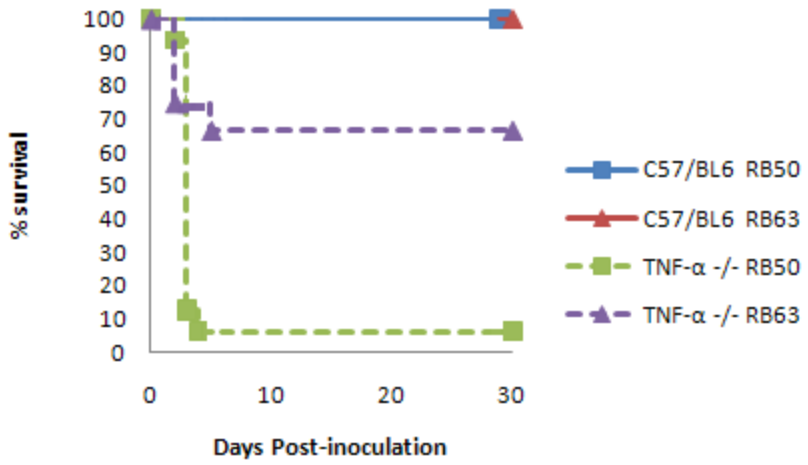
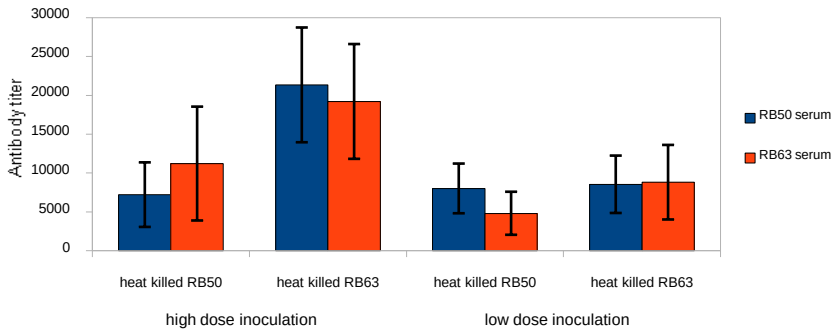


Figure 3: Survival curve of C57/BL6 mice and TNF-$\alpha^{-/-}$ mice inoculated with 5 x 10^5 CFU RB50 or RB63. Survival is represented as the percentage of mice living on the indicated day post-inoculation.

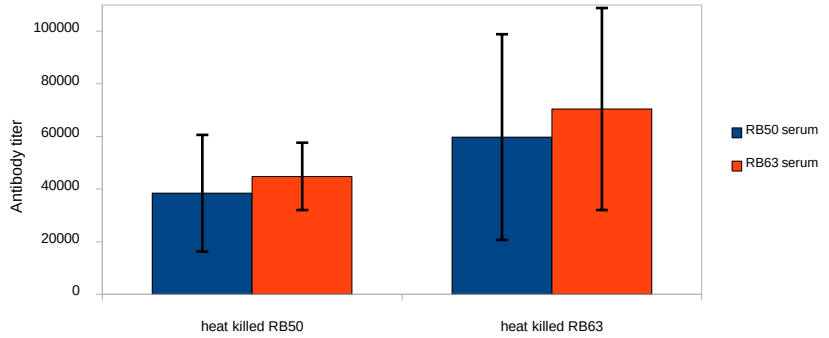
Polyvalent antibody titer ELISA

a



IgG titer ELISA

b



IgG1 titer ELISA

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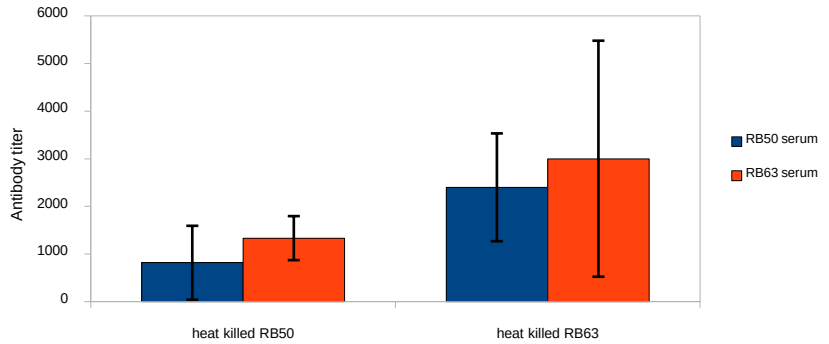

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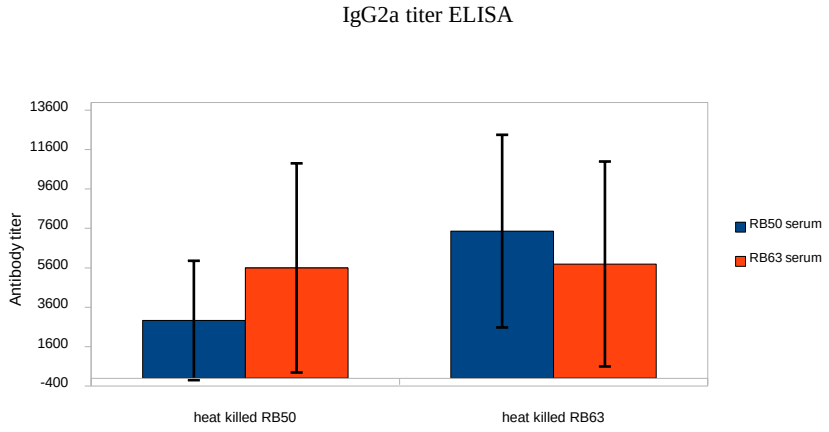

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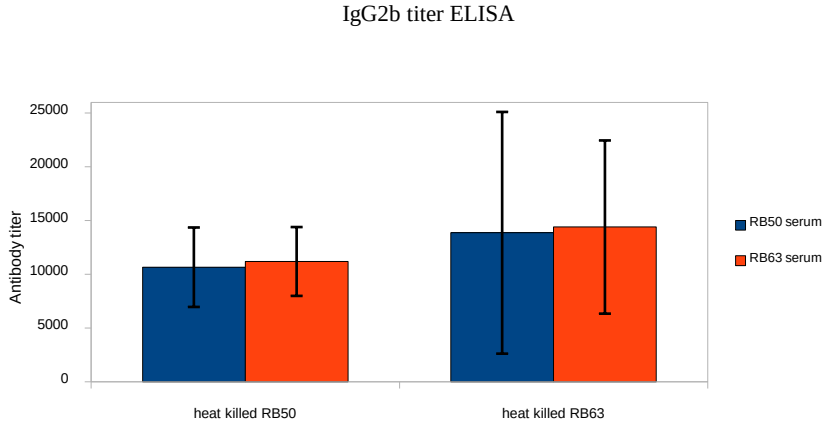

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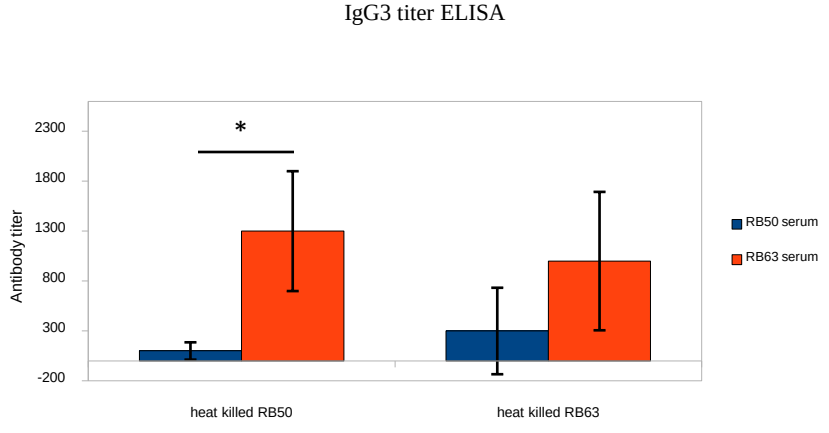

Figure 4: Titer of antibodies in serum recognizing heat killed RB50 and RB63 collected from mice inoculated with 5 x 10^5 CFU (a, b, c, d, e, and f) and 50 CFU (a only) of RB50 and RB63 on day 28 post-infection . Titers were determined for: polyvalent Ig (a), IgG (b), IgG1 (c), IgG2a (d), IgG2b (e), and IgG3 (f). * denotes a statistically significant difference (P value ≤ 0.05)

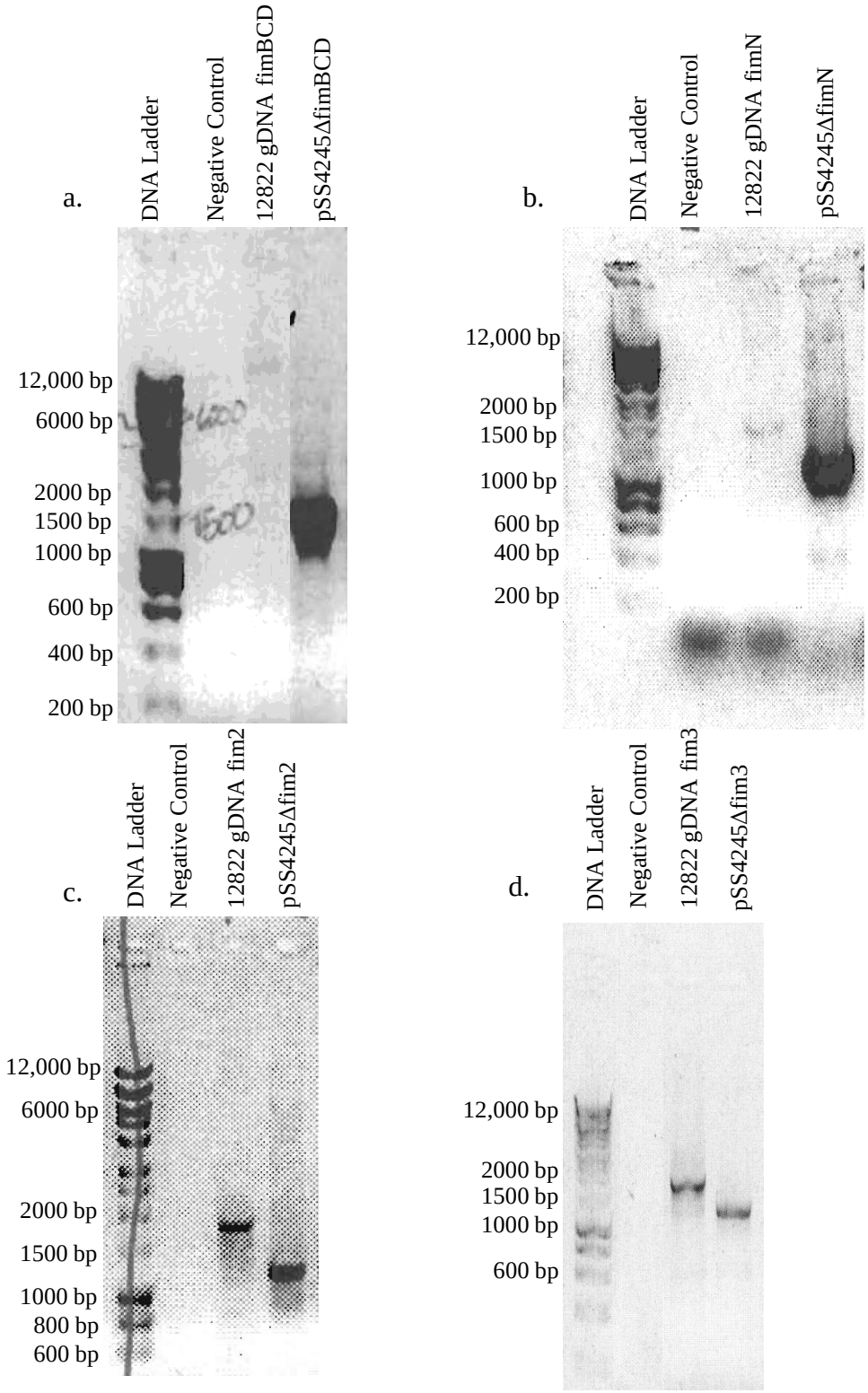

Figure 5. Agarose gels showing PCR amplified knockout constructs of *B. parapertussis fimBCD* (a), *fimN* (b), *fim2* (c), and *fim3* (d).

a.

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ΔFimBCD expected CAATTGCAGATCGAGATAGGAATAGCCGTATTCGTCCGCGGGAACGATGGTGATGTTGCC 60
pSS4545 ΔFimBCD  ----------------------------------------ACGATGGTGATGTTGCC 17
                                                         ****************

ΔFimBCD expected GGTCTTGCCGCCATTGTTGATGTTATAGATCGCCTGGTCGATATCGAATACGTTCAGCAC 120
pSS4545 ΔFimBCD  GGTCTTGCCGCCATTGTTGATGTTATAGATCGCCTGGTCGATATCGAATACGTTCAGCAC 77
                 ************************************************************

ΔFimBCD expected CTTGTCCTGCCAGCCTGGCATGGCCGAGAACACCATCATGCGGTCGCGTGTCCCCTCCAG 180
pSS4545 ΔFimBCD  CTTGTCCTGCCAGCCTGGCATGGCCGAGAACACCATCATGCGGTCGCGTGTCCCCTCCAG 137
                 ************************************************************

ΔFimBCD expected CGGCTTGCCGTCGATCAGCCATCCCTTGATGCGGCCCCATTCCACCTTCAGCTTGAGCAC 240
pSS4545 ΔFimBCD  CGGCTTGCCGTCGATCAGCCATCCCTTGATGCGGCCCCATTCCACCTTCAGCTTGAGCAC 197
                 ************************************************************

ΔFimBCD expected GCCGTCCACCACGCCCGGCGGCACGAAAGTCACGATGCTGGTCGCGTAGCCGCGGTCGTA 300
pSS4545 ΔFimBCD  GCCGTCCACCACGCCCGGCGGCACGAAAGTCACGATGCTGGTCGCGTAGCCGCGGTCGTA 257
                 ************************************************************

ΔFimBCD expected CAGGGCCGCGCTCAATGCCTTGACCAGCAGGAACAGCTGCTCGTTGTCCAGCGGGCGGTT 360
pSS4545 ΔFimBCD  CAGGGCCGCGCTCAATGCCTTGACCAGCAGGAACAGCTGCTCGTTGTCCAGCGGGCGGTT 317
                 ************************************************************

ΔFimBCD expected CAGGTAGTCCTGAACCAACGGCGCGGGGTCGAACAGCCGGCCCTCGACGCCGAAGTCCAG 420
pSS4545 ΔFimBCD  CAGGTAGTCCTGAACCAACGGCGCGGGGTCGAACAGCCGGCCCTCGACGCCGAAGTCCAG 377
                 ************************************************************

ΔFimBCD expected GTCCACGGCCTGTACCGTCACGGTGTGGCCGGACGTGGCGTCCGGCTTGCGCGCCGGCGA 480
pSS4545 ΔFimBCD  GTCCACGGCCTGTACCGTCACGGTGTGGCCGGACGTGGCGTCCGGCTTGCGCGCCGGCGA 437
                 ************************************************************

ΔFimBCD expected AGCGGCCTCGGACTGTGGATTCAATTCCACCGGAGGCCGCGTCAAGGCGCGCTCGATATC 540
pSS4545 ΔFimBCD  AGCGGCCTCGGACTGTGGATTCAATTCCACCGGAGGCCGCGTCAAGGCGCGCTCGATATC 497
                 ************************************************************

ΔFimBCD expected GCGCTGCAGCTGCTCCTTGCGCTGGCGATCGTCGATACGGTTGAGGTCGCGCGCGCCGGG 600
pSS4545 ΔFimBCD  GCGCTGCAGCTGCTCCTTGCGCTGGCGATCGTCGATACGGTTGAGGTCGCGCGCGCCGGG 557
                 ************************************************************

ΔFimBCD expected CAGCAGCTGCGCCTGCGCACAGGCGGCCACGGCGAACAGCAGGCCTGCCCGGACCAGCGC 660
pSS4545 ΔFimBCD  CAGCAGCTGCGCCTGCGCACAGGCGGCCACGGCGAACAGCAGGCCTGCCCGGACCAGCGC 617
                 ************************************************************

ΔFimBCD expected CCGAACAACCAGGCCCGGCCGGTAACGGTTCGTTGCGTCAGTCATA------TGTATTCA 714
pSS4545 ΔFimBCD  CCGAACAACCAGGCCCGGCCGGTAACGGTTCGTTGCGTCAGTCATAAAGCTTTGTATTCA 677
                 ******************************************      ********

ΔFimBCD expected TGATTCAAGAATCGCGGCCGTTCGTAGCTATCGATGCTTTGCATGCATCAAGCTGGCGCT 774
pSS4545 ΔFimBCD  TGATTCAAGAATCGCGGCCGTTCGTAGCTATCGATGCTTTGCATGCATCAAGCTGGCGCT 737
                 ************************************************************

ΔFimBCD expected GCGACCGCGTGAAAAGAAAGAAATGGAAAACAAGAATCTCGTGACAAGCCGACCATCCCG 834
pSS4545 ΔFimBCD  GCGACCGCGTGAAAAGAAAGAAATGGAAAACAAGAATCTCGTGACAAGCCGACCATCCCG 797
                 ************************************************************



ΔFimBCD expected TACCGGGCCCCCCCCGCAATGGGTGGCCCCTCTTTCACGGGCTACGGATACATCACGGAGA 894
pSS4545 ΔFimBCD  TACCGGGCCCCCCCCGCAATGGGTGGCCCCTCTTTCACGGGCTACGGATACATCACGGAGA 857
                 ************************************************************

ΔFimBCD expected ACCCCACCTGACTGCGGAGATTCCCCGCAACGATGGAGCCAGCGCCCGAACGGATGTAGC 954
pSS4545 ΔFimBCD  ACCCCACCTGACTGCGGAGATTCCCCGCAACGATGGAGCCAGCGCCCGAACGGATGTAGC 917
                 ************************************************************
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ΔFimBCD expected GTGCCAGATAGCGCAGGGTCGTCCCGTACGTCGACGACCCTGCCGTCTGCGATACCAGAA 1014
pSS4545 ΔFimBCD  GTGCCAGATAGCGCAGGGTCGTCCCGTACGTCGACGACCCTGCCGTCTGCGATACCAGAA 977
                 ************************************************************

ΔFimBCD expected ACGTCTGAGCAGCCTGCGCACCCTCGTTGCGACCCAAATGAATGGCGGAACCATCCAGAT 1074
pSS4545 ΔFimBCD  ACGTCTGAGCAGCCTGCGCACCCTCGTTGCGACCCAAATGAATGGCGGAACCATCCAGAT 1037
                 ************************************************************

ΔFimBCD expected TGGCAAGCTGGAATTGCACGTTGTCGGCTTTCGTCTGCGGCACCTGTTCTACGCCCTGAC 1134
pSS4545 ΔFimBCD  TGGCAAGCTGGAATTGCACGTTGTCGGCTTTCGTCTGCGGCACCTGTTCTACGCCCTGAC 1097
                 ************************************************************

ΔFimBCD expected TATCGGCCCGGTACGCAAACAGATTTCCCGACGTATAGTCAACCGTGCCGCCAGGTTCGA 1194
pSS4545 ΔFimBCD  TATCGGCCCGGTACGCAAACAGATTTCCCGACGTATAGTCAACCGTGCCGCCAGGTTCGA 1157
                 ************************************************************

ΔFimBCD expected AGAACAATCCCACTTGCTGACCATTCAATGCCTGCGGACACTCGGTCAGTTTCACGTCAA 1254
pSS4545 ΔFimBCD  AGAACAATCCCACTTGCTGACCATTCAATGCCTGCGGACACTCGGTCAGTTTCACGTCAA 1217
                 ************************************************************

ΔFimBCD expected AAACTGTCCCGCCTGCCGTCGACCCTACGTCCTTGAGCGCGGTGCGCGAAATGGTCGGCA 1314
pSS4545 ΔFimBCD  AAACTGTCCCGCCTGCCGTCGACCCTACGTCCTTGAGCGCGGTGCGCGAAATGGTCGGCA 1277
                 ************************************************************

ΔFimBCD expected ATTGCACCGCTATGTTGGTGGGAGGCTCCGCGCCATTGATCTTGCACGTCGTATCGGTGA 1374
pSS4545 ΔFimBCD  ATTGCACCGCTATGTTGGTGGGAGGCTCCGCGCCATTGATCTTGCACGTCGTATCGGTGA 1337
                 ************************************************************

ΔFimBCD expected TGGCGCCGGTAATCACGAGCGTGCCGTCCGCCGCGAAAACCTGATGCTGGTATGTCAATG 1434
pSS4545 ΔFimBCD  TGGCGCCGGTAATCACGAGCGTGCCGTCCGCCGCGAAAACCTGATGCTGGTATGTCAATG 1397
                 ************************************************************

ΔFimBCD expected CACAGGCGGTCAGGCCCCAGTGCGATTCCTGCAAATTTGAGATTCATGATCGACCCCTGT 1494
pSS4545 ΔFimBCD  CACAGGCGGTCAGGCCCCAGTGTGATTCCTGCAAATTTGAGATTCATGATCGACCCCTGT 1457
                 ********************* ***********************************

ΔFimBCD expected TAATCCGAAAACCAGGCGATCGGCGCTCCGTGAAAAACGAATCCAGGAATGGAGCCCGAT 1554
pSS4545 ΔFimBCD  TAATCCGAAAACCAGGCGATCGGCGCTCCGTGAAAAACGA---------------------- 1497
                 ************************************

ΔFimBCD expected GCCTAAGAATATTCA 1569
pSS4545 ΔFimBCD  ---------------
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b.

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ΔFimN expected  TCTCCACTGGCCTTCTTCACGTAGCCGGCCAGGTATTGCAAGGTGAACGATTTCTTCGCC 60
pSS4245 ΔFimN  TCTCCACTGGCCTTCTTCACGTAGCCGGCCAGGTATTGCAAGGTGAACGATTTCTTCGCC 60
               ************************************************************

ΔFimN expected  TGGCCGGCTTGCATGGTGCCGGAAAATGCCGCGGCATGCTGGTTGGGCTCGGTCTCGCCC 120
pSS4245 ΔFimN  TGGCCGGCTTGCATGGTGCCGGAAAATGCCGCGGCATGCTGGTTGGGCTCGGTCTCGCCC 120
               ************************************************************

ΔFimN expected  ATGGGAATCTGCTTGCCGTTCAGGTTCATCAGCCGGATCTGCACCCCCTTGGCTTCGGTC 180
pSS4245 ΔFimN  ATGGGAATCTGCTTGCCGTTCAGGTTCATCAGCCGGATCTGCACCCCCTTGGCTTCGGTC 180
               ************************************************************

ΔFimN expected  ACACCACTGGGCGGCGATTTGAGCAGCGTTGCGGCGTCGACGTACCAAGCCTGCTTATAG 240
pSS4245 ΔFimN  ACACCACTGGGCGGCGATTTGAGCAGCGTTGCGGCGTCGACGTACCAAGCCTGCTTATAG 240
               ************************************************************

ΔFimN expected  GCTTTCAGATCCTTGGTGCCGTAATCCGGTGGTGGGGCCGGGCTCGAAGTACAGCTTGAG 300
pSS4245 ΔFimN  GCTTTCAGATCCTTGGTGCCGTAATCCGGTGGTGGGGCCGGGCTCGAAGTACAGCTTGAG 300
               ************************************************************

ΔFimN expected  AGTGTTGACGGTGGTCGGGCAGTCCTTCAGCTTGATATCGAAGCGAGTGCGCCCCGCCAC 360
pSS4245 ΔFimN  AGTGTTGACGGTGGTCGGGCAGTCCTTCAGCTTGATATCGAAGCGAGTGCGCCCCGCCAC 360
               ************************************************************

ΔFimN expected  GTCGCCGGCGTTCTTCAGCGCGCTCTTGGAGATCGTGGGCAGGTGCACGACCTTGATGTA 420
pSS4245 ΔFimN  GTCGCCGGCGTTCTTCAGCGCGCTCTTGGAGATCGTGGGCAGGTGCACGACCTTGATGTA 420
               ************************************************************

ΔFimN expected  ACCGGGGCTCGGGTCCTCGATCGTGCAGGTCTGGTCGGTGATCGTGCCGGTAATGACAAT 480
pSS4245 ΔFimN  ACCGGGGCTCGGGTCCTCGATCGTGCAGGTCTGGTCGGTGATCGTGCCGGTAATGACAAT 480
               ************************************************************

ΔFimN expected  GGTGCCATCGTCGGCATGCGCCGCGAAGGCAAGGGCGAGGGCACCAGCGCAGACCGAGGC 540
pSS4245 ΔFimN  GGTGCCATCGTCGGCATGCGCCGCGAAGGCAAGGGCGAGGGCACCAGCGCAGACCGAGGC 540
               ************************************************************

ΔFimN expected  GCGAAGAACGGAGGATTTTGCTTGCATACGGCCAATAGGCGGCGCAAAGCGCCGTACATG 600
pSS4245 ΔFimN  GCGAAGAACGGAGGATTTTGCTTGCATACGGCCAATAGGCGGCGCAAAGCGCCGTACATG 600
               ************************************************************

ΔFimN expected  AATCGAAGTGCAAGCATTTTAGAAAGTGCGGGGGGGGGCTTACTTTTGTATAGGACTTAT 660
pSS4245 ΔFimN  AATCGAAGTGCAAGCATTTTAGAAAGTGCGGGGGGGGGCTTACTTTTGTATAGGACTTAT 660
               ************************************************************

ΔFimN expected  CCTTATCAAGCCCCGCGCCTCATATCTAAGGATAGATGACCGAAAAGCCTACCTAAGTGG 720
pSS4245 ΔFimN  CCTTATCAAGCCCCGCGCCTCATATCTAAGGATAGATGACCGAAAAGCCTACCTAAGTGG 720
               ************************************************************

ΔFimN expected  TTATCGCGCT------CTTCATGCTGGTATTGGCGCAAGCGCGCCGGAATTACGGAGGAA 774
pSS4245 ΔFimN  TTATCGCGCTAAGCTTCTTCATGCTGGTATTGGCGCAAGCGCGCCGGAATTACGGAGGAA 780
               **********      ******************************************

ΔFimN expected  GCAAGAATATTAGAGGTGGTCGGGGGGGGGGGCTGATCGGTGTGTAGGAACCCACCTCATT 834
pSS4245 ΔFimN  GCAAGAATATTAGAGGTGGTCGGGGGGGGGGGCTGATCGGTGTGTAGGAACCCACCTCATT 840
               ************************************************************



ΔFimN expected  TTCGGGCGGCCTGCTTAAGGATAGACCACGGAAAAACCCACATAAGTGGTGATGGCGCTG 894
pSS4245 ΔFimN  TTCGGGCGGCCTGCTTAAGGATAGACCACGGAAAAACCCACATAAGTGGTGATGGCGCTG 900
               ************************************************************

ΔFimN expected  GCCTGGATGTCGCCATCCGCTTTTACGTACGAGGCGAGGTAGCGCATGGTCACGGTGCGT 954
pSS4245 ΔFimN  GCCTGGATGTCGCCATCCGCTTTTACGTACGAGGCGAGGTAGCGCATGGTCACGGTGCGT 960
               ************************************************************
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ΔFimN expected  TTGTTGGTGTCGCCGGTTTGTTCCACGGGATTGAACCCCTGTGCGCGCTGGTTGGTGACG 1014
pSS4245 ΔFimN  TTGTTGGTGTCGCCGGTTTGTTCCACGGGATTGAACCCCTGTGCGCGCTGGTTGGTGACG 1020
               ************************************************************

ΔFimN expected  TCGACGCCCATCGGGATTTTGCTGTCGTCTAGGTTGGAGATCCGGATTTCCACGCCGGTG 1074
pSS4245 ΔFimN  TCGACGCCCATCGGGATTTTGCTGTCGTCTAGGTTGGAGATCCGGATTTCCACGCCGGTG 1080
               ************************************************************

ΔFimN expected  GCGGGAGTTGCCGCAACGATGCTGTTGAGCGGGGTGGCCTTGGGGTTGGTGGCGTAGGCC 1134
pSS4245 ΔFimN  GCGGGAGTTGCCGCAACGATGCTGTTGAGCGGGGTGGCCTTGGGGTTGGTGGCGTAGGCC 1140
               ************************************************************

ΔFimN expected  AGCTTGTACGCCTTGAGATCGCCGGTGGCGTAGTTGGTGGTGGGACCGGGCTCGAAATAG 1194
pSS4245 ΔFimN  AGCTTGTACGCCTTGAGATCGCCGGTGGCGTAGTTGGTGGTGGGACCGGGCTCGAAATAG 1200
               ************************************************************

ΔFimN expected  ACCTTGACGCCGTTGCCGAGATCGGCCGGGCAGTCTCTCAGGCTGATCGTGAATGGCGTG 1254
pSS4245 ΔFimN  ACCTTGACGCCGTTGCCGAGATCGGCCGGGCAGTCTCTCAGGCTGATCGTGAATGGCGTG 1260
               ************************************************************

ΔFimN expected  CGGCCGGCCTGGTCGCCTTTGACCTTCAATGCGCTCTTGGAGA 1297
pSS4245 ΔFimN  CGGCCGGCCTGGTCGCCTTTGACCTTCAATGCGCTCTTGGAGA 1303
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C.

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ΔFim2 expected  CTGCTGTCGCATTCCAACTTCGGCAGCCGGCCCACCGATTCGTCGCGCAAGATGGCCCAT 60
pSS4245 ΔFim2  CTGCTGTCGCATTCCAACTTCGGCAGCCGGCCCACCGATTCGTCGCGCAAGATGGCCCAT 60
               ************************************************************

ΔFim2 expected  GCCCGCAAGCTGGTGGCCGAGCGCGCGCCGCATCTGGAGGTCGATGGCGAGATGCACGCC 120
pSS4245 ΔFim2  GCCCGCAAGCTGGTGGCCGAGCGCGCGCCGCATCTGGAGGTCGATGGCGAGATGCACGCC 120
               ************************************************************

ΔFim2 expected  GACGCCGCGCTGTCGGAGTCGATCCGCCTGCAGGCCTACCCGGACAGCACGCTCAAGGGT 180
pSS4245 ΔFim2  GACGCCGCGCTGTCGGAGTCGATCCGCCTGCAGGCCTACCCGGACAGCACGCTCAAGGGT 180
               ************************************************************

ΔFim2 expected  CGCGCCAACCTGCTGGTCATGCCCAACCTCGATACCGGCAACATCACCTACAACATGCTG 240
pSS4245 ΔFim2  CGCGCCAACCTGCTGGTCATGCCCAACCTCGATACCGGCAACATCACCTACAACATGCTG 240
               ************************************************************

ΔFim2 expected  AAGATGACCGGCAGCAACGGGATTGCGATGGGCCCGATCCTGCTGGGTTCGGCCCGCCCG 300
pSS4245 ΔFim2  AAGATGACCGGCAGCAACGGGATTGCGATGGGCCCGATCCTGCTGGGTTCGGCCCGCCCG 300
               ************************************************************

ΔFim2 expected  GTGCACATCCTGACCACCAGCGCCACCGTGCGCCGCATCGTCAACATGACGGCATTGGCA 360
pSS4245 ΔFim2  GTGCACATCCTGACCACCAGCGCCACCGTGCGCCGCATCGTCAACATGACGGCATTGGCA 360
               ************************************************************

ΔFim2 expected  GTGGTGGACGCGCAGCAGGAAGCCGCCGAAGGCTGACGCTGATGCGCCGGCCCGGCGCCG 420
pSS4245 ΔFim2  GTGGTGGACGCGCAGCAGGAAGCCGCCGAAGGCTGACGCTGATGCGCCGGCCCGGCGCCG 420
               ************************************************************

ΔFim2 expected  CCATGGCGCCGGGCCCTGCATGCACGGGTCCAGTCCCGATAAAAGCCGCATGCGACAGGG 480
pSS4245 ΔFim2  CCATGGCGCCGGGCCCTGCATGCACGGGTCCAGTCCCGATAAAAGCCGCATGCGACAGGG 480
               ************************************************************

ΔFim2 expected  CTGTTTCCCACATCGGAATCAGCCCCCCCCCTCCCCCCCCTAAGACCTAAGATCGTGGCT 540
pSS4245 ΔFim2  CTGTTTCCCACATCGGAATCAGCCCCCCCCCTCCCCCCCCTAAGACCTAAGATCGTGGCT 540
               ************************************************************

ΔFim2 expected  CCATAACTCTTCTGGCGCCAAGACGCCCGTGTTACCCATGCAA------ATCTATCCGTA 594
pSS4245 ΔFim2  CCATAACTCTTCTGGCGCCAAGACGCCCGTGTTACCCATGCAAGGATCCATCTATCCGTA 600
               ****************************************      ************

ΔFim2 expected  ACCCCGACTCCTGCCCTCCAGACAGGCCAAGGGGGCTCTCGCGATCCTCCCGTCGTACTA 654
pSS4245 ΔFim2  ACCCCGACTCCTGCCCTCCAGACAGGCCAAGGGGGCTCTCGCGATCCTCCCGTCGTACTA 660
               ************************************************************

ΔFim2 expected  TCGAGACTGAGCGGCGGCTGTCACGGCCGCCGCCCCCATCCGGGAGACCCCATGCTGCAA 714
pSS4245 ΔFim2  TCGAGACTGAGCGGCGGCTGTCACGGCCGCCGCCCCCATCCGGGAGACCCCATGCTGCAA 720
               ************************************************************

ΔFim2 expected  GACGCCCTACTCGCCTGGCTGCACTATCTGGCCATCTTCGTCCTGATCGTCCTCATGACC 774
pSS4245 ΔFim2  GACGCCCTACTCGCCTGGCTGCACTATCTGGCCATCTTCGTCCTGATCGTCCTCATGACC 780
               ************************************************************

ΔFim2 expected  GCCGAAGCCGTGCTGCTGCGGCCCGGCATGTCCGCCCGGTCCGTGGGCCGGCTGGCGCTC 834
pSS4245 ΔFim2  GCCGAAGCCGTGCTGCTGCGGCCCGGCATGTCCGCCCGGTCCGTGGGCCGGCTGGCGCTC 840
               ************************************************************



ΔFim2 expected  TACGACCGCCTGTACCTGGCAAGCGCGCTCGCCGTGCTGGCCACCGGCGTCCTGCGCCTG 894
pSS4245 ΔFim2  TACGACCGCCTGTACCTGGCAAGCGCGCTCGCCGTGCTGGCCACCGGCGTCCTGCGCCTG 900
               ************************************************************

ΔFim2 expected  ACGCTGGGCGCCAAGGGCGCGGCCTTCTACATGGCCAACCCCTGGTTCCACGCCAAGATC 954
pSS4245 ΔFim2  ACGCTGGGCGCCAAGGGCGCGGCCTTCTACATGGCCAACCCCTGGTTCCACGCCAAGATC 960
               ************************************************************
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ΔFim2 expected  GGGCTGTTCGTGCTGATCGCGCTCTGTTCCATCCCGCCCACCCTGGCCTTCCTGAGCTGG 1014
pSS4245 ΔFim2  GGGCTGTTCGTGCTGATCGCGCTCTGTTCCATCCCGCCCACCCTGGCCTTCCTGAGCTGG 1020
               ************************************************************

ΔFim2 expected  AAGAAGCAGTCGCTGTCGCAGCCGGGCTTCACCCCCGCCGATGCCGATATCCGGCGCGCG 1074
pSS4245 ΔFim2  AAGAAGCAGTCGCTGTCGCAGCCGGGCTTCACCCCCGCCGATGCCGATATCCGGCGCGCG 1080
               ************************************************************

ΔFim2 expected  CGCCGCTGGGTGATGATCGAATCGCACCTGTTCATCTTCCTGCCGCTGTTCGCCGTGCTG 1134
pSS4245 ΔFim2  CGCCGCTGGGTGATGATCGAATCGCACCTGTTCATCTTCCTGCCGCTGTTCGCCGTGCTG 1140
               ************************************************************

ΔFim2 expected  ATGGCGCGCGGCATCGGCATCTAGCGCA 1162
pSS4245 ΔFim2  ATGGCGCGCGGCATCGGCATCTAGCGCA 1168
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d.

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ΔFim3 expected  CCGAGATCTACATCACGCACAACCAGATGCTGGAAAAGCGTGTCGGCTCGGATGGCGGCC 60
pSS4245 ΔFim3  -----------------------------------CGTGTCGGCTCGGATGGCGGCC 22
                                                   **********************

ΔFim3 expected  AGGTGCAATGGACCCACGGCAAGGAAGACCCGGGCCTGAACGCGGCCATGCTGGCGCGCC 120
pSS4245 ΔFim3  AGGTGCAATGGACCCACGGCAAGGAAGACCCGGGCCTGAACGCGGCCATGCTGGCGCGCC 82
                ************************************************************

ΔFim3 expected  TGATGGTGTACCTGGGCACCGACGTGGATGCGGCGCGCAAGCTGGTGGCGCAGGCCGAAG 180
pSS4245 ΔFim3  TGATGGTGTACCTGGGCACCGACGTGGATGCGGCGCGCAAGCTGGTGGCGCAGGCCGAAG 142
                ************************************************************

ΔFim3 expected  CGGCCCCGCAGGCGCCCAAGGTGCAGAGCGTGCGCGCCGAAGGCGCGATGCTGGTAGTCG 240
pSS4245 ΔFim3  CGGCCCCGCAGGCGCCCAAGGTGCAGAGCGTGCGCGCCGAAGGCGCGATGCTGGTAGTCG 202
                ************************************************************

ΔFim3 expected  ACGAGTCGTTCGATCGCGCCTGGCGGCGCGTGGGCGTGGCCCTGGATTCGGGCGGCTTCG 300
pSS4245 ΔFim3  ACGAGTCGTTCGATCGCGCCTGGCGGCGCGTGGGCGTGGCCCTGGATTCGGGCGGCTTCG 262
                ************************************************************

ΔFim3 expected  CGGTCGACGATCGCGACCGCAGCGCCGGCGAGTACTTCGTGCGCTACGTGGATACCGACA 360
pSS4245 ΔFim3  CGGTCGACGATCGCGACCGCAGCGCCGGCGAGTACTTCGTGCGCTACGTGGATACCGACA 322
                ************************************************************

ΔFim3 expected  CGGGCGCGCAGAACGAGCAGCCGGGCTTCTTCAGCCGCCTGTTCTCCAGCGACAAGAAGG 420
pSS4245 ΔFim3  CGGGCGCGCAGAACGAGCAGCCGGGCTTCTTCAGCCGCCTGTTCTCCAGCGACAAGAAGG 382
                ************************************************************

ΔFim3 expected  CCCAGGCGCCGCAGTACCGCATCCGCCTGACGGGCTCCGGCACGCAGACGCAGGTCACGG 480
pSS4245 ΔFim3  CCCAGGCGCCGCAGTACCGCATCCGCCTGACGGGCTCCGGCACGCAGACGCAGGTCACGG 442
                ************************************************************

ΔFim3 expected  TGCTCGACGCCAACGGGCAGCGCGACAGCAGCGCAACCGCCCAGCGCATGCTGAGCGTGC 540
pSS4245 ΔFim3  TGCTCGACGCCAACGGGCAGCGCGACAGCAGCGCAACCGCCCAGCGCATGCTGAGCGTGC 502
                ************************************************************

ΔFim3 expected  TGAAGGACAAGATGGTCTGAGTCCCTTCGTCTCCTGACGCCGCGTAGCGGCACAAAAAAA 600
pSS4245 ΔFim3  TGAAGGACAAGATGGTCTGAGTCCCTTCGTCTCCTGACGCCGCGTAGCGGCACAAAAAAA 562
                ************************************************************

ΔFim3 expected  CCGGCAAGCCGCAAGGCTTGCCGGTTTTTTTTGGCTGGCGCGAGAACTCAGGGATAGAC- 659
pSS4245 ΔFim3  CCGGCAAGCCGCAAGGCTTGCCGGTTTTTTTTGGCTGGCGCGAGAACTCAGGGATAGACA 622
                ************************************************************

ΔFim3 expected  -----GGACATGGTGATTCTGAGACTGAGGGGTGCCGTCATGTGCTTGGCGTCGGCATCA 714
pSS4245 ΔFim3  AGCTTGGACATGGTGATTCTGAGACTGAGGGGTGCCGTCATGTGCTTGGCGTCGGCATCA 682
                     ****************************************************

ΔFim3 expected  GAATATTAGGTCCGGGAGGGGGGCTGATGGTTGTGTGGGAATTTGCCGGTAATCGCGCCA 774
pSS4245 ΔFim3  GAATATTAGGTCCGGGAGGGGGGCTGATGGTTGTGTGGGAATTTGCCGGTAATCGCGCCA 742
                ************************************************************

ΔFim3 expected  GATCGGCGGGCGGCTTCGCGCAATGAAAATGGCCTCCGTTACCGGAGGCCATGAGCATGC 834
pSS4245 ΔFim3  GATCGGCGGGCGGCTCCGCGCAATGAAAATGGCCTCCGTTACCGGAGGCCATGAGCATGC 802
                *************** ********************************************



ΔFim3 expected  CTGCCCGCGGGCGAGCATCCCGACGGCGTGCCGGCCGGCACGGGGGCTGGCCGGCGGGGC 894
pSS4245 ΔFim3  CTGCCCGCGGGCGAGCATCCCGACGGCGTGCCGGCCGGCACGGGGGCTGGCCGGCGGGGC 862
                ************************************************************

ΔFim3 expected  CGCGAATCAGGACGACGGCGTCGTGGCGCCGCCGGAGCTGCCCGGCGCCGGTTCGGCGGG 954
pSS4245 ΔFim3  CGCGAATCAGGACGACGGCGTCGTGGCGCCGCCGGAGCTGCCCGGCGCCGGTTCGGCGGG 922
                ************************************************************
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ΔFim3 expected  CGAGGGAGCCGGCGCAGGCGCGGGCGCGGGGGCCGAAGGAGCCGGGGCCGTCGTGGCCGG 1014
pSS4245 ΔFim3  CGAGGGGGCCGGCGCAGGCGCGGGCGCGGGGGCCGAAGGAGCCGGGGCCGTCGTGGCCGG 982
                ****** ************************************************

ΔFim3 expected  GGCGCCGTCGCCAGCGGGGGCGGCCGCGTCTTCGTTCTTGTTGCAGGCCGTGAGGGCGAC 1074
pSS4245 ΔFim3  GGCGCCGTCGCCAGCGGGGGCGGCCGCGTCTTCGTTCTTGTTGCAGGCCGTGAGGGCGAC 1042
                ************************************************************

ΔFim3 expected  TGCCATCACCGAAGCCAGAATCAAGGTTTTGCTCATCATGGTCGACACTCCTGTTGATGA 1134
pSS4245 ΔFim3  TGCCATCACCGAAGCCAGAATCAAGGTTTTGCTCATCATGGTCGACACTCCTGTTGATGA 1102
                ************************************************************

ΔFim3 expected  CGATTTCGTGGCCCCATTAGGGGAAACCAGTAGTCAGGCTACAGGAATCATTCAACGGCT 1194
pSS4245 ΔFim3  CGATTTCGTGGCCCCATTAGGGGAAACCAGTAGTCAGGCTACAGGAATCATTCAACGGC- 1161
                ***********************************************************

ΔFim3 expected  GGCGTTTGGAAATTAAAGCGGTCGCATCCCAAATGAAACAACCCTT 1240
pSS4245 ΔFim3  -------------------------------------------
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Figure 6: DNA sequences of *B. parapertussis fimBCD* (a), *fimN* (b), *fim2* (c), and *fim3* (d) knockout constructs in allelic exchange vector pSS4245 compared to expected knockout construct sequences.

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Bachelor of Science Degree in Microbiology, Penn State University, Spring 2010

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Thesis Title: The Role of Fimbriae in *Bordetella* Colonization

Thesis Supervisor: Eric Harvill

Related Experience:

Laboratory assistant and undergraduate research at Penn State University

Supervisor: Eric Harvill

Summer 2007 to Spring 2010

Awards:

Phi Beta Kappa Honor Society

Dean's List

Hutchings Science Scholarship

Lewman Endowed Scholarship

Academic Excellence Scholarship

National Honor Society

Presentations/Activities

Vice Convention Chair of Setsucon, organized a two day convention with over 1000 attendees and raised $2,500 for charity

Treasurer for the Penn State Chapter American Society for Microbiology

Volunteer for the Conversation Partners Program to help international students improve their English skills.