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DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

THE EFFECTS OF VACCINATION ON TRANSMISSION DYNAMICS OF *BORDETELLA BRONCHISEPTICA*

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ABSTRACT

The communicable nature of infectious diseases requires that public health interventions address them on a population level as well as an individual one. Vaccination has proven effective at preventing disease in vaccinated individuals, but vaccines that prevent transmission between hosts can further reduce the prevalence of disease within a population. *B. bronchiseptica*, an animal respiratory pathogen that is closely related to *B. pertussis*, the etiologic agent of whooping cough, colonizes the respiratory tract of mice and persists in the nasal cavities for the life of the animal. Vaccination with heat-killed bacteria does not prevent colonization of the nasal cavities, but does reduce disease pathology and shedding of bacteria from the nares. Studies of bacterial shedding in several immunodeficient strains of mice determined that both antibodies and a cell-mediated T_H1 response is required to control shedding of *B. bronchiseptica* during post-vaccination challenge. Whole-cell *B. bronchiseptica* vaccination prevented transmission between co-housed mice, but acellular *B. pertussis* vaccination was only partially effective. These results have implications for vaccine development, as the ability of vaccination to reduce transmission and therefore the population-wide burden of disease depends on the immune response generated by the vaccine. Given the widespread use of acellular *B. pertussis* vaccine in humans, its protective ability with regard to transmission also warrants further study.

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Chapter 1
Introduction

The threat of infectious diseases, while somewhat lessened in recent decades by the use vaccines and antibiotics, is still a major concern in public health. While autologous ailments such as cancer and heart diseases are still significant problems in medicine, infectious diseases are unique in their ability to transmit between hosts. This makes the battle against infectious diseases a problem of treating not only the afflicted individual, but the population as a whole.

Whooping cough, a severe, vaccine-preventable disease caused by *Bordetella pertussis,* has recently reemerged as serious epidemic threat.[1] Vaccination can still be relatively protective, preventing severe whooping cough for most individuals, however mild disease and asymptomatic carriage of *B. pertussis* remains prevalent, and widespread surreptitious transmission increases the likelihood of severe or fatal disease spreading to susceptible persons such as infants and a growing population of immunocompromised adults.[2] Estimates based on WHO data conclude that up to 80% of children will become infected by 5 years of age, depending on vaccine coverage, and that pertussis accounted for 12.7 million disability-adjusted life years in 2000, more than double the 5.8 million of meningitis.[3] Continued circulation of *B. pertussis* is therefore a significant threat to public health, especially with waning immunity and vaccine failure.[2,4] Because *B. pertussis* does not colonize the murine respiratory tract efficiently,

it is difficult to study *in vivo*. Fortunately, a closely related animal pathogen allows for some conclusions to drawn about *B. pertussis* from studies in mice.

Bordetella bronchiseptica is a member of the bacterial genus *Bordetella*, a taxon of Gram-negative coccobacilli that cause lower respiratory tract infections in a number of mammals. *B. bronchiseptica* infects several non-human mammals, causing kennel cough in dogs, snuffles in rabbits, and atrophic rhinitis in swine, making it a relevant pathogen in veterinary medicine.[5] This broad host range, combined with its natural capacity to infect mice and its close relation to the human pathogen *B. pertussis* as a purported ancestor, make it an ideal candidate to model respiratory infection in the mouse model.[6]

Vaccines have proven effective in lessening the burden of infectious diseases since their creation, but they are not infallible. Pathogens evolve rapidly, which requires constant monitoring to keep vaccine efficacy high and ensure that the immune responses they generate are protective against strains currently circulating throughout a population. The influenza virus, for example, requires a new vaccine every year to sufficiently prevent widespread epidemics.[7] Other pathogens have acquired certain genes that allow them to escape vaccination-induced immunity and persist even within vaccinated populations.[8,9]

The goal of vaccination is to generate a protective immune response that prevents symptoms of disease when a host encounters the pathogen in the future. The ideal vaccine, then, is one that generates long-lasting protective immunity which prevents pathology in the host, leads to rapid clearance, and reduces the spread of the pathogen to other, possibly susceptible individuals. Since different vaccines, which often exist for a given pathogen, can induce different adaptive responses through differential generation

of memory T cells, understanding the immune signals involved in generating these protective responses is crucial to vaccine development.[10] And in order to elicit a protective response that reduces transmission, knowledge of the role of immunity in the transmission and population dynamics of disease is required.[11]

The goal of this investigation is to elucidate the roles of vaccination and the murine immune system in the transmission dynamics of *B. bronchiseptica*. By monitoring shedding of bacteria from the nasal cavities during a post-vaccination challenge, the ability of different types of vaccination to control bacterial shedding, an indicator of the likelihood of transmission, was determined. To determine which components of the innate and adaptive immune system were required for this effect, shedding from several types of immunodeficient mice was observed. Finally, a vaccinated and challenged initial case, or index, mouse was co-housed with naive mice to observe actual transmission events and determine the ability of different vaccines to prevent transmission. Acellular *B. pertussis* vaccine Adacel was also tested for its ability to prevent transmission, since previous research has demonstrated that *B. pertussis* infection or vaccination is protective against *B. bronchiseptica* infection.[12,13]

A novel method used in these experiments was a low dose, low volume inoculation of mice. Previous immunological studies in mice have used inocula containing high bacterial numbers ($5*10^5$ CFU compared to 150 CFU) in much larger droplets (50 μL compared to 5 μL). While the high dose, high volume inoculation method produces robust results with respect to the immune responses it elicits, it is likely a source of experimental artifacts, as more bacteria are introduced to the respiratory tract than are contracted in a natural infection, and the large droplet ensures bacteria reach the lungs

soon after inoculation. In a low dose, low volume inoculation, RB50 still establishes a persistent infection of the nasal cavities, but almost no colonization is observed in the lungs of even sham-vaccinated mice. In experiments where simulation of natural infection conditions is important, such as in studies of population dynamics, low dose, low volume inoculation is preferable, as it more closely mirrors the conditions found in nature.

Chapter 2
Materials and Methods

Vaccination Schedule

Sham vaccine consisted of 200 uL Stainer-Scholte liquid media. Whole-cell *Bordetella bronchiseptica* (wcBb) vaccine was made by growing *B. bronchiseptica* in Stainer-Scholte liquid media to a concentration of $1 * 10^9$ CFU/mL. Culture was divided into 1 mL aliquots and heat killed at 65°C for 45 minutes. Commercial vaccine Adacel, diluted to 1/5 human dose concentration, was used as acellular *B. pertussis* (aP) vaccine. One mouse dose of Adacel contained 0.5 μg pertussis toxin, 1 μg filamentous hemagglutinin, 0.6 μg pertactin, and 1 μg fimbriae, with 0.3 μg Alum as adjuvant.

Mice were vaccinated by intraperitoneal injection with 200 uL of vaccine on Days 0 and 14. Inoculation occurred on Day 35. Shedding was monitored for 21 days post-inoculation, or 28 days during transmission studies.

Mouse Infections

B. bronchiseptica strain RB50 was grown in Stainer-Scholte liquid media to mid-log phase and diluted to a concentration of 3×10^4 CFU/mL in phosphate-buffered saline (PBS). Mice were anesthetized by exposure to gaseous isofluorane and inoculated by placing a 5 uL droplet containing 150 CFU on the tips of the nares.

Mice were sacrificed at the end of the time course and dissected to extract their nasal cavities and lungs. Organs were homogenized in 1 mL phosphate-buffered saline (PBS) and plated on agar plates containing Bordet-Gengou (BG) media + streptomycin.

Plates were incubated at 37°C and visible colonies of *B. bronchiseptica* were counted after 48 hours.

Shedding and Transmission

Bacterial shedding was measured by swabbing the nares of each mouse with a sterile cotton swab for 10 seconds and placing the tip of the swab into 1 mL PBS. Tubes containing swabs + PBS were vortexed for 30 seconds to ensure distribution of bacteria in PBS, and 200 uL were plated on agar plates containing Bordet-Gengou (BG) media + streptomycin. Plates were incubated at 37°C and visible colonies of *B. bronchiseptica* were counted after 48 hours.

Chapter 3
Results

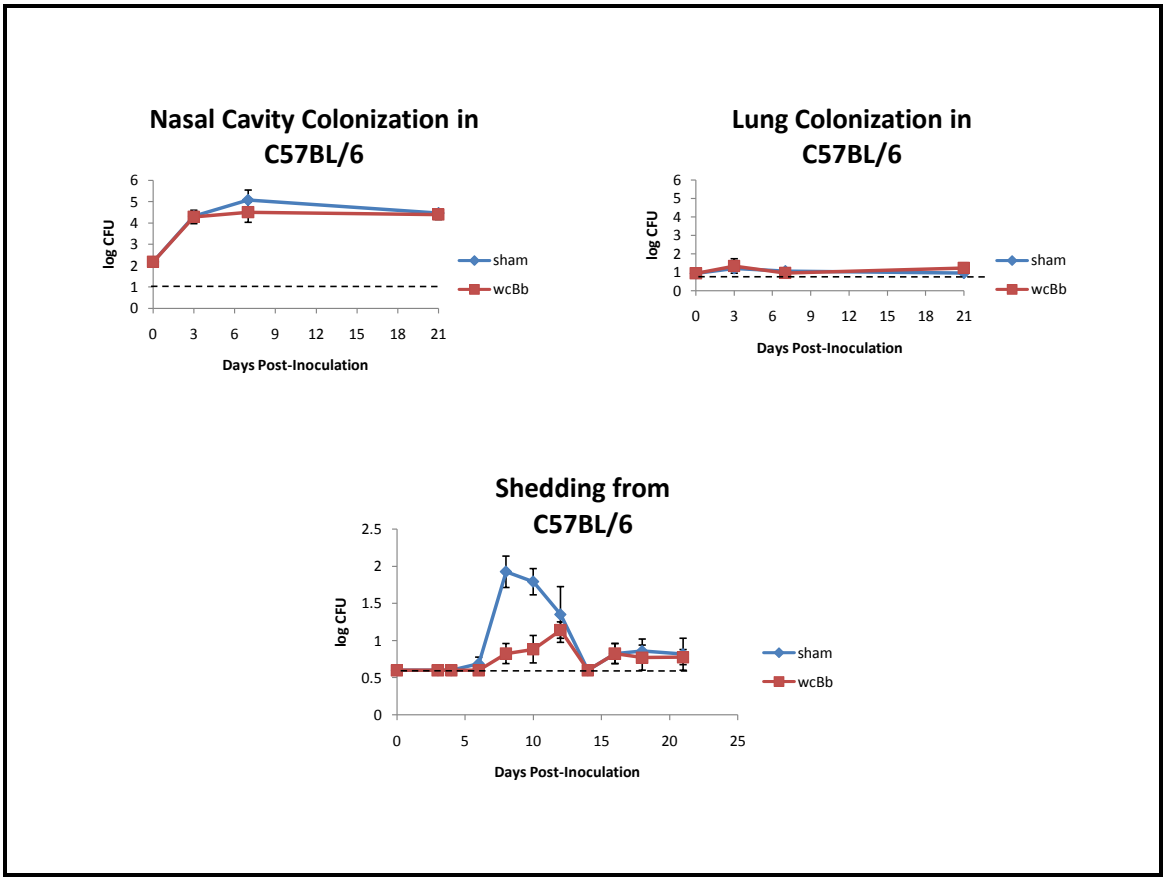

Figure 3-1 Effects of whole-cell *B. bronchiseptica* vaccine on colonization and shedding from wild-type C57BL/6 mice. Colonization was measured in nasal cavities (A) and lungs (B) as well as shedding from murine nares (C).

Whole-cell vaccination reduces shedding

Figure 3-1 provides preliminary data and rationale for the investigation. An important factor in the transmission of any pathogen is the ability to shed from an infected host. To determine whether vaccination resulted in altered shedding of bacteria from the nares, groups of C57/BL6 were vaccinated with whole-cell *B. bronchiseptica* or sham vaccinated with Stainer-Scholte media. Vaccination with whole-cell *B. bronchiseptica* vaccine has no significant impact on the ability of RB50 to colonize the respiratory tract

during a post-vaccination challenge, with a long-lasting infection still being established in the nasal cavities but not in the lungs. However, shedding of RB50 from the nares was significantly lower in mice previously vaccinated with whole-cell *B. bronchiseptica* vaccine as compared to sham-vaccinated mice. Vaccination not only reduced the magnitude of the peak of shedding by a factor of 10, but also delayed the time to reach this peak by two days.

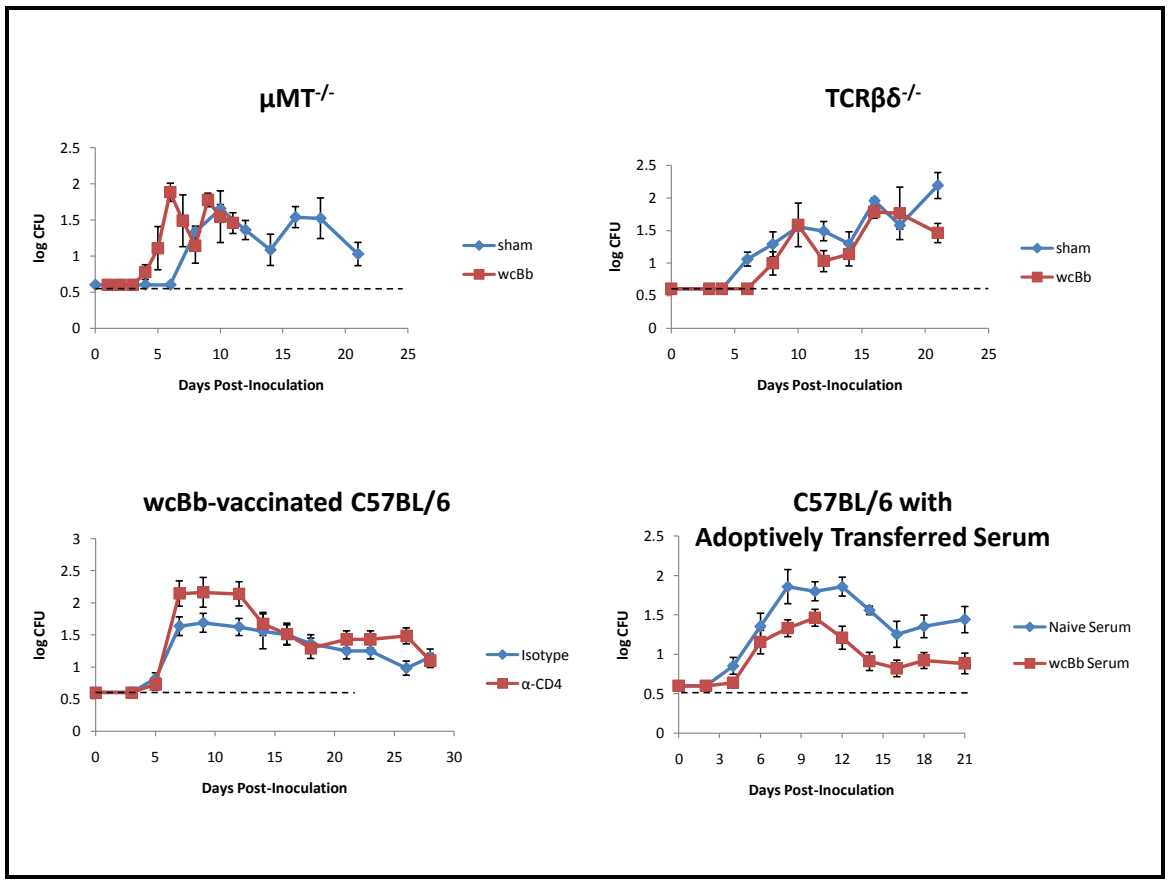

Figure 3-2 Shedding in vaccinated mice lacking components of the adaptive immune system. Mice tested were lacking B cells (A), T cells (B), or CD4$^+$ T-helper cells (C). Mice in (D) were injected with serum to provide passive immunity from antibodies.

B and T cells are required for reduction of shedding by whole-cell vaccination

Since the goal of vaccination is to induce a long-lasting, protective response against a pathogen, which is governed by the adaptive immune system, the role of adaptive immunity in control of shedding was tested, as shown in Figure 3-2. Little to no reduction in shedding was observed in vaccinated mice deficient in B or T cells, suggesting that control of shedding is dependent on both of these cell types. Depletion of CD4$^+$ T-helper cells abrogated the reduction of shedding normally seen in vaccinated mice compared to mice treated with non-functional isotype control antibodies, which

indicates that $CD4^+$ cells specifically are required for reduction in shedding. Passive immunization with serum from a vaccinated mouse was able to partially rescue the reduction in shedding, which also suggests a role for antibodies, particularly late in infection.

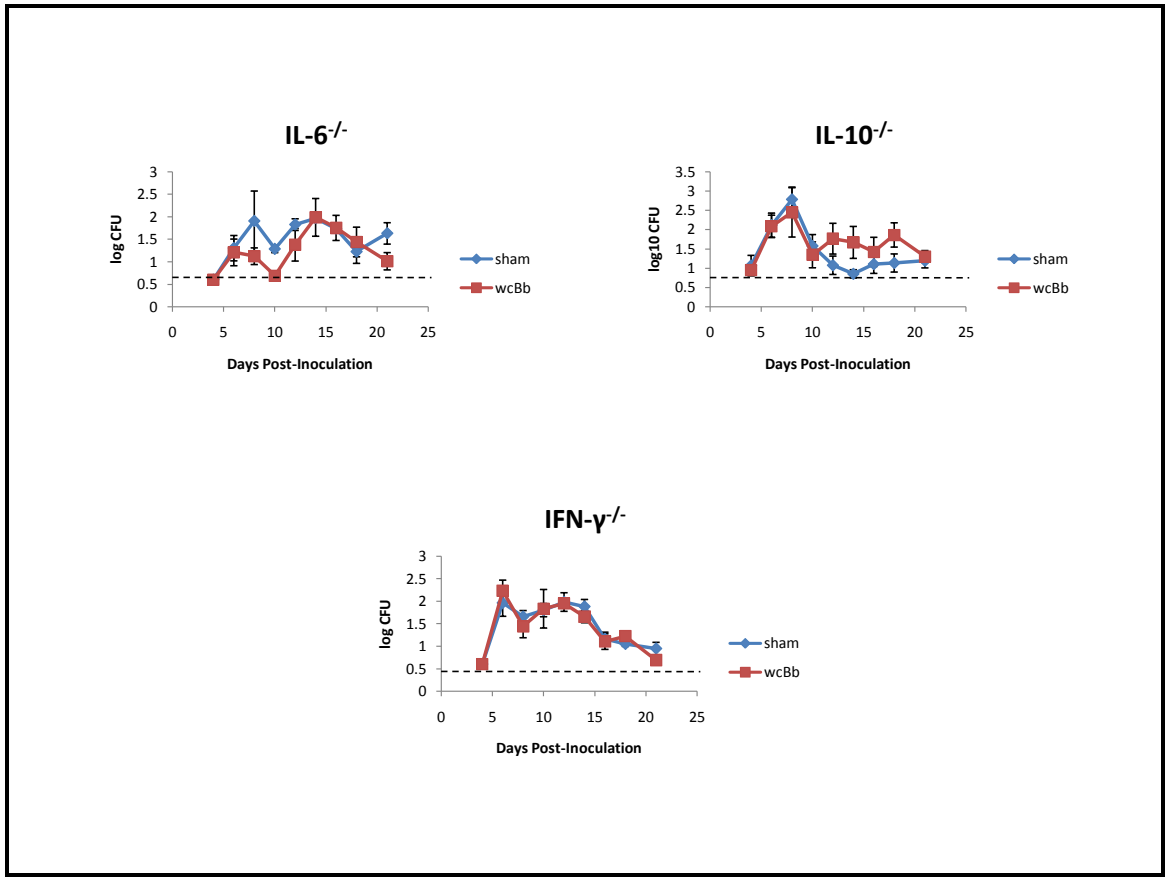

Figure 3-3 Shedding in vaccinated mice lacking innate immune cytokines. Mice tested did not express IL-6 (A), IL-10 (B), or IFN-γ (C).

IFN-γ and IL-10 required for reduction of shedding

Shedding has previously been correlated with inflammation and neutrophil recruitment (data not shown), which can induced by the innate as well as the adaptive immune system, the effect of infection on certain innate immune cytokines were tested and the results are displayed in Figure 3-3. Although vaccination inhibited shedding in

wild type mice, vaccination had no effect on shedding of IFN-$\gamma^{-/-}$ mice, suggesting IFN-γ is required for vaccination to inhibit shedding. The peak of shedding in IL-$10^{-/-}$ mice is the same in vaccinated and non-vaccinated mice, suggesting IL-10 is not involved in vaccine-mediated effect on shedding early in infection, although there may be an inhibitory effect of IL-10 later in infection. The peak of shedding in vaccinated IL-$6^{-/-}$ mice is lower than in sham-vaccinated ones, so a lack of IL-6 does not completely prevent whole-cell vaccination from reducing shedding.

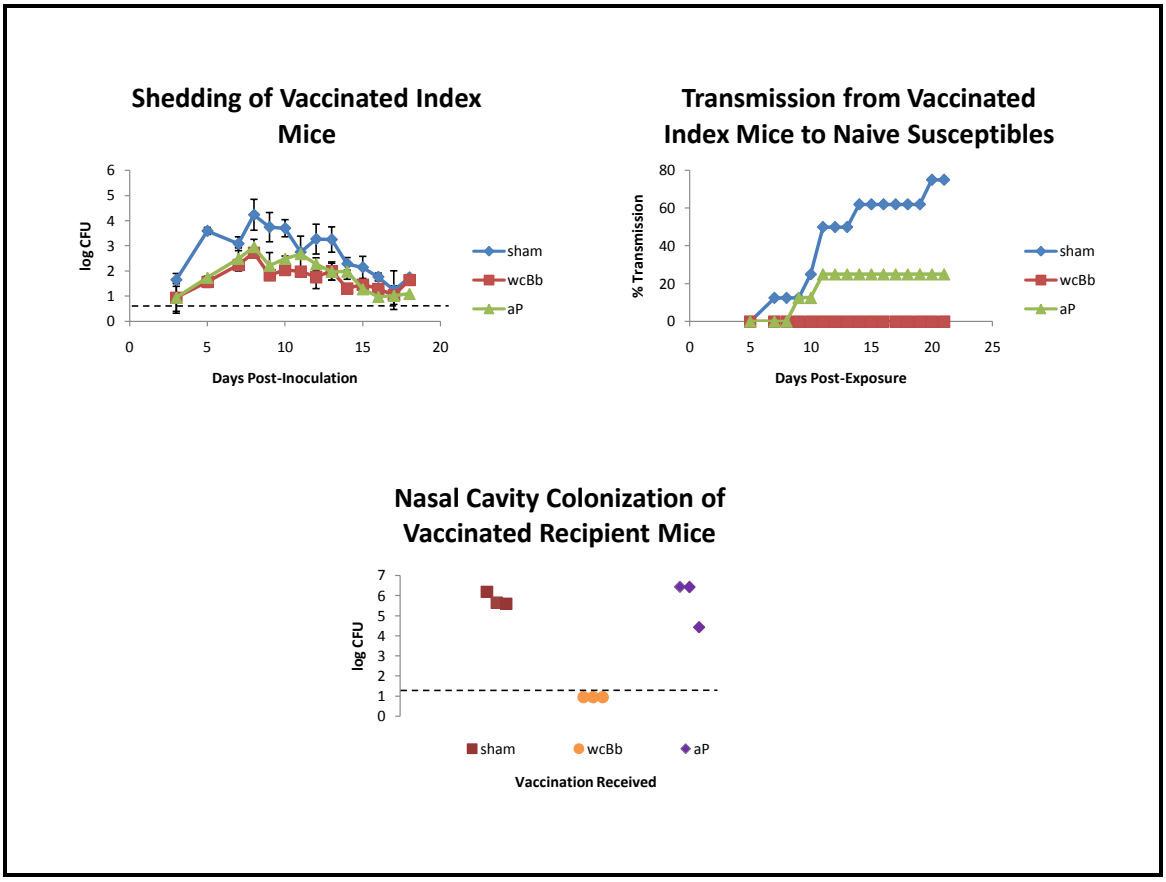

Figure 3-4 Transmission between C3H/HeJ mice. Shedding from vaccinated index mice was monitored (A), as well as from naïve susceptible mice to determine infection status (B). Transmission from naïve index mice to vaccinated recipient mice was determined by colonization after 21 days (C).

Whole-cell vaccination prevents transmission of *B. bronchiseptica*

To test the ability of vaccination to prevent transmission, vaccinated and challenged mice were co-housed with naïve mice in the same cage. Both whole-cell *B. bronchiseptica* and acellular *B. pertussis* vaccines reduced shedding compared to sham vaccination, as shown in Figure 3-4. However, while whole-cell vaccination completely prevented transmission for the duration of the time course, acellular vaccination was only partially protective, with 20% of mice given the acellular vaccine becoming infected.

The ability of vaccination to prevent a host from becoming infected, as opposed to preventing the transmission to other hosts, is another important concern in judging the usefulness of a vaccine. To determine the ability of whole-cell vaccination and Adacel to prevent transmission to recipient mice, mice were vaccinated with whole-cell *B. bronchispetica* vaccine, acellular *B. pertussis* vaccine, or sham-vaccinated and co-housed with a naïve index mouse inoculated with RB50. Nasal cavity colonization by RB50 was measured after 21 days to determine whether mice had become infected by the index mouse. Mice vaccinated with whole-cell *B. bronchiseptica* vaccine were protected from transmission, while sham-vaccinated mice and those vaccinated with acellular *B. pertussis* vaccine were not. These data indicate that the whole-cell vaccine protects against infection while the acellular vaccine does not. These results are consistent with clinical observations of *Bordetella* circulating amongst humans given the acellular vaccine and therefore have important implications, discussed below.

Chapter 4
Discussion

Vaccines have been critical in the fight against infectious diseases, but they are typically judged based on their efficacy at preventing disease in a vaccinated individual. Without evaluation of a vaccine's effects on the population dynamics of the target pathogen, widespread use could have harmful effects such as the evolution of increased virulence[14]. *B. pertussis* vaccination has been studied extensively with respect to the immune system and within-host infection dynamics, but little is known about the effects of vaccination on transmission. Given the importance of disease transmission to public health, the role of vaccination in altering transmission rates is deserving of further study.

The results of this investigation indicate that whole-cell *B. bronchiseptica* vaccine does not decrease colonization during a post-vaccination challenge compared to sham vaccine, but it does significantly reduce shedding. In addition to reducing the peak of shedding, vaccination also delayed the time it took for mice to reach this peak. Delaying the length of the infectious period could increase the duration of an epidemic, as hosts that are infectious for longer can infect more hosts before they recover.[15] This makes the effect of vaccination on the duration of the infectious period another challenge to address in vaccine design. However, peak shedding in whole-cell vaccinated mice on day 10 was lower than shedding in sham-vaccinated mice on the same day, and returned to negligible levels along with sham-vaccinated shedding, so in this case the concern is not relevant.

Figure 3-3 indicates that both B and T cells, major components of the adaptive immune system, are required for control of shedding by whole-cell *B. bronchiseptica* vaccine. In addition to the failure of vaccination to reduce peak shedding in both $\mu MT^{-/-}$ and $TCR\beta\delta^{-/-}$ mice, these mice continued to shed through the end of the time course, indicating that both cell types are required to lower shedding at late time points during infection of naïve mice. Figure 3-3D demonstrates the requirement of antibodies, especially at later time points when shedding has normally decreased to negligible levels, which agrees with the observed requirement of B cells. Antibodies have previously been shown to be required for clearance of *B. pertussis* from mice, so a role of antibodies in controlling shedding would not be surprising.[16] In particular, $CD4^+$ cells are required to for vaccination to control shedding. As *B. bronchiseptica* is a predominantly extracellular pathogen, $CD4^+$ T-helper cells are more likely than $CD8^+$ cytotoxic T cells to be the ones responsible for controlling shedding, as they aid B cells in producing more potent antibodies and enhance cell-mediated responses by other myeloid leukocytes. Memory $CD4^+$ cells have been shown to induce innate immune responses that lead to earlier control of infection in an immune host than a naïve one, and these cells typically belong to the T_H1 or T_H17 subsets of T-helper cells.[17] In conjunction with the observed IFN-γ requirement, this suggests that the action of T_H1 cells and the cell-mediated responses they induce are responsible for control of shedding. T_H1 cells have a demonstrated role in protection against *B. pertussis* in vaccinated mice, so their ability to prevent shedding in challenged mice is an additional benefit of their generation during an adaptive immune response.[18]

The results that indicate a vaccine-induced T_H1 response is responsible for reducing shedding during challenge do not preclude the involvement of other T-helper cells such as T_H2 or T_H17 cells. T_H1 and T_H2 cells each secrete cytokines that suppress the other response, but different factors can force differentiation in a different direction.[19] Indeed, differences in shedding were observed in mice lacking IL-10, a classical T_H2 cytokine, but only on days when depletion of $CD4^+$ cells in vaccinated C57BL/6 mice had no significant effect. Other bordetellae have been shown to induce IL-10, though, as a way to suppress cell-mediated T_H1 immunity.[20] Previous study of vaccine-induced immunity have found that IL-4, another T_H2 cytokine that stimulates B cell expansion, is involved in reducing inflammation and controlling pathology during *B. pertussis* infection.[21] The action of T_H17 cells producing IL-17 has also been shown to be required for efficient clearance of *B. pertussis* in vaccinated mice.[22] Testing the specific roles of IL-4 and IL-17 in this system has the potential to yield more information about the specific T-cell responses that are required for vaccine-mediated control of shedding.

The failure of acellular *B. pertussis* vaccine to completely protect from transmission, as observed in Figure 3-4B, even though it protects against pathology from *B. bronchiseptica*,[13] may be due to its induction of a T_H2 response rather than the T_H1 and T_H17 responses that are usually induced by whole-cell vaccination.[23] Cross-protective immunity to *B. bronchiseptica* characterized by reduced inflammation, tissue damage, and neutrophil infiltration has previously been generated by a live, attenuated *B. pertussis* vaccine.[12,24] The success of this vaccine at generating cross-species immunity suggests that the vaccine being acellular was more likely to be the reason transmission occurred, rather than it being composed of *B. pertussis* antigens. The long-lasting nature

of this immunity has potentially beneficial implications for public health, as it reduces the likelihood that waning immunity allows epidemics to occur.[25]

Vaccines typically include an adjuvant, a compound that is not part of a pathogen but that is able to stimulate the immune system and elicit a response that is more protective than one generated by only the pathogens. By targeting certain receptors, different adjuvants are able to generate different types of immune responses. If a specific immune response is found to be more protective than another, the adjuvant that helps elicit that response could be incorporated into a vaccine to improve efficacy. Clearance of bacteria from mice requires the action of both T_H1 and T_H17 cells, while alum, the adjuvant in current acellular *B. pertussis* vaccines induces a T_H2 response.[26] The immune signals that drive T_H1 and T_H2 differentiation are antagonistic, so incorporating an adjuvant that induces a T_H1 response will dampen a T_H2 response, but that dichotomy does not prevent the development of a T_H17 response, which is often seen together with T_H1 responses. [12,22,26] For example, use of polysaccharide chitosan conjugated to CpG DNA has been shown to enhance both T_H1 and T_H17 responses, making it a candidate adjuvant where cell-mediated immunity is the desired outcome of a vaccine.[27]

Even if more efficacious, a vaccine that is not used is not effective, so public concerns remain a factor in vaccine choice. Because the recommended *B. pertussis* vaccine was changed from a whole-cell to an acellular one in recent decades due to concerns about the safety of using whole bacterial cells, improving the current acellular vaccine to prevent transmission could be preferable to a return of the use of a whole-cell vaccine.[28] If such improvements could be made, then continued use of the acellular

vaccine could also reduce transmission, and therefore the prevalence of disease within a population in addition to protecting vaccinated individuals.

One caveat of this investigation was the use of two different mouse strains and backgrounds. Transmission was originally observed in C3H/HeJ mice, which have non-functional Toll-like receptor 4. TLR-4 is responsible for detection of lipopolysaccharide and is critical to initiation of the innate immune response in bacterial infection, so a mutation in this receptor prevents C3H/HeJ mice from providing an accurate representation of the immune dynamics during *B. bronchiseptica* infection. In addition to its role in natural infection, TLR-4 has been shown to be required for efficacy of both whole-cell and acellular *B. pertussis* vaccine efficacy and the generation of effective T_H1 and T_H17 responses.[29]

C57BL/6 mice are suitable wild-type mice with functional immune systems, but transmission is difficult to observe in these mice due to their lower shedding. The peak of shedding in C57BL/6 mice is approximately 100 CFU (Figure 3-1C), while peak shedding from C3H/HeJ mice can be 100 to 1000 times higher (Figure 3-4A). Despite a lower peak, shedding in C57BL/6 mice follows the same type of curve as shedding in C3H/HeJ mice, allowing for observations to be made about shedding, a strong indicator of transmission likelihood, in a wild-type mouse model. The availability of immunodeficient mutant strains on this background also allows conclusions to be made about the role of various immune system components in vaccine-mediated control of shedding.

Appendix A
Supplemental Data

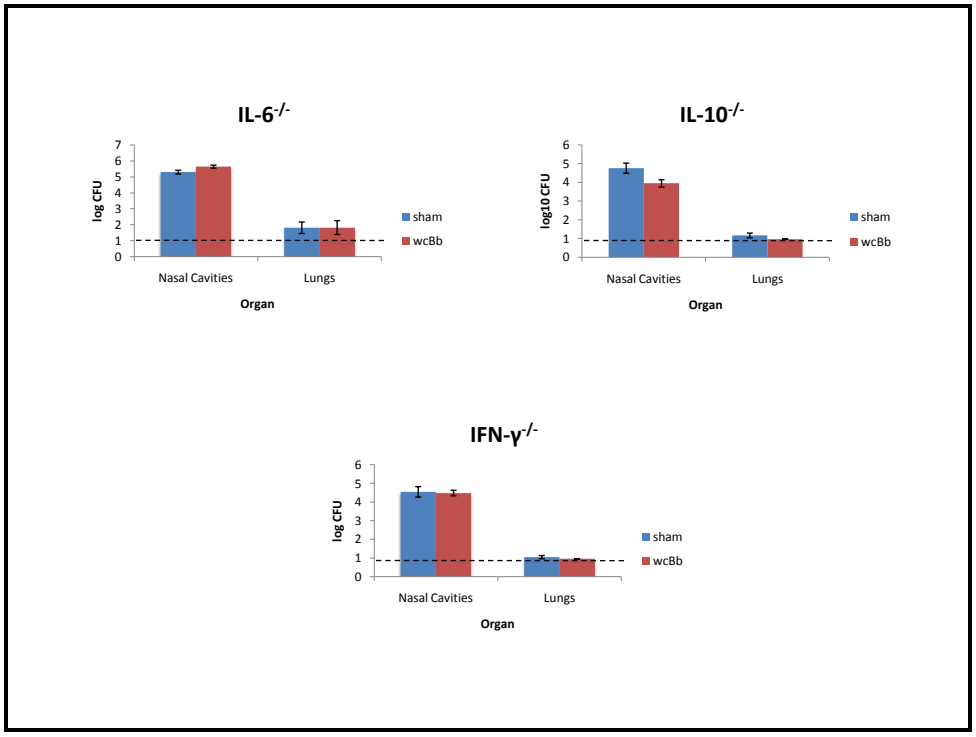

Figure 4-1 Colonization in innate immunodeficient mice. Colonization was measured in lungs and nasal cavities of mice lacking IL-6 (A), IL-10 (B), and IFN-γ (C) at the end of the time course on day 21.

Vaccination does not prevent persistent colonization of mice lacking innate immune cytokines

Figure 4-1 shows colonization by RB50 of sham-vaccinated and wcBb-vaccinated mice by lacking various cytokines at the end of each shedding time course. RB50 persisted in the nasal cavities of each mouse, but very few, if any, bacteria were detected in the lungs. In IL-10$^{-/-}$ mice, vaccination reduced nasal cavity colonization by 10-fold, but that was the only significant observation. Overall, this figure shows that lack of IL-6, IL-10, or

IFN-γ does not prevent long-term colonization of murine nasal cavities, regardless of vaccination status.

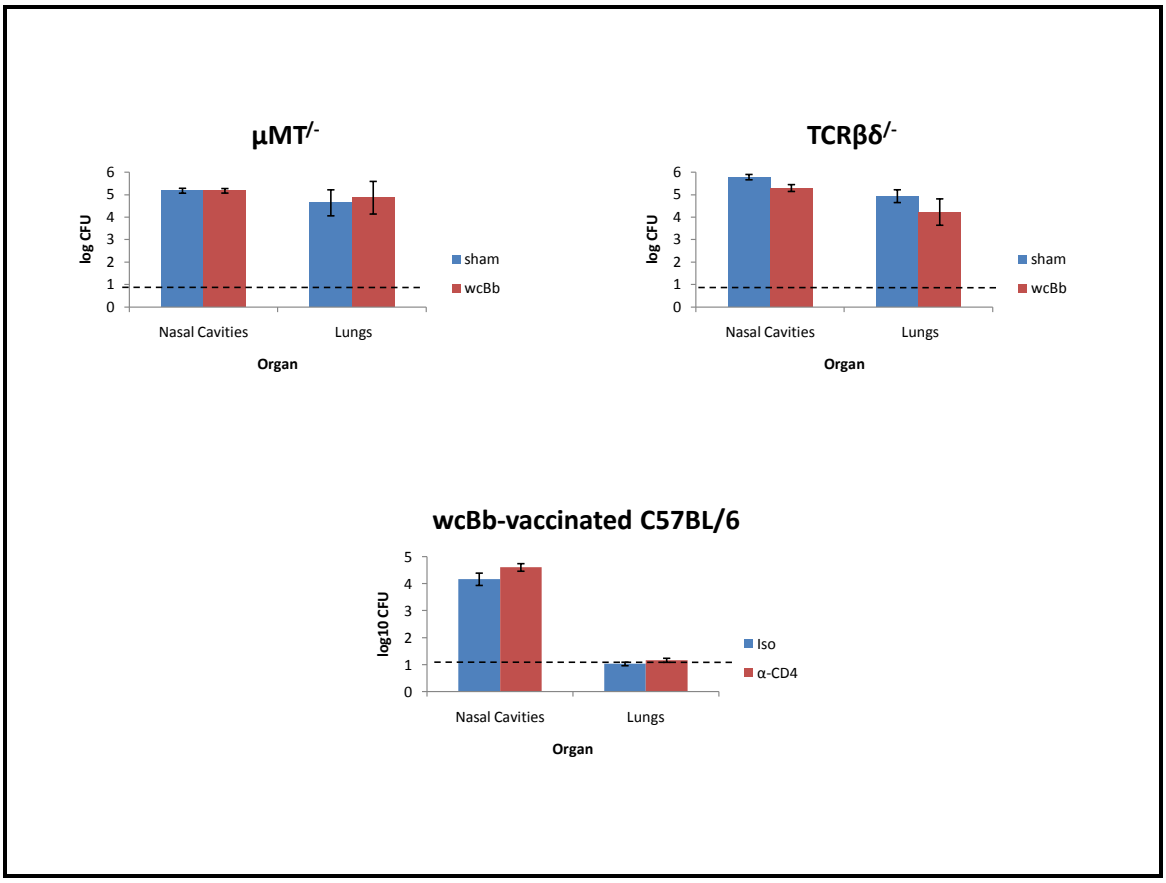

Figure 4-2 Colonization in adaptive immunodeficient mice. Colonization was measured in lungs and nasal cavities of mice lacking B cells (A), T cells (B), or CD4$^+$ T-helper cells (C) at the end of the time course on day 21 (A and B) or day 28 (C).

Vaccination does not prevent persistent colonization of mice lacking B or T cells

Figure 4-2 shows long-term RB50 colonization of mice lacking various components of adaptive immunity. Like the innate immunodeficient mice shown in Figure 4-1, vaccination had little effect on colonization. Significant bacterial numbers were detected in the lungs of mice lacking B or T cells, suggesting that both cell types are required to prevent persistent colonization of the lungs, even in vaccinated hosts. The nasal cavities

of all three groups of mice were colonized with RB50 at the end of the time courses, which indicates the establishment of long-term persistence of RB50 within the nasal cavities of mice regardless of vaccination status or the presence of B and T cells.

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Academic Vita of Nathan T. Jacobs

EDUCATION

The Pennsylvania State University, University Park, Pennsylvania, 2009 – 2013
Schreyer Honors College, 2009 - 2013
B.S. in Biology, concentration in Ecology – May 2013
B.S. in Immunology & Infectious Disease, with honors – May 2013

AWARDS and HONORS

- Barry M. Goldwater Scholarship, 2012 – 2013
- Penn State Undergraduate Summer Discovery Grant, 2012
- Eberly College of Science Hammond Scholarship, 2012 – 2013
- Eberly College of Science Braddock Scholarship, 2009 – 2013
- Schreyer Honors College Academic Excellence Scholarship, 2009 – 2013
- National Merit Scholarship, 2009 – 2013

RESEARCH EXPERIENCE

Undergraduate Honors Research: The Pennsylvania State University
Department of Veterinary and Biomedical Sciences: February 2010 – May 2013 (Thesis Advisor: Dr. Eric T. Harvill)

- Investigation of the effects of vaccination on the transmission dynamics of *B. bronchiseptica* between mice.
- Investigation of the Type VI Secretion System in *Bordetella bronchiseptica* and interactions with the innate immune system.

PUBLICATIONS

Weyrich, L.S., Rolin, O.Y., **Jacobs, N.T.**, Harvill, E.T. 2012. *Bordetella bronchiseptica* Type VI Secretion System Dampens Antibody Production by Modulating CD1d, submitted.

TEACHING EXPERIENCE

Teaching Assistant – Laboratory Instructor:

Biology 220W: Populations and Communities
Biology 110: Introductory Concepts and Biodiversity

- Developed an interactive exercise to help students understand the spread of antibiotic resistance in bacteria.
- Composed a list of recommended reading for students in life sciences.
- Graded quizzes and writing assignments.

PROFESSIONAL MEMBERSHIPS

- Phi Beta Kappa Society
- Gamma Sigma Delta Agricultural Honor Society