Acellular pertussis vaccination facilitates *Bordetella parapertussis* infection in a rodent model of bordetellosis

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Despite over 50 years of population-wide vaccination, whooping cough incidence is on the rise. Although *Bordetella pertussis* is considered the main causative agent of whooping cough in humans, *Bordetella parapertussis* infections are not uncommon. The widely used acellular whooping cough vaccines (aP) are comprised solely of *B. pertussis* antigens that hold little or no efficacy against *B. parapertussis*. Here, we ask how aP vaccination affects competitive interactions between *Bordetella* species within co-infected rodent hosts and thus the aP-driven strength and direction of in-host selection. We show that aP vaccination helped clear *B. pertussis* but resulted in an approximately 40-fold increase in *B. parapertussis* lung colony-forming units (CFUs). Such vaccine-mediated facilitation of *B. parapertussis* did not arise as a result of competitive release; *B. parapertussis* CFUs were higher in aP-relative to sham-vaccinated hosts regardless of whether infections were single or mixed. Further, we show that aP vaccination impedes host immunity against *B. parapertussis*—measured as reduced lung inflammatory and neutrophil responses. Thus, we conclude that aP vaccination interferes with the optimal clearance of *B. parapertussis* and enhances the performance of this pathogen. Our data raise the possibility that widespread aP vaccination can create hosts more susceptible to *B. parapertussis* infection.

**Keywords:** pathogen evolution; *Bordetella parapertussis* disease; acellular vaccination; epidemiology; co-infection

1. INTRODUCTION

Despite decades of worldwide pertussis vaccination, whooping cough is re-emerging in highly vaccinated countries (CDC 2002; Celentano et al. 2005). A rise in non-vaccine alleles coincident with widespread vaccination has been documented for *Bordetella pertussis* (Elomaa et al. 2005; Van Amersfoorth et al. 2005; Van Gent et al. 2009) leading some authors to propose that vaccine-driven epitope-evolution in *B. pertussis* is one factor—among several others (Berbers et al. 2009)—that may contribute to whooping cough re-emergence in humans (Mooi et al. 2001). However, it is not clear how *Bordetella parapertussis*—the other major aetiological agent of human whooping cough—might respond to the selective pressure exerted by large-scale pertussis vaccination. Here, we postulate that the widespread and long-term use of acellular subunit pertussis vaccines creates hosts that are more favourable for *B. parapertussis*.

All commercial whooping cough vaccines currently contain either killed whole cells or purified antigens of *B. pertussis*—herein referred to as whole cell (wP) and acellular vaccines (aP), respectively. Currently, aP vaccines are largely favoured over their wP predecessors owing to their reduced reactogenicity (Anderson et al. 1988). Although aP vaccines are very effective at reducing the incidence of *B. pertussis* infection (Mattio & Cherry 2005), they hold little or no efficacy against *B. parapertussis* (Stehr et al. 1998; Willems et al. 1998; Liese et al. 2003; David et al. 2004). In fact, *B. parapertussis* prevalence is predicted to increase slightly in response to vaccines that are less protective against *B. parapertussis* than natural *B. pertussis* infection (Restif et al. 2008). Thus, analogous to the serotype specificity observed for conjugate vaccines against other infectious diseases and the serotype replacement associated with their use (Obaro et al. 1996; Lipsitch 1997), we hypothesize that the prolonged and widespread use of *B. pertussis*-specific aP vaccines has the potential to increase carriage of species not included in the vaccine, namely *B. parapertussis*.

The rationale to design and employ vaccines that target only *B. pertussis* stems from the assumption that *B. parapertussis* infections are not widely prevalent. Indeed, the vast majority of whooping cough studies do not attempt to identify *B. parapertussis* because differential diagnosis does not affect clinical management and this probably leads to under-reporting. However, when differential diagnosis has been carried out, *B. parapertussis* was found to comprise between 2 and 36 per cent of cases (Watanabe & Nagai 2004) and, in one study, to constitute the major aetiological agent (Borska & Simkovicova 1972). Both mixed and sequential infections of *B. pertussis* and *B. parapertussis* have been reported in epidemiological studies (Mertsofa...
B. parapertussis in mice are consistent with an aP-driven adaptive response on which are based the observation that aP vaccination could generate this selective advantage, all of which is driven by B. pertussis vaccination and a non-significant interaction between vaccination and aP, and the infection type. If enemy release is occurring, we expect B. parapertussis CFUs to be higher in aP-vaccinated relative to sham-vaccinated hosts (tested with the term ‘infection type’, a two-level factor describing the vaccination regime administered, sham or aP, and the infection type). Evidence that aP vaccination interferes with an optimal host immune response against B. parapertussis would further support the ERH. Our results support the enemy release model: aP vaccination interferes with the optimal clearance of B. parapertussis and enhances the performance of this pathogen.

2. MATERIAL AND METHODS

(a) Bacteria strains and growth conditions

Bordetella pertussis 1740 is a derivative of Tohama I (Kasuga et al. 1954), rendered kanamycin resistant by the chromosomal insertion of pSS4266 (Goebel et al. 2008) and was a kind gift from Dr Scott Stibitz (USDA). Bordetella parapertussis 12822 was isolated from German clinical trials (Heininger et al. 2002) and 12822G is a gentamicin-resistant derivative of the parent strain (Wolfe et al. 2005). Bordetellae were maintained on Bordet-Gengou (BG) agar (Difco) containing 10 per cent defibrinated sheep blood (Hema Resources) at 37 °C for approximately 72 h. Supplementing BG plates with kanamycin or gentamicin (50 and 20 μg ml⁻¹, respectively; Sigma Aldrich) allowed differentiation between bacteria in mixed infections. For experimental inocula, liquid culture bacteria were grown overnight at 37 °C and shaken to mid-log phase (optical density at 600 nm of approx. 0.3) in Stainer-Scholte broth.

(b) Hosts, vaccination and inoculation

Four- to six-week-old female C57BL/6 mice (Jackson Laboratories) were maintained in specific pathogen-free rooms at Pennsylvania State University and were handled in accordance with Institutional Animal Care and Use Committee guidelines. In two experiments, a total of 200 mice were divided into eight treatment groups. Half of all mice received two 50 μl subcutaneous injections (on days 0 and 14) of the commercial Adacel vaccine (referred to as aP; Sanofi Pasteur) at one-fifth the human dose, whereas the other half were sham vaccinated sterile phosphate buffered saline (PBS) and both treatments were administered with Imject Alum adjuvant (Thermo Scientific). Using this vaccination protocol, vaccine efficacy in human clinical trials was shown to correlate with bacterial clearance in a murine model of B. pertussis (Mills et al. 1998; Guiso et al. 1999). Adacel vaccines are provided as combined tetanus–diphtheria–pertussis formulation adsorbed to alum and contain the following five B. pertussis antigens: 5 μg ml⁻¹ of in lung colony-forming units (CFUs), cytokine milieu, neutrophil recruitment and pathogen-specific antibody responses. If B. parapertussis is competitively suppressed by B. pertussis infection, B. parapertussis lung CFU will be lower in mixed relative to single infections (tested with the term 'infection type', a two-level factor describing the number of Bordetella species present in an infection; single or mixed). Following from this, competitive release of B. parapertussis would present as a significant interaction between infection type and ‘vaccination’—a two-level factor describing the vaccination regime administered, sham or aP, and the infection type. If enemy release is occurring, we expect B. parapertussis CFUs to be higher in aP-vaccinated relative to sham-vaccinated hosts (tested with the term vaccination), regardless of whether infections were alone or in a mixture (which would present as a significant main effect of vaccination and a non-significant interaction between vaccination and infection type). Evidence that aP vaccination interferes with an optimal host immune response against B. parapertussis would further support the ERH. Our results support the enemy release model: aP vaccination interferes with the optimal clearance of B. parapertussis and enhances the performance of this pathogen.
detoxified pertussis toxin, 10 μg ml⁻¹ filamentous haemagglutinin, 6 μg of pertactin and 10 μg of fimbriate types 2 and 3.

In both vaccinated and sham-vaccinated groups, 27 mice were each infected with B. pertussis alone, B. parapertussis alone or a mixture of both, and 21 were sham infected with sterile PBS (table S1, electronic supplementary material). Mice were challenged intranasally with 5 × 10⁵ CFU three weeks after the second vaccination (day 35), as described (Harvill et al. 1999). For mixed infections, the 50 μl inocula contained 5 × 10⁶ CFU of each of B. pertussis and B. parapertussis. The same dose of each bacterium in mixed and single infections was used as we wanted to compare the dynamics of each bacterium on its own versus in mixed infections. On each day of sacrifice (table S1, electronic supplementary material; experiment 1: days 0, 3, 7, 14 and 35 post-infection (p.i.) and experiment 2: days 0, 3 and 7 p.i.), three to four mice per group were sacrificed in experiments 1 and 2, respectively. In both experiments, lungs were aseptically removed and homogenized in 1 ml of sterile PBS. Serial dilutions of organ homogenate were plated on BG agar plates containing the relevant antibiotics and cells were incubated for 3–5 days at 37°C to quantify the number of viable bacteria. In experiment 1, blood was collected on each day of sacrifice for the assessment of Bordetella-specific serum antibodies.

(c) Lung cytokines, neutrophil numbers and antibody enzyme linked immunosorbent assays (ELISAs)

In experiment 1, levels of the lung cytokines interleukin (IL)-4, IL-5, interferon gamma (IFN-γ) and granulocyte macrophage-colony stimulating factor (GM-CSF) were quantified using a flow cytometric cytokine assay, according to the manufacturer’s instructions (Bio-Plex Mouse Cytokine T₄1/T₄2 Panel Cytokine Assay and a Bio-Plex cytokine reagent kit, Bio-Rad).

In experiment 2, lung leukocyte numbers were quantified by performing lung perfusions on days 0, 3 and 7 p.i. Briefly, lungs were perfused with sterile PBS on day of sacrifice to remove red blood cells before homogenizing through sterile cell strainers (BD Biosciences). Homogenates were laid on a Histopaque gradient 1119 (Sigma Aldrich), centrifuged at 1500 g for 30 min and the leukocyte layer collected and counted on a hemocytometer at ×40 magnification. Aliquots of cells were stained with fluorescein labelled antibodies (FITC)-labelled anti-Ly-6G to detect neutrophils (eBioscience). The percentage of FITC-positive cells was multiplied by the total number of leukocytes to calculate neutrophil numbers.

Bacteria were grown overnight (optical density of 0.7 at 600 nm), diluted in carbonate buffer and 200 μl added to each well of a 96-well plate. Serum from experiment 1 was diluted and serially diluted across the plates to a final dilution of 1:102400. Incubation, wash and development steps were carried out as detailed (Wolfe et al. 2005). Total immunoglobulin (Ig) titre was quantified using biotin-conjugated anti-mouse Ig (Southern Biotechnology Associates) and peroxidase-conjugated streptavidin (BD Pharmingen). Results were reported as endpoint titres.

(d) Statistical analyses

All analyses were performed in R v. 2.7.0 (http://www.R-project.org) using generalized linear models (GLM; Crawley 2007; R 2008). Analyses focused on 200 mice, with experimental groups as detailed above and in table S1, electronic supplementary material. We assumed lognormal errors in CFU, cytokine and antibody titres and carried out the analysis on the log₁₀ transformed data, using least squares with normal errors and the identity link. Data from duplicate bioassay and triplicate antibody enzyme linked immunosorbent assay (ELISA) plate wells were averaged and the respective titres induced by naive animals were subtracted from experimental animals before being log₁₀((a + 1) transformed to satisfy homogeneity-of-variance and normality-of-error assumptions of models used.

CFU, lung cytokine and antibody data were analysed from days 3 to 35 p.i. inclusive in order to capture the full post-peak dynamics of infection. Main effects were vaccination (aP versus sham vaccinated), infection type (single versus mixed infection) and day p.i. (fitted as a categorical variable). The main effects of infection, vaccination and the infection by vaccination interaction terms explicitly test the main hypothesis of this study. To control for the dynamic kinetics of Bordetella infection, the main effect of day—as well as all two-way interactions between day and vaccination or infection type—was included in all analyses. In no cases were any of the three-way interactions significant and so they are not reported. Qualitative differences owing to infection type and vaccination were strong and consistent across experimental blocks and quantitative differences were controlled for by including experimental block as a factor in all analyses. Maximal models were first fit to the data and minimal models reached by removing non-significant terms (p > 0.05), beginning with the highest level interaction. Reported parameter estimates were taken from the relevant minimal models.

3. RESULTS

(a) Vaccine-mediated interactions and mixed infection

As expected, aP vaccination significantly reduced the CFU of B. pertussis (figure 1a,b; CFU days 3–35 inclusive, vaccination (acellular or sham): F₁,₇₂ = 145.9, p < 0.0001; vaccination × day: F₁,₇₂ = 6.5, p = 0.001). The average bacterial abundance produced throughout the infection was approximately 700-fold lower in aP-vaccinated relative to sham-vaccinated hosts. By contrast, aP vaccination significantly increased B. parapertussis CFU (figure 1c,d; CFU days 3–35 inclusive, vaccination: F₁,₇₂ = 16.9, p < 0.0001; vaccination × day: F₁,₇₂ = 5.5, p = 0.002). The average bacterial abundance produced throughout infection was approximately 40-fold higher in aP-vaccinated relative to sham-vaccinated hosts.

We found no evidence to support within-host competitive suppression of either pathogen species by the other. For B. pertussis, the vaccine-driven decrease in bacterial abundance was observed independent of whether infections were alone or in a mixture with B. parapertussis (figure 1a,b; CFU days 3–35 inclusive, infection type (mixed versus single) and infection type × vaccination, infection type × day, all p > 0.05) and was of similar magnitude in both experimental blocks (block × vaccination: p > 0.05). Likewise, the increase in B. parapertussis CFU was unaffected by the presence of B. pertussis (figure 1c,d; CFU days 3–35 inclusive, infection type, infection type × vaccination, infection
type × day and block × vaccination, all p > 0.05). Thus, we found no support for the competitive release hypothesis: there was no competition and hence no expansion of B. parapertussis when B. pertussis was selectively suppressed by aP vaccination.

Consistent with this absence of competition, mixed infections had an approximately twofold higher average CFU relative to single infections (CFU days 3–35 inclusive, infection type: B. pertussis, F1,75 = 67.9, p < 0.0001; B. parapertussis, F1,75 = 39.4, p < 0.0001, respectively), implying that there is no constrained ‘niche space’ over which the two species were competing. Thus, aP vaccination enhanced B. parapertussis CFUs in the lung, reversing the dominance from B. pertussis to B. parapertussis independent of the multiplicity of infection, consistent with the ERH.

(b) Lung cytokines and neutrophil recruitment

The lung immune response was skewed from a TH1 towards a predominantly TH2 response by aP vaccination. Specifically, aP-vaccinated mice produced significantly lower IFN-γ and higher lung IL-5 and IL-4 levels—a cytokine profile characteristic of TH2 cells—relative to sham-vaccinated mice (figure 2a–c; cytokine between days 3 and 35, inclusive: IFN-γ vaccination, F1,64 = 23.75, p < 0.0001 and vaccination × day, F3,64 = 8.9, p < 0.0001; IL-5 vaccination, F1,67 = 14.5,
and vaccination \( / \text{C}_2 \) day, \( p \approx 0.05 \); IL-4 vaccination, \( F_{1,70} = 6.0, p = 0.03 \) and vaccination \( / \text{C}_2 \) day, \( p \approx 0.05 \) and this was true for both \textit{Bordetella} species (infection type, infection type \( / \text{C}_2 \) vaccination and infection type \( / \text{C}_2 \) day terms, all \( p \approx 0.05 \)). In addition, lung GM-CSF levels were significantly reduced from day 3 p.i. onwards in aP-vaccinated hosts, regardless of the \textit{Bordetella} species or multiplicity of infection (figure 2d; cytokine) between days 3 and 35 inclusive: vaccination, \( F_{1,64} = 23.3, p < 0.0001 \); vaccination \( \times \) day, \( F_{5,64} = 6.0, p = 0.03 \); infection type, infection type \( / \text{C}_2 \) vaccination and infection type \( / \text{C}_2 \) day terms, all \( p < 0.05 \).

Although the number of neutrophils recruited to the lungs early in infection was significantly lower in aP- relative to sham-vaccinated hosts, the extent of this aP-driven reduction in neutrophils depended on \textit{Bordetella} species; aP-vaccinated hosts infected with \textit{B. parapertussis} (either as a single or mixed infection) had significantly lower neutrophil numbers compared with \textit{B. pertussis}-infected individuals (figure 3; lung neutrophil numbers on days 3–7: vaccination, \( F_{1,58} = 4.2, p = 0.04 \); vaccination \( \times \) day, \( p > 0.05 \); infection type, \( F_{2,58} = 0.46, p = 0.6 \); infection type \( \times \) day, \( F_{2,58} = 3.1, p = 0.02 \); infection type \( / \text{C}_2 \) vaccination, \( F_{2,58} = 3.8, p = 0.03 \)).

### (c) Pathogen-specific antibody response
Acellular vaccination enabled both \textit{B. pertussis}- and \textit{B. parapertussis}-infected hosts to mount more rapid anti-\textit{B. pertussis}- and anti-\textit{B. parapertussis}-specific Ig responses, respectively, relative to their sham-vaccinated counterparts (figure 4a,b; \textit{B. pertussis}: vaccination, \( F_{1,37} = 23.3, p < 0.001 \); vaccination \( \times \) day, \( F_{3,37} = 4.9, p = 0.006 \); figure 4c,d; \textit{B. parapertussis}: vaccination, \( F_{1,37} = 24.2, p < 0.0001 \); vaccination \( \times \) day, \( F_{3,37} = 3.9, p = 0.02 \), respectively). The extent to which aP vaccination affected the anamnestic responses depended on whether the infection was single or a mixture (figure 4a–d; \textit{B. pertussis}...
4. DISCUSSION

Here we show that aP vaccination accelerated the clearance of *B. pertussis* from the lower respiratory tract (LRT) of mice (figure 1a,b), but delayed *B. parapertussis* clearance, resulting in approximately 40-fold higher total *B. parapertussis* lung CFUs (figure 1c,d). Importantly, no evidence to support competitive interactions between *B. pertussis* and *B. parapertussis* was found in either sham- or aP-vaccinated co-infected hosts (figure 1a–d). An aP vaccine-driven reduction in inflammatory cytokine responses (figure 2) as well as neutrophil recruitment to the lung in response to *B. parapertussis* infection (figure 3)—two key players in the clearance of this pathogen (Kirimanjeswara et al. 2005; Mann et al. 2005; Wolfe et al. 2009)—correlated with delayed *B. parapertussis* clearance. In addition, antibody responses in vaccinated *B. parapertussis*-infected hosts, although robust, were likely to have reduced efficacy relative to non-vaccinated hosts owing to species differences in prominent surface molecules preventing immune cross-protection (Wolfe et al. 2007; Zhang et al. 2009a,b).

Thus aP vaccination, by priming the host response against *B. pertussis* clearance, confers an advantage to *B. parapertussis* by interfering with optimal immune clearance and resulting in increased lung CFUs, consistent with the ERH outlined in §1 (Mitchell & Power 2003).

*Bordetella parapertussis* and *B. pertussis* have been thought to compete directly with one another as they exploit the same respiratory tract niche (Bjørnstad & Harvill 2005). However, we found no evidence of within-host competition between *B. parapertussis* and *B. pertussis* in our study: CFUs of each species appeared to be unaffected by the presence of the other (figure 1a–d). The lack of competition probably arose as total infection densities were not constrained in the lung: infection with two species resulted in total pathogen densities twice that of single-species infections. Indeed, by colonizing discrete areas in the LRT, these distinct infections may avoid direct interaction. Another possibility is that by focusing solely on the LRT, we failed to capture within-host competition between *B. parapertussis* and *B. pertussis* in the upper respiratory tract (URT). *Bordetella* infection is initiated by the attachment of organisms to epithelial cell cilia of the URT, a respiratory area that is thought to act as an important reservoir of *Bordetella* infection (Matto & Cherry 2005). Experiments examining the localization of distinct bacterial populations in both the URT and LRT, as well as transmission of bacteria from the respiratory tract (which can be carried out experimentally in rat or rabbit models of bordetellosis), would increase our understanding of colonization and shedding processes respectively, and how these may vary with vaccination status or *Bordetella* species.

What mechanisms are behind the ‘enemy release’ of *B. parapertussis* under aP vaccination and why does wP vaccination or prior exposure to *B. pertussis* not drive similar increases in *B. parapertussis* CFU (Wolfe et al. 2007; Zhang et al. 2009b)? First, robust T_{H1} inflammatory responses and neutrophil recruitment to the LRT are required for optimal anamnestic responses against *B. parapertussis* (Kirimanjeswara et al. 2005; Mann et al. 2005; Wolfe et al. 2005, 2009). Here, we show that aP vaccination skew the host immune response towards a T_{H2} response (Barnard et al. 1996; Ryan et al. 1997) and it is likely that this lack of inflammatory help—reduced lung inflammatory responses and neutrophil recruitment—enables *B. parapertussis* to evade rapid antibody-mediated clearance in our study (Wolfe et al. 2009). Second, omission of the critical protective O-antigen from aP vaccine preparations is also likely to reduce aP vaccine efficacy against *B. parapertussis* (Zhang et al. 2009b) and could contribute towards enhanced infection. Third, aP vaccination may have the potential to provoke immune interference in the form of original antigenic sin. Of those *B. pertussis* antigens contained in the aP vaccine expressed by *B. parapertussis*, antigenic differences exist between the *Bordetella* species and so individuals exposed to a *B. parapertussis* antigen similar, but not identical to one encountered previously, may induce an immune response to the latter antigen directed against the first (Francis 1953; Webster 1966; Klenerman & Zinkernagel 1998). Thus, subunit vaccines with limited epitopes—such as the aP vaccine—may have the potential to prevent appropriate immune responses against challenging *B. parapertussis* bacteria whose epitopes are divergent from those of the vaccine variant and lead to sub-optimal clearance and perhaps enhanced infection.

Importantly, following the effects of aP vaccination on infection dynamics over time allowed us to resolve previously conflicting results concerning the effect of aP on *B. parapertussis* (David et al. 2004; Zhang et al. 2009b).
Specifically, we show that the effect of aP vaccination on *B. parapertussis* infection varied temporally—aP vaccination did not affect *B. parapertussis* lung CFU on day 3 p.i. consistent with Zhang et al. (2009b), but enhanced CFU on day 7 p.i. consistent with David et al. (2004) (figure 1a–d)—which resolves these previously conflicting studies and highlights the importance of tracking dynamics throughout infection in order to capture full effects of the treatment of interest. It is possible that these findings may be relevant only to the specific strains we have examined and further studies should be carried out to determine if our results hold across *B. pertussis* and *B. parapertussis* strains.

As always, it is important to be cautious about extrapolating from animal models to humans. The dynamics of *B. pertussis* and *B. parapertussis* infection in rodent hosts shares many similarities with human infection, but like all experimental models, differs from the human situation in a number of key ways (Elahi et al. 2007). However, the relative efficacies of pertussis vaccines in the rodent model correspond to those obtained in clinical trials (Mills et al. 1998; Guiso et al. 1999), and we note that epidemiological evidence in human whooping cough infections is consistent with an enhancement effect for *B. parapertussis* (Bergfors et al. 1999; Liese et al. 2003). Directly proving aP vaccination puts treated people at risk of acquiring *B. parapertussis* is very difficult, but we hope our study highlights the need for more thorough *B. parapertussis* epidemiological data and encourages further work in this neglected area. If our experiments are capturing the phenomenology of what is happening under aP vaccination in humans, it may be important to consider the introduction of vaccines that better

Figure 4. *Bordetella* species-specific serum Ig titres. Timeline showing titres of (a) anti-*B. pertussis* total Ig and (c) anti-*B. parapertussis* total Ig during single and mixed *B. pertussis* and *B. parapertussis* infections of sham or vaccinated hosts. (a and c) Filled (sham) and open (aP) triangles represent *B. parapertussis*, filled (sham) and open (aP) squares represent *B. pertussis*, and filled (sham) and open (aP) circles represent mixed infections. Shown is the log_{10} transformed mean endpoint titre ± s.e.m. Average Ig titre between days 3–35 p.i. (least-squares mean ± s.e.m.) from (b) GLMs of anti-*B. pertussis* Ig and (d) anti-*B. parapertussis* Ig. The means from 120 independent infections are presented. The x-axis is jittered for clarity and dotted grey lines indicate limit of detection.

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protect against both bordetellae; for example, live attenuated *B. pertussis* nasal vaccines (Mienczak et al. 2006), WP vaccines containing both *B. pertussis* and *B. parapertussis* (Burianova-Vysoka et al. 1970), or supplementation of AP vaccines with *B. parapertussis* protective antigens (Zhang et al. 2009a). An enhanced understanding of the evolutionary consequences of widespread AP vaccination is needed in order to optimize the next generation of vaccination strategies and fully reap the benefits of this powerful medical intervention.

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