

Demonstration of 1-Year Duration of Immunity for Attenuated *Bordetella bronchiseptica* Vaccines in Dogs*

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CLINICAL RELEVANCE

Three groups of healthy dogs with low antibody titers to *Bordetella bronchiseptica* (Bb), canine parainfluenza virus (CPI), and canine adenovirus type 2 (CAV-2) were used in this study. One group was vaccinated with a single dose of monovalent attenuated Bb vaccine and one group with a trivalent vaccine containing attenuated Bb, CPI, and CAV-2; dogs were vaccinated intranasally with a single dose of the respective vaccines. The third group served as unvaccinated controls. All vaccinated dogs subsequently developed serum antibody titers to Bb that persisted for at least 1 year. Following Bb challenge 1 year after vaccination, all vaccinated dogs, regardless of group, showed significantly fewer clinical signs and shed significantly fewer challenge organisms than unvaccinated controls. These results demonstrate that intranasal administration of a single dose of monovalent attenuated Bb vaccine or trivalent vaccine containing attenuated Bb, CPI, and CAV-2 provides 1 year of protection against Bb.

■ INTRODUCTION

Kennel cough is a multifactorial, contagious upper respiratory disease of dogs. *Bordetella bronchiseptica* (Bb) is considered to be the primary etiologic agent,¹⁻³ although other infectious agents, including canine parainfluenza virus (CPI), canine adenovirus type 2 (CAV-

2), and *Mycoplasma* spp, are frequently involved.^{4,5} Vaccination plays a key role in preventing disease. Vaccines containing attenuated, inactivated, or subunit antigens of Bb have been used to control the disease with varying degrees of success.⁶⁻¹² A local immune response in the upper respiratory tract is considered to

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be of primary importance in providing protection against infections with Bb and other upper respiratory pathogens.^{2,7,13} Intranasal vaccination with attenuated Bb vaccines has been shown to be significantly more effective in inducing an antigen-specific mucosal antibody response than a subcutaneously administered antigen extract vaccine, and dogs with mucosal antibodies were protected against virulent Bb challenge.¹² Intranasal vaccination has two additional advantages: It can be administered to young puppies, and maternal antibodies are less likely to interfere with immunization by this route.¹³

Less information is available on the duration of immunity induced by intranasal vaccination. Duration of immunity is an important factor in selecting a vaccine for immunization. Typically, many killed vaccines provide protection for a shorter duration than do attenuated vaccines (with the exception of adjuvanted killed vaccines, such as canine rabies vaccine, which has an immune duration of 3 years or more). Jacobs and colleagues showed that intranasal attenuated Bb vaccination provided a duration of immunity of 1 year.¹¹ Our study extends that finding by comparing a monovalent and three-way combination vaccine for protection against virulent Bb challenge 1 year after vaccination.

■ MATERIALS AND METHODS

Animals and Vaccines

Forty-four 6- to 7-week-old healthy puppies with no history of vaccination with Bb, CPI, or CAV-2 and having low antibody titers to these organisms were randomly divided into three groups:

- One group ($n = 14$) was vaccinated by intranasal administration of a monovalent attenuated Bb vaccine (Intrac, Schering-Plough Animal Health; batch K377A).

- A second group ($n = 15$) was vaccinated with a trivalent vaccine containing attenuated Bb, CPI, and CAV-2 (Intra-Trac 3, Schering-Plough Animal Health; serial 54139).
- A third group ($n = 15$) received no treatment and served as unvaccinated controls.

Dogs in the vaccinated groups were immunized on study day 0 by instilling 0.5 ml of vaccine into one nostril. All dogs were handled under an IACUC-approved protocol and were housed in an isolation facility under controlled environmental conditions throughout the study.

Serum Antibody Measurement

Blood samples were collected from each puppy on study days -15, 0 (before vaccination), 21, 42, 103, 227, 307, and 364, and sera were separated and evaluated for antibody titers to Bb for all three groups. Serial twofold dilutions of test serum, along with known positive and negative sera, were made in a U-bottom microtiter plate, and 0.1 ml of heterologous Bb antigen was added to each well and mixed for 15 to 30 seconds on a microtiter plate shaker. The plates were incubated at $36^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 to 4 hours, stored for 36 to 72 hours at 2°C to 7°C , and read visually for agglutination on a mirror stand. The titer is expressed as the reciprocal of the highest dilution showing complete agglutination.

Challenge

Dogs in all three groups were challenged intranasally with 1.0 ml of a live virulent Bb (strain D-2) culture on 2 consecutive days (days 371 and 372) after vaccination. The challenge dose contained 3.9×10^{10} CFU/ml on first day of challenge and 1.1×10^{10} CFU/ml on second day of challenge. The challenge culture was originally isolated from an infected dog.

Clinical Observation

Dogs were monitored daily for clinical signs for 21 days after challenge. Clinical observations were made at the same time each day. Each dog was observed and assigned a cough score using a previously published numeric scale¹²:

- 0 = No coughing
- 1 = Coughing induced with gentle tracheal palpation
- 2 = Spontaneous or frequent coughing
- 3 = Spontaneous coughing with retching or frequent coughing with retching

Nasal Swab Collection for *Bordetella* Isolation

Nasal swabs were collected from both nostrils of each dog before challenge and on days 6, 9, 16, and 20 after challenge using commercially available calcium alginate culture swabs and transport media. Swabs were frozen immediately on dry ice and stored at -50°C or colder until testing. Thawed nasal swabs were serially diluted 10-fold in tryptose phosphate broth and plated on MacConkey agar (with nitrofurantoin at a concentration of 50 $\mu\text{g}/\text{ml}$) then incubated at $36^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 to 72 hours. Resulting colonies with Bb morphology were counted to determine the CFU/ml. Nitrofurantoin was included in MacConkey agar to differentiate challenge strain from attenuated vaccine strain. The challenge strain is resistant to nitrofurantoin, whereas attenuated vaccine strain is susceptible.

Statistical Analysis

Statistical analysis was performed using SAS version 9.1.3 (SAS Institute, Cary, NC) and

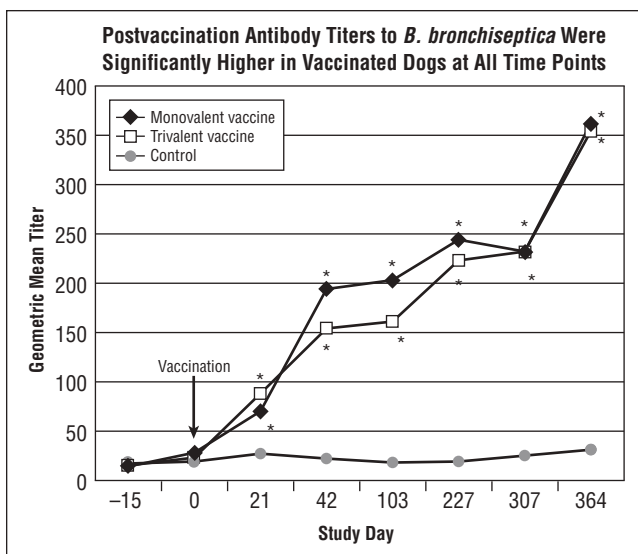


Figure 1. Geometric mean postvaccination antibody titers to *B. bronchiseptica*. *Significantly higher than control ($P < .0001$).

StatXact 6.2.0 (Cytel Corporation, Cambridge, MA). Statistical significance was declared for P values $< .05$.

Clinical cough scores for Bb and bacterial shedding were evaluated using Wilcoxon exact rank sum tests for pairwise comparisons and the Kruskal–Wallis test for overall comparisons. Serology was analyzed using \log_2 values.

RESULTS

Postvaccination antibody titers to Bb were significantly higher ($P < .0001$) at all time points in dogs vaccinated intranasally with either the monovalent attenuated Bb vaccine or the trivalent vaccine containing attenuated Bb, CPI, and CAV-2 compared with controls (Figure 1). Titers persisted in the vaccinated dogs for the entire duration of the 12-month study. Antibody titers in the control group remained low throughout the study period. Steady increases in antibody titers were noticed until day 42 in the vaccinated groups; increases then became more

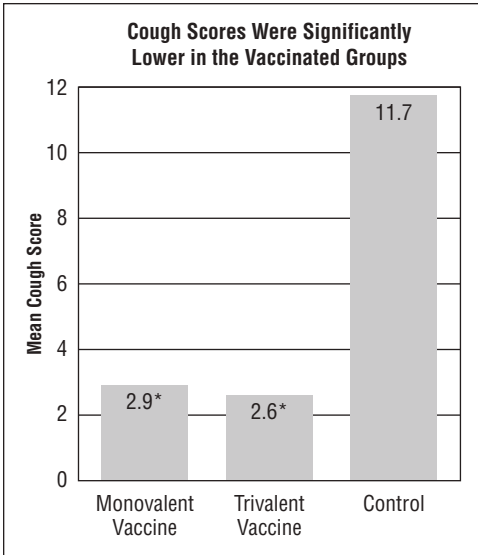


Figure 2. Mean cough scores after *Bordetella* challenge. *Significantly lower than control ($P \leq .0001$).

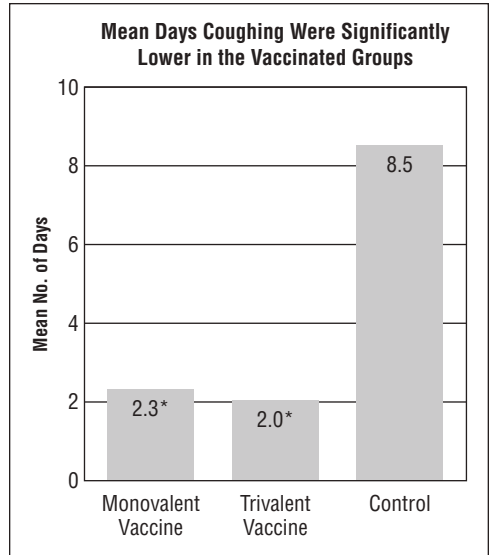


Figure 3. Mean number of days coughing after *Bordetella* challenge. *Significantly fewer than control ($P < .0001$).

gradual until the time of challenge. The increase in antibody titer from day 42 until challenge is not considered significant because the increase is less than twofold. The dogs in this study were kept in isolation (biosafety level 2) to prevent natural Bb exposure. The sustained antibody response over the 1-year period appears to be due to vaccination and not to a booster effect as a result of low-level natural exposure; this was supported by lack of isolation of Bb in nasal swabs collected before challenge.

After challenge, 93% of unvaccinated control dogs developed coughing and one dog progressed to a persistent cough with retching (score, 3). The coughing persisted in 10 dogs (67%) in the control group through the end of 21-day observation period. Four of 14 dogs (29%) in the monovalent vaccine group and 3 of 15 dogs (20%) in the trivalent vaccine group showed no coughing at any time after challenge, and none of the vaccinated dogs had a cough score of 3. Five dogs (33%) in the

trivalent vaccine group coughed on palpation (score, 1) on only 1 day during the entire observation period. Only two dogs in each vaccinated group showed coughing on day 21, and that was only on palpation. Total mean cough scores during the 21-day postchallenge observation period were 2.9, 2.6, and 11.7 for dogs in the monovalent vaccine group (Intrac), trivalent vaccine group (Intra-Trac 3), and control group, respectively (Figure 2). Cough scores for both vaccinated groups were significantly lower compared with controls ($P \leq .0001$).

Vaccinated dogs coughed for an average of 2 days compared with 9 days for unvaccinated controls (Figure 3). The mean days coughing for both vaccinated groups was significantly lower compared with controls ($P < .0001$).

Dogs in both vaccinated groups shed significantly fewer Bb organisms after challenge compared with the controls ($P < .0001$; Figure 4). Significantly ($P < .0001$) more dogs in the control group (100%) were still shedding chal-

challenge organisms at the conclusion of the 21-day observation period compared with 21% of dogs in the monovalent vaccine group and 0% of the dogs in the trivalent vaccine group.

DISCUSSION

These results demonstrate that intranasal vaccination of dogs with a monovalent attenuated Bb vaccine or a trivalent vaccine containing attenuated Bb, CPI, and CAV-2 provided at least a 1-year duration of immunity against Bb. The protection was demonstrated by significantly lower coughing scores, fewer days of coughing, and reduced shedding of challenge organisms compared with unvaccinated control dogs. This finding is consistent with findings from a previous study that used an attenuated vaccine.¹¹

This study also shows that intranasally vaccinated dogs develop serum antibody titers that persist for at least 1 year after vaccination. These results confirm an earlier study showing that this attenuated vaccine induced high levels of circulating antibodies that persisted for 9 weeks after vaccination¹² and suggest that intranasal administration of these attenuated vaccines induces a systemic immune response as indicated by the presence of circulating antibodies. An earlier study also found that intranasal vaccination induced high levels of antigen-specific mucosal IgA in nasal secretions, whereas a subcutaneously injected antigen extract vaccine failed to induce a similar mucosal response.¹² There seems to be a good correlation between Bb-specific IgA in nasal secretions and protection against Bb.^{7,12} The mucosal immune response is believed to play a key role in providing protection against respiratory surface pathogens.^{2,7,13}

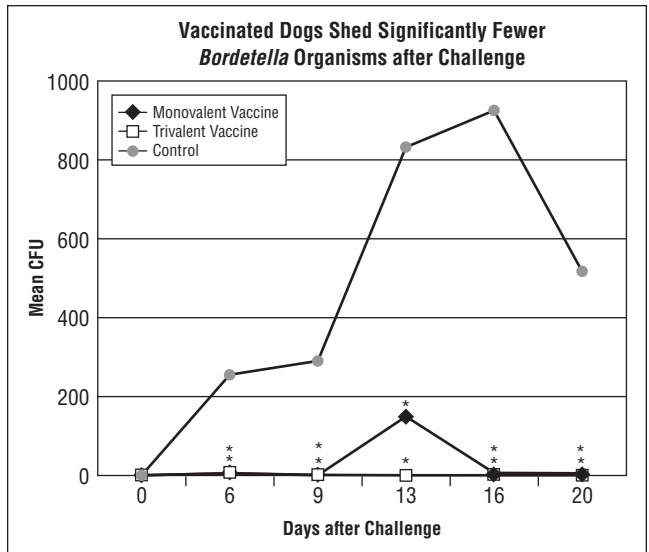


Figure 4. Nasal shedding of challenge organisms after *Bordetella* challenge. *Significantly lower than control ($P < .0001$).

Bb is considered to be one of the primary etiologic agents involved in the respiratory disease complex in dogs.^{1,3} The disease is exacerbated by co-infection with other infectious agents such as CAV and CPI.^{4,5} Therefore, a combination vaccine comprising all key antigens involved in the respiratory disease complex provides a more complete approach to the management of kennel cough.

Investigators have shown that the Bb organisms can be shed for 3 months after infection.¹³ In our present study, all control dogs were still shedding high levels of bacteria 3 weeks after challenge compared with 3 dogs in the monovalent vaccine group and none in the trivalent vaccine group. Our findings suggest that a single vaccination can minimize the colonization of virulent bacteria when challenged 1 year after vaccination, which is consistent with an earlier published study¹¹ and extends that research by demonstrating duration of immunity with a trivalent formulation. The mecha-

nism of protection is likely associated with increased levels of nasal mucosal antigen-specific immunoglobulins preventing colonization of the pathogen, thereby preventing replication of bacteria. Colonization and subsequent shedding are likely to be important factors in propagation of a canine infectious tracheobronchitis disease outbreak, and it has been recommended that affected animals be isolated.⁶ Shedding of virulent organisms could also potentially increase the risk for Bb exposure in animal owners, who may be immunologically compromised. Therefore, intranasal vaccination appears to provide greater disease protection and to reduce the risk of exposure to susceptible dogs and people by reducing the environmental contamination with virulent Bb after a disease challenge.

■ CONCLUSION

This study provides clear evidence that intranasal vaccination with attenuated Bb alone or in combination with CPI and CAV-2 provides at least a 1-year duration of immunity.

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