Comparison of the Mucosal Immune Response in Dogs Vaccinated with Either an Intranasal Avirulent Live Culture or a Subcutaneous Antigen Extract Vaccine of *Bordetella bronchiseptica*

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**INTRODUCTION**

Infectious canine tracheal bronchitis (also known as canine cough or kennel cough) is a common upper respiratory disease of dogs. It may occur as a result of *Bordetella bronchiseptica* infection only but is more frequently a complex disease involving upper respiratory infection with various potential pathogens.\(^1\) However, *B. bronchiseptica* is considered to be a common cause of canine upper respiratory infections,\(^2\) and vaccination to induce protection against this organism is a cornerstone in the

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**CLINICAL RELEVANCE**

Healthy dogs with low antibody titer to *Bordetella bronchiseptica* were vaccinated intranasally with an avirulent live vaccine, subcutaneously with an antigen extract vaccine, or subcutaneously and intranasally with a placebo. Intranasally vaccinated dogs developed *B. bronchiseptica*–specific IgA titers in nasal secretions that remained at high levels until the end of the study; dogs vaccinated subcutaneously with the antigen extract or placebo did not develop measurable antigen-specific IgA titers in nasal secretions. Dogs were challenged with virulent live *B. bronchiseptica* 63 days after vaccination. Intranasally vaccinated dogs had significantly lower cough scores (\(P \leq .0058\)) and shed significantly fewer challenge organisms (\(P < .0001\)) than dogs in either of the other groups. Cough scores of subcutaneously vaccinated dogs were not significantly different from placebo-vaccinated dogs.

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prevention of infectious canine tracheal bronchitis. Currently, veterinarians in the United States may select from two general approaches when immunizing dogs against this specific organism: an avirulent live culture inoculated intranasally or an antigen extract vaccine administered subcutaneously.

Local immunity is considered to be of primary importance in providing protection against infections with *B. bronchiseptica* and other upper respiratory pathogens, and intranasal vaccination has been thought to be more effective in inducing local mucosal immunity. For example, intranasal vaccination with an avirulent live culture induced detectable clinical protection against challenge with virulent *B. bronchiseptica* as early as 48 hours after administration in dogs. This study also reported indirect immunofluorescence evidence of *B. bronchiseptica*–specific secretory IgA in nasal secretions at postvaccination day 4. Furthermore, intranasal vaccination against a different canine upper respiratory pathogen, canine parainfluenza virus, with a modified-live virus vaccine has been shown to reduce postchallenge shedding of virus compared with subcutaneous or intramuscular vaccination.

In contrast, two other studies raise questions regarding the comparative efficacy of intranasally and parenterally (intramuscular in one study and subcutaneous in the other) administered vaccines against *B. bronchiseptica* in dogs. One study found no significant difference in clinical signs on postchallenge days 4 to 10 between puppies vaccinated 14 days previously with either an intranasal avirulent live *B. bronchiseptica* or an intramuscularly administered *B. bronchiseptica* bacterin. The other study reported that intranasal vaccination of *B. bronchiseptica*–seropositive dogs induced a significantly lower serum IgG response than vaccination with a subcutaneously administered *B. bronchiseptica* bacterin. The latter study also reported that dogs receiving the subcutaneous vaccine had significantly higher serum IgA levels than dogs receiving an intranasal vaccine; the significance of this finding is unclear because serum IgA concentrations are thought to be poor indicators of mucosal secretion and IgA is considered to have evolved primarily to protect body surfaces.

When administered by the intranasal route, avirulent live vaccines are expected to colonize nasal mucosa, which could result in stimulation of mucosal IgA response. The relationship between intranasal administration of a live vaccine and parenteral administration of an inactivated vaccine in inducing IgA response and protection has not been clearly established in a controlled study. Therefore, the study reported here was designed to evaluate whether an avirulent live vaccine administered by intranasal route induces a similar humoral and mucosal IgA response compared with an inactivated vaccine administered parenterally. An additional objective of this study was to evaluate whether any correlation exists between protection and mucosal IgA response.

**MATERIALS AND METHODS**

**Animals and Vaccines**

Thirty-five 9- to 10-week-old healthy beagle puppies with no history of *B. bronchiseptica* vaccination and with a low level of antibodies against this organism on a serum agglutination assay were randomly assigned to one of three groups. A nasal swab and a nasal lavage sample were collected from each dog before vaccination. Dogs in each group were then vaccinated on day 0 with one of the following products:

- A modified-live trivalent intranasal vaccine (Intra-Trac 3, Schering-Plough Animal Health; serial 54119; expiration date: February 04, 2007) containing avirulent live *B. bronchiseptica* culture, modified-live canine
parainfluenza virus, and modified-live canine adenovirus type 2

- A killed subcutaneous antigen extract vaccine (Bronchicine CAe; Pfizer Animal Health; serial B570229A; expiration date: April 18, 2007), followed by a second vaccination 21 days later as recommended on the product's package insert
- A saline placebo administered both intranasally and subcutaneously

No personnel making clinical observations had any knowledge of the treatment group assignments. All dogs were handled under an Institutional Animal Care and Use Committee approved protocol.

**Mucosal IgA Measurement**

Nasal lavage samples were collected from each puppy 5 days before vaccination (day −5) and on days 14, 21, 28, 42, and 56 after vaccination. These samples were collected by administering light anesthesia to each dog; then, with the dog in sternal recumbency, a flexible rubber catheter was inserted into the nasal cavity and 5.0 ml of warm sterile normal saline was flushed into the nasal cavity and the effluent collected. Samples were centrifuged (2,500 ×g at 2˚C to 10˚C for 10 minutes) and the supernatant was saved. Preservatives were added to achieve a final concentration of 0.01% thimerosal and less than 0.25 mg/ml gentamicin, and the samples were dispensed into 0.2- to 1.0-ml aliquots and frozen at −50˚C or below until testing. At testing, IgA antibody titers to the heterologous D-2 *B. bronchiseptica* strain were determined by indirect ELISA. Briefly, two-fold dilutions of lavage fluid were added to a microtiter plate coated with washed, sonicated *B. bronchiseptica* antigen. After washing, the plate was incubated with peroxidase-conjugated anti-dog IgA and dye substrate was added. The reaction was stopped and the optical density read at 450 nm. Titer was determined based on the reciprocal of the highest dilution showing optical density above background and was then normalized based on the response of a positive-control nasal lavage sample on each plate.

**Serum Antibody Measurement**

Serum samples were collected from all dogs in the study on days 0 (before vaccination), 28, 42, 62, and 84 (the last sample was taken 21 days after challenge) for measurement of heterologous serum titers to *B. bronchiseptica*. Two-fold serial dilutions of test serum, along with known positive and negative serum were made in a U-bottom microtiter plate; 0.1 ml of heterologous *B. bronchiseptica* antigen was added to each well and mixed for 15 to 30 seconds on a microtiter plate shaker. The plates were incubated at 36˚C (±2˚C) for 2 to 4 hours, stored for 36 to 72 hours at 20˚C to 28˚C, and read visually on a mirror stand for agglutination. The titer is expressed as the reciprocal of the highest dilution showing complete agglutination.

**Challenge**

All dogs were challenged intranasally with 1.0 ml of a live virulent *B. bronchiseptica* (strain D-2) culture on day 63 after vaccination.
Clinical Observation
Dogs were observed before challenge, on the day of challenge, and then daily for 21 days, with clinical observations made at the same time each day. Each dog was observed and assigned a cough score as follows:

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

Nasal Swab Collection for Bacterial Isolation
Nasal swabs were collected from both nostrils of each dog on postchallenge days 3, 7, 10, 14, 17, and 21 using commercially available calcium alginate culture swabs and transport media. Swabs were frozen immediately on dry ice and stored at −50°C or below until testing for isolation. Thawed nasal swabs were serially diluted 10-fold in tryptose phosphate broth and plated on MacConkey agar (with nitrofurantoin) then incubated at 36°C (±2°C) for 24 to 72 hours. Resulting bacterial colonies were counted to determine the CFU/ml.

Statistical Analysis
Clinical cough scores and bacterial shedding were statistically compared using the Wilcoxon exact rank sum tests for pairwise comparisons and the Kruskal-Wallis test for overall comparisons. Serology was analyzed using log₂ values. Statistical analyses were performed using SAS (version 8.2, SAS Institute, Cary, NC). Statistical significance was declared for P values < .05.

RESULTS
Dogs vaccinated intranasally with a modified-live avirulent culture developed a significantly higher B. bronchiseptica–specific mucosal IgA response to heterologous antigen (Figure 1) that persisted for the duration of the study. The mucosal IgA response in nasal secretions was significantly higher in the intranasally vaccinated group than in either the antigen extract or placebo vaccine on days 42 and 56 (P ≤ .0002). Dogs vaccinated with either the antigen extract vaccine or the placebo failed to develop a measurable increase in their nasal mucosal B. bronchiseptica IgA levels, and there were no significant differences between these two groups at any time point.

Figure 1. B. bronchiseptica–specific IgA antibody in nasal secretions of dogs vaccinated with either an intranasal avirulent live, subcutaneous antigen extract, or placebo vaccine. *Asterisks indicate a significant difference in the intranasal group compared with the placebo or antigen extract vaccine group.

Dogs receiving the intranasal vaccination also developed a significant increase ($P < .0001$) in serum antibody titer against a heterologous *B. bronchiseptica* strain following vaccination, whereas antigen extract– and placebo-vaccinated dogs had no change in serum titer (Figure 2) until after the challenge was administered on day 63.

Mean postchallenge cough scores (Figure 3) were significantly lower (i.e., coughing was less severe) in the group vaccinated with the intranasal avirulent live vaccine compared with the groups receiving either antigen extract vaccine ($P = .0009$) or placebo ($P = .0058$). Cough scores in the antigen extract–vaccinated dogs were not significantly different from scores in the placebo group ($P = .5120$). The proportion of dogs that showed the highest coughing scores (frequent coughing and/or coughing with retching [score $\geq 2$]) was 8.3% for intranasally vaccinated dogs, 63.6% for antigen extract–vaccinated dogs, and 58.3% for placebo-vaccinated dogs. The total days of coughing (Figure 4) were also significantly lower for the intranasal vaccine group (mean: 1.3 days) compared with either the antigen extract (mean: 5.9 days; $P = .0016$) or placebo (mean: 4.8 days; $P = .0090$) vaccinated group. There were no significant differences between the placebo- and antigen extract–vaccinated groups ($P = .4370$).

Dogs vaccinated with the intranasal vaccine shed significantly fewer organisms following challenge compared with the other groups, with only four dogs (33%) shedding low levels ($<10^2$ CFU/ml) of bacteria in nasal swab samples at one time point each, and had a significantly lower geometric mean shedding CFU/ml (Figure 5) compared with the other two groups ($P < .0001$). All dogs from the antigen extract–vaccinated group and 11 of 12 (92%) placebo-vaccinated dogs shed *B. bronchiseptica* organisms at one or more time points after challenge, with the greatest number of organisms ($10^5$ CFU/ml) detected in a subcutaneously administered antigen extract–vaccinated dog at 21 days postchallenge.

**DISCUSSION**

These results show that intranasal vaccination of dogs with avirulent live *B. bronchiseptica* leads to the production of a significantly higher level of antigen-specific mucosal IgA immunoglobulins compared with vaccination with a subcutaneously injected antigen extract vaccine. Additionally, intranasal vaccination...
provides significant protection against challenge compared with vaccination with a subcutaneously administered antigen extract vaccine, as shown by significantly lower coughing scores, fewer days of coughing, and less bacterial shedding following intranasal challenge with a virulent *B. bronchiseptica* culture. These results suggest that there is good correlation between *Bordetella*-specific IgA in nasal secretions and protection against challenge with this organism. This finding is consistent with the documented role of mucosal IgA in providing protection against respiratory surface pathogens.\(^\text{11}\)

Antigen-specific IgA levels were measured in canine nasal secretions using an ELISA technique developed and validated for this study. However, a previous study\(^\text{5}\) using an immunofluorescence assay detected antigen-specific IgA 4 days after intranasal vaccination in dogs. The early detection of specific IgA might have occurred because dogs in the previous study had higher nasal secretion titers as a consequence of prior exposure to *B. bronchiseptica*. An additional study is under way to evaluate the timing of nasal secretion antibody detection in dogs vaccinated intranasally with antigens available in other avirulent live intranasal vaccines commercially available in the United States.

A previous study\(^\text{5}\) reported that dogs could be protected as early as 48 hours after intranasal vaccination, suggesting that protection against challenge can occur sooner than the onset of the measurable adaptive immunologic response seen in this study. This may indicate that induction of nonspecific protective mechanisms is responsible for the early onset of protection.

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**Figure 3.** Mean cough scores following challenge with virulent *B. bronchiseptica*. *Asterisk indicates a significant difference in the intranasal group compared with the placebo or antigen extract vaccine group.**
after intranasal vaccination; however, the present study was not designed to determine the timing of onset of immunity. Evidence for rapid development of nonspecific protective mechanisms following intranasal vaccination has been seen in association with live attenuated influenza vaccination of humans, as shown by a significant postvaccinal increase in production of interferon-γ, a cytokine thought to play an important role as the first line of defense against influenza infection.

Subcutaneous vaccination of dogs with two doses of antigen extract failed to induce mucosal antibody in nasal secretions or an antibody titer in serum (Figures 1 and 2). The reason for this failure is unknown, although it is unlikely to be a result of maternal antibody interference because the dogs in this study had a low *B. bronchiseptica* titer before vaccination and a booster vaccination was administered 3 weeks after the initial immunization. However, the lack of specific antibody response is consistent with the observation that subcutaneously vaccinated dogs were not protected against *B. bronchiseptica* infection because they fared no better after challenge than placebo-vaccinated dogs as measured by postchallenge cough scores. The lack of protection observed is further support for the established immunologic roles of mucosal IgA in protecting the canine upper respiratory tract against infection and disease caused by mucosal surface pathogens. The protective value of subcutaneously administered antigen extract vaccine could not be demonstrated during this study. Parenteral immunization has generally been considered to be ineffective at eliciting a secretory IgA response. Further work is needed to evaluate the mucosal response following parenteral vaccination in previously immunized animals.

The results of this study are not consistent with data reported in two previous investigations that compared immunity against *B. bronchiseptica* induced by either an intranasal or injectable vaccine. However, these earlier studies used a bacterin vaccine (CoughGuard, Pfizer Animal Health; no longer commercially available in the United States) that likely has significant formulation differences. Additionally, one of these studies used an intramuscular administration route that may have affected the results, whereas the antigen extract vaccine used in this study is labeled for subcutaneous administration only.

The earlier studies comparing intranasal and bacterin vaccination of dogs found inconsistent effects of administration of these vaccines on *B. bronchiseptica*–reactive salivary IgA (nasal mucosal IgA levels were not evaluated). One study found no significant differences in salivary IgA over time or among groups after vaccination of dogs previously exposed to *B. bronchiseptica*, however, the other study found
significantly higher salivary IgA concentrations following either intramuscular bacterin vaccination or intranasal vaccination. The significance of salivary IgA levels for protection is not clear; considering the evidence for the compartmentalization of mucosal immune responses, it is likely that salivary IgA levels do not correlate with IgA levels in nasal secretions.

The data in the present study do not assess the duration of immunity following vaccination or the immunologic effects of intranasal vaccine administration to previously exposed dogs. This study shows, however:

- Intranasal vaccination induced elevated levels of antigen-specific IgA in nasal secretions.
- IgA levels in intranasally vaccinated dogs remained high for at least 56 days after vaccination (Figure 1), the last sampling time point in this study.

- Intranasal vaccination provided good protection against challenge 63 days after vaccination.

The present study showed that dogs challenged following intranasal vaccination shed significantly fewer challenge bacteria after challenge (Figure 5; \( P < .0001 \)) when compared with bacterial shedding from dogs that received the subcutaneous antigen extract vaccine. This finding is consistent with earlier published studies comparing the level of virus shedding following challenge in dogs vaccinated with either an intranasal or an injectable avirulent live canine parainfluenza virus vaccine. The mechanism is likely associated with increased levels of nasal mucosal antigen-specific immunoglobulins preventing pathogen colonization and replication. Bacterial colonization and subsequent shedding is likely to be an important factor in propagation of a canine infectious tracheobronchitis disease outbreak, and it has been recommended that affected animals be isolated. Therefore, intranasal vaccination appears to offer a greater benefit in outbreak management by reducing shedding and subsequently reducing the risk of exposure for susceptible animals.

**CONCLUSION**

This study presents clear evidence that intranasal vaccination with an avirulent live culture of *B. bronchiseptica* provides a specific local immune response that is significantly more effective at inducing protection against challenge with virulent organisms than is subcutaneous vaccination with an antigen extract vaccine.
REFERENCES


