Comparative efficacy of an injectable vaccine and an intranasal vaccine in stimulating *Bordetella bronchiseptica*-reactive antibody responses in seropositive dogs

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Objective—To compare antibody responses to intranasal and SC *Bordetella bronchiseptica* vaccines in seropositive dogs.

Design—Randomized controlled study.

Animals—40 young adult Beagles vaccinated against *B bronchiseptica*.

Procedure—Dogs were randomly assigned to 1 of 4 groups (intranasal vaccine, SC vaccine, intranasal and SC vaccines, no vaccine) and vaccinated on day 0. Serum and salivary *B bronchiseptica*-reactive antibody responses were measured on days 0 through 7, 10, 14, 21, and 28.

Results—Dogs that were vaccinated with the SC vaccine, alone or in combination with the intranasal vaccine, had a significant increase in serum concentration of *B bronchiseptica*-reactive IgG beginning on day 5 and persisting through day 28. Dogs that were vaccinated with the intranasal vaccine alone had a significant increase in serum concentration of *B bronchiseptica*-reactive IgG beginning on day 10 and persisting through day 28, but serum IgG concentration in these dogs was significantly less than concentration in dogs that received the SC vaccine. Neither vaccine had a demonstrable effect on salivary concentrations of *B bronchiseptica*-reactive IgA or IgG. On day 10, all vaccinated groups had significantly higher serum IgA concentrations than did unvaccinated control dogs.

Conclusions and Clinical Relevance—Results suggest that the SC *B bronchiseptica* vaccine may be used to stimulate antibody responses in seropositive dogs. There was no apparent benefit to administering these vaccines simultaneously. Intranasal vaccines may not be effective for booster vaccination of dogs previously exposed to or immunized against *B bronchiseptica*. Dogs should be vaccinated at least 5 days prior to exposure to *B bronchiseptica*. (*J Am Vet Med Assoc* 2002;220:43–48)

Bordetella bronchiseptica is recognized as an important cause of respiratory tract disease in dogs.[1-3] Infectious tracheobronchitis or "kennel cough" caused by *B bronchiseptica* acting alone or together with respiratory viruses and other bacterial pathogens occurs worldwide, and the condition is particularly common when dogs are group housed, such as in kennels and veterinary hospitals.[1-3]

Single and combination vaccines to control *B bronchiseptica* infection have been available for more than 20 years and have generally been shown to be efficacious, at least in reducing the severity of disease associated with infection.[4-8] Results of a recent challenge study[9] using a field isolate of *B bronchiseptica* suggested that administration of intranasal and IM vaccines in sequence engendered superior immunity in naïve puppies, compared with administration of either vaccine alone. These results, as well as data from a recent study[10] in humans indicating that combining SC or IM vaccine administration with intranasal vaccine administration can enhance the overall immune response against influenza, suggest that efficacy of *B bronchiseptica* vaccines could be improved by administering intranasal and SC or IM vaccines simultaneously, especially in instances when rapid development of immunity is desired.

The duration of vaccine-induced immunity is currently an area of great interest and concern in veterinary medicine.[11] At present, little is known about the duration of immunity induced by most veterinary vaccines, including those for *B bronchiseptica*, although it is generally assumed that intranasal *B bronchiseptica* vaccines confer a shorter duration of clinical protection than do SC or IM vaccines.[12] From a practical standpoint, the interval between vaccination and the development of protective immunity is often as much of a concern as the duration of immunity, especially in situations when dogs are kenneled with little advance notice. It has generally been assumed that intranasal vaccines are more effective at rapidly stimulating immunity; however, there are virtually no comparative studies examining this assumption in seropositive dogs. The purposes of the study reported here were to compare antibody responses to intranasal and SC *B bronchiseptica* vaccines in seropositive dogs and to determine whether simultaneous administration of these vaccines potentiated the rate and magnitude of serum and mucosal antibody responses.

Materials and Methods

Dogs—Forty young adult (approx 1 year old) male and female Beagles from a laboratory colony were used in the study. Dogs had been vaccinated against canine distemper,

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parvovirus infection, adenovirus infection, parainfluenza infection, and leptospirosis at regular intervals but had not received any vaccinations during the 21 days prior to initiation of the study. There was a variable history of vaccination against *B bronchiseptica*, but none of the dogs had received any *B bronchiseptica* vaccines during the 90 days prior to initiation of the study. Dogs had no history of respiratory tract disease (defined as persistent coughing with or without ocular or nasal discharge or a rectal temperature > 39.7 C [103.5 F] for > 3 consecutive days).

Dogs were individually housed in stainless steel cages in 2 biosafety level-1 containment rooms. General procedures for dog care and housing met current Association for Assessment and Accreditation of Laboratory Animal Care standards, guidelines published in the *Guide for Care and Use of Laboratory Animals*, and requirements of the Animal Welfare Act. Dogs had ad libitum access to water and were fed a dry commercial canine diet once daily in quantities sufficient to meet the nutritional requirements of an average Beagle. Dogs were returned to the colony at the end of the study.

Experimental design—On study day –7, a physical examination, including thoracic auscultation, measurement of rectal temperature, and assessment of the general physical condition, was performed by a veterinarian to ensure that dogs were healthy and free of respiratory tract disease. A venous blood sample was collected for determination of serum concentration of IgG against *B bronchiseptica*. Deep pharyngeal (tonsillar) swab specimens were collected and submitted for isolation of *B bronchiseptica*. Saliva swab specimens were collected and submitted for determination of salivary (mucosal) concentrations of IgA and IgG against *B bronchiseptica*. Saliva was collected on cotton-tipped swabs that were placed in 0.5 ml of saline (0.9% NaCl) solution, as described.[13]

Dogs were randomly assigned to 10 blocks of 4 dogs each on the basis of serum concentration of *B bronchiseptica*-reactive IgG and pharyngeal carriage of *B bronchiseptica* on day –7. Dogs were assigned to treatment groups of 10 dogs each and to rooms on day –1 in a randomized complete block design. Group-1 dogs were not vaccinated. Group-2 dogs were vaccinated with an injectable *B bronchiseptica* vaccine[a] that was administered SC. Group-3 dogs were vaccinated with an intranasal *B bronchiseptica* vaccine.[b] Group-4 dogs were vaccinated with the SC vaccine followed by the intranasal vaccine. Dogs in groups 2, 3, and 4 were vaccinated in group order to minimize the chances of accidental exposure to live *B bronchiseptica* in the intranasal vaccine. All vaccines were administered according to label directions. The SC vaccine was administered in the dorsal thoracic area. Latex examination gloves were worn by all personnel during the vaccination process and were changed after handling and vaccination of each dog. To further minimize the possibility of transmission of *B bronchiseptica* in the intranasal vaccine, all dogs in each room on days –1 through 3 were from the same treatment group. To minimize the possibility of a room effect, dogs were moved after day 3 so that on days 4 through 28 both rooms contained dogs from all 4 treatment groups. On days –1 and 4, dogs were randomly assigned to cages in the 2 rooms.

Dogs were observed clinically before and approximately 8 hours after vaccination on day 0. Dogs were specifically observed for evidence of coughing, sneezing, vomiting, diarrhea, abnormal nasal or ocular discharges, urticaria, pruritus, erythema, edema, lethargy, tremors, and seizures, but any abnormality that was observed was recorded. Dogs were observed once daily on days –7 through –1 and days 1 through 28, and observations were recorded. General health observations included, but were not limited to, observations of general physical appearance and behavior, abnormalities of food and water consumption, and appearance of urine and feces. Any abnormalities were recorded. A physical examination was performed at the discretion of an attending veterinarian. Results of all physical examinations were recorded for each dog.

Pharyngeal swab specimens were collected on day 28 and submitted for isolation of *B bronchiseptica*. Blood samples for determining serum antibody concentrations were obtained by means of jugular or cephalic venipuncture prior to vaccination on day 0 and on days 1 through 7, 10, 14, 21, and 28. Swab samples of saliva were collected prior to vaccination on study day 0 and on study days 1 through 7, 10, 14, 21, and 28 and submitted for determination of concentrations of mucosal IgA and IgG reactive with *B bronchiseptica*. Serum and saliva samples were stored at –20 C until tested. All assays were performed in a single set at the termination of the study. Persons conducting testing procedures had no knowledge of treatment group.

Quantitation of *B bronchiseptica*-reactive antibodies—Concentrations of IgG and IgA reactive with *B bronchiseptica* were measured with ELISA performed as described, with minor modifications.[9,14] Briefly, 96-well flat-bottomed microtitration plates[c] were coated overnight at 20 C with washed sonicated *B bronchiseptica* in carbonate coating buffer (7.5 µg/well). The bacterial antigen was prepared from confluent 24-h cultures of the Regina-1 isolate[9]; organisms had been suspended in saline solution, and aliquots had been frozen at –70 C until used. The optimal dilution of antigen had been determined in a standard checkerboard design, using serum from immune and nonimmune dogs.[9] The coating antigen was removed from the wells, and the plates were washed by immersion in double-distilled water containing 0.05% Tween 20. The wells were filled with **phosphate-buffered saline (PBS)** solution containing 0.05% Tween 20 and 0.1% gelatin, and plates were incubated for 30 min at 37 C. Test serum, diluted 1:50 with PBS solution containing 0.05% Tween 20 and 0.2% gelatin, was added to replicate wells. Saliva samples were added undiluted to wells. Plates were incubated at 37 C for 1 hour and washed, and peroxidase-conjugated goat anti-canine IgG or IgA[d] diluted in PBS solution with 0.05% Tween 20 and 0.2% gelatin was added to the wells. Plates were again incubated for 1 hour and washed, and peroxidase substrate was added to the wells according to the manufacturer's instructions.[e] Controls included serum and saliva from unvaccinated dogs (negative controls) and serum from dogs vaccinated against and challenged with *B bronchiseptica* (positive controls)[9]; blanks wells containing PBS solution with 0.05% Tween 20 and 0.2% gelatin were also used.

Optical density (OD) values for test samples were converted to ELISA units, using a modification of a described procedure.[15] Calculations were performed with a computerized program[f]; the following formulas were used:

$$\text{Net OD} = \text{Mean OD of control or test sample wells} - \text{mean OD of blank wells.}$$

$$\text{ELISA units} = 100 \times \frac{\text{Net OD of sample well} - \text{net OD of negative control well}}{\text{Net OD of positive control well} - \text{net OD of negative control well}}$$

Bacterial culture—Standard selective and semiquantitative bacterial culture methods were used to isolate *B bronchiseptica* from pharyngeal swab specimens.[9,16,17] Briefly, swabs were used to inoculate 1 quadrant of complete blood agar, MacConkey agar, and peptone agar plates. A sterile bacteriologic loop was then used to sequentially streak the 4 quadrants of the plate, without flaming the loop between quadrants. Plates were incubated at 37 C and observed after 24 and 48 hours for growth of microorganisms. Colonies that

grew as nonlactose fermenters on MacConkey agar and as blue colonies on peptone agar were considered likely to be *B bronchiseptica*. To confirm that colonies were *B bronchiseptica*, typical colonies were subcultured on blood agar plates and tested with identification strips.[g]

Statistical analyses—Response variables (serum IgG concentration and salivary IgA and IgG concentrations on days 4 through 28 and serum IgA concentration on days 4, 6, and 10) were analyzed with commercial software,[h] using general linear repeated-measures mixed models[18] that included terms for block, treatment, block by treatment, day of study, treatment by day of study interaction, and residual as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_{j(i)} + \alpha_i\beta_j + \delta_k + \beta\delta_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} = response variable, μ = overall constant, α_i = random effect of ith block, β_j = fixed effect of jth treatment, $\alpha_{j(i)}$ = random effect for testing treatment (animal within treatment combination), δ_k = fixed effect of kth day of study, $\beta\delta_{ik}$ = fixed effect of treatment by day of study interaction, ε_{ijk} = random residual for testing day of study treatment by day of study. One-degree-of-freedom contrasts among treatments were made for each study day if significant ($P < 0.05$) effects for treatment or treatment by day of study interaction were detected.

Values for ELISA units were transformed to the natural logarithm scale before analysis, and the treatment by day of study least square means were back transformed to geometric means. This transformation resulted in residuals that were more normally distributed and for which the variance was stabilized.

Bacterial growth on peptone agar plates was categorized as follows: 0 = no growth; 1 = growth in the first quadrant; 2 = growth in the first and second quadrants; 3 = growth in the first, second, and third quadrants; 4 = growth in all quadrants. Results for bacterial growth were analyzed with the Cochran-Mantel-Haenszel statistic, which essentially is a χ^2 test adjusting for blocking. If treatment effects were significant ($P < 0.05$), pairwise treatment differences were examined with the Cochran-Mantel-Haenszel statistic.

Descriptive statistics (mean and range) were calculated for continuous physical examination response variables (rectal temperature, pulse rate, and respiratory rate), and frequency distributions were constructed for categorical physical examination response variables (clinical observations, general health observations, pharyngeal swab specimens).

Number of days from the last *B bronchiseptica* vaccination to the start of the present study (day –7) was calculated for each dog; values were analyzed with a general linear mixed model to test for differences among treatment groups. Values were also tested for a correlation with initial *B bronchiseptica*-reactive serum IgG concentrations.

To determine whether there was a relationship between serum IgG or IgA concentration at the time of vaccination and peak IgA and IgG responses, product moment correlations were calculated within each treatment group. Four correlations were examined: IgG concentration at vaccination with IgA concentration at vaccination, IgG concentration at vaccination with peak IgA concentration, peak IgG concentration with IgA concentration at vaccination, and peak IgG concentration with peak IgA concentration.

Results

Clinical signs—No adverse effects were identified following administration of the vaccines to the dogs. None of the dogs had signs of respiratory tract disease or of any other clinically important disease during the course of the study.

Quantitation of *B bronchiseptica*-reactive antibodies—On days –7 and 0, dogs had variable serum concentrations (range, 33 to 158 units) of *B bronchiseptica*-reactive IgG. The general linear model analysis indicated no difference among treatment groups in regard to number of days since the last *B bronchiseptica* vaccination (range, 287 to 320 days), and number of days since the last *B bronchiseptica* vaccination was not significantly (r^2= –0.4; $P \leq 0.817$) correlated with initial serum *B bronchiseptica*-reactive IgG concentration.

Beginning on day 5 and day 6, respectively, and continuing to the end of the study, dogs in groups 2 and 4 had significantly higher serum concentrations of *B bronchiseptica*-reactive IgG than did dogs in groups 1 and 3 (**Fig 1**). Beginning on day 10 and continuing through day 28, dogs in group 3, which had received the intranasal vaccine alone, had significantly higher serum concentrations of *B bronchiseptica*-reactive serum IgG than did the unvaccinated dogs in group 1 but still had significantly lower concentrations than dogs in groups 2 and 4.

As with serum IgG concentrations, dogs had variable salivary concentrations of mucosal IgA (range, 4 to 218 units) at the beginning of the study (**Fig 2**). There were no significant differences over time or among groups in regard to salivary concentration of

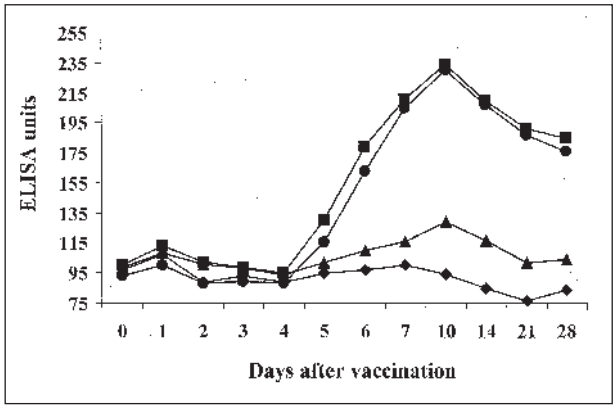

Figure 1—Serum *Bordetella bronchiseptica*-reactive IgG concentrations in dogs (n = 10/group) before and after vaccination with an intranasal vaccine (triangles), a SC vaccine (squares), or the intranasal vaccine and the SC vaccine (circles) and in unvaccinated control dogs (diamonds). Values represent least square group means.

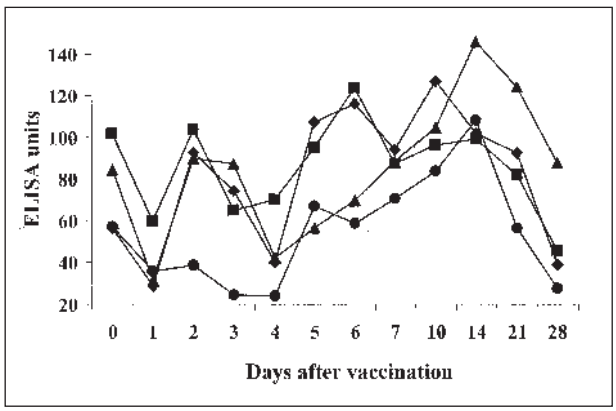

Figure 2—Salivary *B bronchiseptica*-reactive IgA concentrations in dogs before and after vaccination. *See* Figure 1 for key.

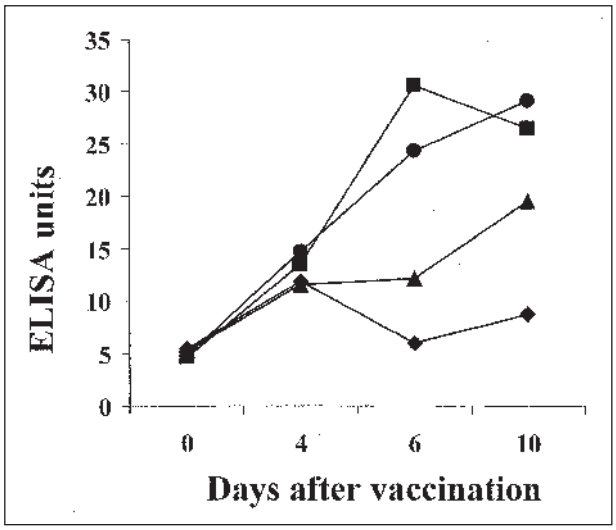

Figure 3—Serum *B bronchiseptica*-reactive IgA concentrations in dogs before and after vaccination. *See* Figure 1 for key.

IgA after vaccination, regardless of vaccine type. Salivary concentrations of IgG were low, and there were no consistent significant changes over time or among groups after vaccination.

Analysis of serum IgA concentrations on days 0, 4, 6, and 10 revealed that by day 6, dogs that had been vaccinated with the SC vaccine, alone or in combination with the intranasal vaccine, had significantly higher serum IgA concentrations than did unvaccinated dogs and dogs that had received the intranasal alone (**Fig 3**). On day 10, dogs that had received the intranasal vaccine alone had significantly higher serum IgA concentrations than did the unvaccinated dogs, and serum IgA concentrations in dogs that received the intranasal vaccine alone were not significantly different from concentrations for dogs in the other 2 vaccinated groups. Serum IgA and IgG concentrations at the time of vaccination were not significantly correlated with peak serum IgA or IgG concentration, regardless of treatment group, indicating there was no relationship between preexistent immunoglobulin concentrations and the anamnestic antibody response.

Bacterial culture—Low numbers of *B bronchiseptica* were isolated from the pharyngeal swab specimen collected from 1 dog on day –7; this dog was randomly assigned to group 2. Bacterial culture of pharyngeal swab specimens collected from the remaining dogs on day –7 and from all dogs on day 28 did not yield *B bronchiseptica*.

Discussion

The present study was designed to address a practical concern of veterinarians in private practice who care for dogs that are kenneled, dogs that are taken to shows or sporting events, and dogs that have the potential to otherwise be exposed to *B bronchiseptica*. In contrast to what has been implied in textbooks[2,3,12] concerning a generic development of immune responses to *B bronchiseptica* vaccines, administration of an intranasal vaccine to previously vaccinated, and possibly exposed, seropositive dogs in this study did not induce a more rapid, or quantitatively greater, *B bronchiseptica*-reactive antibody response than did administration of the SC vaccine. Moreover, there was no apparent immunologic benefit to administering the intranasal and SC vaccines simultaneously. These data, together with those from previous studies,[4-9] suggest that the immune status of the host, as well as the route of vaccine administration, affects the response to *B bronchiseptica* vaccines.

As in many previous studies[4-9] involving *B bronchiseptica* vaccines and studies of other canine vaccines, this study was conducted with laboratory Beagles. Although this experimental approach cannot directly address potentially important environmental factors or genetic factors within the general canine population that may affect vaccine efficacy in individual dogs, it does control environmental and management factors, including exposure to *B bronchiseptica* and antigenically related bacteria, that could confound analysis of the data. In the present study, previously vaccinated young adult dogs, as opposed to seronegative puppies, were specifically chosen, because they represent a large proportion of the potential candidates for vaccination against *B bronchiseptica* and because there are no controlled studies that address the early development of anamnestic responses in these dogs.

This study did not involve a challenge of immunity (ie, inoculation of dogs with virulent *B bronchiseptica*) and, therefore, did not directly address the controversial issue[1-3,9,12] of protective immunologic mechanisms in ongoing *Bordetella* infections. Previous studies[4-9] have documented the efficacy of *B bronchiseptica* vaccines and, directly or indirectly, correlated various systemic and mucosal immune responses with protection. In humans, the clinical efficacy of *B pertussis* and other vaccines has been determined[19] without challenging vaccinated individuals. Therefore, these immunologic results are relevant to a discussion of clinical protection.

It is commonly assumed that maternal antibodies and, by extension, circulating antibodies induced by active immune responses will inhibit the response to SC and IM *B bronchiseptica* vaccines but not to intranasal vaccines.[1-3,12] Although we cannot exclude the possibility that passive (maternal) and active antibodies have differential blocking effects or the possibility that primary and secondary (anamnestic) antibody responses may have different susceptibilities to blocking, results of this study clearly indicate that dogs with moderate to high serum *B bronchiseptica*-reactive antibody concentrations can mount a significant anamnestic antibody response to this SC vaccine. These results suggest that the generalization[1-3,12] concerning the negative effects of maternal antibodies on the protective effects of SC and IM *B bronchiseptica* vaccines in young puppies should be reexamined, especially because it appears to have been based an extrapolations from the experiences with nonadjuvanted modified-live virus vaccines[20] and not specific experiments with this bacterium.

Results of the present study demonstrate that administration of this intranasal *B bronchiseptica* vaccine was significantly less effective, both temporally

and quantitatively, at stimulating systemic anamnestic responses than was SC administration of the same antigen. This may be due simply to the higher dose of antigen and the presence of an adjuvant in the SC vaccine. Alternatively, the significantly reduced anamnestic response to the intranasal vaccine may have been due to an inadequate delivery of vaccinal *B bronchiseptica* to the immune system as a result of preexistent mucosal antibody. This would be compatible with the concept that the primary immunologic mechanism of mucosal IgA is "immune exclusion" or removal of pathogens, including in this case vaccine antigens.[21] This would decrease the effective amount of antigen presented to the immune system. There are some data from a study[22] of the interaction between various antigens complexed with IgA antibodies and intestinal M cells (specialized epithelial cells in mucosal lymphoid tissue) that suggest that a preexisting mucosal IgA response may enhance presentation of at least some antigens to mucosal (intestinal) lymphoid tissue. However, our data, including the lack of a significant correlation between IgA and IgG concentrations at the time of vaccination and peak IgA and IgG concentrations, suggest that the large particulate nature of the modified-live bacteria that are the antigen in this vaccine may abrogate the potentially immunoenhancing effect of preexisting IgA antibody that has been documented for some antigens in other species. According to current immunologic concepts,[21] mucosal delivery of antigens is expected to be less effective at stimulating secondary (vs primary) immune responses, and our data in Beagles support this. Furthermore, these results question the generic recommendation[1-3,12] for use of intranasal vaccines for booster vaccination of previously vaccinated or exposed animals that have IgA on mucosal surfaces, especially in the case of antigens (ie, modified-live bacteria) that are physically similar or identical to the pathogen that would normally be removed by immune exclusion.

Neither vaccine in the present study had an appreciable effect on salivary concentrations of *B bronchiseptica*-reactive IgA and IgG. In part, this apparent lack of effect may have been related to variation in the data. In contrast to assessment of antibody responses in serum, it is difficult to standardize the amount of salivary secretions that is collected, and this inconsistency in sampling may have led to underestimation of IgA concentrations with the ELISA. On the basis of results of a comparative study[23] of the biophysical characteristics of IgA in canine blood and intestinal lymph, it is currently thought[24] that in dogs serum IgA originates primarily from mucosal lymphoid tissue. Therefore, the serum IgA concentration is thought to be a direct reflection of a mucosal (IgA) antibody response. To address the problem of variation in salivary samples, serum IgA concentration was measured on days 0, 4, 6, and 10 in the present study. These days were chosen to reflect early and peak IgG responses. Similar to results for serum IgG concentration, results for serum IgA concentration indicated that there was no benefit to administering this intranasal vaccine alone or with the SC vaccine with regard to stimulating a more rapid IgA response or a response of greater magnitude. The low salivary IgG concentrations detected before and after vaccination were likely due to low numbers of IgG-secreting plasma cells in mucosal lymphoid tissue,[21] as the lack of overt disease and inflammation meant that IgG would not leak into the saliva from the blood. Another important factor is the possibility that *B bronchiseptica*-reactive IgA in the nasal cavity effectively removed vaccinal bacteria and reduced antigen presentation to the mucous-associated lymphoid tissue.[21] In a previous study,[9] there was immunologic and clinical benefit to administering intranasal and IM *B bronchiseptica* vaccines in sequence to seronegative puppies. Results of the present study, along with results of the previous study,[9] suggest that sequential or simultaneous vaccination may be most useful for primary vaccination of dogs, whereas the most effective method for stimulating secondary responses may be SC or IM delivery of the antigen. We recognize the limitations of interspecific extrapolations with regard to responses to vaccines and infectious agents; nevertheless, it is of interest that earlier work[16,25,26] with *B bronchiseptica* infection in pigs indicated that SC or IM administration of vaccines had similar effects on limiting bacterial growth in the respiratory tract and disease sparing as has recently been documented in dogs.[9] Moreover, these disease sparing responses were highly correlated with concentrations of actively[25] or passively[26] derived serum antibodies. In addition, the clinical effects of infection with the related and more virulent *B pertussis* in humans have been effectively controlled with SC or IM vaccines alone.[19]

Although a reduction in growth of *B bronchiseptica* in the respiratory tract and, presumedly, clinical immunity can be associated with the serum concentration of *B bronchiseptica*-reactive IgG,[9] the concentration of serum antibodies needed to afford protection is not precisely known. Therefore, it is unclear whether vaccination of seropositive dogs would affect either efficacy or duration of clinical immunity. An estimate of protective immunity can be derived from concentrations of specific immunoglobulin in serum, and this method has been used extensively to monitor *B pertussis* immunity in humans.[27] In general, higher systemic antibody concentrations are likely to confer a longer duration of immunity, because a higher initial concentration of antibody would effectively prolong the time for the concentration to decay to the minimal protective concentration. A recent investigation[28] has also documented the importance of *B pertussis*-reactive T cells in immunity to pertussis in humans. These cells secrete cytokines, such as interferon-γ, which activate phagocytic cells, and serve as long-lived memory cells.[28] The role of T cell responses in immunity to *B bronchiseptica* has not been examined in dogs, but T cell memory is likely to affect the duration of immunity following administration of *B bronchiseptica* vaccines and would not be measured by serologic assays.

Detection of moderate to high concentrations of *B bronchiseptica*-reactive antibodies in many of the dogs in the present study, which had not been vaccinated for 9 to 10 months, and the failure to detect a significant relationship between number of days since the last *B bronchiseptica* vaccination and the *B bronchiseptica*-

reactive IgG concentration at the start of the study suggest that individual variation in responses to vaccination, as well as unidentified environmental factors such as exposure to *B bronchiseptica* or antigenically related bacteria, affects duration of immunity.[29] Because only low numbers of *B bronchiseptica* were isolated on day –7 from only 1 dog and the organism was not isolated from any dogs on day 28, it is unlikely that endemic or acute *B bronchiseptica* infection contributed to the observed immunologic results in these dogs. Clearly, additional seroepidemiologic and challenge studies are needed to address the issue of duration of immunity induced by *B bronchiseptica* vaccines and to further understand the best vaccination strategies for protection against this pathogen.

[a]CoughGuard B, Pfizer Animal Health, Exton, Pa.
[b]NasaGuard-B, Pfizer Animal Health, Exton, Pa.
[c]Immunlon-4, Bio Merieux, St Laurent, QC, Canada.
[d]Bethyl Laboratories Inc, Montgomery, Tex.
[e]Kirkegaard & Perry Laboratories Inc, Gaithersburg, Md.
[f]Microplate Manager, Bio-Rad Laboratories, Hercules, Calif.
[g]API, Bio Merieux, St Laurent, QC, Canada.
[h]PROC GLM, SAS Institute Inc, Cary, NC.

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