Article

Comparative efficacy of intranasal and injectable vaccines in stimulating *Bordetella bronchiseptica*-reactive anamnestic antibody responses in household dogs

John A. Ellis, Sheryl P. Gow, Lindsey B. Lee, Stacey Lacoste, Eileen C. Ball

Abstract — In order to determine the comparative efficacy of injectable and intranasal vaccines to stimulate *Bordetella bronchiseptica (Bb)*-reactive anamnestic antibodies, a trial was conducted using 144 adult household dogs of various breeds and ages, which had been previously administered intranasal *Bb* vaccine approximately 12 months before enrollment. Dogs were randomized into 2 groups and blood, nasal swabs, and pharyngeal swabs were collected prior to the administration of single component *Bb* vaccines intranasally or parenterally. Ten to 14 days later all dogs were resampled to measure changes in systemic and local antibody to *Bb.* There were no differences in the changes in *Bb*-reactive serum IgG and nasal IgA between the groups, whereas intranasally vaccinated dogs had significantly higher *Bb*-reactive serum IgA. These data indicate that both of the current generation of intranasal (modified-live) and injectable (acellular) *Bb* vaccines can stimulate anamnestic local and systemic antibody responses in previously vaccinated, *Bb*-seropositive adult household dogs.

Résumé — Efficacité comparative des vaccins intranasaux et injectables pour stimuler les réponses des anticorps anamnestiques réagissant à *Bordetella bronchiseptica* chez les chiens domestiques. Afin de déterminer l'efficacité comparative des vaccins injectables et intranasaux pour stimuler les anticorps anamnestiques réagissant à *Bordetella bronchiseptica (Bb),* un essai a été réalisé à l'aide de 144 chiens domestiques adultes de diverses races et d'âges différents, auxquels l'on avait déjà administré le vaccin *Bb* intranasal environ 12 mois avant le recrutement. Les chiens ont été assignés au hasard à deux groupes et des échantillons sanguins, et écouvillons nasaux et pharyngés ont été prélevés avant l'administration de vaccins *Bb* à composant unique soit par voie intranasale ou parentérale. Dix à 14 jours plus tard, on a prélevé de nouveaux échantillons pour tous les chiens afin de mesurer les changements dans les anticorps systémiques et locaux pour *Bb.* Il n'y avait aucune différence au niveau des changements pour l'IgG sérique et l'IgA nasal réactif à *Bb* entre les groupes, tandis que les chiens vaccinés par voie intranasale présentaient un niveau significativement supérieur d'IgA sériques réactives à *Bb.* Ces données indiquent que les deux générations actuelles de vaccins *Bb* intranasal (vivant modifié) et injectable (acellulaire) peuvent stimuler les réponses locale et systémique des anticorps *Bb* chez les chiens adultes domestiques antérieurement vaccinés.

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Conflict of interest statement

One of the authors (EB) is an employee of Zoetis. Zoetis played no direct role in the acquisition of data, or in the analysis of data. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

Introduction

Bordetella bronchiseptica (Bb) is a Gram-negative bacterium that is one of about 12 pathogens that have been causally associated with the canine infectious respiratory disease complex (CIRDC) (1). Various parenteral and mucosal vaccines against *Bb* are available and have frequently been used in veterinary practices for more than 30 y (2). Throughout this period there has been controversy about the relative efficacy of these vaccines in stimulating primary protective immune responses and in their comparative utility as "booster shots" (2).

Environmental co-factors, such as natural exposure to *Bb,* that could provide a "boosting" effect for iatrogenically primed immune responses likely significantly contribute to vaccine efficacy and duration of immunity (DOI) in client-owned dogs (3,4). The involvement of these cofactors, including dose and frequency of *Bb* exposure in settings such as boarding kennels and grooming operations, is virtually impossible to model in a laboratory setting, requiring the use of household dogs to best gauge the Gestalt of immunity to *Bb* and other pathogens. For various reasons, perhaps most notably logistical difficulties related to owner participation and compliance, there are few studies that have sequentially examined immune responses to *Bb* vaccines in real-world dogs (5). Neither are there many recent data concerning the carriage of *Bb* in clinically normal household dogs, that could affect responses to vaccination and DOI (6,7). The purpose of this study was to extend extant laboratory findings related to the immunogenicity of *Bb* vaccines by comparing the anamnestic systemic and mucosal antibody responses induced by the current generation of injectable or intranasal single component *Bb* vaccines in adult household dogs presenting for their annual "booster shots."

Materials and methods

Study population and experimental design

Clinically normal client-owned household dogs of various ages and breeds (Table 1) with a documented history of intranasal vaccination for *Bb* approximately 1 y before enrollment (a common and often recommended interval between vaccinations for *Bb*) (2) were subjects, and had written owner consent. Owners were questioned regarding their dogs' lifestyle as related to potential exposures to other dogs. Patients were randomized into 2 groups using a computerized random number generator. Dogs in 1 group received a single injectable *Bb* vaccine; those in the other received a single intranasal vaccine. When there were 2 or more dogs in a household, all dogs received the same treatment. Venous blood (for serum), nasal swabs (sterile polyester tipped), and pharyngeal swabs were collected on day 0 prior to vaccination and again 10 to 14 d later. Individual swabbing was performed in both nares, left first, and then in the deep pharynx (including tonsil whenever possible). All sampling was done away from owners, and fractious dogs were mildly sedated, if necessary. Only pharyngeal swabbing was done in dogs with stenotic nares (i.e., too small to insert swab). Nasal swabs were placed in 1 mL, and pharyngeal swabs in 2 mL, of transport medium and frozen at −20°C, then −80°C prior to analysis.

Vaccines

Single component injectable (Bronchicine; Zoetis, Parsippany, New Jersey, USA) and intranasal (Vanguard B; Zoetis) *Bb* vaccines were obtained from a distributor.

Quantitation of *Bb*-reactive antibodies

Enzyme-linked immunosorbent assays (ELISAs) to measure IgG and IgA reactive with *Bb* were performed as previously described (8) using *Bb*-antibody positive and negative sera and saliva as controls.

Polymerase chain reaction (PCR)

A real time PCR for *Bb* (and other respiratory pathogens; RealPCR test code 2524; Idexx Reference Laboratories, Calgary, Alberta) was performed on deep pharyngeal swabs.

Statistical analysis

Statistical analyses were performed using a commercial software package (SPSS Statistics 23.0; IBM, Markham, Ontario). Changes in *Bb*-reactive serum IgG, serum IgA, and nasal IgA between the enrollment/vaccination visit and the follow-up visit were the 3 outcome variables examined. Non-parametric Mann-Whitney U-tests were used to determine the differences between treatment groups (9). Baseline data collected from the primary visit were also used to examine the secondary hypothesis that activities which increase the likelihood of interaction with other dogs increase the chance of natural exposure to *Bb,* and subsequently provide a "boosting" effect for iatrogenically primed immune responses. A score was created for each dog by categorizing the potential risk of natural *Bb* exposure based on activities (boarding, grooming, etc.) which could increase interaction with other dogs in the 12 mo prior to the onset of the study (Table 2). These categories were then summed for each dog in order to create a total risk score. The risk for natural *Bb* exposure was considered greater for dogs with a higher total score for these parameters. The relationships between baseline IgG or IgA and the total risk score for potential natural *Bb* exposure were examined using a Spearman's correlation (10).

Results

A total of 144 dogs were enrolled between September 4, 2014 and October 28, 2015. Seventy-seven dogs were randomly assigned to the injectable *Bb* vaccine group and 67 to the intranasal *Bb* vaccine group. Before initiation of the study, historical data revealed that there were 22 dogs in the injectable *Bb* vaccine group which were vaccinated for *Bb* between January and November of 2013, and 55 dogs vaccinated between January and October of 2014. In the intranasal *Bb* vaccine group, there were 16 dogs vaccinated between July and December of 2013, 50 dogs vaccinated between January and October of 2014, and 1 dog in May of 2012. There was no statistical difference in the previous vaccination dates between the 2 treatment groups ($P = 0.87$).

There were also no statistical differences between breed classification (large, medium, small) between the 2 treatment groups (Table 1, $P = 0.24$). The dogs in the injectable *Bb* vaccine group and the intranasal *Bb* vaccine group were also not different from

Table 1. Dog breeds categorized by treatment group and breed size

Dog breeds	Treatment group: Injectable *Bb* vaccine	Treatment group: Intranasal *Bb* vaccine	Total
Large breed dogs			
Alaskan malamute	0	1	1
American foxhound	1	0	1
Boxer mix	1	1	2
Bullmastiff	0	1	1
Dalmatian	0	1	1
Doberman	0	1	1
Flat-coated retriever	1	0	1
Fox terrier (wire-haired)	2	0	2
German shepherd	7	1	8
German shepherd mix	0	2	2
Great Dane mix	1	0	1
Greyhound	0	1	1
German shorthaired pointer	0	1	1
Hound mix	1	0	1
Husky	0	1	1
Husky mix	0	1	1
Labrador	3	8	11
Labrador mix	2	0	2
Old English sheepdog	0	1	1
Rhodesian ridgeback	2	0	2
Schnauzer	0	2	2
Weimaraner	1	0	1
Total	**24**	**23**	**47**
Medium breed dogs			
Australian shepherd	1	0	1
Australian shepherd mix	1	0	1
Bassett hound	0	2	2
Beagle	2	2	4
Beagle mix	1	1	2
Border collie	0	1	1
Brittany spaniel	0	1	1
Cocker spaniel	1	1	2
Collie	0	1	1
French bulldog	1	0	1
King Charles spaniel	1	0	1
Pitbull	1	1	2
Pitbull Mix	1	0	1
Pointer Mix	0	1	1
Shar Pei	1	0	1
Shetland sheepdog	0	1	1
Total	**11**	**12**	**23**

Breeds	Treatment group: Injectable *Bb* vaccine	Treatment group: Intranasal *Bb* vaccine	Total
Small breed dogs			
Bichon frisé	0	3	3
Boston terrier	0	1	1
Cairn terrier	1	0	1
Chihuahua	1	1	2
Chihuahua mix	1	0	1
Dachshund	1	3	4
Dachshund mix	0	2	2
Jack Russell terrier	1	1	2
Maltese	0	1	1
Maltese mix	1	1	2
Miniature dachshund	1	0	1
Miniature schnauzer	3	0	3
Papillion	1	0	1
Pekingese	1	0	1
Pomeranian	1	0	1
Pomeranian mix	0	1	1
Poodle mix	0	1	1
Pug	0	1	1
Scottish terrier	1	0	1
Shih tzu	3	4	7
Terrier mix	3	1	4
Toy poodle	1	2	3
West Highland terrier	1	2	3
West Highland terrier mix	0	1	1
Welsh corgi	2	0	2
Welsh corgi mix	1	0	1
Yorkshire terrier	2	1	3
Yorkshire terrier mix	1	0	1
Total	**28**	**27**	**55**
No classification			
Mix	14	5	19

each other for any of the other potential confounding variables explored (*Bb* exposure variables, Tables 2, 3 and baseline *Bb*-reactive antibodies, Table 4).

In a subset of dogs the *Bb*-reactive serum IgG (n = 9 dogs), serum IgA (n = 12 dogs) and nasal IgA (n = 30 dogs) decreased between the enrollment/vaccination visit and follow-up visits. Therefore, when calculating the change in these parameters from baseline a negative value was obtained. Since, it is biologically less probable to have these parameters decrease in the 10- to 14-day period between the initial vaccination and follow-up visit there was uncertainty as to the best way to manage these data. To ensure that either including or excluding dogs that had lower serum IgG, IgA, and/or nasal IgA after vaccination did not bias the outcomes, the data were analyzed using 5 approaches; dogs that had a lower serum IgG on the post-vaccination visit excluded, dogs that had a lower serum IgA on the post-vaccination visit excluded, dogs that had a lower nasal IgA on the post-vaccination visit excluded, dogs with a lower serum IgG, IgA, or nasal IgA on the post-vaccination visit excluded and no dogs excluded. Interpretation of the analyses for both the explanatory variables or primary outcomes of interest did not change regardless of the dataset used; therefore, for brevity only the analysis for all of the enrolled dogs is presented.

The changes in *Bb*-reactive serum IgG and nasal IgA were not significantly different between the 2 vaccination types (Table 5; Figures 1, 2); whereas the change in *Bb*-reactive serum IgA was significantly higher in the intranasally vaccinated (median = 32, range −42 to 154) versus the injected dogs (median 16, range −32 to 103) (Table 5; Figure 3) (P = 0.007).

There was no statistical association between the calculated total lifestyle risk score for potential natural *Bb* exposure and baseline IgG (P = 0.12) or IgA (P = 0.93).

Pharyngeal swabs from 4/101 dogs from 2014 (3 injectable-vaccinated/1 intranasally vaccinated) were positive for *Bb* DNA. Because of the low prevalence and variable baseline and post-vaccination responses, the pharyngeal swab data were not further analyzed.

Table 2. Categorization of potential risk factors for being naturally exposed to *Bordetella bronchiseptica*

Potential *Bordetella* exposure variables	*Bordetella* exposure risk category
Boarded (previous 12 months)	
a. None	0
b. 1 time	1
c. 1 to 2 times	2
d. 2 times	3
e. 2 to 3 times	4
f. 3 times	5
g. 3 to 4 times	6
h. > 4 times	7
i. Other	8
Groomed (last 12 months)	
a. None	0
b. Monthly	6
c. Every other month	5
d. 4 to 5 times per year	4
e. 2 to 3 times per year	3
f. 1 time per year	2
g. Other (stated regularly but not specified)	1
Other factors in last 12 months for risk of exposure	
a. None	0
b. Dog park	1
c. Travel with family	1
d. Interact with other dogs	1
e. Fostering other dogs (75 dogs in a home)	2

Table 3. Counts and associated *P*-values for potential *Bordetella bronchiseptica* exposure variables

	Treatment group		
Bordetella exposure variables	Injectable *Bb* vaccine Number of dogs	Intranasal *Bb* vaccine Number of dogs	*P*-value
Boarded (previous 12 months)			0.98
a. None	21	17	
b. 1 time	4	6	
c. 1 to 2 times	1	1	
d. 2 times	21	17	
e. 2 to 3 times	10	12	
f. 3 times	2	3	
g. 3 to 4 times	5	2	
h. > 4 times	7	5	
i. Other	6	4	
Total	**77**	**67**	
Groomed (previous 12 months)			0.87
a. None	47	40	
b. Monthly	6	5	
c. Every other month	8	9	
d. 4 to 5 times per year	4	7	
e. 2 to 3 times per year	6	4	
f. 1 time per year	0	0	
g. Other (regularly)	6	2	
Total	**77**	**67**	
Other exposure factors (previous 12 months)			0.64
a. None	67	60	
b. Dog park	2	1	
c. Travel with family	4	3	
d. Interact with other dogs	0	1	
e. Fostering other dogs	4	2	
Total	**77**	**67**	
Potential exposure (1 month prior to sample)			0.40
a. None	55	52	
b. Boarded	5	1	
c. Groomed	7	11	
d. Foster dogs	3	1	
e. Other (travel, dog day care, dog show, extended family dog interaction)	7	2	
Total	**77**	**67**	
Potential exposure (between 2 test periods)			0.84
a. None	61	54	
b. Boarded	8	7	
c. Groomed	2	5	
d. Foster dogs	3	0	
e. Other (travel, dog day care, dog show, extended family dog interaction)	3	1	
Total	**77**	**67**	

Discussion

The results of this study extend our previous investigations related to the immunogenicity of the current generation of parenteral vaccines for *Bb* in dogs, an acellular nonadjuvanted filtrate preparation similar to the vaccines used against *Bordetella pertussis (Bp)* in humans (5,10). To our knowledge, this is the first study to compare the ability of injectable and intranasal vaccines to stimulate anamnestic mucosal and systemic antibody responses in a large cohort of previously intranasally vaccinated, variably *Bb* seropositive adult household dogs.

To the extent that there were no significant differences in the change in *Bb*-reactive IgG responses in serum between groups that received the injectable versus intranasal vaccines, these results are in contrast to a previous study that documented significantly higher systemic antibody responses, that developed more rapidly in parenterally vaccinated *Bb*-seropositive adult laboratory beagles compared to a similar group of intranasally vaccinated dogs (8). That study examined a whole cell alum-adjuvanted bacterin; therefore, it is perhaps not surprising that the latter vaccine was apparently more immunogenic since it contained orders of magnitude more of the protein antigen (5,8,10). As well, the whole cell bacterin contained more and different pathogens associated molecular patterns (PAMPs) or "danger signals" that likely had adjuvant activity (5,10,11,12) in addition to the inclusion of aluminum hydroxide.

In human medicine, until about the early 1990's, various whole cell *Bp* vaccines were used to successfully control whooping cough in vaccinated populations (10). However, these vaccines were associated with an approximately 50% incidence of adverse reactions, most often local inflammatory reactions and/or transient malaise and pyrexia (10). It is most likely that the PAMPs in the whole cell vaccines were responsible for not only adjuvant effects but also for inducing the inflammatory responses that constituted the majority of the adverse reactions (10,12). These adverse reactions were a main instigator in the development of less reactive acellular vaccines containing both less PAMPs and less potential antigens (10). The adverse reaction rate to the previously widely used whole cell *Bb* vaccines in dogs was apparently considerably less than with the *Bp* vaccines according to available, primarily anecdotal data; however, there is a dearth of reliable prevalence data on adverse reactions

Table 4. Descriptive statistics and associated *P*-values for dog gender, age, baseline *Bordetella bronchiseptica (Bb)*-specific serum IgG, baseline *Bb*-specific serum IgA and baseline *Bb*-specific nasal IgA

	Treatment group						
	Injectable *Bb* vaccine			Intranasal *Bb* vaccine			
Variable	Number of dogs	Female	Male	Number of dogs	Female	Male	*P*-value
Gender (male/female)	77	44	33	67	40	27	0.8
	Number of dogs	Median	Range	Number of dogs	Median	Range	*P*-value
Age (years)	77	6.5	1 to 15	67	6	1 to 13	0.85
Baseline *Bb*-specific serum IgG	77	72.1	5.5 to 111.1	67	75.1	−3.5 to 128.9	0.62
Baseline *Bb*-specific serum IgA	77	67	0 to 169	67	63.5	0 to 173	0.99
Baseline *Bb*-specific nasal IgA	62	66	3 to 116	58	73	0 to 137	0.41

Table 5. Descriptive statistics and associated *P*-values for the 3 outcomes of interest; change in *Bordetella bronchiseptica (Bb)*-specific serum IgG, change in *Bb*-specific serum IgA, and change in *Bb*-specific nasal IgA between initial and final sampling

	Treatment group						
	Injectable *Bb* vaccine			Intranasal *Bb* vaccine			
Variable	Number of dogs	Median	Range	Number of dogs	Median	Range	*P*-value
Change in *Bb*-specific serum IgG	77	15	−39 to 69	67	13	−16 to 95	0.31
Change in *Bb*-specific serum IgA	77	16	−32 to 103	67	32	−42 to 154	0.007
Change in *Bb*-specific nasal IgA	60	6.5	−83 to 76	55	12	69 to 134	0.23

to *Bb* vaccines in dogs. Nevertheless, a similar desire to reduce perceived reaction rates was a major reason for the development of the current acellular *Bb* vaccine. Therefore, a safety-driven "parallel-evolution" has occurred in human and veterinary medicine related to vaccines for relevant *Bordetella* spp., resulting in less reactive acellular vaccines. However, as part of that process it is important to acknowledge the expected "trade-off" between overall immunogenicity and less reactogenicity; it is generally not biologically possible to have both (10,12,13).

It was of interest that in some dogs, *Bb*-reactive antibodies in the serum and nasal secretions apparently decreased 10 to 14 d after vaccination. In the case of the nasal secretions, this could simply be a sampling "artifact" related to the volume of nasal secretion collected on the swabs prior to placement into a standard amount of transport medium; there is a high degree of variation in collection of these samples. This factor is difficult to control, especially when collecting from client-owned dogs, with variably sized, often small, nares. This constraint alludes to the logistical difficulty of conducting minimally invasive studies in household dogs, and warrants the use of the immunological surrogate, serum IgA, as an indicator of a relevant mucosal response (14). In the case of serum antibodies this possible dilution effect is not relevant, as a standard dilution of serum is tested in the ELISAs. A decrease in antibody 10 to 14 d after antigen exposure in serum or nasal secretions could be an indicator of a variable lack of response to the vaccine in an already antibody positive animal, in combination with a decay in antibody related to the expected half-life of those proteins in plasma and on mucosal surfaces, and/or immune complexing of antibody with vaccinal antigens (15). Alternatively, an apparent decrease in antibody measured in the ELISAs which used a whole cell preparation of *Bb* as antigen, could be due to the failure to accurately measure responses to immunodominant *Bb* antigens or different IgG subtype antibody responses in different dogs (13,16,17). In other words, after boosting with vaccine some dogs may respond preferentially to particular antigens *versus* others, which could then be detected in an apparent overall decrease in reaction with the standardized constellation of antigens in the whole cell preparation used as the ELISA antigen.

The site of production and immunological relevance of canine serum IgA has been controversial (18,19). The finding that most adult dogs in this study that received intranasal vaccine had increases in *Bb*-reactive IgA in the serum post-vaccination is consistent with the concept, based primarily on studies in the gut, that mucosal production following antigen exposure is the source of the dimeric IgA in the serum (20–22). This concept is supported by our recent finding of substantial increases in serum IgA subsequent to the administration of a single dose of oral *or* intranasal *Bb,* to young *Bb*-seronegative beagle puppies (14). Perhaps more controversial is the ability of parenteral immunization to boost measurable IgA on non-diseased mucosal surfaces, and there are few comparative data that address this issue. Although the numbers of nasal swab samples were decreased compared to serum, the finding that there were no significant differences in mucosal *Bb*-reactive IgA in the groups of dogs that received injectable or intranasal vaccines is consistent with the concept that parenteral administration of vaccine can result in increases in antigen-specific IgA in nasal secretions in mucosally-primed individuals (23). More relevant to *Bb,* it has been demonstrated that parenteral vaccination of previously naturally exposed human adolescents with acellular *Bp* (DTaP) stimulated not only anamnestic *Bp*-specific IgG responses, but IgA as well (24). This was not the case following primary parenteral immunization with the DTaP vaccine indicating

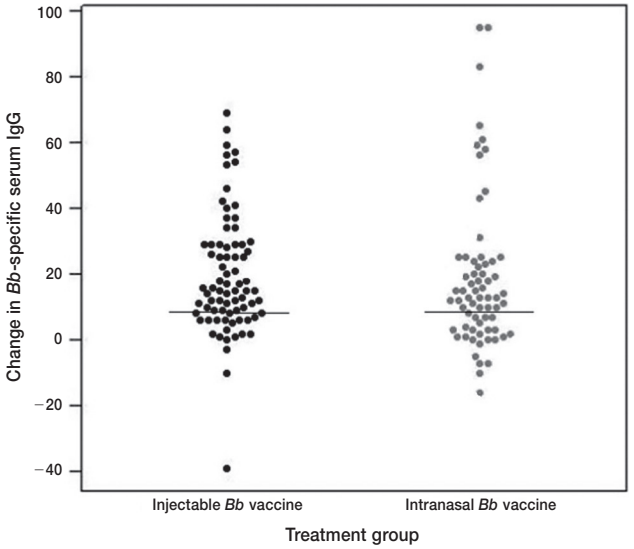

Figure 1. Beeswarm plot of change in *Bordetella bronchiseptica*-reactive serum IgG concentrations between initial and final sampling. Solid lines depict the median values for each treatment group.

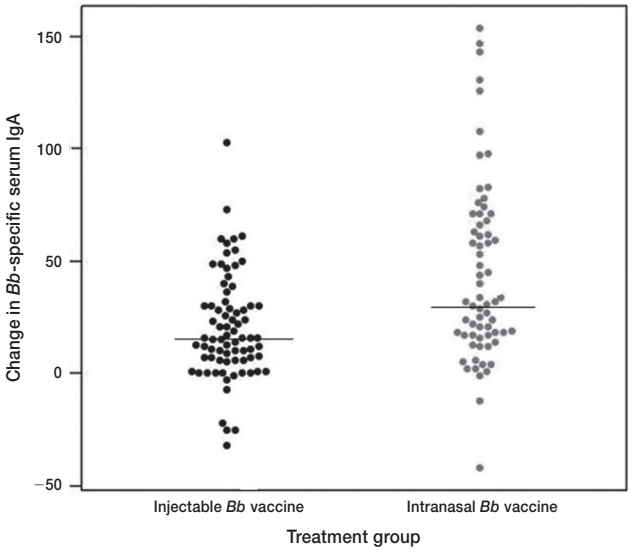

Figure 3. Beeswarm plot of change in *Bordetella bronchiseptica*-reactive serum IgA concentrations between initial and final sampling. Solid lines depict the median values for each treatment group.

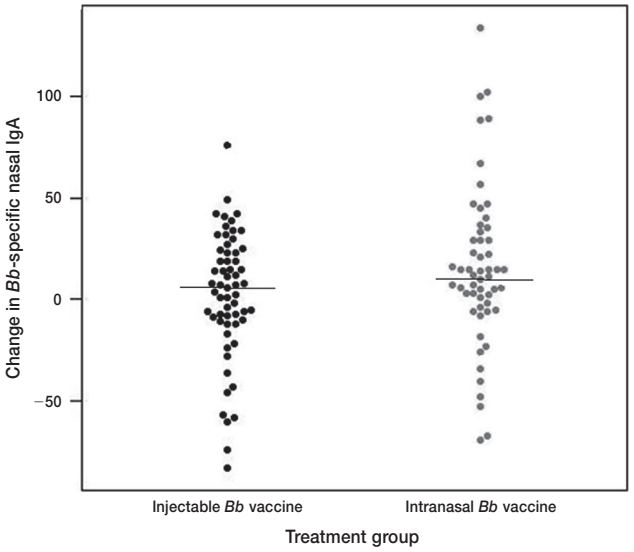

Figure 2. Beeswarm plot of change in *Bordetella bronchiseptica*-reactive IgA in nasal secretions between initial and final sampling. Solid lines depict the median values for each treatment group.

that mucosal priming was required to affect the latter response (24). The practical implication from these observations is that priming puppies with intranasal vaccine followed by parenteral delivery in an initial series achieves boosting of both systemic and mucosal antibody responses (25,26).

It was suggested 40 y ago that natural transmission of *Bb* from clinically affected, convalescent, or asymptomatic carrier dogs could contribute to the duration of immunity to *Bb,* and that this effect could vary with a dog's "lifestyle" (3). We were unable to associate the lifestyle co-factor of potential exposure to other dogs with baseline antibody responses to *Bb* in this cohort of dogs. This may be due to limitations of the data such as recall bias of owners, inadvertently missing or misclassifying key exposure risk(s), or an inability to quantify the exposure risk appropriately; for example, a lack of knowledge related to the "circulation" of *Bb* in subsets of the canine population. Data concerning the carriage of *Bb* by asymptomatic dogs are somewhat conflicting. Early studies based on culture reported no isolation of *Bb* in 2 populations of laboratory beagles [n = 467 (27); n = 25 (28)], and 3/50 (6.0%) asymptomatic dogs entering, or housed in a university veterinary clinic (29) were *Bb* culture positive. In recent studies using potentially more sensitive PCR, 2/22 (9.1%) normal dogs presenting at veterinary clinics (7) and 98/503 (19.5%) asymptomatic dogs presenting at shelters were positive for *Bb* DNA (30). Unfortunately, the immune status to *Bb* was not known/reported in any of those studies. Based on the low prevalence of *Bb* positive dogs (4%) in this population of routinely vaccinated dogs, carriage of *Bb* could not be implicated as an important co-factor in the response to *Bb* vaccines in this study. Nevertheless, exposure to *Bb* from acutely infected individuals, carrier animals, or fomites is undoubtedly an important, if difficult to measure co-factor in the response to *Bb* vaccines (4), which begs further investigation.

In conclusion, these data demonstrate that both intranasal and parenteral administration of current vaccines for *Bb* to previously intranasally vaccinated variably *Bb* seropositive adult household dogs can stimulate anamnestic systemic and mucosal antibody responses that have been associated with disease sparing in *Bordetella* infections (10,25). Therefore, these data suggest that either vaccine could be considered for use as a booster in animals with immunological memory established by previous immunization (25) and/or natural exposure.

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