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Comparative efficacy of intranasal and oral vaccines against *Bordetella bronchiseptica* in dogs

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ABSTRACT

In order to determine the comparative efficacy of vaccines administered intranasally or orally to protect puppies from disease subsequent to experimental infection with *Bordetella bronchiseptica* (*Bb*), a randomized controlled trial was performed using 48 approximately 8-week-old specific pathogen free, *Bb* naive Beagle puppies. Puppies were randomized into three groups and administered vaccines containing *Bb* intranasally or orally, or a placebo intranasally. Twenty-one days later, all dogs were challenge exposed via aerosol administration of *Bb*. Clinical signs, nasal bacterial shedding and immune responses were monitored for 28 days after challenge. Intranasally vaccinated puppies had significantly lower rates of coughing, nasal discharge, retching and sneezing (i.e. were less sick clinically) than control puppies. The distinction between the orally vaccinated puppies and the control puppies was less consistent. The orally vaccinated puppies had less coughing and less retching than the control puppies, but nasal discharge and sneezing did not differ from control animals. Orally vaccinated puppies had higher rates of coughing, nasal discharge, retching and sneezing than the intranasally vaccinated puppies. Although both intranasal and oral *Bb* vaccines stimulated immune responses associated with disease sparing following *Bb* infection, the intranasal route of delivery conferred superior clinical outcomes. The observed difference in clinical efficacy suggests the need to question the rationale for the use of currently available orally administered *Bb* vaccines.

Introduction

Bordetella bronchiseptica (*Bb*) is a Gram negative bacterium recognized as one of a constellation of agents etiologically associated with the canine respiratory disease complex (CIRD) (M'Gowan, 1911; Ford, 2006). Once its role as a canine respiratory pathogen was definitively established in the early 1970s (Wright et al., 1973), single component and combination vaccines for the agent, first parenteral, then intranasal, were developed (Ellis, 2015). Recently a single component oral *Bb* vaccine was licensed for commercial use (Hess et al., 2011; Ellis, 2015).

Given the relative ease of administration, the oral *Bb* vaccine has replaced vaccines for this pathogen administered by other routes in many veterinary practices. Since *Bb* vaccines first became available and commonly used in dogs, there have been differing opinions regarding the efficacy and mechanisms of protection of the various routes of administration (Ellis, 2015). Recently, there has been controversy regarding the relative efficacy of the oral and intranasal routes for mucosal administration; vaccines administered by these routes have been used as both primary immunogens and as 'last minute' prophylactics prior to commingling. The aim of this study was to compare the efficacy of representative intranasal and oral vaccines for *Bb*, and to examine immune responses, including those at the mucosa at the earliest documented onset of clinical immunity (72 h; Gore et al., 2005).

Materials and methods

Experimental subjects

Forty-eight (24 male, 24 female) weaned, specific pathogen free Beagle dogs, aged 56–62 days, were obtained from a commercial breeder (Ridglan Farms) and acclimated for 7 days at the study site. The puppies had received a single component vaccine against canine parvovirus (NeoPar, NeoTech) at 6 weeks of age. All dogs had low or no antibodies against *Bb* (<1:16 by microagglutination test, MAT; Ellis et al., 2001) and were determined to be free of *Bb* by deep nasal swab cultures on day 0 prior to vaccination. All dogs were maintained and handled using procedures consistent with the United States Department of Agriculture 9CFR, and approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and had access to ad libitum dry food and water.

Vaccines

A single component oral *Bb* vaccine (Bronchi-shield ORAL, Boehringer Ingelheim Vetmedica) was obtained commercially from a distributor. A triple component

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Table 1
Clinical scoring rubric.

Clinical sign	Score	Description
Nasal discharge	0	Absent: Includes normal, moist nose
	1	Mild: Serous (clear, watery) discharge, must be extending approximately half way down the nasal philtrum
	2	Moderate: Serous discharge extending more than half way down the nasal philtrum, or evidence of mucopurulent discharge
	3	Severe: Mucopurulent discharge extending more than half way down the nasal philtrum, or bloody discharge, or a combination of mucopurulent and bloody discharge
Ocular discharge	0	Absent
	1	Mild: Evidence of excessive tear production (brimming and/or flowing out of the eye), such as some secretion in the corner of the eye or brimming with tears
	2	Moderate: Serous discharge extending more than half way down the nasal philtrum, or evidence of mucopurulent discharge
	3	Severe: Mucopurulent discharge extending more than half way down the nasal philtrum, or bloody, or bloody discharge, or a combination of mucopurulent and bloody discharge
Cough	0	Absent
	1	Mild: One cough episode
	2	Moderate: Spontaneous and frequent coughing; two or more coughing episodes
	3	Severe: Spontaneous coughing with frequent retching; animal had persistent and prolonged cough
Sneezing	0	Absent
	1	Mild: Animal sneezed once or twice
	2	Moderate: Animal sneezed repeatedly
	3	Severe: Animal presented paroxysmal sneeze
Depression	0	Absent
	1	Mild: Animal is slow to rise, lost interest in playing but still somewhat active
	2	Moderate: Animal is able to rise and move, but inactive other than to eat or drink
	3	Severe: Animal is recumbent, unable to rise, and refuses food and/or drink
Retching	0	Absent
	1	Mild: Animal retches or vomits once briefly or occasionally
	2	Moderate: Animal retches or vomits for a prolonged period
	3	Severe: Animal retches or vomits multiple times for a prolonged period
Respiration	0	Normal respiration
	2	Moderate: Small clicking, bubbling, or rattling sounds in the lung (rales)
	3	Severe: Difficult or labored breathing; shortness of breath (dyspnea)

('3-way') intranasal vaccine (Vanguard Rapid Resp, Zoetis) containing *Bb*, canine parainfluenza virus (CPIV) and canine adenovirus-2 (CAV-2) was obtained from the manufacturer. Both vaccines contain 'live avirulent cultures' of *Bb*; however, the specific isolates of the bacteria and the dose in the vaccines are considered proprietary.

Experimental design and housing

Dogs were randomized using a software program (SPSS, SAS Institute). Puppies were blocked in groups of six by date of birth and dam, and assigned to treatments within blocks (two per treatment). Treatments were randomly assigned to rooms for the vaccination phase of the study. Within vaccination rooms, blocks were randomly assigned to pens. During the vaccination phase, the 48 puppies were divided into three treatment groups: (1) the control group received 0.5 mL of sterile diluent (distilled water) intranasally; (2) the second group received 1.0 mL of the single component vaccine orally between the cheek and lateral gum; and (3) the third group received 0.5 mL of the three-way vaccine in one nare. The dogs were housed two per pen in three separate biosecure isolation rooms. To further reduce the chances for exposure to *Bb*, the room housing the control puppies was entered first; therefore, observers were not masked to treatment groups during the vaccination phase. On day 20 (the day before the challenge on day 21), the dogs were co-mingled in three rooms; three blocks were randomly assigned to two challenge rooms and two blocks to one challenge room. Within each room, there were six puppies per double pen (two dogs from each treatment group) (see Appendix: Supplementary Fig. S1). All personnel performing clinical evaluations (during the challenge phase), laboratory testing and analyses were 'masked' (unaware of treatment groups). This protocol was approved by IACUC committee at the study site (approval number KZ-1894e2013-10-ajb; year of approval 2013).

Bordetella bronchiseptica *inoculum and experimental infection*

The virulent Bihr (feline origin) *Bb* strain was used as the inoculum and was cultured on selective (Bordet Gengou, BG) agar from stock as previously described (Ellis et al., 2001). The number of bacteria was adjusted nephelometrically (optical density, OD, 600) to approximately 4×10^{10} colony forming units (CFUs)/mL. Dogs were challenged six at a time via aerosolization of 25 mL of inoculum containing approximately 6×10^{8} CFUs *Bb* (target of 1×10^{8} CFUs per dog) into a chamber. Dogs remained in the chamber for a total of 30–35 min.

Clinical assessment and sampling

General health observations were completed on all puppies from the day of arrival (day –7) to study completion (day 49). Puppies were observed prior to and approximately 3 h post-vaccination for any adverse reactions. Puppies were observed twice on day 20 (prior to and approximately 3–4 h after co-mingling), twice on day 21 (prior to and approximately 4–5 h post-challenge), then twice daily (morning and afternoon for 30 min per group) on days 22–48, and once on day 49. During the challenge phase, puppies were clinically scored according to a predetermined rubric (Table 1) focusing on the primary outcome variable, spontaneous coughing. Rectal temperatures were recorded during the morning observation period on days –1 and 0, and on days 20–49.

Nasal swabs for bacterial culture were collected on day 0, twice weekly (Tuesday, Thursday) until day 20, on day 21 and then thrice weekly (Monday, Wednesday, Friday) on days 21–49, and placed in tryptose phosphate broth transport medium. Nasal and oropharyngeal swabs were collected on days 0 and 3, and placed in 1 mL Dulbecco's modified Eagles transport medium for measurement of mucosal immunoglobulin (Ig) A (IgA) and interferon α (IFNα). Serum was obtained on days 0, 3, 20 and 49.

Bacteriological culture

Nasal swabs were streaked onto BG agar plates as previously described (Ellis et al., 2001). The identity of suspect colonies was confirmed by matrix-assisted laser desorption/ionization (MALDI; Patel, 2015) using a commercial apparatus (Microflex LT, Bruker Daltonics).

Quantitation of Bordetella bronchiseptica*-reactive antibodies*

MATs and ELISAs to measure *Bb* reactive IgG and IgA were performed as previously described (Harris and Switzer, 1972; Ellis et al., 2001).

Quantitation of canine interferon α

A capture ELISA for canine IFNα (Cloud Clone) was performed according to the manufacturer's instructions. The protein contents of nasal swab samples were determined using a colorimetric assay (Bradford, 1976) for comparison with OD values obtained in the capture (and IgA) ELISAs.

Statistical analyses

Descriptive statistics were conducted for the pre-challenge (vaccination) phase of the trial and descriptive and inferential statistics were conducted on the post-challenge data. In all inferential analyses, each treatment was compared to every other treatment (control versus oral vaccinates, control versus intranasal vaccinates, oral vaccinates versus intranasal vaccinates).

Post-challenge, the puppies were observed and scored for seven clinical signs using a Likert scale (score range 0–3; Table 1). Scoring was performed twice daily