Evaluation of Efficacy of Oral Administration of *Bordetella bronchiseptica* Intranasal Vaccine When Used to Protect Puppies from Tracheobronchitis Due to *B bronchiseptica* Infection

Thomas J. Hess, BS[1]

Dana S. Parker, BS[1]

Alan J. Hassall, BS[1]

Yu-Wei Chiang, MS, PhD[1]

[1]*Biological Research and Development*
Boehringer Ingelheim VetMedica, Inc.
Fort Dodge, Iowa 50501

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ABSTRACT

This study evaluated the efficacy of oral administration of two *Bordetella bronchiseptica* vaccines[a,b] regarding prevention of canine infectious tracheobronchitis (ITB). Puppies free of *B bronchiseptica* by culture and serological testing were used in this study. At 8 weeks of age the puppies were randomly distributed into three groups. Group A received the placebo vaccine. Group B received the multivalent vaccine.a Group C received the monovalent vaccine.b The puppies were orally immunized with a single dose of vaccine in the buccal pouch. Five weeks after vaccination, they were challenged with virulent *B bronchiseptica* using an aerosol challenge model. They were observed for 30 minutes twice daily for 14 days. After challenge none of the multivalent-vaccinated puppies, only 1 of the monovalent-vaccinated puppies, and all of the placebo-vaccinated puppies had tracheobronchitis. Results of this study demonstrate excellent efficacy for prevention of ITB due to *B bronchiseptica* infection for these orally administered vaccines containing avirulent live B bronchiseptica.

INTRODUCTION

Currently USDA-licensed *B bronchiseptica* vaccines are labeled for either injectable or intranasal administration to aid in the prevention of ITB. The efficacy of intranasal, subcuticular, and intramuscular routes of *B bronchiseptica* vaccine administration have been studied regarding antibody response

and protection against disease.[1,2] The relative effectiveness of modified live intranasal vaccines as compared to injected vaccines is not clear due to conflicting results from different challenge studies. [1,2,3] Results from a study have shown that the use of intranasal vaccines reduced shedding of B. bronchiseptica while a killed vaccine did not. [3] In addition, intranasal vaccines are known to stimulate local immune responses. It can be speculated that these local responses, especially to the major pathogen Bordetella bronchiseptica, would confer effecicacy advantages over injected vaccines (Personal communication with Dr. John A. Ellis, University of Saskatchewan). Intranasal vaccines may be difficult to administer especially to aggressive dogs or dogs with unusual nasal anatomy. Oral administration into the buccal cavity of a modified live vaccine might maintain the advantages of live vaccine administration while reducing the administration disadvantages of intranasal vaccination. To date the efficacy of oral administration of an intranasal-labeled canine vaccine for prevention of ITB caused by *B bronchiseptica* has not been documented.

B bronchiseptica is considered one of the primary causes of canine ITB and also causes serious disease in pigs (atrophic rhinitis and pneumonia).[4] In addition, it causes illness in cats, horses, humans, and other species. *B bronchiseptica* is a gram-negative, aerobic coccobacillus that attaches to respiratory epithelia and cilia where it replicates, causes inflammation, and produces toxins that inhibit phagocytosis and movement of the cilia. The rapidity with which tracheal cilia are affected after exposure to *B bronchiseptica* is astounding. Normally cilia beat at a frequency of about 9 beats/second. The beat frequency decreases by 2-3 beats/sec just 5 minutes after tracheal exposure to B bronchiseptica. By 30 minutes after exposure, beat frequency is reduced almost in half. In 3 hours 80-90% of ciliated cells are completely inhibited from beating.[5]

B. bronchiseptica is the primary etiologic agent associated with ITB. Ciliostasis due to *B bronchiseptica* infection paves the way for concurrent and secondary infection by a variety of viruses, e.g., canine parainfluenza viruses, canine adenoviruses, bacteria, e.g., *Streptococcus sp., Pasteurella sp., Pseudomonas*, and mycoplasmas.[5,6,7] It is reasonable to speculate that immune defenses against B.bronchiseptica should be of major importance in the prevention of ITB disease.

The objective of this study was to determine if oral administration of *B bronchiseptica* vaccines labeled for intranasal administration would be effective in preventing signs of ITB in puppies challenged with virulent B bronchiseptica.

MATERIALS AND METHODS

Animals & Animal Care

Institutional Animal Care and Use Committee approval was obtained before the initiation of the study. Fifty male and female beagles, sero- and culture-negative for *B bronchiseptica* and 8 weeks of age at time of vaccination were used initially. Animals were blocked by litter and randomly allotted to one of three treatment groups. Group A received placebo vaccine. Group B received a combination vaccine containing modified live canine parainfluenza virus (CPI), canine adenovirus type 2 (CAV2), and live avirulent B bronchiseptica. Group C received a vaccine containing live avirulent B bronchiseptica.

During the vaccination phase of the study, the puppies were segregated into housing units by treatment group to prevent puppies in Groups B and C from contaminating puppies in Group A due to shedding modified live virus and avirulent B bronchiseptica. During the challenge phase of the study, the puppies were randomly distributed to house a proportional number of puppies from each group in each room. During both the challenge and vaccination phases of the study, littermates within a group were housed in the same isolation facility.

Food and water were available ad libitum. The dogs were provided with appropriate minimum floor space as defined by the Code of Federal Regulations (9 CFR 3.6.C).

***Table 1**:Study Groups*

Group	Number of Puppies	Vaccine	Vaccination Route	Challenge
A	17	Placebo	oral	*B bronchiseptica*
B	17	Multivalent vaccine	oral	*B bronchiseptica*
C	16	Monovalent vaccine	oral	*B bronchiseptica*

Animals that required medical attention were treated as deemed necessary by the plant veterinarian after consultation with the study investigator.

Study Design

This study used a randomized complete block design, blocked by litter. The puppies were randomly divided into three groups as shown in Table 1.

Vaccines and Vaccination

Three vaccines were used in this study: 1. A placebo vaccine consisting of stabilizer and blending diluents; 2. A multivalent lyophilized vaccine,a consisting of stabilizer, blending diluent, modified live canine adenovirus type 2, canine parainfluenza, and avirulent live B bronchiseptica; and 3. A monovalent lyophilized vaccineb consisting of stabilizer, blending diluent, and avirulent live B bronchiseptica. The puppies were immunized at 8 weeks of age, with a single dose of test or placebo vaccine by the oral route in the buccal pouch.

Experimental Challenge

At 5 weeks postvaccination, the puppies were challenged with virulent *B bronchiseptica* using an aerosol challenge model. The challenge material was a pool of two strains of B. bronchiseptica. The strains were cultivated on Bordet-Gengou agar plates for 40 to 48 hours at 35 ± 2°C. Bacteria were harvested from the surface of the plates using sterile cotton swabs and peptone saline. Each strain was dispensed and stored in liquid nitrogen without any additional stabilizer.

The challenge dosage was 20 mL for a target concentration of 5 x 10^8 CFU/m.3 The challenge material was aerosolized using a nebulizer for at least 15 minutes in a 1m^3 isolation chamber. Twenty-five mL of challenge material was added to the nebulizer dosage cup and run until approximately 20 mL was aerosolized. The residual 5 mL was discarded. The puppies remained in the chamber for an additional 5 minutes.

Clinical Observation

The puppies were monitored on Days 4, 3, and 2 pre-challenge to establish a baseline for rectal temperatures, coughing (both spontaneous and upon palpation), purulent nasal discharge, sneezing, and any other clinical signs or injuries. The protocol stated any puppy with elevated rectal temperature, severe injury, or abnormal clinical signs during this time would be eliminated from the study after veterinary evaluation.

Tracheal Swab Sample Collection and Culture

Tracheal swab samples were collected from puppies to detect the presence or absence of B. bronchiseptica. Samples were collected 20 days pre-vaccination, 1 day pre-vaccination, 21 days post-vaccination, and 2 days pre-challenge. Swabs were placed in transport medium, stored on ice, and processed as quickly as possible. Swabs were vortexed and expressed to remove as much fluid as possible. Samples were inoculated onto MacConkey agar plates, 100 µL per plate. The plates were incubated for 48 hours at 35 ± 2°C and examined for typical colonies of B. bronchiseptica. Suspect colonies were identified using the Vitek 2 Compact Automated Microbial Identification System (BioMérieux, Inc., Durham, NC).

Serum Sample Collection and Serological Assay

Serum agglutinating antibodies to *B bronchiseptica* whole cell antigen were measured

Table 2: *Microscopic Agglutination Test Results*

Group	One day prior to vaccination	Two days prior to challenge
A (Placebo)	<3±2*	<2±0
B (Multivalent vaccine)	<2±1	<47±2
C (Monovalent vaccine)	<2±2	<28±3

**Titer values expressed as geometric mean ± standard deviation*

using a Microscopic Agglutination Test (MAT) of serum samples collected 1 day pre-vaccination and 2 days pre-challenge. Serial 2-fold dilutions of sera were prepared in round bottom microtiter plates. An equal volume of killed *B bronchiseptica* suspension was added to each serum dilution. The plates were shaken for 2 minutes at room temperature, incubated at 35 ± 2°C for 2 hours, and held at 2-7°C for up to 40 hours before reading. The titer of each serum sample was reported as the reciprocal of the highest dilution of the serum that produced distinctive agglutination.

Data Analysis

All statistical analyses were performed using the SAS system (SAS Institute, Inc., Cary, NC). The level of significance was set at $p<0.05$. The primary outcome studied was the occurrence of tracheobronchitis, which was compared between treatment groups by Fisher's Exact test. Group comparisons were made using the Bonferroni adjustment for multiple comparisons. The risk ratios were estimated from the 2x2 tables. The vaccine efficacy statistics were estimated from the risk ratio. The secondary outcome, *B bronchiseptica* antibody titers, was tabulated for the two time points measured, 1 day prior to vaccination and 2 days prior to challenge, and reported as a geometric mean.

RESULTS

All pups were free of *B bronchiseptica* prior to vaccination. All placebo-vaccinated puppies remained free of *B bronchiseptica* prior to challenge. Six puppies (two from each group) were removed from the study and did not complete the challenge phase of the study. One animal died due to a complication unrelated to the study. After vaccination and prior to challenge, 1 litter of 4 dogs was randomly removed from the study. This resulted in 15 dogs in each group for a total of 45 dogs. Later one dog in Group C had a fever during the base-line observation and was removed from the study after vaccination and prior to challenge. Thus results are reported on 14 puppies in Group C and 15 puppies in each of Groups A and B.

The primary outcome for this study was the occurrence of tracheobronchitis. A puppy was considered positive for tracheobronchitis when observed coughing on any 2 days (not necessarily consecutive) during the 14-day observation period. Group A (placebo vaccinated) puppies were all (15 of 15) positive for tracheobronchitis, while none (0 of 15) of the Group B (multivalent vaccinated - Bronchi-Shield III) puppies ($p \leq 0.0001$) and only 1 of 14 of the Group C (monovalent vaccinated - Bronchi-Shield) puppies ($p \leq 0.0001$) were positive for tracheobronchitis. The vaccine efficacy was estimated at 1.0 (95% CI 0.78, 1.00) and 0.92 (95% CI 0.66, 1.00) respectively for the puppies vaccinated with the multivalent vaccine and monovalent vaccine compared to controls. Other observed respiratory clinical signs were sneezing and mucopurulent ocular discharge. In the placebo-vaccinated puppies 7 of 15 were observed sneezing compared to only 2 of 15 multivalent-vaccinated pups and 1 of 14 monovalent-vaccinated pups. Only two puppies were observed with mucopurulent ocular discharge, one puppy in the placebo-vaccinated Group A, and one in the Bronchi-Shield III-vaccinated Group B.

A febrile response for this study was defined as rectal temperature >103.4ºF and 1.0ºF above the average prechallenge baseline. Four placebo-vaccinated puppies

had a febrile response. None of the Group B (multivalent-vaccinated) pups were febrile. Two of the Group C (monovalent-vaccinated) pups had single febrile events.

The results of the MAT antibody titers (see Table 2) demonstrate that all pups were free of *B bronchiseptica* prior to vaccination and that placebo control pups remained free prior to challenge. The results also demonstrate a positive serological response to vaccination in all Group B and C pups.

DISCUSSION

Results of this study support the new label claim of oral administration of the multivalent vaccine (Bronchi-Shield III) for use as an aid in the prevention of ITB caused by B bronchiseptica. These data demonstrate efficacy against virulent *B bronchiseptica* challenge. The multivalent vaccine had similar results to monovalent indicating the other vaccine fractions did not interfere with *B bronchiseptica* efficacy.

The results show that the oral route of administration is efficacious. Oral use efficacy is also supported by the *B bronchiseptica* isolation data and the MAT antibody serological response results. These data demonstrate that none of the pups were exposed to *B bronchiseptica* prior to vaccination and that the placebo-vaccinated pups remained free of *B bronchiseptica* until challenge. Pups in both of the B bronchiseptica-vaccinated groups (B & C) had a positive serological response to vaccination and had colonization of the trachea with *B bronchiseptica* following vaccination.

Tracheal colonization with *B bronchiseptica* is expected after intranasal vaccination with avirulent live *B bronchiseptica* bacteria, which may stimulate mucosal secretory IgA. One *B bronchiseptica* challenge study showed that dogs vaccinated intranasally with avirulent live *B bronchiseptica* vaccine had higher B bronchiseptica-specific IgA titers in nasal secretions, less coughing, and shed fewer challenge organisms than dogs vaccinated subcutaneously with a killed antigen extract vaccine.[3] B bronchiseptica-specific IgA titers in nasal secretions following oral *B bronchiseptica* vaccination has not been studied, but oral vaccination can induce secretory antibody response in other mucosal sites. Oral immunization can protect the intestinal and respiratory mucosa against poliovirus and adenovirus, respectively. Also oral immunization with Chlamydia trachomatis induces a secretory antibody response in genital mucosa of mice.[8]

Further research is indicated to evaluate the duration and degree of mucosal immunity that results after oral vaccination for B bronchiseptica. It would also be interesting to determine if oral administration of the CPI, CAV2, *B bronchiseptica* combination vaccine imparts immunity and protection against CPI or CAV2 infection. In addition, more research will be needed to determine how the efficacy of oral *B bronchiseptica* vaccination compares with that of other routes of administration and if combining the oral route of administration with other routes for primary and booster vaccination will improve efficacy even further.

Oral administration of this vaccine will prove useful in aggressive and frightened dogs that are difficult to handle during administration of parenteral and intranasal vaccines. While both the American and Canadian Veterinary Medical Associations have issued position statements regarding canine vaccination and the American Animal Hospital Association has issued in-depth guidelines including differentiation between core and noncore vaccines, ultimately decisions of what diseases to vaccinate against and which vaccines to use are left to the primary care veterinarian and the dog owner. The novel oral administration of a vaccine previously labeled for intranasal use only is yet another tool for the practitioner to use as an insurance policy against the consequences of ITB.

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Footnotes

a Bronchi-Shield III manufactured by Boehringer Ingelheim Vetmedica, Inc. (Canine Adenovirus Type 2 - Parainfluenza - *Bordetella Bronchiseptica* Vaccine Modified Live Virus and Avirulent Live Culture)

b Bronchi-Shield previously manufactured by Fort Dodge Animal Health, Inc. (Monovalent *Bordetella Bronchiseptica* Vaccine Avirulent Live Culture)

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