

A Comparative Study of Protective Immunity Provided by Oral, Intranasal and Parenteral Canine *Bordetella bronchiseptica* Vaccines

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ABSTRACT

The present study was designed to determine if an oral live *Bordetella bronchiseptica* vaccine protects dogs from developing signs of respiratory disease after challenge with virulent *Bordetella bronchiseptica*. The study was also designed to compare the level of protection induced by the oral vaccine with the protection provided by an intranasal live vaccine and an injected killed vaccine. Forty 6-8 week old beagles, culture and antibody negative to *Bordetella bronchiseptica*, were randomly distributed to 4 groups. Group 1 (O) received a single dose of attenuated oral *Bordetella* vaccine at study day 14; Group 2 (IN) received a single dose of attenuated intranasal *Bordetella* vaccine at study day 14; Group 3 (SCu) received two doses of killed *Bordetella* vaccine administered subcutaneously at study days 0 and 14; and Group 4 (C) received saline both

intranasally and subcutaneously. All dogs were challenged with virulent *Bordetella bronchiseptica* via nebulization chamber at study day 42. Blood and nasal swabs were collected weekly thru-out the study for serology and bacterial culture. After challenge, daily clinical assessments included body temperature and a weighted score of coughing and other respiratory disease signs. Severe signs of disease in the control group proved the validity of our challenge model. Results of this study showed that the oral *Bordetella bronchiseptica* vaccine protects dogs from challenge. This protection provided by the oral vaccine was equivalent to that induced by intranasal vaccine, and was superior to protection provided by the killed, subcutaneously administered vaccine.

INTRODUCTION

Bordetella bronchiseptica (*B bronchiseptica*) is the most important bacterial pathogen associated with Canine Infectious Respiratory Disease Complex (CIRDC) (Kiel et al. 1998). However, this complex disease is

also caused by a variety of viruses including: Canine Distemper Virus (CDV), Canine Adenovirus type 2 (CAV-2), Canine Parainfluenzavirus 5 (CPI-5), Canine Influenza virus (CIV) and possibly others (Appel et al 1978; Erles et al 2004; Wagener et al 1984). In addition to *Bordetella bronchiseptica*, bacteria such as haemolytic *E. coli*, *Klebsiella* sp., *Staphylococcus* sp., *Pasteurella multocida*, *Streptococcus canis*, *Streptococcus equi zooepidemicus* and *mycoplasma* sp. may also contribute to disease (Bemis et al 1977; Mannering et al 2008; Mochizuki et al 2008; Roycroft et al 2006; Ueland 1990). Stress, poor ventilation, inadequate hygiene, dust and aerosols created by power washers also play a role (Priestnall et al 2012). Constant barking is another important factor when dogs are placed in boarding kennels or animal shelters with other dogs.

Currently there are three different types of *Bordetella bronchiseptica* vaccines licensed in the United States for use in dogs: a killed injectable vaccine for subcutaneous use, a live attenuated vaccine for intranasal administration, and most recently, a live attenuated vaccine to be given orally. The practical advantage of the oral vaccine is its ease of administration as compared to the intranasal vaccine. The oral vaccine is also able to provide local mucosal immunity similar to the intranasal vaccine. The present study was designed to compare the different types of *B bronchiseptica* vaccines.

MATERIALS AND METHODS

Animals

Institutional Animal Care and Use Committee approval was obtained for this study. Forty beagle pups (Ridglan Farms, Inc.) aged 6 to 8 weeks were screened to be culture negative for *Bordetella bronchiseptica*, and to have low or no antibody against *B bronchiseptica* via ELISA tests at time of vaccination. Selected pups were randomly assigned to one of four groups and housed in the isolation units of the Charmany Instructional Facility, UW-Madison School of Veterinary Medicine.

Vaccines

Group 1 (O) was vaccinated orally with a single dose of Bronchi-Shield®ORAL (Boehringer Ingelheim Vetmedica, Inc [BIVI]). Group 2 (IN) was vaccinated intranasally with a single dose of Bronchi-Shield®III (BIVI). Group 3 (SCu) was vaccinated subcutaneously with two doses of Bronchicine®CAe (Zoetis). Group 4 (C) was vaccinated subcutaneously and intranasally with saline.

Challenge Organism

Challenge included a pool of 3 strains of virulent *B bronchiseptica*. The bacteria were grown on Bordet-Gengou agar for 48 hours at 36 ±2° C. Bacteria were harvested from the plates using sterile glass rods and inoculated into peptone broth. The culture was then held at 35°C until used for challenge, which was administered within 2 hours after the culture was placed in the peptone broth. The challenge dosage was approximately 4.5x10¹⁰ cfu. The bacterial culture was aerosolized using a mechanical nebulizer and attached holding chamber. The chamber was primed with challenge material immediately before using. All dogs were exposed to challenge by placing them in the chamber where they inhaled continuously aerosolized *bordetella* for 15 to 20 minutes each.

Experimental Design

Sera and nasal swabs were collected from all pups weekly throughout the duration of the study. Dogs in Group 3 (SCu) were vaccinated at study days 0 and 14. Dogs in all other groups were vaccinated at study day 14 only. Additional nasal swabs were collected for ELISA to determine mucosal immunity at study days 35, 49, and 56. Nebulized challenge was administered at study day 42. Pups were monitored daily for clinical signs on study days 35 thru 40 (pre-challenge) and then daily after challenge until end of study. At study day 55, half of the dogs in groups which showed no clinical signs post-challenge were released. All remaining dogs were humanely euthanized at study day 56. Lung consolidation scores were recorded and samples were collected for culture and serology.

Clinical Signs

Clinical observations included 1) rectal temperatures, 2) coughing (spontaneous and induced), 3) nasal discharge, 4) sneezing, 5) difficult breathing, and 6) decreased activity. Fever was defined as body temperature $\geq 39.5^{\circ}\text{C}$. See Table 1 for weighted scoring rubric.

Serology

Serum anti-*Bordetella* antibody was determined via ELISA (Dees et al., 1982.) Mucosal anti-*Bordetella* IgA present in nasal

Table 1. Clinical Score Rubric

Sign	Qualifier	Score
Cough	Mild	1
Cough	Induced	3
Cough	Spontaneous	5
Nasal Discharge	Thick, mucus	2
Sneezing	Multiple	1
Lethargy		10
Dyspnea		10

secretions was also determined by ELISA (Bey et al., 1981.)

Bacterial Culture

Weekly nasal swabs were streaked onto McConkey's agar, and incubated for 24 hours. Lung swabs collected at necropsy were streaked similarly. *Bordetella* was confirmed by typical growth, metabolic pattern, and microscopic morphology. Quantitative growth level was reported as last quadrant of the plate that the organism was able to be streaked to. A low level of *bordetella*, for example, would be found only in the first quadrant and would be reported as 1. High level of growth would be reported as 4 for the fourth quadrant.

Gross Pathology

Lung consolidation was assessed by a pathologist who was blinded to study groups. Lung pathology was determined by direct palpation of lung tissues, and recorded as

percent consolidation per lobe. (Larson et al. 2011)

RESULTS

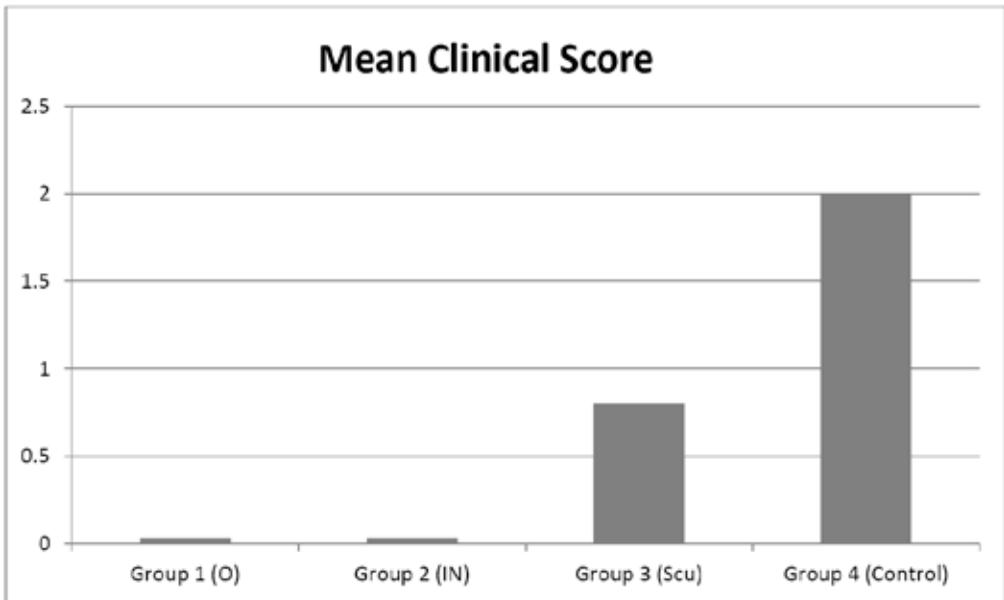
Bacterial Culture

All dogs remained culture negative for *Bordetella bronchiseptica* through study day 14. Beginning on study day 21, *bordetella* was detected sporadically in nasal swabs at a low level in Groups 1 (O) and 2 (IN), which received attenuated live vaccines. Groups 3 (SCu) and 4 (C), which received killed vaccine and saline respectively, remained culture negative until after challenge. At study day 56 (necropsy) *B bronchiseptica* was found in nasal swabs of all groups; however it grew to a lower level in swabs collected from Group 1 (O) than from the other groups. Tracheal swabs collected at necropsy (study day 56) were positive for *Bordetella bronchiseptica* in every dog tested, and grew to equivalently high levels. In general, lung tissues were less likely to harbor *bordetella* than nasal and tracheal samples. Group 4 (C) dogs harbored slightly more *bordetella* in lung tissues than vaccinates.

Clinical Signs

Dogs in both Group 1 (O) and Group 2 (IN) showed very few signs of disease post challenge. These groups both had average cumulative scores of 0.03, with the most common clinical sign being occasional mild cough with no further complications. Many dogs (70%) in both these groups did not cough at all. Group 3 (SCu) had an average cumulative score of 0.8. All dogs in this group showed some level of morbidity. Seven of 10 dogs (70%) in the SCu group showed signs of spontaneous, harsh cough that continued for several days. Only three of the ten dogs had clinical signs that could be described as mild cough. Group 4 (C) (unvaccinated control) showed signs of severe respiratory disease, and had a cumulative average score of 2.0. Although one dog in this group had signs of only mild cough, all the remaining nine dogs showed harsh, ongoing severe cough. Two dogs in this group required euthanasia due to severity of

Graph 1. Cumulative Mean Clinical Score (Higher number indicates more significant clinical disease)



disease. See Graph 1

Body Temperature

Groups 1 (O), 2 (IN), and 3 (SCu) showed body temperatures in the normal range throughout the post challenge period. In Group 4 (C), however, half of the group developed body temperatures at or above 39.5°C for at least one day. (Data not shown.)

Serology

Average serum IgG levels in all groups were very low and were reported at OD = 0.3 at one week before the present study began. This represents seronegativity in our serum ELISA. By study day 35 (post vaccination, but before challenge) all groups showed significant increases in serum IgG. Groups 1 (O) and 2 (IN) (oral and intranasal vaccines) both had average OD = 0.7. Groups 3 (SCu) and 4 (C) (SQ and control) both had average OD = 0.8. (Data not shown)

Nasal swab IgA ELISA at study day 35 (post vaccination) showed the oral group average OD = 0.4; intranasal group average OD = 0.2; parenteral group average OD = 0.2; and control group average OD = 0.1.

Gross Pathology

Group 1 (O) showed very little consolidation, and scored an average cumulative score of 2.0. Group 2 (IN) included an outlier pup which skewed the cumulative average of this group to a score of 12.2. With the outlier dog removed, the average score for this group was 7. Group 3 (SCu) showed an average cumulative score of 10. The control Group 4 (C) showed an average cumulative consolidation score of 46 due primarily to two dogs that required euthanasia due to severity of disease. See Graph 3.

DISCUSSION

Many previous studies have demonstrated that *B bronchiseptica* plays a significant role in canine infectious respiratory disease complex (CIRDC) (Appel et al 1978; Bemis et al 1977; Keil et al 1998). It has also been demonstrated that *B bronchiseptica* vaccines reduce the severity of clinical signs associated with CIRDC (Edinboro et al 2004; Glickman et al 1981; Kontor et al 1981; Lehar et al 2008).

The first *B bronchiseptica* vaccine to be licensed was a killed injectable product, which was shown to significantly reduce clinical disease signs (Ellis et al 2001,

2002).

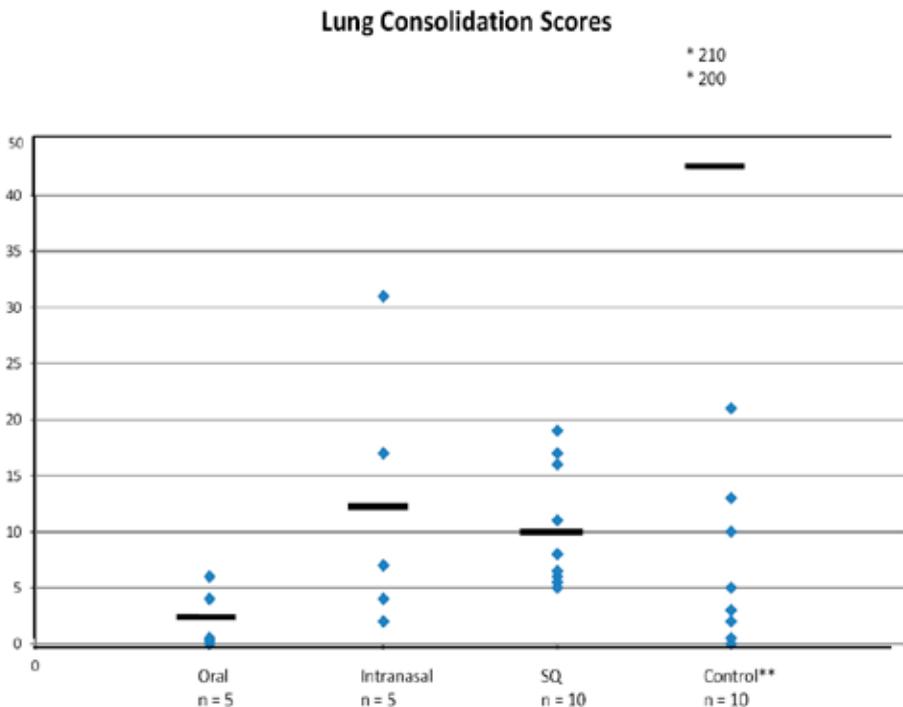
The killed vaccine was followed by the development of a live attenuated intranasally administered vaccine. The efficacy of the intranasal product in reducing clinical disease was considered superior to the killed injectable products (Chladek et al 1981; Davis et al 2007). A further advantage of the live attenuated vaccine over the killed product is that it has been shown to induce immunity after a single dose, and has been shown to provide protection after only 72 hours (Gore et al 2005).

However, intranasal vaccines are more difficult to administer than an injectable product, therefore are less popular with veterinarians. Parenteral vaccine remains the most commonly used *bordetella* product. Recently a live attenuated oral *Bordetella* vaccine was licensed. The oral *Bordetella* vaccine provided immunity that was similar to that induced by the intranasal product, but it is more easily administered than the intra-

nasal product (Hess et al 2011). Importantly, in the present study we demonstrated that local secretory IgA immunity was induced by both the oral and intranasally administered vaccines.

The immunologic mechanism of protection provided by vaccination has not been well defined for *Bordetella bronchiseptica* (Chalker et al 2003). It is known that bacterial endotoxins play a role in causing pathology in both the upper respiratory tract as well as in the lung. Neutralization of those endotoxins by locally produced secretory antibody, most likely of the IgA class, as well as systemically produced IgG antibody that can reach the lung via the serum, would be important in reducing pathology caused by the toxins. It is also expected that secretions containing IgA and serum containing primarily IgG (but also some IgA) antibody to *Bordetella bronchiseptica* would provide protection in the lungs through transudation. Secretory antibody is induced by the intrana-

Graph 3. Lung Consolidation Scores (Bars indicate average scores)



sal and oral vaccines given locally, whereas killed vaccines given systemically would be expected to produce serum IgG and IgA, but not produce local secretory IgA. Secretory IgA is produced by dogs that have been vaccinated with live *bordetella* which replicates primarily in the upper respiratory and/or intestinal tract.

In the present challenge study, and also in previous vaccine studies where intranasally administered live *bordetella* vaccines have been compared with systemically administered killed vaccines, protection from clinical disease has been greater with the live vaccines (Davis et al, 2007). In the present study, dogs challenged with highly virulent *Bordetella bronchiseptica*, the severity of disease was shown to be most severe in control (unvaccinated) dogs. Clinical disease was less severe in the group vaccinated with the parenterally administered killed *Bordetella bronchiseptica* vaccine than the control group. However, the live bacterial vaccines administered orally or intranasally provided the greatest protection from development of clinical disease. Administration of live *bordetella* vaccine intranasally has been shown previously by others to be superior to the killed vaccine administered parenterally (Davis et al 2007). In the present study as well as in a previous study (Hess et al 2011) a live vaccine administered orally provided a level of protection from clinical disease superior to that provided by parenteral killed vaccine, and equivalent to that provided by the same live vaccine given intranasally. Although, the exact mechanism of the enhanced protection from oral vaccine remains to be determined, dogs that received the oral vaccine in the present study had both IgG and IgA antibody to *bordetella* in serum and nasal secretions similar to dogs vaccinated intranasally with live vaccines. Studies are in progress to determine the mechanism for the greater protection provided by the live vaccines given orally or intranasally compared to the killed vaccine given parenterally. It is speculated that the greater protection provided by the live vaccines given orally

or intranasally is due to the heterogeneity of the antibody produced to multiple antigenic epitopes provided by the replicating *Bordetella bronchiseptica* versus the more restrictive antigenic epitopes presented with a killed parenterally administered product. In addition to protection provided by systemically produced IgG and IgA antibodies and locally produced secretory IgA antibodies, other protective proteins are likely produced by the live bacterial vaccines which are most like protection provided after recovery from disease.

In the present study, *bordetella* was cultured from dogs that received the live orally and live intranasally vaccinated dogs prior to challenge demonstrating vaccine *bordetella* organisms were present. In contrast, *bordetella* was not cultured from the killed parenterally vaccinated group or the control group prior to challenge, as expected. Another possible explanation for the difference in protection from clinical disease provided by the live locally administered IN and O vaccines and the parenteral vaccine is based on the difference between secretory IgA and non-secretory IgA. Secretory IgA is a dimeric immunoglobulin containing a secretory component. Non-secretory IgA is a monomeric immunoglobulin without a secretory component. The secretory component enables the immunoglobulin to be more effective in controlling infection or neutralizing the endotoxins produced by *Bordetella bronchiseptica*. Studies are in progress in an attempt to determine the precise mechanisms that provide increased protection seen when live locally administered vaccines are used. Unfortunately, the mechanisms of protective immunity to *Bordetella bronchiseptica* are poorly understood (Erles et al 2010).

In the present study the three *bordetella* vaccines currently licensed in the US (oral, intranasal, and injectable) were compared. The results show that the oral *bordetella* vaccine compared very favorably with the intranasal *bordetella* vaccine and both provided greater protection than the killed injectable *bordetella* vaccine in reducing

clinical disease. The oral vaccine has the advantage of being more easily administered than the intranasal product. Both oral and intranasal live vaccines provide better protection from disease caused by *Bordetella bronchiseptica* in the dog than the protection provided by the injectable killed product.

CONCLUSION

Results of this study show that oral attenuated *Bordetella bronchiseptica* vaccine protects dogs from challenge which created significant disease in non-vaccinated and challenged control dogs. This protection was equivalent to that induced by intranasal *Bordetella bronchiseptica* vaccine, and was superior to protection provided by the killed, subcutaneously administered vaccine. Oral *Bordetella bronchiseptica* vaccine offers a convenient alternative to the intranasal vaccines due to the ease of administration and an alternative to killed injectable due to improved efficacy.

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