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Comparative onset of immunity of oral and intranasal vaccines against challenge with *Bordetella bronchiseptica*

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ABSTRACT

Three groups of approximately eight-week-old beagles were vaccinated once with 1 ml of placebo vaccine (oral, n=9), 1 ml of Recombitek® Oral Bordetella (oral, n=10) or 1 ml Nobivac® Intra-Trac$_3$ (intranasal, 0.5 ml/nostril, n=10). Seven days after vaccination, the three groups were challenged with virulent *Bordetella bronchiseptica* via aerosolisation. Eight of nine dogs in the placebo group and no dogs in the Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ vaccine groups developed spontaneous cough of two or more consecutive days (disease case definition). Dogs in the Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ groups had a significantly lower incidence of disease (P<0.0001) with a 100 per cent preventable fraction. The study demonstrated that vaccination with either Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ is effective in preventing disease seven days after vaccination when compared with dogs vaccinated with a placebo.

INTRODUCTION

Bordetella bronchiseptica is an important bacterial component in canine infectious respiratory disease complex.[1 2] To date, there are injectable, oral and intranasal monovalent and combination vaccines available for vaccination of dogs to reduce or avoid the clinical signs of infection with *B bronchiseptica*. Several studies, some comparative, have evaluated the efficacy of these vaccines against *B bronchiseptica* challenge with varying results.[1–9]

The majority of the studies used a scoring system to assess the clinical signs after challenge, and intranasal vaccines tended to have better efficacy than vaccines administered by other routes. Intranasal vaccines have been noted to be difficult to administer, and injectable vaccines have the potential to cause injection site reactions.[7] Injectable *Bordetella* vaccines also have a longer onset to protection since they require two doses separated by a few weeks to initiate immunity. A particular advantage of oral vaccines is the ease of administration while maintaining the local mucosal immunity of an intranasal administration.[2]

Since intranasal vaccines were most frequently observed to have the best efficacy, this study was designed to compare the efficacy of a newly developed oral vaccine with an established intranasal vaccine against a *B bronchiseptica* challenge seven days after vaccination. Rather than using a scoring system, the clinical signs in this study were evaluated as present or absent to provide the most stringent comparison of the vaccines.

MATERIALS AND METHODS

All animals were handled in compliance with Institutional Animal Care and Use Committee guidelines and approval was obtained prior to the initiation of the study. The study was conducted in accordance with Good Clinical Practice guidelines. Three groups of approximately eight-week-old beagles were vaccinated once with 1 ml of placebo vaccine (oral, n=9), 1 ml of Recombitek® Oral Bordetella (oral, n=10) (Merial) or 1 ml Nobivac® Intra-Trac$_3$ (intranasal, 0.5 ml/nostril, n=10) (Merck Animal Health). Thirty-one days prior to vaccination, all dogs were screened and determined to be serologically negative for antibodies to *B bronchiseptica* and to be negative for the presence of *B bronchiseptica* by tracheal culture. Dogs were tested again on day 0 and any serologically or culture positive dogs were excluded. All of the dogs were housed in an isolation building and were randomised to group by litter and gender. The dogs were separated by group (one pen per group) at the time of vaccination and were housed separately by group until the day of challenge to prevent cross-contamination from shedding. Approximately 30 minutes prior to challenge, the dogs from all three groups were commingled in the challenge room to maintain blinding of personnel performing clinical observations and sample collection. Personnel involved with sample analysis were also unaware of treatment group assignments. Personnel involved with vaccination were not involved with clinical observations, sample collection or sample analysis. The statistician

accessed the data after the database was locked by data management and then merged the randomisation (group assignments) with the data and ran the statistical analysis according to the data analysis plan.

The Recombitek® Oral Bordetella group received a monovalent, avirulent modified live, industrial scale, prelicense serial at the targeted commercial dose. The Nobivac® Intra-Trac$_3$ group received a commercial canine adenovirus type 2, parainfluenza, *B bronchiseptica*, modified live virus and avirulent live culture vaccine. Dogs in the placebo group received sterile water.

Seven days after vaccination, all of the dogs were challenged with a mixture of two strains of virulent *B bronchiseptica* via aerosolisation. The challenge isolates were grown on Bordet-Gengou blood agar plates and incubated at 37°C for 24 hours. Bacterial growth on plates for each isolate was harvested and pooled in PBS. The challenge process was similar to that described by Hess *et al*[7] and Larson *et al*.[2] Dogs were randomised to challenge chamber with dogs from each group represented in each chamber. Dogs of different groups were comingled after challenge and were randomised to two pens so that each vaccination group was represented in each pen.

During the 14-day postchallenge phase, dogs were observed for spontaneous cough, malaise, nasal discharge, ocular discharge and other signs of respiratory infection. Malaise was defined as a dog with the appearance of generalised illness, weakness or fatigue. Consecutive days of respiratory clinical signs were considered clinically relevant and described. Rectal temperatures were recorded daily. Fever was defined as a rectal temperature of at least 39.7°C and 0.5°C above the day 0 rectal temperature. Tracheal swabs were collected under light sedation with propofol prior to vaccination and on the final day of the study and were evaluated for the presence of *B bronchiseptica* using a procedure previously described.[7] Tracheal swabs were collected in a manner that avoided contamination from the oral cavity. Serum samples were collected on days 0, 7 (prior to challenge), 14 and 21 and analysed for the presence of *B bronchiseptica* antibodies by microagglutination assay.[7]

For this study, a dog was classified as having disease due to *B bronchiseptica* if it developed spontaneous cough for two or more consecutive days. This case definition was selected to provide a robust comparison of the vaccines while avoiding over-representing isolated instances of cough. Incidence of disease was compared between each test vaccine group and the placebo vaccine group using Fisher's exact test. The prevented fraction in each test vaccine and its 95% CI were also calculated. All statistical analyses were performed using SAS V.9.4 Enterprise Guide (SAS Institute) and PF package in R V.3.1.1. All tests were two sided, and statistical significance was declared at a P value of 0.05 or less. Statistical analyses were not performed for other clinical signs of infection, serology titres or tracheal swab isolation.

RESULTS

Vaccine safety

No animal experienced any adverse vaccine reactions during the study.

Spontaneous cough and incidence of disease

Prior to challenge, no dogs in any group showed clinical signs of disease. Eight of 10 dogs in the placebo group and no dogs in the Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ vaccine groups developed spontaneous cough of two or more consecutive days (Table 1). The prevented fraction was 1.00 and the 95% CI was (0.67 to 1.00) for both vaccine groups.

Other signs of infection

Two dogs in the placebo group were observed with one day of malaise each. No malaise was observed in either the Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ vaccine group. The Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ vaccine groups each had one dog with one day of fever while there were five dogs in placebo group with one day of fever. One dog in the placebo group had fever on two consecutive days (Table 2).

No dogs in either the Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ vaccine group had consecutive days of mucopurulent ocular discharge while it was observed in four dogs in the placebo group. Serous ocular discharge was observed on consecutive days in six dogs in the Recombitek® Oral Bordetella group, four dogs in the Nobivac® Intra-Trac$_3$ group and six dogs in the placebo group.

No dogs in the Recombitek® Oral Bordetella group were observed with consecutive days of serous or mucopurulent nasal discharge. Four dogs in the

TABLE 1: The incidence of positive disease due to *Bordetella bronchiseptica* infection by group with P values of the Fisher's exact test, prevented fraction and 95% CI

Group	Dogs with two or more cumulative days of cough	Number of dogs with disease (consecutive days of cough)*	Per cent of dogs with disease	Prevented fraction (95% CI)
Recombitek® Oral Bordetella (n=10)	2	0	0.00	1.00 (0.67 to 1.00)
Placebo vaccine (n=9)	8	8	88.9	
Nobivac® Intra-Trac$_3$ (n=10)	1	0	0.00	1.00 (0.67 to 1.00)

*Significant difference between groups (Fisher's exact test P<0.0001).

TABLE 2: Number of dogs expressing clinical signs for two or more days after challenge

	Dogs with two or more cumulative days of clinical sign					Dogs with consecutive days of clinical sign				
		Nasal discharge		Ocular discharge			Nasal discharge		Ocular discharge	
Vaccine	Fever	S	MP	S	MP	Fever	S	MP	S	MP
Recombitek® Oral Bordetella (n=10)	0	2	0	10	2	0	0	0	6	0
Nobivac® Intra-Trac$_3$ (n=10)	0	6	1	6	0	0	4	1	4	0
Placebo vaccine (n=9)	1	3	3	9	6	1	2	3	6	4

MP, mucopurulent; S, serous.

Nobivac® Intra-Trac$_3$ and two dogs in the placebo group were observed with consecutive days of serous nasal discharge. Three dogs in the placebo group and one dog in the Nobivac® Intra-Trac$_3$ group were observed with mucopurulent nasal discharge on consecutive days.

Serum agglutination titres

All dogs in the Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ vaccine groups were seronegative prior to vaccination (Table 3). On day 7, two dogs in the Recombitek® Oral Bordetella vaccine group were seropositive for antibodies to *Bordetella* with a maximum of five dogs on day 21. None of the animals in the Nobivac® Intra-Trac$_3$ group were seropositive for *Bordetella* antibodies during the study. Two dogs in the placebo group were seropositive on day 21 (14 days after challenge). Dogs in the Recombitek® Oral Bordetella vaccine group had consistently higher geometric mean titres than dogs in the Nobivac® Intra-Trac$_3$ or placebo group (Table 4).

B bronchiseptica isolation from tracheal swabs

Tracheal swabs for all dogs were negative for *B bronchiseptica* prior to vaccination (Table 5). All of the dogs (100 per cent) in the Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ vaccine groups and eight dogs (88.9 per cent) in the placebo group had positive tracheal isolation on day 21 (14 days after challenge). The dog in the placebo group with the negative day 21 tracheal swab was observed with serous ocular discharge and mucopurulent ocular discharge on non-consecutive days, with consecutive days of serous nasal discharge, mucopurulent nasal discharge and cough, and was seropositive on day 21.

TABLE 3: Dogs seropositive for antibodies to *Bordetella bronchiseptica* (titre >8) by microagglutination assay

	Day			
Group	0	7*	14	21
Recombitek® Oral Bordetella (n=10)	0	2	4	5
Nobivac® Intra-Trac$_3$ (n=10)	0	0	0	0
Placebo vaccine (n=9)	0	0	0	2

*Day of challenge.

DISCUSSION

Vaccination with Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ provided protection against a virulent two-strain *B bronchiseptica* challenge seven days after vaccination. Based on the case definition of two consecutive days of spontaneous cough, eight of nine dogs (88.9 per cent) in the placebo group developed disease while no dogs (0.0 per cent) vaccinated with the Recombitek® Oral Bordetella or Nobivac® Intra-Trac$_3$ developed disease. These results are consistent with those reported by Larson *et al*[2] where an oral *B bronchiseptica* vaccine provided similar protection as an intranasal vaccine when challenged 42 days after vaccination.

Clinical signs of respiratory disease were considered clinically relevant when they occurred on two or more consecutive days. Forty per cent of dogs in the Nobivac® Intra-Trac$_3$ were observed with serous nasal discharge compared with 22.2 per cent of dogs in the placebo group and no (0 per cent) dogs in the Recombitek® Oral Bordetella group. It is hypothesised that the intranasal vaccination may have irritated the nasal mucosa to some extent making serous nasal discharge more frequent after challenge in the Nobivac® Intra-Trac$_3$ group. No dogs in the Recombitek® Oral Bordetella group had consecutive days of mucopurulent nasal discharge compared with one dog

TABLE 4: Microagglutination assay geometric mean titres for *Bordetella bronchiseptica* antibody

	Day			
Group	0	7*	14	21
Recombitek® Oral Bordetella (n=10)	8.00	9.19	12.13	13.93
Nobivac® Intra-Trac$_3$ (n=10)	8.00	8.00	8.00	8.00
Placebo vaccine (n=9)	8.00	8.00	8.00	10.08

*Day of challenge.

TABLE 5: Dogs with *Bordetella bronchiseptica* positive tracheal isolation

	Number of dogs with positive tracheal isolation	
Group	**Day 0**	**Day 21**
Recombitek® Oral Bordetella (n=10)	0	10
Nobivac® Intra-Trac$_3$ (n=10)	0	10
Placebo vaccine (n=9)	0	8

in the Nobivac® Intra-Trac$_3$ group and three dogs in the placebo group.

While disease protection from the two vaccines was clearly demonstrated, neither of the vaccines prevented colonisation of the upper respiratory tract by *B bronchiseptica* after challenge, as detected by reisolation of the challenge organisms from tracheal swabs.

Interestingly, mean serum antibody titres to *B bronchiseptica* were higher in the Recombitek® Oral Bordetella group than in the Nobivac® Intra-Trac$_3$ or placebo group following both vaccination and challenge. The total number of seropositive dogs was also higher in the Recombitek® Oral Bordetella group with 50 per cent of dogs seropositive 14 days after challenge compared with 22.2 per cent in the placebo group and 0.0 per cent in the Nobivac® Intra-Trac$_3$ group. It is unknown why the intranasal group did not show a serological response after vaccination as it was anticipated that the Nobivac® Intra-Trac$_3$ group would have a similar serologic response as the Recombitek® Oral Bordetella group and as previously reported in the literature.

This study demonstrates that the newly developed Recombitek® Oral Bordetella vaccine is equivalent to the Nobivac® Intra-Trac$_3$ intranasal vaccine in preventing disease due to *B bronchiseptica* when compared with dogs vaccinated with a placebo. Dogs vaccinated with Recombitek® Oral Bordetella and Nobivac® Intra-Trac$_3$ had a significantly lower incidence of disease ($P<0.0001$) with a 100 per cent preventable fraction. The results of this study provide clear evidence that vaccination with Recombitek® Oral Bordetella vaccine is effective in preventing cough in dogs when challenged with *B bronchiseptica* seven days after vaccination and offers an alternative to the more difficult intranasal administration without the potential injection site reactions of a parenterally administered product.

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Data sharing statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

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