Veterinary Record Open

OPEN ACCESS

Thirteen-month duration of immunity of an oral canine vaccine against challenge with *Bordetella bronchiseptica*

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To cite: Scott-Garrard M, Wang X, Chiang Y-W, *et al*. Thirteen-month duration of immunity of an oral canine vaccine against challenge with *Bordetella bronchiseptica*. *Veterinary Record Open* 2020;**5**:e000423. doi:10.1136/vetreco-2020-000423

Received 01 June 2020
Revised 20 August 2020
Accepted 15 September 2020

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ABSTRACT

Background Very few studies have evaluated the duration of immunity of *Bordetella bronchiseptica* vaccines in dogs, and to date, no studies have been published on the duration of immunity of oral canine *Bordetella bronchiseptica* vaccines. This study was designed to determine the effectiveness of a single dose of an oral *B bronchiseptica* vaccine in dogs when challenged 13 months after vaccination.

Methods Two groups of approximately eight-week-old beagles were vaccinated once with 1 ml of placebo vaccine (oral, n=17) or 1 ml of Recombitek Oral Bordetella (oral, n=17). Thirteen months after vaccination, both groups were challenged with virulent *B bronchiseptica* via aerosolisation.

Results Thirteen of 17 dogs in the placebo group (76.5 per cent) and no dogs in the Recombitek Oral Bordetella vaccine group (0.0 per cent) developed spontaneous cough of two or more consecutive days (disease case definition). Dogs in the Recombitek Oral Bordetella group had a significantly lower prevalence of disease with prevented fraction of 1 (100 per cent prevention). In addition, the number of days coughing, duration of cough and prevalence of tracheal and nasal shedding were significantly lower for dogs vaccinated with Recombitek Oral Bordetella.

Conclusions The study demonstrated that vaccination with Recombitek Oral Bordetella is effective in preventing disease and reducing shedding 13 months after vaccination when compared with dogs vaccinated with a placebo.

INTRODUCTION

Canine infectious respiratory disease complex (CIRD) is an upper respiratory tract disease caused by several different pathogens,[1] and vaccination is important in the prevention and reduction of clinical signs associated with CIRD.[2] With *Bordetella bronchiseptica* being recognised as the most important bacterial component of CIRD,[2 3] several injectable, oral and intranasal monovalent and combination vaccines are used to vaccinate dogs against infection with *B bronchiseptica*.

While several studies have been published evaluating the onset of immunity of vaccines against *B bronchiseptica*,[4 5] very few studies have evaluated their duration of immunity.[2 6] In studies by Jacobs and others[2] and Lehr and others,[6] monovalent and combination intranasal vaccines were reported to have at least a one-year duration of immunity. Both studies used a scoring system to assess the clinical signs.

To date, no studies have been published on the duration of immunity of oral canine *B bronchiseptica* vaccines. This study was designed to determine the effectiveness of a single dose of an oral *B bronchiseptica* vaccine in dogs when challenged 13 months after vaccination.

MATERIALS AND METHODS

The study was conducted in accordance with good clinical practice guidelines. Two groups of approximately eight-week-old beagles were vaccinated once with 1 ml of placebo vaccine (oral, n=17) or 1 ml of Recombitek Oral Bordetella (oral, n=17) (Boehringer Ingelheim Animal Health). Thirty-one days before vaccination, all dogs were screened and determined to be serologically negative for antibodies to *B bronchiseptica* by microagglutination assay and to be negative for the presence of *B bronchiseptica* by culture of tracheal swabs. Dogs were tested again one day before vaccination, and all dogs remained serologically and culture negative. All of the dogs were housed in an isolation building and were randomised to vaccination groups and housing units using litter as a randomisation factor. The dogs were separated by group at the time of vaccination and were housed separately by group until the day of challenge to prevent cross-contamination from shedding. As the dogs aged, the number of pens and animals per pen were adjusted to account for size and gender. During the 13-month prechallenge period, swabs (tracheal or nasal) and blood were collected monthly.

Table 1 The prevalence of positive disease due to *Bordetella bronchiseptica* infection by group with prevented fraction and 95 per cent CI

Vaccine	Dogs with one or more days of cough	Dogs with two or more cumulative days of cough	Number of dogs with disease (consecutive days of cough)*	Percent of dogs with disease	Prevented fraction (95 per cent CI)
Placebo vaccine (n=17)	15	13	13	76.5	1.000
Recombitek Oral Bordetella (n=17)	3	0	0	0.00	(0.773 to 1.000)

*Significant difference between groups.

The Recombitek Oral Bordetella group received a monovalent, avirulent, modified live vaccine at the targeted minimum protective dose, which is below the commercial dose. This dose was selected as a dose that demonstrates clinically relevant protection and assures that a dog receiving a commercial formulation would never receive a lower dose. Dogs in the placebo group received a similarly formulated vaccine with no active ingredient.

Approximately 30 minutes before challenge, the dogs from both 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.

Four hundred and six 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 heterologous to the vaccine strain and 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 phosphate buffered saline. The challenge process was similar to that described by Hess and others[7] and Larson and others.[3] For the challenge phase, dogs from both groups were commingled and housed with littermates in three rooms.

During the 14-day postchallenge phase, dogs were observed twice per day for spontaneous cough, nasal discharge, ocular discharge and other signs of respiratory infection. 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. In addition to the samples collected during the prechallenge period, tracheal swabs, collected under light sedation with propofol, and nasal swabs were collected the day before challenge and on the final day of the study. Blood was collected weekly postchallenge. The swabs were cultured for the presence of *B bronchiseptica* using a procedure previously described.[6] Tracheal swabs were collected in a manner that avoided contamination from the oral cavity. Swabs were placed directly in the trachea and withdrawn without touching the oral mucosa. All serum samples in the study were 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. Prevalence of positive tracheal and nasal shedding, the number of days of cough and the duration of cough were also analysed.

		Placebo Vaccine																	Recombitek® Oral Bordetella														
Dog		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Day	407	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	408	1	0	1	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	409	1	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	410	1	1	1	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	411	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	412	1	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	413	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	414	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	415	1	1	1	0	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	416	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	417	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	418	1	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	419	1	1	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	420	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 = dog observed with cough

Figure 1 Individual animal data for cough postchallenge.

Table 2 Summary statistics for total number of days of cough

Vaccine	Mean	Minimum	25th percentile	Median	75th percentile	Maximum
Placebo vaccine (n=17)	7.8	0.0	4.0	10.0	12.0	14.0
Recombitek Oral Bordetella (n=17)	0.2	0.0	0.0	0.0	0.0	1.0

Prevalence of disease and of positive tracheal and nasal shedding were compared between the test vaccine group and the placebo vaccine group. The prevented fraction (PF) and its 95 per cent CI were calculated using the Two One-sided Scores Test method in the Proc Binomial procedure in StatXact. For the number of days of cough (total number of days observed with cough) and duration of cough (number of days from onset (first observation) to last occurrence of cough), the mitigated fraction (MF) and 95 per cent CI were estimated, using the Highest Density Interval method applied to the bootstrap distribution.[8] Statistical analyses were performed using SAS V.9.4 (SAS Institute) and STATXACT V.11.1 (Cytel), and statistical significance was declared if the 95 per cent CI did not include 0. Statistical analyses were not performed for other clinical signs of infection or serology titres.

RESULTS

Vaccine safety

No animal experienced any adverse vaccine reactions during the study.

Spontaneous cough and prevalence of disease

Before challenge, no dogs in either group showed clinical signs related to disease. Thirteen of 17 dogs in the placebo group and no dogs in the Recombitek Oral Bordetella vaccine group developed spontaneous cough of two or more consecutive days (table 1). The PF was 1.000, and the 95 per cent CI was 0.773 to 1.000. Individual animal data are presented in figure 1.

Number of days and duration of cough

Dogs in the placebo vaccine group had a higher total number of days of cough and duration of cough compared with dogs in the Recombitek Oral Bordetella vaccine group (tables 2 and 3). The medians for the total number of days of cough and duration of cough for dogs in the placebo vaccine group were 10.0 and 11.0 days, respectively, while the medians for dogs in the Recombitek Oral Bordetella vaccine group were 0.0 days for both. The MFs for number of days of cough (0.841) and duration of cough (0.841) were statistically significant (table 4).

Table 3 Summary statistics for duration of cough

Vaccine	Mean	Minimum	25th percentile	Median	75th percentile	Maximum
Placebo vaccine (n=17)	8.6	0.0	4.0	11.0	12.0	14.0
Recombitek Oral Bordetella (n=17)	0.2	0.0	0.0	0.0	0.0	1.0

Table 4 MF and 95 per cent CI for number of days and duration of cough

MF for total number of days of cough (95 per cent CI)	MF for duration of cough (95 per cent CI)
0.841 (0.668 to 1.000)	0.841 (0.668 to 1.000)

MF, mitigated fraction.

Other signs of infection

Four dogs in the placebo group had fever on a single day postchallenge while fever was not observed for any dogs in the Recombitek Oral Bordetella vaccine group. No dogs in either group were observed with nasal discharge, ocular discharge or other signs of respiratory infection.

B bronchiseptica isolation from tracheal and nasal swabs

Tracheal and nasal swabs were negative for *B bronchiseptica* for all dogs through day 405 (one day before challenge) (tables 5 and 6). Fourteen days after challenge (day 420), 88.2 per cent of dogs vaccinated with a placebo vaccine had positive tracheal isolation and 64.7 per cent had positive nasal isolation. For the Recombitek Oral Bordetella group, one dog (5.9 per cent) was tracheal isolation positive, and one dog (5.9 per cent) was nasal isolation positive for *B bronchiseptica*. These were two different dogs, and neither dog was observed with cough. The PFs for tracheal shedding (0.933) and nasal shedding (0.909) were statistically significant (table 7).

Serum agglutination titres

All dogs were seronegative before vaccination and on day 25 (table 8). Over the course of the study, 14 dogs (82.4 per cent) in the Recombitek Oral Bordetella and

Table 5 Dogs with *Bordetella bronchiseptica* positive tracheal isolation

	Number of dogs with positive tracheal isolation									
	Day									
Vaccine	−1*	53	102	166	222	278	327	362	405†	420
Placebo vaccine (n=17)	0	0	0	0	0	0	0	0	0	15
Recombitek Oral Bordetella (n=17)	0	0	0	0	0	0	0	0	0	1

*Day before vaccination.
†Day before challenge.

Table 6 Dogs with *Bordetella bronchiseptica* positive nasal isolation

	Number of dogs with positive nasal isolation								
	Day								
Vaccine	−1*	25	81	131	194	243	306	405†	420
Placebo vaccine (n=17)	0	0	0	0	0	0	0	0	11
Recombitek Oral Bordetella (n=17)	0	0	0	0	0	0	0	0	1

*Day before vaccination.
†Day before challenge.

six dogs (35.3 per cent) in the placebo group became seropositive. Overall, geometric mean titres were consistently higher for dogs in the Recombitek Oral Bordetella vaccine group compared with dogs in the placebo group (table 9).

On days 102, 131 and 166, one dog in the control group (same dog for all days) had a seropositive titre. The titre on each day was 16, one dilution above the seronegative cut-off of 8, and seemed to be the result of assay variability due to a non-specific background reaction. This dog was seronegative on day 194 and remained seronegative through day 405. This dog became seropositive again after challenge on day 413. There were no signs of illness related to *B bronchiseptica* infection before challenge, and the dog was observed with coughing postchallenge and developed disease. Additionally, the dogs that were housed with this dog before challenge did not become seropositive. Consequently, the dog was considered to be susceptible to challenge and was not excluded from the study.

Of the four dogs in the control group that were seropositive on day 413, two were also seropositive on day 420. Two other dogs that were not seropositive on any other day were seropositive on day 420. Only one of the six seropositive dogs in the placebo group did not develop disease. This dog was observed with cough on one day. Of the three dogs with one day of cough in the Recombitek Oral Bordetella group, one dog was seropositive on one day (day 53), and two dogs were seropositive on two days during the study (days 53 and 420 for one dog and days 306 and 405 for the other dog).

DISCUSSION

A single dose of Recombitek Oral Bordetella provided protection against a virulent two-strain *B bronchiseptica* challenge 13 months after vaccination. Based on the case definition of two consecutive days of spontaneous cough, 13 of 17 dogs (76.5 per cent) in the placebo group developed disease while no dogs (0.0 per cent) vaccinated with the Recombitek Oral Bordetella developed disease. In addition to a lower prevalence of disease, dogs vaccinated with Recombitek Oral Bordetella had a significantly shorter duration of cough (mean=0.2 day) and total number of days of cough (mean=0.2 day) compared with dogs in the placebo vaccine group (mean=8.6 days and 7.8 days, respectively).

Other than cough, clinical signs of respiratory disease in this study were sparse with four dogs in the placebo group developing one day of fever. This is not unexpected as cough is the primary clinical sign associated with disease due to *B bronchiseptica*.[9]

It should be reiterated that before challenge, the dogs were housed by group, and respiratory shedding of modified live *Bordetella* vaccines can occur. While an effect of housing on the efficacy of the vaccine cannot be ruled out completely, the potential impact of a revaccination effect was considered low in this study as the vaccinates remained negative for nasal and tracheal isolation of *B bronchiseptica* before challenge.

Reducing shedding is an important aspect of preventing spread of *B bronchiseptica*,[10] and vaccination with Recombitek Oral Bordetella resulted in significant reduction of shedding (tracheal PF=0.933 and nasal PF=0.909). Shedding was evaluated at a single time point postchallenge to avoid interfering with collection of clinical signs during the postchallenge observation period. Collection of nasal and tracheal swabs can be irritating and lead to potential increased coughing and inflammation of nasal mucosa that are not desired when attempting to evaluate clinical protection of a vaccine. Additionally, tracheal swabs are collected under sedation, and frequent sedation can have unintended adverse effects. Since the main goal of the study was to identify whether the vaccine protected against disease due to *B bronchiseptica*, the limited evaluation of shedding was accepted. While more dogs in the placebo vaccine group had positive tracheal (88.2 per cent) and nasal (64.7 per cent) isolation 14 days after challenge compared with the Recombitek Oral Bordetella vaccine group (5.9 per cent for both), additional studies would be needed to fully evaluate the extent of shedding inhibited by the vaccine.

The total number of seropositive dogs and mean serum antibody titres to *B bronchiseptica* were higher

Table 7 Prevented fractions and 95 per cent CI for positive tracheal and nasal shedding

PF for tracheal shedding (95 per cent CI)	PF for nasal shedding (95 per cent CI)
0.933 (0.694 to 0.998)	0.909 (0.501 to 0.997)

PF, prevented fraction.

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

	Number of dogs seropositive																
	Day																
Vaccine	–1*	25	53	81	102	131	166	194	222	243	278	306	327	362	405†	413	420
Placebo vaccine (n=17)	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	4	4
Recombitek Oral Bordetella (n=17)	0	0	9	6	3	2	3	4	4	3	4	7	2	1	6	4	6

*Day before vaccination.
†Day before challenge.

in the Recombitek Oral Bordetella group than in the placebo group throughout the study. It is not known why the vaccinated dogs fluctuated serologically, but this is not an unexpected finding. Generally, we do not see 100 per cent seroconversion in animals vaccinated with Recombitek Oral Bordetella (as seen in this study and a previously published study).[5] This observation, however, does not impact the effectiveness of the vaccine.[5] Additionally, while an increase in serum *Bordetella* antibody concentrations is suggestive of immunity, the antibody concentration needed to provide protection is not known.[10] It has been demonstrated that mucosal *Bordetella* vaccines produce lower serum IgG titres than injectable vaccines[11] and that protective mucosal immunity is mediated by secretory IgA.[12] Mucosal antibodies were not measured in this study; however, mucosal IgG and IgA antibody responses to this vaccine have been measured (unpublished data) and indicate a favourable response to vaccination.

There are two published studies on the duration of immunity provided by intranasal monovalent and multivalent *B bronchiseptica* vaccines, Jacobs and others[2] (13 months) and Lehr and others[6] (one year), and Ellis[4] discussed limitations of these studies in a review article. While it would be interesting to compare the results of the intranasal studies to this study using an oral vaccine, there are many study design differences that make this difficult. Both intranasal vaccine studies had a 21-day postchallenge observation period and used scoring systems to evaluate clinical signs. Both scoring systems incorporated the use of tracheal palpation to induce cough. Individual animal clinical sign data by day postchallenge is not presented in either publication. The Lehr and others[6] study used an experimental intranasal *B bronchiseptica* challenge, and the Jacobs and others[2] study used a combination aerosol *B bronchiseptica* and intranasal, oral and aerosol canine parainfluenza virus challenge.

Since the coughing data cannot be isolated from the other clinical signs in the Jacobs and others[2] study, no relative comparison can be made with our oral vaccine study. Similarly for the Lehr and others[6] study, while the number of dogs with cough can be determined, a comparison of cough would not be appropriate due to the differences in length of postchallenge observation period and the use of induced cough. The mean days coughing is presented and was approximately two days for vaccinated dogs and nine days for unvaccinated control dogs. The mean days coughing for controls was similar to what is reported in this study (7.8 days) and was higher for vaccinates than what was observed in this study (0.2 day).

Similar to the other studies evaluating the duration of immunity of mucosal *Bordetella* vaccines, this is a laboratory study using an experimental challenge. While field efficacy studies are desired, an initial evaluation of an oral vaccine in a controlled environment is needed to establish a baseline of protection afforded by the vaccine. The Recombitek Oral Bordetella vaccine prevented disease in 100 per cent of the vaccinated animals in this study and provides a good basis for going into the field.

Mucosal immunity is considered highly important in protecting dogs against respiratory pathogens,[12] and the Recombitek Oral Bordetella vaccine has been previously shown to provide an onset of immunity comparable with intranasal vaccination.[5] The data presented in this study add to the evidence that mucosal vaccines, specifically oral vaccines, can provide long-term immunity. A single dose of Recombitek Oral Bordetella vaccine is effective in preventing cough, reducing duration and number of days of cough and reduces tracheal and nasal shedding in dogs challenged 13 months after vaccination and is suitable for annual vaccination. As oral vaccines are easier to administer than intranasal vaccines and have fewer adverse reactions than

Table 9 Microagglutination assay geometric mean titres for *Bordetella bronchiseptica* antibody (seronegative titre ≤8)

	Day																
Vaccine	–1*	25	53	81	102	131	166	194	222	243	278	306	327	362	405†	413	420
Placebo vaccine (n=17)	8.00	8.00	8.00	8.00	8.33	8.33	8.33	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	9.42	10.22
Recombitek Oral Bordetella (n=17)	8.00	8.00	13.59	13.05	10.22	9.04	10.22	9.42	10.22	9.81	10.22	11.09	9.42	8.33	13.05	9.81	11.09

*Day before vaccination.
†Day before challenge.

injectable vaccines, this vaccine provides an effective alternative to intranasal and parenterally administered vaccines.

Acknowledgements The authors would like to acknowledge Ashley Higginbotham, Nicollette Poole, Kris Dewitt, Dana Parker, Summer Walker-Escoe and Brian Fergen for their assistance.

Contributors All of the authors made substantial contributions to the design of the study and interpretation of the data. MS-G drafted the initial version of the manuscript, and the other authors were involved with editing the manuscript and approved the final version. All authors agree to be accountable for all aspects of the work and ensure that any questions involving the integrity or accuracy of the study will be investigated and addressed.

Funding This study was sponsored by Boehringer Ingelheim Animal Health, and the authors are employees of Boehringer Ingelheim Animal Health.

Competing interests None declared.

Ethics approval All animals were handled in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines and approval was obtained prior to the initiation of the study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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