Effectiveness of two intranasal vaccines for the control of bovine respiratory disease in newborn beef calves: A randomized non-inferiority multicentre field trial

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Original Article

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Abstract

Bovine respiratory syncytial virus (BRSV) and bovine parainfluenza-3 virus (bPI3V) are major causes of bovine respiratory disease (BRD) in newborn calves worldwide. Vaccination is widely used to prevent BRD, and intranasal vaccines for BRSV and bPI3V were developed to overcome interference from BRSV and bPI3V-specific maternally derived antibodies. Many experimental challenge trials have demonstrated that intranasal vaccines for BRSV and bPI3V are efficacious, but effectiveness under field conditions has been demonstrated less often, especially for newborn beef calves. The objective of this field trial was to compare the effectiveness of a newly available commercial BRSV-bPI3V intranasal vaccine with that of a benchmarked one in newborn beef calves reared in a cow-calf system. A total of 935 calves from 39 farms were randomized into two vaccine groups (Bovalto Respi Intranasal [Vaccine A], $n = 468$; Rispoval RS + PI3 Intranasal [VaccineB], $n = 467$), and monitored during the in-house risk period up to three months after vaccination. Non-inferiority analysis was performed by calculating the difference in BRD prevalence between the two vaccine groups.

No significant differences were observed between vaccines regarding clinical outcomes of morbidity, mortality, duration between vaccination and BRD occurrence, or treatments required. Because the upper limit of the 2-sided 95% confidence interval of the difference in BRD prevalence between the two treatment groups (0.8%) was less than the margin of non-inferiority ($\delta = 5\%$), a non-inferiority of Vaccine A was concluded. In conclusion, Vaccine A is at least as effective as Vaccine B for the prevention of BRD in newborn beef cattle in a cow-calf system under field conditions.

41 *Keywords:* Bovine; Bovine respiratory syncytial virus; Bovine parainfluenza-3 virus; Calf;
42 Intranasal vaccine; Vaccination
43

Introduction

Bovine respiratory disease (BRD) is one of the main health issues encountered in non-weaned beef calves, and can lead to high economic losses (Assié et al., 2004a; Wang et al., 2018). Viral infections generally initiate BRD and predispose animals to secondary bacterial infections (Mosier, 2014). Bovine respiratory syncytial virus (BRSV), an *Orthopneumovirus* of the *Pneumoviridae* family, is a major virus involved in the BRD complex and is highly prevalent in both dairy and beef herds (Brodersen, 2010; Sacco et al., 2014; Valarcher and Taylor, 2007). Likewise, bovine parainfluenza-3 virus (bPI3V), a *Respirovirus* of the *Paramyxoviridae* family, is another virus involved in the BRD complex, widely prevalent in herds (Ellis, 2010). Vaccines against BRSV and bPI3V are widely used to control BRD, especially in beef calves. In a French study of 165 cow-calf herds in 2000, 116/186 (62%) batches of beef calves were vaccinated against BRSV (Assié et al., 2009).

The neonatal period is a significant risk period for BRD. The immune system of newborn calves differs from that of adults in several respects (Chase et al., 2008; Cortese, 2009). Although functional at birth, the immune system of a calf remains immature until six months of age (Hauser et al., 1986; Tizard, 2018), and the immune response during this time is weak, slow and more easily overcome by pathogenic microorganisms. In addition, maternally derived antibodies (MDA), which are transmitted through colostrum and remain present for up to six months, can interfere negatively with immunization of newborn calves after vaccination (Ellis et al., 2014; Kimman et al., 1989). To overcome interference between parenteral vaccines and MDA, intranasal vaccination strategies using modified live vaccines for respiratory diseases have been developed and used widely for many years (Windeyer and Gamsjäger, 2019). Intranasal vaccination is able to induce protective immunity in newborn calves despite the presence of MDA by priming mucosal immunization of the upper

69 respiratory tract whereas protective immunity is inconsistent after parenteral vaccinations
70 (Osman et al., 2018).
71
72 Veterinary vaccine efficacy is mainly evaluated in challenge trials under controlled
73 conditions (Knight-Jones et al., 2014). The efficacy of BRSV intranasal vaccines has been
74 proven in many challenge trials under controlled conditions even when vaccinations are
75 performed in the presence of MDA (Ellis, 2017; Osman et al., 2018). However, these studies
76 generally do not consider variations that occur under field conditions, such as exposure to
77 other pathogens, or host and environmental factors. Field trials are therefore needed to
78 reliably evaluate vaccine effectiveness (Knight-Jones et al., 2014). To our knowledge, only
79 one study dedicated to BRSV intranasal vaccination effectiveness has been carried out under
80 field conditions in newborn dairy calves. In that study, no decrease in BRD incidence or lung
81 lesions associated with pneumonia was demonstrated, but an increase in average daily gain
82 was observed (Ollivett et al., 2018). It should be noted, however, that the management of
83 dairy calves is quite different from that of beef suckler calves. Indeed, in cow-calf systems,
84 animals of different susceptibilities to respiratory diseases or with different immune statuses
85 are mixed in collective barns, whereas dairy calves are classically housed in individual pens
86 during the first eight weeks of life before being sorted and mixed into groups of similar age in
87 collective barns.
88
89 One BRSV-bPI3V intranasal vaccine authorized for use in newborn calves to prevent
90 BRD has been available for over 10 years in Europe (Vaccine B, Rispoval RS + PI3
91 Intranasal, Zoetis). The efficacy and the safety of Vaccine B have been demonstrated in
92 several experimental studies (Vangeel et al., 2009, 2007). With this vaccine, nasal shedding of
93 BRSV and bPI3V in vaccinated calves with or without MDA was reduced after challenges

with BRSV and bPI3V respectively. Additionally, the severity of clinical disease was also reduced after BRSV in vaccinated calves with BRSV MDA. Moreover, this vaccine has been widely used in Europe and is now a benchmark for BRSV-bPI3V intranasal vaccines. Recently, several new BRSV intranasal vaccines have been launched in Europe. Our study aimed to compare the effectiveness of a new intranasal vaccine against BRSV and bPI3V (vaccine A, Bovalto Respi Intranasal, Boehringer Ingelheim) with that of the benchmarked vaccine (vaccine B) in terms of decreasing BRD morbidity in newborn beef calves reared in a cow-calf farming system. As these two vaccines were very similar in their composition (i.e. bivalent modified live vaccines against BRSV and bPI3V) and their indication for use (i.e. active immunization), a non-inferiority study was performed.

Materials and methods

The trial was carried out under the agreement of the Ethics Committee for Clinical and Epidemiological Veterinary Research of Oniris (CERVO, Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering, France; Approval number, CERVO-2018-8-V; Approval date, 8 October, 2018).

Vaccines

Vaccines A and B contain BRSV and bPI3V strains administered as a single dose of 2 mL with an intranasal applicator. A dose of vaccine A (evaluated vaccine) contains between $10^{4.0}$ and $10^{6.0}$ $TCID_{50}$ of BRSV Bio 24/A strain and between $10^{5.0}$ and $10^{7.5}$ $TCID_{50}$ of bPI3V strain Bio 23/A reconstituted with phosphate buffered saline. A dose of vaccine B (benchmarked vaccine) contains between $10^{5.0}$ and $10^{7.2}$ 50% tissue culture infective doses ($TCID_{50}$) of BRSV 375 strain and between $10^{5.0}$ and $10^{8.6}$ $TCID_{50}$ of temperature-sensitive mutant bPI3V strain RLB 103 reconstituted with saline.

Study design

Type of trial

A randomized non-inferiority multicentre trial was carried out to assess whether vaccine A was at least as effective as vaccine B, with a pre-stated margin of non-inferiority (δ) for the prevention of BRD in newborn beef cattle. The null hypothesis (H_0) was that vaccine A was inferior to vaccine B in preventing BRD, whereas the alternative hypothesis (H_a) was that vaccine A was not inferior by more than the predefined non-inferiority margin. The hypothesis statements may be summarized as follows:

H_0: $(P_{BRD}[\text{vaccine A}] - P_{BRD}[\text{vaccine B}]) \geq \delta$

H_a: $(P_{BRD}[\text{vaccine A}] - P_{BRD}[\text{vaccine B}]) < \delta$

where P_{BRD} was the prevalence rate of calves treated for BRD during the study period and δ the non-inferiority margin.

Determination of the non-inferiority margin

Due to the lack of published field trials of the effectiveness of vaccine B, it was not possible to determine the non-inferiority margin using a 2-step approach as described by Freise et al. (2013). However, efficacy and the safety of vaccine B has been demonstrated in controlled challenge trials, and this vaccine has been until now been considered as the benchmarked BRSV-bPI3V IN vaccine. Thus, based on clinical judgment, because 5% was the largest loss of effectiveness of vaccine A that would be considered clinically insignificant, the non-inferiority margin δ was defined as 5%.

Sample size determination

Sample size was determined using the package 'TrialSize' in R[1] based on a non-inferiority trial with the prevalence of calves treated for BRD during the study period as the primary outcome. Four hundred and forty-six (446) calves per group were needed to demonstrate non-inferiority assuming $\alpha = 0.05$, $\beta = 0.20$, $\delta = 0.05$ and a prevalence of BRD in the active control vaccine group B of 10%. As it was a stratified multicentre individually randomized trial with two equal group sizes (A/B) and an equal number of calves in the two groups on each farm, the inflating factor was defined as $1- \rho$, with ρ the intraclass correlation coefficient (Vierron and Giraudeau, 2009, 2007). After assigning this inflation factor with $\rho = 0.14$ (Hendrick et al., 2013) and assuming 20% of loss to follow-up, it was decided to enrol at least 920 calves in the study.

Animals

Herd selection

Forty cow-calf farms with 110 ± 55 calvings (mean ± standard deviation, SD), located in four areas of France (Auvergne-Rhône-Alpes, Bretagne, Nouvelle-Aquitaine, Pays de La Loire), were selected for this study. Initial herd selection by veterinarians was based on the following criteria: (1) pure Charolais breed herds, (2) at least 30 calvings between December 2018 and April 2019, (3) calvings in stalls and a housing period as long as possible, ideally at least three months after vaccination, (4) ability to detect and treat sick animals and to record health events, and (5) no other BRD vaccination program during the study period (from birth to 3 months after enrolment). Herds were then enrolled in the study after farmers agreed to participate.

Calf selection

[1] See: R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org (Accessed 16 August 2020).

On each farm, 1 to 3 blocks of 10 calves were enrolled (3, 6 and 32 farms had respectively 1, 2 or 3 blocks). As soon as there were more than 10 calves over 10 days of age, a clinical examination of each calf was performed by a veterinarian. Only calves in good health (no intercurrent illness detected during the clinical examination) at the time of enrolment were included. If a calf had been ill and treated for BRD or other diseases before the day of inclusion, enrolment was possible only after full clinical recovery of the animal.

Randomization and vaccination protocols

Randomization was performed with a 1:1 allocation ratio. In each block of 10 enrolled calves from each farm, calves were sorted by decreasing age. A predefined randomized spreadsheet was prepared for each block: a random drawing was performed using the Rand function of MS Excel to assign the vaccine to the oldest calf, then the following calves were vaccinated alternately with one of the two vaccines.

Vaccines were administered by one veterinary investigator with calves restrained by the farmer, while another veterinary investigator reconstituted vaccines and recorded data. The vaccines were administered according to the manufacturer's recommendations: for Vaccine A, 1 mL in each nostril with the specific intranasal applicator (cannula and disk) provided by the manufacturer; for Vaccine B, 2 mL in one nostril with the specific intranasal applicator (cannula) provided by the manufacturer.

Intranasal applicators were changed between each calf. Due to the different administration modalities for the two vaccines, it was not possible to carry out a blinded vaccination. However, data were collected and registered by another veterinary investigator who kept randomization sheets and was not involved in the monitoring of calves after

vaccination. To avoid potential bias in later husbandry, farmers were asked to not read the tag numbers of calves during vaccination. After vaccination, cow-calf couples were raised in the same pen according to the usual rearing conditions on each farm.

Outcomes and data collection

The protocol started with vaccination and ended three months later. The primary outcome for analysis was BRD events, defined by at least one respiratory sign (such as cough, dyspnea, and/or nasal discharge) associated with at least one general clinical sign (hyperthermia, depression, and/or anorexia). Secondary outcomes included the time between vaccine administration and the occurrence of BRD, calf mortality, and the number of calves treated with antibiotics or anti-inflammatory drugs during the study.

For each calf, demographic data (tag number, date of birth, sex, and parity of the dam) were extracted from the official identification databases, and medical history was gathered from animal health records of the farm. For three months following vaccination, the farmers, under supervision of the veterinary investigators, had to monitor and record all observations and treatments carried out: date, clinical signs, diagnosis (respiratory disorders and others) and drug administration (antibiotic and/or anti-inflammatory preparations).

In the event of mortalities during the study, a veterinary investigator performed required necropsy examinations to identify the cause of death. In the case of bronchopneumonia, lung samples (approximately 5 cm^3) were frozen at -20°C for PCR analysis. Multiplex real-time PCR assays (Pack Respiratory 8 Bio-T kit, Biosellal) designed for the detection of eight BRD agents (BRSV, bPI3V, bovine Coronavirus, Influenza D,

Mannheimia haemolytica, *Pasteurella multocida*, *Mycoplasma bovis*, *Histophilus somni*) were performed in Agrivalys 71.

Post-admission exclusion

Calves with incomplete data, vaccinated before 10 days or after 60 days of age, or treated for BRD without at least two clinical signs being recorded, or as part of a metaphylaxis protocol, or vaccinated after the beginning of the grazing period were excluded.

Statistical analysis

The characteristics of calves assigned to the two vaccine groups were compared to assess homogeneity: Student's *t*-test was used for continuous variables (i.e., *age at vaccination*, *risk period*) after checking normality, and chi-squared or Fisher tests were used for categorical variables (i.e., *parity of dam*, *sex*, and *disease occurrence before vaccination*). The statistical analysis of the primary outcome was performed using a mixed logistic regression model (with *'calf'* as the statistical unit). The primary explanatory variable of interest was *vaccine*. Other variables were also tested (*sex*, *parity of dam*, *age at vaccination*, *risk period*) and kept in the model if $P < 0.2$ in the univariable analysis. A backward stepwise elimination of variables was then performed until all explanatory variables with $P < 0.05$ were included in the final model, taking into account potential confounders. *Herd* (categorical variable) was included as a random effect. The variable *risk period* was kept in the model to adjust BRD occurrence to the variation of the duration of exposition to pathogens between calves. The variables *vaccine* and *risk period* were forced in the final model, written as:

$$BRD_{ij} \sim Bernoulli(p_{ij})$$

$$Logit(p_{ij}) = \ln\left(\frac{p_{ij}}{1-p_{ij}}\right) = \beta_0 + vaccine_{ij}\beta_1 + risk\ period_{ij}\beta_2 + X_{ij}\beta_k + \nu_j$$

$$\nu_j \sim Normal(0, \sigma_\nu^2)$$

where BRD_{ij} is the occurrence of a BRD case diagnosed during the study *Risk Period* for the calf i of the herd j with a probability of occurrence p_{ij}, β_0 is the intercept, $vaccine_{ij}$ is either vaccine A or B, $risk\ period_{ij}$ is the duration of the in-house risk period, X_{ij} is *sex*, *parity of dam* and *age at vaccination* variables and ν_j is the random effect for herd j. Herd random effect followed a normal distribution with mean 0 and variance σ_ν^2.

The difference in BRD prevalence between vaccine groups and its 95% confidence interval (CI) was calculated from the model. Non-inferiority of vaccine A compared with vaccine B was concluded if the upper bound of the 2-sided 95% CI of the difference of BRD prevalence between the two vaccines was smaller than the non-inferiority margin δ (Fig. 1). Secondary outcomes were compared between the two vaccine groups using the chi-squared test, Fisher test or Student's *t*-test. All analyses were carried out using R software [1] and a statistical significance at $P \leq 0.05$ was used.

Results

Descriptive results

A total of 1,120 calves from 40 farms were enrolled in the study. Data from 185 calves were excluded: 40 because of incomplete data, 85 for date of vaccination before 10 days or after 60 days of age, 30 for being treated for BRD without at least two clinical signs or in a metaphylaxis protocol, and 30 for being vaccinated after the beginning of the grazing period (no housing period). Thus, a total of 935 calves from 39 herds were used in the study analysis: 24 ± 7 calves (mean ± SD) per herd. The two experimental groups were homogeneous in regard to age at vaccination, duration of in-house risk period, parity of dams, sex ratio, and occurrence of diseases before vaccination (Table 1).

Primary outcome

The occurrence of BRD during the in-house risk period between the two vaccine groups was similar (Table 2). Using least squares means of model outcome (BRD events), the difference P_{BRD}(vaccine A) - P_{BRD}(vaccine B) was estimated at -0.4% with a 95% CI between -1.6% and 0.8%. Non-inferiority of Vaccine A compared to Vaccine B was concluded since the upper limit of the 2-sided 95% CI (0.8%) of the difference in prevalence of calves diagnosed with BRD between the two vaccines was smaller than δ (Fig. 1). BRD incidence in our study was 0.74 cases per 1,000 calf-days at risk (Table 3).

Secondary outcome

The two experimental groups were similar in regard to time between vaccination and occurrence of BRD, treatments and mortality (Table 4). For the six calves which died during the study period, BRSV was not detected from the samples collected during the necropsy procedure (Table 5).

Discussion

The objective of this study was to compare the effectiveness of the newly available BRSV-bPI3V intranasal vaccine to the benchmarked vaccine under field conditions in newborn beef calves reared in a cow-calf system. Based on our results, non-inferiority of Vaccine A compared to Vaccine B was concluded. Due to the lack of studies dedicated to the effectiveness of Vaccine B compared to a placebo, the non-inferiority margin δ was defined as 5% only, based on a clinical judgment. This margin is narrow compared to the one used in most vaccine trials, with δ usually fixed at 10% as reviewed by Donken et al. (2015). Choosing a more conservative non-inferiority margin required an increased sample size and improved the clinical significance of the trial. Indeed, based on the experiences of the authors,

291 an increase of more than 5% of BRD events (i.e., the primary outcome) in the vaccine A
292 group compared to the vaccine B group was considered unfavorable.
293
294 In this study, calves from the two vaccine groups were mixed together in order to
295 homogenize as much as possible environmental conditions and exposure to pathogens. This
296 design is often chosen in field studies dealing with vaccine effectiveness (Schunicht et al.,
297 2003; Stilwell et al., 2008; Wildman et al., 2008). Moreover, in a cow-calf system, this design
298 enables the absence of separation of paired calves of the two vaccine groups after
299 randomization, and improves blind assessment of calf health in a single group. However, a
300 bias in vaccine effectiveness evaluation could be introduced with this method. The reduction
301 of virus shedding after vaccination of the calves of one vaccine group contributes to the
302 protection of the calves of the other vaccine group reared in the same environment (Smith,
303 2019, 2014; Stokka, 2010). Indeed, apparent effectiveness of the test vaccine could be
304 improved if the comparison involved a reference vaccine with a better shedding reduction
305 efficacy. Moreover, Vaccines A and B are both modified live vaccines. Cross-immunization
306 thus could occur between the two vaccine groups. In previous studies using commercial
307 vaccines including the reference vaccine, nasal shedding of vaccine-origin viruses was
308 detected by PCR in nasal swabs during 14 days in most of the vaccinated calves after
309 vaccination and was detected up to 28 days post vaccination in a few calves (Timsit et al.,
310 2009; Walz et al., 2017).
311
312 The study was designed to determine the effectiveness of two commercial vaccines
313 under conditions as close as possible to those encountered by calves reared in beef herds.
314 Although the minimum age at vaccination recommended by the manufacturers is 9 and 10
315 days of age for Vaccines B and A, respectively, under our conditions the mean age (± SD) at

316 vaccination was 26 ± 12 days. This delay in administrating vaccines was due to the
317 distribution of births on each farm and the packaging of the vaccines in 5-dose bottles. Since
318 both vaccines are available in a 5-dose bottle, 10 calves had to be over 10 days of age before
319 being vaccinated in order to randomize them into two equal groups of five calves. However,
320 this difference between the recommended and actual age at vaccination was the same in both
321 groups and is common in French cow-calf systems.
322
323 Most of the efficacy studies for BRSV and bPI3V intranasal vaccination include a
324 controlled challenge, but challenges may not reproduce natural exposure under variable host
325 and environmental factors (Knight-Jones et al., 2014). It has been observed that many BRSV
326 infection models failed to reproduce the severe clinical signs of the disease, complicating the
327 evaluation of vaccine efficacy (Belknap et al., 1995; Blodörn et al., 2015; Taylor, 2013). In
328 these studies, efficacy was demonstrated in newborn calves both in the absence of BRSV-
329 specific MDAs (Ellis et al., 2007; Vangeel et al., 2007) and in their presence (Ellis et al.,
330 2013; Hill et al., 2012). However, the absence of maternal antibody interference is not always
331 observed (Ellis et al., 2010). In our study, colostral immunity in calves was not systematically
332 controlled for practical reasons, and the BRSV and bPI3V serological status of calves at the
333 time of vaccination was unknown. Due to the high prevalence of BRSV in France, most of the
334 vaccinations were likely to have been performed in the presence of BRSV-specific MDAs.
335
336 As observed in a previous study, BRSV and bPI3V infections were found in 71% and
337 80% of cow-calf farms respectively (Assié et al., 2004b). Furthermore, challenge trials do not
338 reproduce the variability of host and environmental conditions that may be encountered in the
339 field, such as variable passive immune transfer (Raboisson et al., 2016), variable calf housing
340 (Assié et al., 2009; Dubrovsky et al., 2019a; Maier et al., 2019) and variable seasonal or

weather conditions (Buczinski et al., 2018; Dubrovsky et al., 2019b). For these reasons, a multicentre study was chosen in order to reproduce this variability of environmental factors. Both efficacy assessment in experimental challenges and effectiveness assessment in field trials have limits but are complementary.

Contrary to challenge trials, the exposure of calves to pathogens, in particular to BRSV and bPI3V, is rarely controlled in a field study (Ellis, 2017; Ollivett et al., 2018). The authors acknowledge that monitoring BRSV and bPI3V exposure (by means such as PCR or virus isolation on deep nasal swabs or fluid of transtracheal aspiration or bronchoalveolar lavage, or serology on sentinels) would have allowed us to assess specifically the effectiveness of vaccines against BRSV and bPI3V, and not only the prevention of BRD. As previously reported, monitoring exposure to pathogens in a vaccinated population is very difficult for both practical and economic reasons (Ellis, 2017). Indeed, the short viremia would require repeated samplings of a large population. In a recent field trial evaluating BRSV and bPI3V intranasal vaccination in dairy calves, Ollivett et al. (2018) similarly did not assess the exposure of calves to BRSV and bPI3V, and monitored BRD morbidity alone.

However, the exposure of calves to respiratory pathogens in our study can be attested by the measurement of BRD incidence, which was 0.74 cases per 1,000 calf-days at risk. Although this BRD incidence was low, it remains consistent with the incidence observed in another French study in a comparable breeding system in which respiratory vaccination was inconsistent: 1.89 cases per 1,000 calf-days at risk in 137 farms (Assié et al., 2004a). Circulation of respiratory pathogens in the study farms can also be attested by the viruses (bPI3V, bovine Coronavirus) and bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*) identified at necropsy in dead animals. To overcome the variability in the

exposure to pathogens under field conditions, our study would need to be repeated.

Conclusions

The effectiveness of a newly available commercial BRSV-bPI3V intranasal vaccine to control BRD has been demonstrated under field conditions. To the authors' knowledge, this is the first study under field conditions assessing BRSV and bPI3V intranasal vaccination effectiveness in newborn beef calves in a cow-calf system. Data from challenge studies or from dairy calf field studies cannot be extrapolated to beef calves. Beef cattle from different age groups with different immune statuses against respiratory pathogens are mixed together in a specific in-house environment, in contrast to dairy calves which are typically housed in individual pens or in collective pens with animals of the same age.

Conflict of interest statement

This study was funded by Boehringer Ingelheim, which supports the European College of Bovine Health Management (ECBHM) residency program of the first author. Boehringer Ingelheim played no role in the study design, in data collection, analysis or interpretation, or in the writing of the report and the decision to submit the manuscript for publication. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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590 **Table 1**

591 Calf characteristics in vaccine groups A and B

Variable	Vaccine group (n = 935)		P
	A (n = 468)	B (n = 467)	
Age at vaccination in days (mean ± SD)	25.97 ± 11.62	25.85 ± 11.54	0.88
Duration of in-house risk period in days (mean ± SD)	56.52 ± 28.25	57.00 ± 27.93	0.80
Parity			
1 (n = 279)	143	136	
2 (n = 157)	72	85	0.51
3 and more (n = 499)	253	246	
Sex			
Male (n = 447)	235	212	
Female (n = 488)	233	255	0.14
Occurrence of diseases before vaccination			
No disease (n = 873)	437	436	1
Respiratory (n = 6)	3	3	
Other than respiratory [a] (n = 56)	28	28	

592 SD, Standard deviation

593 [a] All neonatal diseases were diagnosed and treated (i.e., septicaemia, diarrhoea, umbilical
594 infection, others)

595

596 **Table 2**

597 Multivariable results of mixed logistic regression model of bovine respiratory disease (BRD)

598 prevalence after intranasal vaccination of non-weaned beef calves.

Variable	Category	Number of calves evaluated	Odds ratio of BRD occurrence	95% Confidence interval	*P* [a]
Vaccine	Vaccine B	467	Reference		
	Vaccine A	468	0.61	0.30-1.25	0.17
Duration of in-house risk period in days	(0-45)	313	Reference		
	(45-67.5)	243	8.88	1.07-73.66	0.04
	(67.5-90)	379	6.61	0.86-50.99	0.07

599 [a] For each variable, refers to level of significance between the category under consideration
600 and the reference category.
601

602 **Table 3**

603 Incidence of bovine respiratory disease cases in the two vaccine groups

Vaccine group	Number of		Incidence rate [a]
	Calf-days at risk	Cases	
Vaccine A	25,538	15	0.59
Vaccine B	26,031	23	0.88
Total	51,569	38	0.74

604 [a] per 1,000 calf-days at risk
605

606 **Table 4**

607 Comparisons of secondary outcomes between the two vaccine groups

Outcome	Vaccine group (n = 935)		P
	A (n = 468)	B (n = 467)	
Calves treated for BRD with			
Antibiotics (%)	15 (3.2)	23 (4.9)	0.23
Non-steroidal anti-inflammatories (%)	12 (2.6)	17 (3.6)	0.34
Steroidal anti-inflammatories (%)	1 (0.2)	2 (0.4)	0.50
Mortalities (%)	1 (0.2)	5 (1.1)	0.11
Time between vaccination and occurrence of BRD in days (mean ± SD)	33 ± 20	28 ± 22	0.45

608 BRD, Bovine respiratory disease; SD, Standard deviation

609

610 **Table 5**

611 Results of multiplex real-time PCR on lung samples for detection of eight respiratory

612 pathogens from necropsies of dead calves

Calf number	Vaccine group	Herd number	Pathogen detected in multiplex real-time PCR [a]							
			BRSV	bPI3V	Mh	Pm	Mb	Hs	bCo	ID
14	B	1	No sample							
244	B	13	No sample							
913	B	37	-	+	+	+	-	+	-	-
941	A	38	-	-	-	+	-	+	+	-
955	B	38	-	-	-	+	-	-	+	-
967	B	38	-	+	-	+	-	-	-	-

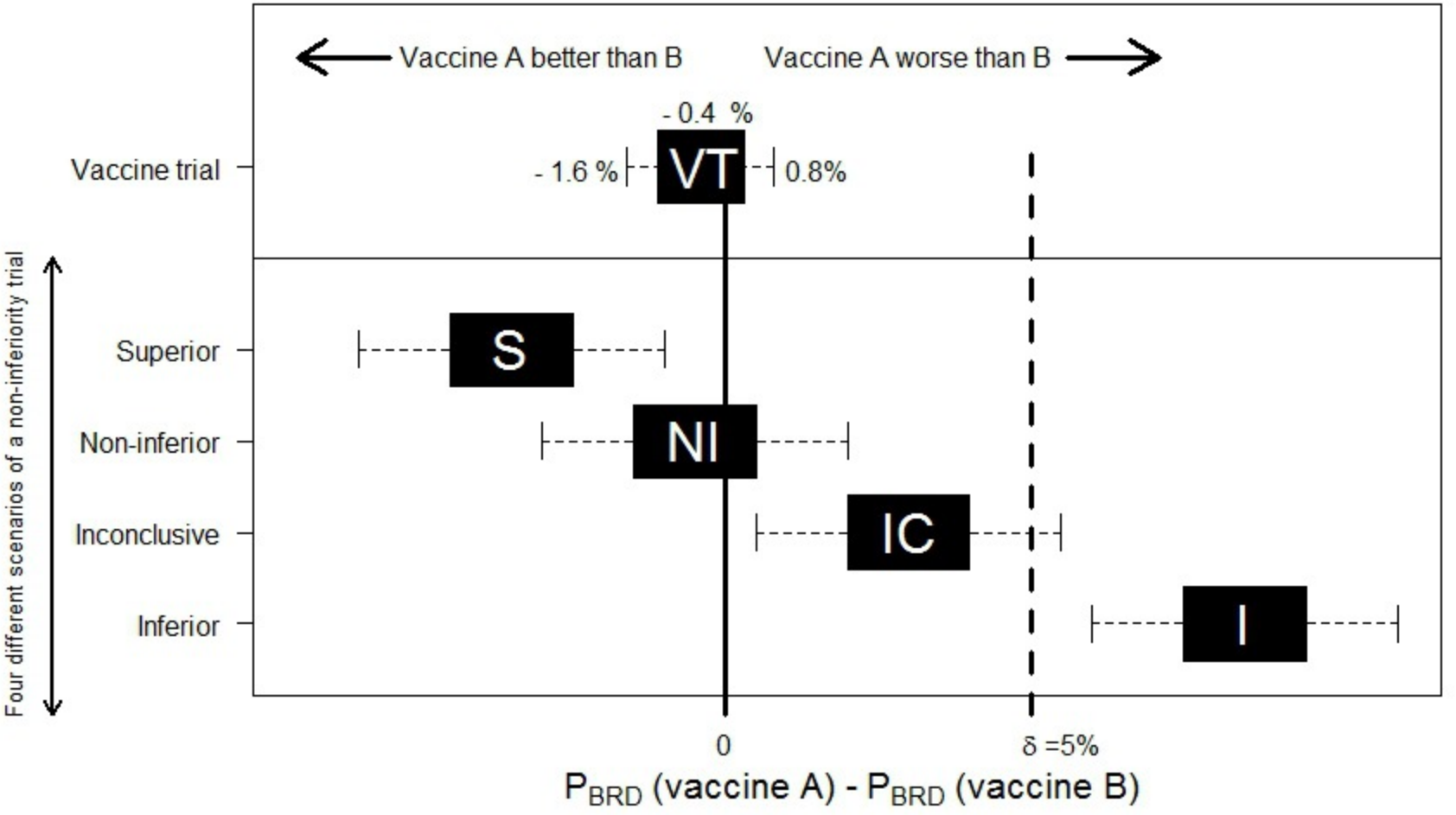
613 BRSV, Bovine respiratory syncytial virus; bPI3V, bovine Parainfluenza Virus type 3; Mh,
614 *Mannheimia haemolytica*; Pm, *Pasteurella multocida*; Mb, *Mycoplasma bovis*; Hs, *Histophilus somni*;
615 bCo, bovine Coronavirus; ID, Influenza D virus
616 [a] Pack Respiratory 8 Bio-T kit, Biosellal
617

618 **Figure legends**

619

620 Fig.1. Four possible scenarios of a non-inferiority trial comparing vaccine A to vaccine B for
621 preventing BRD. The margin of non-inferiority (δ) is drawn by a vertical dashed line. P_{BRD} is
622 the prevalence of BRD cases diagnosed during the study risk period of housing after
623 vaccination. Error bars indicate 2-sided 95% confidence interval (CI) of the difference in
624 BRD incidence (Piaggio et al., 2012). S: if the CI lies wholly to the left of zero, vaccine A is
625 superior. NI: if the CI lies to the left of δ and includes zero, vaccine A is non-inferior. IC: if
626 the CI includes δ and zero, the difference is non–significant but the result regarding non-
627 inferiority is inconclusive. I: if the CI is wholly above δ, vaccine A is inferior. VT is the
628 representation of the main outcome of this non-inferiority trial. The black block indicates the
629 difference in BRD incidence between vaccine A group and vaccine B group. Non-inferiority
630 of vaccine A compared to vaccine B at a margin of 5% is demonstrated because the 95% CI
631 lies to the left of δ (=5%) and includes zero.

632


Vaccine A better than B
Vaccine A worse than B
- 0.4 %
Vaccine trial
- 1.6 %
VT
0.8%
Four different scenarios of a non-inferiority trial
Superior
S
Non-inferior
NI
Inconclusive
IC
Inferior
I
0
δ =5%
P_BRD (vaccine A) - P_BRD (vaccine B)