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# 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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## ► To cite this version:

Nicolas Masset, François Meurens, Maxime Marie, Pauline Lesage, Anne Lehébel, et al.. Effectiveness of two intranasal vaccines for the control of bovine respiratory disease in newborn beef calves: A randomized non-inferiority multicentre field trial. *Veterinary Journal*, 2020, 263, pp.105532. 10.1016/j.tvjl.2020.105532 . hal-02934750

**HAL Id: hal-02934750**

**<https://hal.inrae.fr/hal-02934750>**

Submitted on 30 Aug 2022

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1 **Original Article**

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

6

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16

17 **Abstract**

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

32

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

40

41 *Keywords:* Bovine; Bovine respiratory syncytial virus; Bovine parainfluenza-3 virus; Calf;

42 Intranasal vaccine; Vaccination

43

## 44 **Introduction**

45           Bovine respiratory disease (BRD) is one of the main health issues encountered in non-  
46 weaned beef calves, and can lead to high economic losses (Assié et al., 2004a; Wang et al.,  
47 2018). Viral infections generally initiate BRD and predispose animals to secondary bacterial  
48 infections (Mosier, 2014). Bovine respiratory syncytial virus (BRSV), an *Orthopneumovirus*  
49 of the *Pneumoviridae* family, is a major virus involved in the BRD complex and is highly  
50 prevalent in both dairy and beef herds (Brodersen, 2010; Sacco et al., 2014; Valarcher and  
51 Taylor, 2007). Likewise, bovine parainfluenza-3 virus (bPI3V), a *Respirovirus* of the  
52 *Paramyxoviridae* family, is another virus involved in the BRD complex, widely prevalent in  
53 herds (Ellis, 2010). Vaccines against BRSV and bPI3V are widely used to control BRD,  
54 especially in beef calves. In a French study of 165 cow-calf herds in 2000, 116/186 (62%)  
55 batches of beef calves were vaccinated against BRSV (Assié et al., 2009).

56  
57           The neonatal period is a significant risk period for BRD. The immune system of  
58 newborn calves differs from that of adults in several respects (Chase et al., 2008; Cortese,  
59 2009). Although functional at birth, the immune system of a calf remains immature until six  
60 months of age (Hauser et al., 1986; Tizard, 2018), and the immune response during this time  
61 is weak, slow and more easily overcome by pathogenic microorganisms. In addition,  
62 maternally derived antibodies (MDA), which are transmitted through colostrum and remain  
63 present for up to six months, can interfere negatively with immunization of newborn calves  
64 after vaccination (Ellis et al., 2014; Kimman et al., 1989). To overcome interference between  
65 parenteral vaccines and MDA, intranasal vaccination strategies using modified live vaccines  
66 for respiratory diseases have been developed and used widely for many years (Windeyer and  
67 Gamsjäger, 2019). Intranasal vaccination is able to induce protective immunity in newborn  
68 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

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

104

## 105 **Materials and methods**

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

110

### 111 *Vaccines*

112 Vaccines A and B contain BRSV and bPI3V strains administered as a single dose of 2  
113 mL with an intranasal applicator. A dose of vaccine A (evaluated vaccine) contains between  
114  $10^{4.0}$  and  $10^{6.0}$  TCID<sub>50</sub> of BRSV Bio 24/A strain and between  $10^{5.0}$  and  $10^{7.5}$  TCID<sub>50</sub> of bPI3V  
115 strain Bio 23/A reconstituted with phosphate buffered saline. A dose of vaccine B  
116 (benchmarking vaccine) contains between  $10^{5.0}$  and  $10^{7.2}$  50% tissue culture infective doses  
117 (TCID<sub>50</sub>) of BRSV 375 strain and between  $10^{5.0}$  and  $10^{8.6}$  TCID<sub>50</sub> of temperature-sensitive  
118 mutant bPI3V strain RLB 103 reconstituted with saline.

119

## 120 *Study design*

### 121 Type of trial

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

$$128 H_0: (P_{BRD}[\text{vaccine A}] - P_{BRD}[\text{vaccine B}]) \geq \delta$$

$$129 H_a: (P_{BRD}[\text{vaccine A}] - P_{BRD}[\text{vaccine B}]) < \delta$$

130 where  $P_{BRD}$  was the prevalence rate of calves treated for BRD during the study period and  $\delta$   
131 the non-inferiority margin.

132

### 133 Determination of the non-inferiority margin

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

141

### 142 Sample size determination



143 Sample size was determined using the package ‘TrialSize’ in R<sup>1</sup> based on a non-  
144 inferiority trial with the prevalence of calves treated for BRD during the study period as the  
145 primary outcome. Four hundred and forty-six (446) calves per group were needed to  
146 demonstrate non-inferiority assuming  $\alpha = 0.05$ ,  $\beta = 0.20$ ,  $\delta = 0.05$  and a prevalence of BRD in  
147 the active control vaccine group B of 10%. As it was a stratified multicentre individually  
148 randomized trial with two equal group sizes (A/B) and an equal number of calves in the two  
149 groups on each farm, the inflating factor was defined as  $1 - \rho$ , with  $\rho$  the intraclass correlation  
150 coefficient (Vierron and Giraudeau, 2009, 2007). After assigning this inflation factor with  $\rho =$   
151 0.14 (Hendrick et al., 2013) and assuming 20% of loss to follow-up, it was decided to enrol at  
152 least 920 calves in the study.

153

#### 154 *Animals*

##### 155 Herd selection

156 Forty cow-calf farms with  $110 \pm 55$  calvings (mean  $\pm$  standard deviation, SD), located  
157 in four areas of France (Auvergne-Rhône-Alpes, Bretagne, Nouvelle-Aquitaine, Pays de La  
158 Loire), were selected for this study. Initial herd selection by veterinarians was based on the  
159 following criteria: (1) pure Charolais breed herds, (2) at least 30 calvings between December  
160 2018 and April 2019, (3) calvings in stalls and a housing period as long as possible, ideally at  
161 least three months after vaccination, (4) ability to detect and treat sick animals and to record  
162 health events, and (5) no other BRD vaccination program during the study period (from birth  
163 to 3 months after enrolment). Herds were then enrolled in the study after farmers agreed to  
164 participate.

165

##### 166 Calf selection

---

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

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

173

#### 174 *Randomization and vaccination protocols*

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

180

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

187

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

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

195

#### 196 *Outcomes and data collection*

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

203

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

210

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

216 *Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis, Histophilus somni*)

217 were performed in Agrivalys 71.

218

219 *Post-admission exclusion*

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

223

224 *Statistical analysis*

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

238 
$$BRD_{ij} \sim \text{Bernoulli}(p_{ij})$$

239 
$$\text{Logit}(p_{ij}) = \ln\left(\frac{p_{ij}}{1 - p_{ij}}\right) = \beta_0 + \text{vaccine}_{ij}\beta_1 + \text{risk period}_{ij}\beta_2 + X_{ij}\beta_k + v_j$$

240 
$$v_j \sim \text{Normal}(0, \sigma_v^2)$$

241 where  $BRD_{ij}$  is the occurrence of a BRD case diagnosed during the study *Risk Period* for the  
242 calf  $i$  of the herd  $j$  with a probability of occurrence  $p_{ij}$ ,  $\beta_0$  is the intercept,  $vaccine_{ij}$  is either  
243 vaccine A or B,  $risk\ period_{ij}$  is the duration of the in-house risk period,  $X_{ij}$  is *sex, parity of*  
244 *dam* and *age at vaccination* variables and  $v_j$  is the random effect for herd  $j$ . Herd random  
245 effect followed a normal distribution with mean 0 and variance  $\sigma_v^2$ .

246

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

254

## 255 **Results**

### 256 *Descriptive results*

257 A total of 1,120 calves from 40 farms were enrolled in the study. Data from 185 calves  
258 were excluded: 40 because of incomplete data, 85 for date of vaccination before 10 days or  
259 after 60 days of age, 30 for being treated for BRD without at least two clinical signs or in a  
260 metaphylaxis protocol, and 30 for being vaccinated after the beginning of the grazing period  
261 (no housing period). Thus, a total of 935 calves from 39 herds were used in the study analysis:  
262  $24 \pm 7$  calves (mean  $\pm$  SD) per herd. The two experimental groups were homogeneous in  
263 regard to age at vaccination, duration of in-house risk period, parity of dams, sex ratio, and  
264 occurrence of diseases before vaccination (Table 1).

265

266 *Primary outcome*

267           The occurrence of BRD during the in-house risk period between the two vaccine  
268 groups was similar (Table 2). Using least squares means of model outcome (BRD events), the  
269 difference  $P_{BRD}(\text{vaccine A}) - P_{BRD}(\text{vaccine B})$  was estimated at -0.4% with a 95% CI between  
270 -1.6% and 0.8%. Non-inferiority of Vaccine A compared to Vaccine B was concluded since  
271 the upper limit of the 2-sided 95% CI (0.8%) of the difference in prevalence of calves  
272 diagnosed with BRD between the two vaccines was smaller than  $\delta$  (Fig. 1). BRD incidence in  
273 our study was 0.74 cases per 1,000 calf-days at risk (Table 3).

274

275 *Secondary outcome*

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

280

281 **Discussion**

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

316 vaccination was  $26 \pm 12$  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, colostrum 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 (Raboison et al., 2016), variable calf housing  
340 (Assié et al., 2009; Dubrovsky et al., 2019a; Maier et al., 2019) and variable seasonal or



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

345

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

357

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

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

367

## 368 **Conclusions**

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

377

## 378 **Conflict of interest statement**

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

385

## 386 **Acknowledgements**

387 The authors acknowledge the assistance of the veterinarians of the '*Réseau Cristal*'  
388 and the farmers participating in this study.

389

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590 **Table 1**

591 Calf characteristics in vaccine groups A and B

Variable	Vaccine group ( <i>n</i> = 935)		<i>P</i>
	A ( <i>n</i> = 468)	B ( <i>n</i> = 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 ( <i>n</i> = 279)	143	136	
2 ( <i>n</i> = 157)	72	85	0.51
3 and more ( <i>n</i> = 499)	253	246	
Sex			
Male ( <i>n</i> = 447)	235	212	
Female ( <i>n</i> = 488)	233	255	0.14
Occurrence of diseases before vaccination			
No disease ( <i>n</i> = 873)	437	436	1
Respiratory ( <i>n</i> = 6)	3	3	
Other than respiratory <sup>a</sup> ( <i>n</i> = 56)	28	28	

592 SD, Standard deviation

593 <sup>a</sup> 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	<i>P</i> <sup>a</sup>
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 <sup>a</sup> 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 <sup>a</sup>
	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 <sup>a</sup> per 1,000 calf-days at risk

605

606 **Table 4**

607 Comparisons of secondary outcomes between the two vaccine groups

Outcome	Vaccine group ( <i>n</i> = 935)		<i>P</i>
	A ( <i>n</i> = 468)	B ( <i>n</i> = 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 <sup>a</sup>							
			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 <sup>a</sup> 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 ( $\delta$ ) 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  $\delta$  and includes zero, vaccine A is non-inferior. IC: if  
626 the CI includes  $\delta$  and zero, the difference is non-significant but the result regarding non-  
627 inferiority is inconclusive. I: if the CI is wholly above  $\delta$ , 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  $\delta$  (=5%) and includes zero.

632

Four different scenarios of a non-inferior trial

