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Efficacy of a live intranasal vaccine against parainfluenza type 3 and bovine respiratory syncytial virus in young calves with maternally derived antibodies

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ABSTRACT

Trial design Two randomised controlled vaccination trials with artificial challenges were carried out in addition to a serological survey of levels of maternally derived antibodies (MDA) to parainfluenza type 3 virus (PI3V) and bovine respiratory syncytial virus (BRSV) in European calves.

Participants Ten-day-old calves with and without MDA were included in the two vaccine trials.

Interventions Intranasal administration of a bivalent modified live (PI3V/BRSV) vaccine followed by artificial challenge approximately three months post vaccination.

Objective The study aimed to assess the efficacy of a modified live respiratory vaccine, Bovalto Respi Intranasal (Boehringer Ingelheim). In order to assess the interference of MDA, both seropositive and seronegative calves were used.

Randomisation PI3V and BRSV serological status was determined seven days before vaccination; calves without maternal antibodies became the MDA– vaccinates. Calves with MDA were ranked according to individual titres and allocated alternately to MDA+ vaccinate and MDA+ control groups.

Blinding Treatment was carried out by the unblinded study director. Animal care and veterinary examinations were conducted by personnel unaware of the treatments received. The serological survey used blood samples obtained from calves on commercial farms in five European countries, Germany, Spain, Italy, Ireland and the UK, to determine the levels of MDA to PI3V and BRSV in calves approximately two weeks of age.

Results A total of 36 calves were included in the two challenge studies and 32 of these completed the challenge studies. Twenty-one calves were included in the PI3V challenge study, with six of six MDA– and six of seven MDA+ vaccinated calves and five of five MDA+ unvaccinated control calves being challenged with PI3V. Fifteen calves were included in the BRSV challenge study, with five of five MDA– and five of five MDA+ vaccinated calves and five of five MDA+ unvaccinated control calves being challenged with BRSV.

Outcome For both challenges, clinical scores and nasal shedding were significantly higher in control animals compared with vaccinates (PI3V challenge: clinical scores P=0.001, nasal shedding P=0.001; BRSV challenge: clinical scores P=0.016, nasal shedding P=0.002) and not significantly different between MDA+ and MDA– vaccinated animals for both challenges (P>0.05). A total of 254 samples from six countries were tested in the serological survey of MDA.

Conclusion The results of the challenge studies demonstrated the efficacy of the vaccine in the presence of BRSV and PI3V MDA under laboratory conditions. The field assessment confirmed that the MDA titres in the MDA+ calves corresponded to those typically found on farms.

INTRODUCTION

Bovine respiratory disease (BRD) poses a major challenge to the cattle industry and results in significant economic loss. Vaccination against BRD pathogens is used to control disease at all stages of life, particularly during the pre-weaning period when young calves are often exposed to multiple risk factors, resulting in a high prevalence of disease. Increasing intensification of both meat and dairy operations may increase the risk of BRD early in life.[1 2] In addition, pressure to reduce antimicrobial use has resulted in a drive to increase the number of cattle vaccinated against BRD pathogens in Europe.[3] These factors, and an increasing recognition of the impact of BRD on the short-term and long-term productivity of cattle,[4] may prompt farmers to vaccinate calves at an early age, when maternally derived antibodies (MDA) are still present.

In practice, inducing protective immunity against respiratory pathogens through vaccination of young calves may be difficult, due both to immune system immaturity and to the potential interference of MDA.[5 6] Intranasal modified live vaccines (MLV) induce both an adequate systemic response and at the same

Table 1 Summary of study activities and the number of calves

	PI3V challenge study		BRSV challenge study	
	Day in the study	Calves (n)	Day in the study	Calves (n)
Calves received into facility, aged 0–3 days, and blood sampled for serology	D0–D7	21	D0–D7	15
Enrolment and selection (health, weight)	D0–D3	21	D0–D2	15
Randomisation and separation of MDA+ control animals to avoid cross contamination from vaccinal virus	D0–D1	21	D0–D1	15
Vaccination and blood sampling (animals are 10±3 days old)	D0	21	D0	15
Control animals moved back in the same air space as vaccinates	D21	20	D21	15
(Regular serological evaluation every two weeks post vaccination)				
Challenge	D92	17	D84	15
(Postchallenge monitoring—clinical signs and nasal swabs—and daily for 14 days)				
End of study	D106	17	D98	15

BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

time a local antibody response.[7] Indeed, when vaccine is delivered to the nasopharyngeal mucosal surface, the calf rapidly produces a mucosal immune response that provides a first line of defence against respiratory pathogens.[8 9] This approach provides a means of overcoming the potential negative effect of MDA on the immune response to parenteral vaccines.[10] Similar studies have been published by Ellis and others,[11–13] with particular focus on bovine respiratory syncytial virus (BRSV). These studies used calves reared to be with or without specific BRSV MDA, which were vaccinated intranasally with BRSV-combination MLV vaccines and artificially challenged at seven weeks, nine weeks or up to 14 weeks post vaccination. The authors concluded that protective immunity could indeed be induced by intranasal vaccination in the presence of MDA for a period of up to four months.

The study described here provides an initial assessment, by artificial challenge, of the efficacy of Bovalto Respi Intranasal (Boehringer Ingelheim), a modified live respiratory vaccine containing two BRD pathogens, BRSV and parainfluenza type 3 virus (PI3V). In order to assess the potential interference of MDA, both seropositive and seronegative calves were used in two separate studies, one for PI3V and one for BRSV. To assess the relevance of the MDA levels observed in the study calves, they were compared with levels in a panel of PI3V and BRSV antibody titres from field samples obtained from young (less than two weeks old) calves sampled in several European countries (serological field sampling).

MATERIALS AND METHODS

Informed consent was obtained from the owners of all calves included in the serological field sampling. The artificial challenge studies satisfied the efficacy requirements of immunogenicity tests of the corresponding European Pharmacopoeia monographs (Directive 2001/82/EC as amended by Directive 2004/28/EC); notably, a vaccine formulated at minimum titre was used.

Animals and care

Male calves of Czech-pied, Holstein breed, 10 days old and approximately 30 kg at the time of vaccination, were obtained from a commercial farm in the Czech Republic. They were stabled in stalls (two to three animals per stall) and provided with rubber mats or straw bedding. Table 1 provides a summary of study activities and the number of calves involved at each stage within the two trials. Control animals were separated from vaccinates until 21 days

Table 2 Clinical signs scoring table

Parameter	Absent	Mild	Moderate	Severe	Lethal
Depression/general appearance	0	1	2	3	10
Cough	0	1	2	3	N/A
Nasal discharge	0	1	2	3	N/A
Ocular discharge	0	1	2	3	N/A
Dyspnoea	0	1	2	3	N/A
	Normal (38°C–40°C)		Abnormal (<38°C or >40°C)		
Rectal temperature	0		1		N/A

N/A, not applicable.

Table 3 Specific IgG titres on day of vaccination and day of challenge

	PI3V			BRSV		
Group	**Animal**	**D0 (vaccination)**	**D92 (challenge)**	**Animal**	**D0 (vaccination)**	**D84 (challenge)**
Control MDA+	632181	2	1	641479	4	<2
	643429	1024	1	643771	4	<2
	643433	1024	1	643774	16	<2
	689106	128	1	643778	8	<2
	689109	256	1	643781	4	<2
	643424	1024	512*	–	–	–
	643427	256	8*	–	–	–
	643432	512	64*	–	–	–
	Mean	235	1	Mean	6	<2
Vaccinated MDA–	637676	1	128	641480	<2	2
	643435	1	512	643765	<2	4
	643436	1	32	643766	<2	4
	643437	1	128	643767	<2	8
	643439	1	16	643768	<2	4
	643440	1	16	–	–	–
	Mean	1	64	Mean	<2	4
Vaccinated MDA+	643425	512	128	643769	8	8
	643426	256	64	643770	8	4
	643428	1024	64	643772	2	4
	643430	128	32	643773	8	8
	643431	256	32	643780	16	2
	689108	32	†	–	–	–
	689107	128	8	–	–	–
	Mean	210	40	Mean	7	5

*Still seropositive, excluded from challenge.
†Died on day 7 from sepsis following omphalitis.
BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

post vaccination to avoid vaccinal virus spread. Thereafter, all animals were housed together. Heat, lighting (12 hours of light/day) and ventilation were controlled and monitored. All calves were fed 5–6 litres per day of a milk replacer (Telmilk), three times daily for the first 10 days, then twice a day until eight weeks of age; in addition they had access to supplementary feeding mixture (Telmix) ad libitum from the start and hay ad libitum from one month of age. All calves received a preventive antibiotic treatment by intramuscular injection (1 ml/20 kg amoxicillin-clavulanic acid) once a day for five days as soon as possible after they were obtained, and a probiotic treatment (Progal plv) was given once daily for seven days.

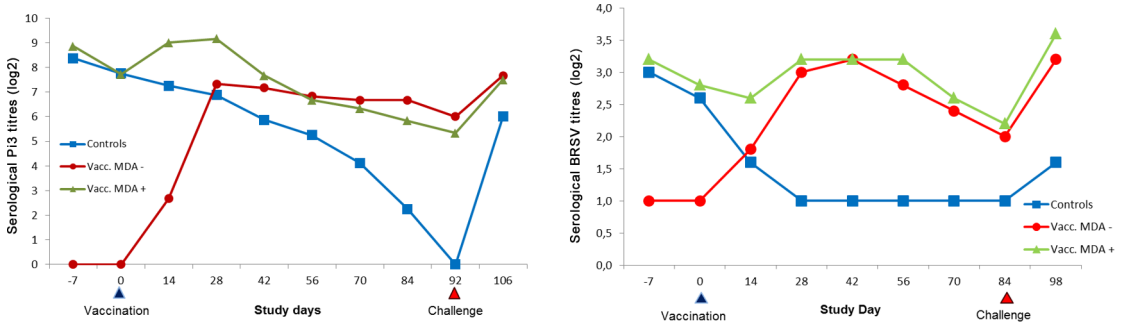

Figure 1 Mean specific IgG titres (log-transformed) per group against PI3V and BRSV. Mean IgG titres (log_2) against PI3V (left) and BRSV (right). BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

Table 4 Number of animals challenged in each test group based on MDA status before vaccination against and challenge by PI3V or BRSV

	PI3V	BRSV	Total
Vaccinated MDA–	6	5	11
Vaccinated MDA+	6	5	11
Control MDA+	5	5	10
Total	17	15	32

BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

Allocation to treatment groups

PI3V and BRSV serological status of all calves was determined seven days before vaccination. Calves without MDA (MDA–) would become the MDA– vaccinates. Calves with MDA were ranked according to their individual titres and allocated alternately to MDA+ vaccinate and MDA+ control groups to ensure a similar distribution of MDA in both these groups. This design followed the recommendation of the European Medicines Agency (EMA) reflection paper regarding the possible impact of MDA on vaccine efficacy in young animals.

Vaccination and bias reducing methods

On study day 0 (SD0, vaccinated calves (MDA+ and MDA–) received 2-ml Bovalto Respi Intranasal (Boehringer Ingelheim), with a 1-ml dose administered into each nostril using an intranasal applicator. Animals in the control group received vaccine diluent. Treatment was carried out by the study director, who was unblinded. Animal care and veterinary examinations were conducted by trained personnel unaware of the treatments received by the animals.

Serology

Blood samples were collected from all calves seven days before the start of the study, on the day of vaccination and at two-week intervals until two weeks after challenge. Serology was performed on blood samples to determine the level of antibodies using a virus neutralisation assay (using MDBK cells and homologous virus) and the results were expressed as PD50 (serum dilution protecting 50 per cent of the infected wells). Serological data were used to support allocation to treatment groups; detect when MDA levels in the MDA+ control animals had declined to low levels; and verify that no intercurrent infection to the specific pathogen of interest occurred during the vaccination-challenge interval.

Table 5 Challenge strain titres, dose and route

	Titre	Dose and route of administration
PI3V	$10^{7,8}$TCID50/ml	4 ml sprayed into nostrils using an intranasal applicator
BRSV	$10^{5,8}$TCID50/ml	

BRSV, bovine respiratory syncytial virus; PI3V, parainfluenza type 3 virus.

Challenge

Based on the EMA reflection paper on the demonstration of a possible impact of MDA on vaccine efficacy in young animals (EMA 2010), the PI3V challenge was conducted after MDA titres in the control group animals had decayed to low levels (92 days or 13 weeks post vaccination). For BRSV, MDA titres in the control group animals decreased faster, but it was thought appropriate to seek a similar duration of immunity as for PI3V (84 days or 12 weeks), and all animals enrolled into the study were challenged at the same time.

Both challenge strains were acquired from the Research Institute of Veterinary Medicine in Brno. Both strains were field-isolated in 2003 from a nasal swab taken from a clinically ill calf.

Postchallenge observations

After challenge, clinical observations were made daily for 14 days and any signs of respiratory disease were recorded and scored (table 2). Rectal temperature was measured daily, with temperatures below 38°C and above 40°C considered to be out of normal range for calves this age. For each animal a daily score was calculated by summing all parameter scores, and a total clinical score calculated by summing all daily scores.

Nasal swabs were collected from each animal immediately before challenge strain administration and then daily for the next 14 days. Nasal swabs were collected and placed in sterile tubes and immediately suspended in supplemented Eagle's minimum essential medium. The elution material was inoculated into MDBK cell line suspension on 96-well plates. After incubation for seven days, PI3V titre was determined based on its specific cytopathogenic effect, completed when necessary with immunofluorescence staining, while immunofluorescence staining was used to reveal and titrate BRSV. Results were expressed in TCID50. TCID50 titres of nasal swabs collected daily post challenge provided an estimate of viral load. Total virus shedding was calculated as the sum of the daily viral loads (TCID50/ml) measured during the 14-day postchallenge period.

Total clinical scores, total virus shedding and number of days shedding were analysed for treatment differences using non-parametric analysis of variance (Kruskal-Wallis, adjusted for ties). Where a significant difference was observed among the three groups, this was further explored with post-hoc pairwise analyses.

Serological survey of MDA level in EU

In order to assess the relevance of the levels of MDA to PI3V and BRSV in the calves used in the challenge study, a survey was performed to determine the serological status of young calves aged around two weeks. Blood samples were obtained from calves on commercial farms in five European countries: Germany, Spain, Italy, Ireland and the UK. The results from calves in the Czech Republic that were sampled during field trials carried out as part of the vaccine development were also included. Antibody

Table 6 Clinical scores following PI3V challenge

PI3V	Clinical scores in control animals: days post PI3V challenge														
Animal ID	0–>5	6		7		8		9		10		11		12–>14	Total clinical score
	0	ND	OD	ND	OD	ND	OD	ND	OD	ND	OD	ND	OD	0	
632181		0	0	0	0	0	0	1	0	1	0	0	0		2
643429		0	0	0	0	0	0	1	0	0	0	0	0		1
643433		1	0	1	2	1	2	1	1	1	0	0	0		10
689106		1	1	1	2	1	2	1	1	0	1	1	0		12
689109		0	0	0	0	0	1	1	1	1	0	1	0		5
	Clinical scores in vaccinated MDA– animals: days post PI3V challenge														
637676							0								0
643435							0								0
643436							0								0
643437							0								0
643439							0								0
643440							0								0
	Clinical scores in vaccinated MDA+ animals: days post PI3V challenge														
643425	1 (day 5, ND)*						0								1
643426							0								0
643428							0								0
643430							0								0
643431							0								0
689107							0								0

*Clinical score 1: mild nasal discharge in one vaccinated MDA+ calf (on day 5 post challenge).
MDA, maternally derived antibodies; ND, nasal discharge; OD, ocular discharge; PI3V, parainfluenza type 3 virus.

Table 7 Clinical scores following BRSV challenge

BRSV	Clinical scores in control animals: days post BRSV challenge														
Animal ID	0–>3	4		5		6		7		8		9		10–>14	Total clinical score
	0	ND	OD	ND	OD	ND	OD	ND	OD	ND	OD	ND	OD	0	
641479		1	0	1	0	0	0	0	0	0	0	0	0		2
643771		0	0	2	0	2	0	0	0	0	0	0	0		4
643774		0	0	0	0	0	0	1	0	0	0	1	0		2
643778		0	0	1	0	1	0	0	0	1	0	0	0		3
643781		0	0	0	0	0	0	0	0	0	0	0	0		0
	Clinical scores in vaccinated MDA– animals: days post BRSV challenge														
641480							0								0
643765							0								0
643766							0								0
643767							0								0
643768	1 (day 2, ND)*						0								1
	Clinical scores in vaccinated MDA+ animals:days post BRSV challenge														
643769							0								0
643770							0								0
643772							0								0
643773							0								0
643780							0								0

*Clinical score 1: mild nasal discharge in one vaccinated MDA– calf (on day 2 post challenge).
BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; ND, nasal discharge; OD, ocular discharge.

Table 8 Nasal shedding following PI3V challenge

PI3V	PI3V titres in nasal swabs (log TCID50): control animals Days post PI3V challenge											Days of shedding	Total nasal viral shedding (TCID50)
Animal ID	0	1	2	3	4	5	6	7	8	9	10 → 14		
632181	2.1	2.1	2.6	4.8	6.6	6.1	4.3	2.3	2.1	2.1	← 2.1 →	6	26.7
643429	2.1	2.1	2.3	5.3	5.1	7.3	3.6	3.3	2.8	2.1		7	29.7
643433	2.1	2.3	5.8	7.6	6.3	7.3	5.8	3.1	2.3	2.3		8	40.5
689106	2.1	2.1	2.1	5.6	5.3	7.3	5.3	2.8	2.3	2.1		6	28.6
689109	2.1	3.3	2.6	3.8	4.6	4.8	2.3	2.1	2.1	2.1		6	21.4
	PI3V titres in nasal swabs (log TCID50): vaccinated MDA– animals Days post PI3V challenge												
637676						← 2.1 →						0	0
643435												0	0
643436												0	0
643437												0	0
643439												0	0
643440												0	0
	PI3V titres in nasal swabs (log TCID50): vaccinated MDA+ animals Days post PI3V challenge												
643425	2.1	3.1					← 2.1 →					1	3.1
643426						← 2.1 →						0	0
643428												0	0
643430												0	0
643431												0	0
689107												0	0

PI3V titres ≤2.1 considered negative.
MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

titres to PI3V and BRSV were determined in a single laboratory using the same virus neutralisation technique. The results are expressed in $\log_2$ units.

RESULTS

Animals

A total of 36 calves were enrolled in the PI3V (n=21) and BRSV (n=15) challenge studies, respectively.

Allocation to treatment groups

A total of 36 calves were assigned to the two virus challenge studies; respectively, six, seven and eight calves (PI3V) and five, five and five calves (BRSV) were allocated to the MDA– vaccinate, MDA+ vaccinate and MDA+ control groups.

Serology

Individual titres on the day of vaccination and on the day of challenge are shown in table 3, clarifying the range of observations and cut-off values. Mean specific IgG titres (log-transformed) per group against PI3V and BRSV are shown in figure 1. Vaccination caused titres to increase in all vaccinates (MDA– and MDA+), while challenge caused titres in all groups to rise sharply.

Challenge and postchallenge observations

The number of calves challenged in each study is shown in table 4. Titres, volume and route of administration used for the challenge are detailed in table 5. After challenge, none of the animals in any of the groups (controls and vaccinates) showed any dyspnoea, depression or rectal temperature outside of the range considered normal (tables 6 and 7). After PI3V challenge, mild nasal discharge was observed in one vaccinated MDA+ calf (on day 5 post challenge), and no other clinical signs were recorded in any of the vaccinates. Clinical scoring was significantly different among the three groups (P=0.001) due to the scores observed in the control animals. The difference between MDA+ and MDA– animals was not significant (P=0.3). After BRSV challenge, a mild nasal discharge was observed in one vaccinated MDA– calf (on day 2 post challenge), and no other clinical signs were recorded in any of the vaccinates. Positive clinical scores were observed in four out of five control animals. Clinical scoring was significantly different among

Table 9 Nasal shedding following BRSV challenge

BRSV	BRSV titres in nasal swabs (log TCID50): control animals Days post BRSV challenge									Days of shedding	Nasal shedding
Animal ID	0→2	3	4	5	6	7	8	9	10→14		
641479	0	0	2.3	2.3	2.6	2.3	0	0	0	4	9.5
643771		2.3	3.3	3.3	2.6	2.1	2.1	0		6	15.7
643774		0	0	2.3	3.3	2.1	0	0		3	7.7
643778		0	2.3	2.6	3.6	2.3	0	2.1		5	12.9
643781		0	2.3	2.3	2.1	0	0	0		3	6.7
	BRSV titres in nasal swabs (log TCID50): vaccinated MDA– animals Days post BRSV challenge										
641480						← 0 →				0	0
643765										0	0
643766										0	0
643767										0	0
643768										0	0
	BRSV titres in nasal swabs (log TCID50): vaccinated MDA+ animals Days post BRSV challenge										
643769						← 0 →				0	0
643770										0	0
643772										0	0
643773										0	0
643780		←0→	2.3				← 0 →			1	2.3

BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies.

the three groups (P=0.02) due to the scores observed in the control animals. The difference between MDA+ and MDA– animals was not significant (P=0.3).

Viral excretion was significantly reduced in vaccinated calves even in the presence of MDA specific for PI3V and BRSV after PI3V and BRSV challenges (tables 8 and 9). Nasal virus excretion post challenge was observed in all control animals, lasting for six to eight days for PI3V and three to six days for BRSV. By contrast, vaccinated MDA– showed no viral excretion, and only one vaccinated MDA+ animal in each study showed transient expression at a low level for a single day. Differences in both the duration of excretion and the viral loads among the three groups were statistically significant (P=0.001, PI3V; P=0.002, BRSV) due to the observations in the control animals. The differences between vaccinated MDA– and MDA+ animals were not significant (P>0.05) in both challenge studies. PI3V and BRSV challenges were fully validated, as confirmed by the clinical signs and nasal shedding observed in the control animals.

In the PI3V challenge study, three control calves were excluded because their antibody levels remained high, and one vaccinated MDA+ animal died on day 7 post vaccination and was therefore not challenged (table 3).

Table 10 Source of blood samples by country

Country of origin	Animals sampled (n)
Czech Republic	129
Germany	26
Ireland	38
Italy	40
Spain	15
UK	6

Serological survey of MDA level in EU

The total number of samples from each country is shown in table 10. In total, 254 samples from six countries were tested. MDA levels for these field calves were compared with the initial serological status of the MDA+ calves included in the challenge studies. The MDA titres from calves in the field are summarised in figure 2. It can be seen that 98 per cent of the PI3V titres were less than 10 $\log_2$ and 80 per cent of the BRSV titres were less than 4 $\log_2$. The starred columns indicate the titres of the MDA+ vaccinated calves in the challenge studies, at the time of vaccination, and this gives a visual indication of how representative of field levels these titres were. The level of MDA present in the calves of the challenge study before vaccination was consistent with that observed in the field.

Overall, the results of the challenge studies demonstrate the efficacy of the vaccine in the presence of BRSV and PI3V MDA under laboratory conditions, while the field sample antibody determinations confirmed that the MDA titres in the MDA+ calves corresponded to those typically found on farms.

DISCUSSION

The studies described here demonstrate good efficacy of a modified live (MLV) intranasal one-shot vaccination against challenges with PI3V and BRSV, two major BRD pathogens.[14] This efficacy was unaffected by the MDA status of calves at the time of vaccination, thus confirming the ability of the intranasally administered MLV vaccine to induce early immunity in young calves. As stated by Ellis,[15] 'currently available data from the last 10 years confirm earlier observations and indicate that IN [intranasal] administration of MLV BRSV (in combination) can prime for protective immunity in the face of maternally-derived immunity, but that the duration of that immunity is rather short-lived'. The question remains whether this relatively short duration of immunity results from the immaturity of the calf immune system or from the administration mode that triggers mainly a short local immunity.[15] In the present case, challenges were carried out after calves in the control group had become seronegative for the relevant pathogen, that is, at 84 days for BRSV and at 92 days for PI3V. The late challenges provided an assessment of the efficacy of the vaccine when administered in the presence of MDA and supported a duration of immunity of at least three months against both pathogens.

Efficacy was demonstrated for both pathogens, based on viral shedding and clinical signs. BRD clinical signs were evaluated using a scoring system, a method which may be criticised for the heterogeneity of its components. However, this methodology has long been used in a BRD context, either for deciding when to start antimicrobial treatment,[16] or evaluating efficacy in animals receiving antimicrobial treatment,[17] or in the development of a challenge model.[18] In addition, evaluation of respiratory disease prevention methods in other species (influenza vaccines in horses[19] and treatment and vaccine evaluation in pigs[20]) also used scoring systems. The difficulties associated with reproducing BRD following a single pathogen challenge during vaccine efficacy studies are well recognised.[15] The challenge models used in this study were able to induce typical, although mild, BRD clinical signs and consistent viral excretion in control animals. This validated the challenge models (from a European Pharmacopoeia monograph perspective) and provided evidence of the viral and clinical protection provided when the vaccine is administered in the presence of MDA.

The strains used in the challenge studies were genetically analysed and compared with a panel of European field isolates; over 93 per cent genetic similarity was demonstrated for both the PI3V and the BRSV strains.[21] The findings of the described challenge studies, in addition to this phylogenetic information, strongly suggest that the vaccine will protect calves from the genetic diversity of PI3V and BRSV strains currently circulating in Europe. Demonstrating protection after vaccination in the presence of MDA is relevant when the MDA levels are comparable with those encountered in the field. PI3V and BRSV are endemic viruses in Europe and are recognised as circulating in the majority of cattle herds. Therefore, young calves receiving colostrum from cows that are seropositive due to either vaccination and/or natural exposure will also be positive. This was indeed reflected in the field assessment of 254 calves that was conducted as part of this study.

There are limited published data available on MDA levels for PI3V and BRSV in calves in Europe,[22] with most studies having been carried out in North America.[23] These studies demonstrated that a high proportion of young calves had antibodies to PI3V and BRSV, with considerable variation in individual levels. However, comparison of data generated in different studies is difficult in view of the different serological tests/methods used. In the field serological survey, while the number of field samples was relatively small and 51 per cent of these were obtained from a Czech field trial, they nevertheless originated from commercial farms in six European countries and provided good context and external validation for the antibody levels observed in MDA+ calves, especially given that the antibody determinations were conducted in the same laboratory.

CONCLUSION

Vaccination of calves as young as 10 days old with a modified live intranasal vaccine against the BRD pathogens PI3V and BRSV resulted in a significant reduction in viral

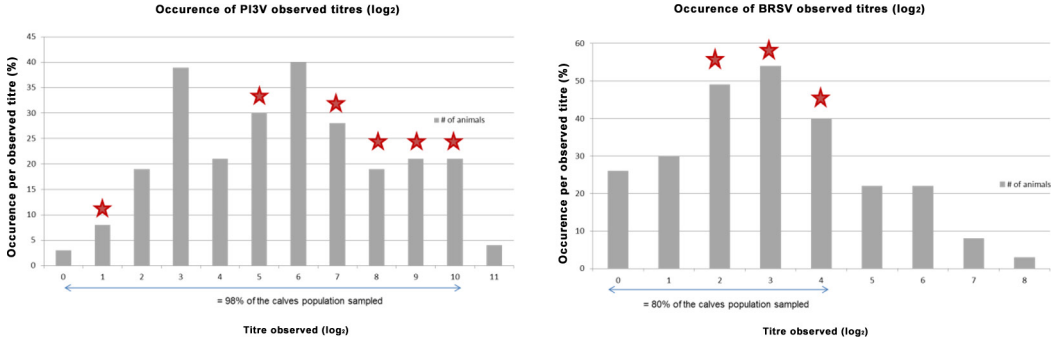

Figure 2 Summary of MDA titres from calves in the field. Results of the field serology survey of PI3V (left) and BRSV (right) MDA. The starred columns indicate the titres of the MDA+ vaccinated calves in the challenge studies, at the time of vaccination. BRSV, bovine respiratory syncytial virus; MDA, maternally derived antibodies; PI3V, parainfluenza type 3 virus.

shedding and clinical signs when calves were subjected to artificial challenges three months after vaccination. The presence of MDA at the time of vaccination did not adversely affect vaccine efficacy.

Finally, it has been demonstrated that the levels of MDA present in the calves in the challenge studies were comparable with those encountered in the field, and that the challenge strains were closely related to circulating wild strains, thus supporting the clinical relevance of these challenge studies.

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Contributors MH was involved in the trial process. CP-R was responsible for the European survey. All authors were involved in the reporting and writing of the publication.

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Competing interests LM, MC, EJ, ST and CP-R are employees of Boehringer Ingelheim. M-PT reports consultancy fees from Boehringer Ingelheim for work in writing the manuscript.

Ethics approval An ethical review was conducted before the study. The studies were carried out in accordance with the Act on Animal Health and Animal Welfare of the Czech Republic.

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Data availability statement No data are available. All data were submitted to the relevant authorities in order to obtain marketing authorisation. For reasons of compliance, data other than that included in the article are not available.

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