Shedding course of bovine respiratory syncytial virus and bovine parainfluenza 3 virus in calves vaccinated intranasally

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Abstract

Shedding time of bovine respiratory syncytial virus (BRSV) and bovine parainfluenza virus 3 (BPIV3) in calves vaccinated intranasally with modified live Rispoval RS-PI3 vaccine was determined. Blood and nasal swabs were collected on selected days before and after vaccination. Antibodies against BRSV and BPIV3 were tested by Respiratory ELISA Pentakit and the viral RNA was detected by RT-PCR. Twenty eight days after administration of the vaccine, a marked increase of specific antibody titres to BRSV and BPIV3 was detected in vaccinated calves. All animals were RT-PCR positive both for BRSV and BPIV3. Both viruses were excreted with nasal discharges within 8 d after vaccination but the course of shedding in individual calves was variable.

Key words: calves, live vaccine, BRSV, BPIV-3, shedding.

Introduction

Respiratory tract diseases in calves are responsible for major economic losses in cattle industry all over the world (6). Viral infections are the main cause of these diseases often associated with other pathogens. Several studies have shown that bovine respiratory syncytial virus (BRSV) and bovine parainfluenza virus 3 (BPIV3) are connected with respiratory diseases in cattle (1, 2, 4, 9, 10). BRSV can infect cattle of all age and breeds although the disease is most commonly observed in calves one to six months-old. In susceptible animals infection with this virus usually results in severe disease characterised by a high fever, depression, anorexia, cough, mucous to seropurulent nasal discharge, increased respiratory rate, and interstitial pneumonia (7). Infection with BPIV3 causes less serious disease symptoms. Only mild clinical signs such as slight fever, coughing, and nasal discharge are observed. However, under natural conditions uncomplicated cases are rare, because BPIV3 infections are usually accompanied by bacteria (12). Although clinical signs may indicated infection with BRSV, and to a lesser extent with BPIV3, laboratory confirmation is necessary to provide a definitive diagnosis. Nasal swabs or lung lavage samples are preferred specimens for diagnosis. Calves with the most severe clinical picture may not be the best candidates for sampling since the highest amount of virus is found about 2 to 3 d before the onset of the disease (7). Moreover, BRSV is labile and samples should be transported to the laboratory cooled, using an appropriate transport medium as quickly as possible. Therefore, the quality of collected specimens is essential for the final test result.

The aim of the study was to investigate the time of shedding of BRSV and BPIV3 in nasal discharges of calves vaccinated with modified live Rispoval RS-PI3 intranasal vaccine. The analyses were performed using RT-PCR.
Material and Methods

Animals and collection of samples. Six clinically healthy calves of black-white lowland breed, 6 to 8 weeks old, were selected for the experiment. Four calves were given a single 2 mL dose of Rispoval RS-PI3 vaccine (Zoetis) containing minimum of 10^5.0 TCID₅₀ of BRSV strain 375 and minimum of 10^5.0 TCID₅₀ of BPIV3 thermosensitive strain RLB103. The vaccine was administered intranasally with the applicator provided by the manufacturer. Two control calves received intranasally 2 mL of sterile PBS. Both groups of calves were housed separately in isolation to prevent the spread of the vaccine viruses.

The nasal swabs were collected from the vaccinated and control calves at -1, 0, 1, 2, 6, 8, 9, 14, 21, and 28 days post vaccination (dpv). Swabs were collected from the caudal part of the nasal cavity with a sterile plastic applicator (UTM-RT Copan system) and placed in a liquid transport medium. Then, the medium was shaken, centrifuged (300 × g, 10 min, 4°C), and the supernatants were stored at -70°C for the further examinations.

Blood samples were collected from each animal used in the experiment. The first sampling was performed before administration of vaccine and the second one 28 d after vaccination.

Serological examination. To determine the presence of antibodies to BRSV and BPIV3, Respiratory ELISA Pentakit (Bio-X diagnostics) was used. The test was performed according to the manufacturer’s instruction. The optical density (OD) of each sample was measured at 450 nm with a microplate spectrophotometer (Multiskan EX, Thermo Labsystems). Percentage of positivity (PP) was calculated by dividing OD value of the sample by corresponding positive control serum signal, and multiplying the result by 100. A sample was considered positive when the calculated PP value was greater than 12.62% for BRSV and 10.87% for BPIV3.

RNA extraction and RT-PCR. Total RNA was extracted from the supernatant of nasal swabs using TRI reagent (Sigma) according to the producer’s instructions. RT-PCR was performed using Titan One-Step RT-PCR System (Roche). Reaction mix consisted of: 5 μL of 5x reaction buffer (with 7.5 mM of MgCl₂ and DMSO); 0.5 μL of AMV/RT enzyme mix; 2 μL of 10 mM of sense and antisense primer; 0.5 μL of 10 mM dNTP mix; 1 μL of MgCl₂ (1.5 mM); 1.5 μL of extracted RNA, and water to a final volume of 25 μL per sample. Primers enabling amplification of a 136 bp fragment within gene encoding nucleoprotein N of BRSV: (N-F 5'-GTC AGC TTA ACA TCA GAA GTT CAA G-3' and N-R 5'-ACA TAGCAC CAC TAT CAT ACC ACA ATC A-3') were chosen, based on previous work by Zulauf (13). Reverse transcription was performed for 1 h at 48°C, followed by 2 min denaturation at 94°C. The amplification was done in 40 cycles according to the following thermal profile: 45 s denaturation at 94°C; 45 s hybridisation at 51°C, and 1.5 min elongation at 72°C. Reaction was finished by final elongation for 7 min at 72°C.

Reaction mix for amplification of BPIV3 consisted of: 5 μL of 5x reaction buffer (with 7.5 mM of MgCl₂ and DMSO); 0.5 μL of AMV/RT enzyme mix; 1 μL of 10 mM of sense and antisense primer; 0.5 μL of 10 mM dNTP mix; 1.5 μL of extracted RNA, and water to a final volume of 25 μL per sample. Amplification reaction of BPIV3 was performed using pair of primers specific to nucleoprotein gene: N-F: 5'-GGG AGT GAT CTT GAG TAT GAT CAA GA-3' and N-R: 5'-TGG ATT ATA AGG GCT CCA AGA CA-3' amplifying a 97 bp product (13). The following thermal profile was used: a single cycle of reverse transcription for 1 h at 48°C, 2 min denaturation at 94°C, followed by 40 amplification cycles of: 15 s denaturation at 94°C; 30 s hybridisation at 52°C, 45 s elongation at 72°C, and a final elongation for 7 min at 72°C.

All PCR products were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualised under UV light.

Results

All tested calves were serologically negative to BRSV and positive to BPIV3 before vaccination. Twenty eight days after administration of the vaccine a marked increase of specific antibody titre to BRSV was detected in vaccinated calves. The highest increase was observed in calves No. 3 (107.17% PP) and 4 (107.69% PP), moderate in calf No. 1 (62.08% PP) and the lowest in calf No. 2 (17% PP). The average PP value of control calves increased by 2.37% (Fig. 1A).

In the case of BPIV3, the highest increase in PP value was observed in calf No. 3 (81.64% PP) and the lowest in calf No. 4 (12.01% PP). The degree of positivity of the serum sample from calf No. 1 increased by 36.42% and from calf No. 2 by 17.1%. The average PP value of control calves increased by 7.96% (Fig. 1B).

All four calves vaccinated with the vaccine Rispoval RS-PI3 were RT-PCR positive for both BRSV and BPIV3 when nasal swabs were tested. The vaccine strain of BRSV was found in 15 out of 40 swabs collected. In calf No. 1, the virus was present in nasal discharges continuously for 8 d, in calves No. 2 and No. 4 it virus shedding lasted from the 2nd to 8th dpv, and in calf No. 3 from the 1st to 8th dpv (Table 1, Fig. 2).

BPIV3 was detected in 14 out of 40 swabs collected. In calves No. 1 and 2, BPIV3 was excreted with nasal discharges since 1st dpv till 8th dpv , in calf No.3 virus shedding was detected only on 8th dpv, and in calf No. 4 it was present in nasal discharges continuously till 8th dpv (Table 2, Fig. 3). Shedding of the virus was not accompanied with any clinical symptoms. The control calves remained PCR negative for both BRSV and BPIV3 throughout the study.
Fig. 1. Specific humoral immune response in calves for BRSV (A) and BPIV3 (B) after vaccination. In blue - values measured before vaccination, in red - values measured on day 28 after vaccination, K1/K2 - non-vaccinated control calves

Table 1. Presence of BRSV vaccine strain in nasal discharges

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Fig. 2. Electrophoresis of RT-PCR products for BRSV. Lanes: M- pUC mix marker 8 (19-1118), 1-4 of vaccinated calves, K1-K2 non-vaccinated control calves, K+ BRSV strain 375, K- non-template control

Table 2. Presence of BPIV3 vaccine strain in nasal discharges

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Discussion

The use of RT-PCR as a diagnostic method allowed detection of shedding of both BRSV and BPIV3 in calves vaccinated with a modified live Rispoval RS-PI3 vaccine. Shedding of viruses in most cases started before 2 dpv and lasted till 8 dpv. Presence of BPIV3 in swabs collected from calf no 4 and BRSV from calf no 1 at day 0, was probably not associated with early shedding but traces of the vaccine used for inoculation. In general, shedding period observed in our study, was slightly shorter than that observed in the case of infections with wild strains of viruses (1, 4, 9), for which shedding was detected up to 14 d after infection. However, in opposition to the results of those studies, no clinical symptoms were present in vaccinated calves in our experiment. Observed difference in shedding period cannot be explained solely by the fact that the calves were inoculated with attenuated strains of the viruses instead of field strains. Challenge studies involving vaccination with the same vaccine have been already described (8, 11), showing that the period of shedding for BRSV vaccine strain could last till 11 dpv (11) or up to 17 dpv (8); whereas, BPIV3 shedding after vaccination, as determined by Vangeel et al. (12) lasted only till 7 dpv.

The discrepancy observed in the duration of BRSV shedding could be explained by different factors. First of all different diagnostic methods were used in each study. Long period of shedding observed by Timsit et al. (8) could result from the use of real time RT-PCR, which has higher sensitivity than both viral isolation test used by Vangeel et al. (11) and RT-PCR used in our study. Length of shedding can also depend on individual susceptibility of animals used in inoculation experiment. However, previous data shows that susceptibility mostly depends on the age of animals (10); whereas, in all studies analysed above, calves were between 2 and 9 weeks old.

Finally, sample collection is an important part of all studies, and can affect results obtained, which can vary depending on the type of applicator used for collection and area of nasal cavity from which sampling was performed. It was proved that BRSV virus replicates mainly in deeper regions of the upper respiratory tract thus in the case of low titre, virus can remain undetectable if nasal swabs are not collected properly (3).

Efficiency of Rispoval RS-PI3 vaccine as a stimulant of humoral response was confirmed by the increase of the titre of antibodies specific to BRSV and BPIV3 in all of the vaccinated animals between the day of vaccination and 28 dpv. Seroconversion was especially distinct in the case of BRSV, where the signal calculated from QC sheet of the test, has risen from negative (-) to triple positive (+++), or quadruple positive (++++) in the case of three out of four animals tested. Relatively lower increase observed in the case of BPIV3 could be explained by the fact that the tested animals were already positive for BPIV3 before vaccination. This could result from either previous contact with BPIV3, which is unlikely, or the presence of maternally derived anti-bodies. Although there is no previous data on the influence of maternal BPIV3 antibodies on vaccination efficiency, a similar study on BRSV showed that their presence can interfere with protective antibodies induced after vaccination (5). Nevertheless, seroconversion in respect to BPIV3 observed in our study was still much higher than the change of antibody titres observed in control calves, which was insignificant.

Overall, the results of our study show that it is possible to detect both viruses for up to 8 d using RT-PCR. Although shedding of the viruses after vaccination was not correlated with appearance of any clinical signs, a period of virus excretion was not different to what was observed after infection with virulent strain. The results of the experiment indicate difficulty of identification of BRSV and BPIV3 infections, and underline the importance of an early sampling, performed as soon as suspicion of infection with these viruses emerges.
References