Original article

Use of rapid human respiratory syncytial virus strip tests for detection of bovine respiratory syncytial virus in experimentally vaccinated calves

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Abstract

Three different rapid strip tests: TRU RSV, BinaxNOW RSV and RSV Respi-strip were compared with RT-PCR and ELISA BRSV Ag for the ability to detect bovine respiratory syncytial virus (BRSV) in nasal swabs collected from calves experimentally vaccinated with live vaccine Rispoval RS-PI3. The reference strains of BRSV (375 and A51908) were detected by ELISA BRSV Ag whereas the strains of human respiratory syncytial virus (HRSV) and bovine parainfluenza virus type 3 (BPIV-3) were not. All rapid strip tests as well as RT-PCR reacted positively both to HRSV and BRSV reference strains and negatively to BPIV-3. The detection limit for RT-PCR was 39.1 $TCID_{50}$ (strain 375 of BRSV), whereas for each of the rapid tests it was approximately 156 $TCID_{50}$ and 312 $TCID_{50}$ for antigen ELISA. Diagnostic sensitivity in detecting BRSV in nasal swabs for TRU RSV and RSV Respi-strip tests was 33% and 50% for BinaxNOW RSV. Diagnostic specificity of TRU RSV was 100%, whereas for both BinaxNOW and Respi-strip it was 87%. We concluded that TRU RSV could be used as a supportive rapid test for BRSV screening in nasal swabs taken directly on a farm. However, due to the small group of animals used in the experiment, the results should be regarded as preliminary and the study should be repeated on a larger number of animals.

Key words: BRSV, diagnosis, strip tests

Introduction

Bovine respiratory syncytial virus (BRSV) is an enveloped, negative-stranded RNA virus belonging to the *Pneumovirus* genus of the *Paramyxoviridae* family. It is one of the major viral pathogens responsible for respiratory tract diseases in cattle worldwide. Infection with BRSV can affect cattle of all ages and breeds and is characterized by increased respiratory rate, nasal discharge, fever and cough. The morbidity is high (60 to 80%), whereas the mortality rate seldom exceeds 5-10%, although occasionally in some outbreaks it can reach up to 20% (Valarcher and Taylor 2007). Clinical signs observed during acute disease may raise the suspicion of BRSV infection but to make a definitive diagnosis laboratory confirmation is needed. The most widely used diagnostic methods for direct detection of BRSV in field specimens are the virus isolation test, antigen enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase

polymerase chain reaction (RT-PCR) (Larsen 2000). Although virus isolation in cell cultures is considered as the "gold standard" among virological methods, in the case of BRSV infection this test can be unsuccessful. Attempts at virus isolation often fail because of the lability of the virus even in optimally stored samples. Moreover, BRSV replicates slowly and several blind passages are often required before any cytopathic effect can be seen. This makes the virus isolation test laborious and time consuming and therefore not suitable for routine diagnostics.

Recently, various RT-PCR assays targeting fusion (F), glycoprotein (G) and nucleoprotein (N) genes of BRSV were developed for detection of the virus (Vilcek et al. 1994, Socha and Rola 2011). Although RT-PCR and especially real time RT-PCR has been shown to be more sensitive than other tests, each of these methods is relatively complicated and requires a well equipped laboratory and trained personnel. Therefore there is a need for a simple and rapid diagnostic test that would allow detection of BRSV in field conditions.

Rapid immunochromatographic strip tests detecting viral antigens have been developed for HRSV (Popow-Kraupp and Aberle 2011). HRSV is the prototype of the *Pneumovirus* genus and BRSV is closely related to it with a high degree of genetic, antigenic and epidemiological similarities. These features, could theoretically make it possible to adapt rapid HRSV strip tests for the diagnosis of BRSV.

The aim of this study was to evaluate the possibility of using rapid HRSV immunoassay tests for detection of BRSV.

Materials and Methods

Reference strains

Two BRSV strains: 375 (VR-1339) and A51908 (VR-794) and two other paramyxoviruses, HRSV strain A2 (VR-1540) and BPIV3 strain SB (VR-739), were used in the study as the positive and negative controls for RT-PCR, ELISA BRSV Ag, rapid TRU RSV, BinaxNOW RSV and RSV Respi-strip tests, respectively.

Experimental infection and collection of nasal swabs

Blood samples were taken before infection from all tested calves and examined using the indirect ELISA BRSV SVANOVIR test (Svanova) to detect BRSV-specific antibodies. Five clinically healthy and serologically negative calves approximately 6-8 weeks old were finally selected for experiment. Three calves were inoculated intranasally with 2 ml of live Rispoval RS-PI3 (Pfizer) vaccine containing no less than $10^{5.0}$ $TCID_{50}$ of attenuated strain 375 of BRSV. The vaccine was administrated intranasally using an applicator provided by the manufacturer of the vaccine. Control calves received 2 ml of sterile water. Both groups of calves were housed separately in isolation to prevent the spread of the vaccine virus.

Nasal swabs were taken from the inoculated and control calves at -1, 0, 1, 2, 6, 8, 9, 14, 21 and 28 days post inoculation (dpi). Swabs were collected from the caudal part of the nasal cavity with a sterile plastic applicator (UTM-RT Copan system) and placed in liquid transport medium. In the laboratory swabs were shaken, centrifuged (300 x g, 10 min) and used directly for testing.

Strip tests

Three different rapid strip tests: RSV Respi-strip (Coris), TRU RSV (Meridian Bioscience), Binax-NOW RSV (Inverness Medical) designed for diagnostics of HRSV were evaluated in the study. The Binax-NOW RSV and RSV Respi-strip tests detect F protein antigen, whereas the TRU RSV test detects F and N antigens. All the tests were run according to the manufacturer's instructions. Interpretation of the final result was similar for each of the strip tests. A positive test result was obtained when both a sample line and a control line were visible. If only a control line was visible the test was negative. If control line was not visible the test was invalid.

ELISA BRSV Ag

Pulmotest BRSV Antigen Detection ELISA (Euroclone) designed for the detection of BRSV antigens was used in the study. The test was performed according to the manufacturer's instructions. Briefly, the optical density of the sample was measured using a microplate spectrophotometer at 450 nm. For each sample the percent of positivity was calculated. The sample was positive if this value was higher than the value given in the Quality Control data sheet of the test batch.

Analytical sensitivity and specificity

The detection limit of all tests was determined by analysing a 2-fold dilution series of BT (bovine

Table 1. Specificity of RSV strip tests compared with ELISA BRSV Ag and RT-PCR.

Controls	Test				
	RT-PCR	ELISA BRSV Ag	RSV Respi-strip Cit – Coris	TRU RSV	BinaxNOW RSV
BRSV A51908	+	+	+	+	+
BRSV 375	+	+	+	+	+
HRSV A2	+	–	+	+	+
BPIV 3	–	–	–	–	–
Negative Swab	–	–	–	–	–

Table 2. Shedding of BRSV in calves experimentally vaccinated with live vaccine Rispoval RS-PI3 determined by RT-PCR.

Calf no.	Days post vaccination									
	-1	0	1	2	6	8	9	14	21	28
1.	–	+	+	+	+	+	–	–	–	–
2.	–	–	–	+	+	+	–	–	–	–
3.	–	–	+	+	+	+	–	–	–	–
Controls	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–

Table 3. Results of rapid strip tests for detection of BRSV in nasal swabs*.

Test	Diagnostic sensitivity (%)	Diagnostic specificity (%)	Positive predictive value (%)	Negative predictive value (%)	True positives	False positives	True negatives	False negatives
RSV Respi-strip	33	87	57	71	4	3	20	8
TRU RSV Test	33	100	100	74	4	0	23	8
BinaxNOW RSV	50	87	67	77	6	3	20	6

* Specimens were defined as true positive if BRSV was detected by RT-PCR.

turbinate) cell cultures infected with BRSV strain 375. These dilutions contained from 20 000 $TCID_{50}$ to 19.5 $TCID_{50}$ of the virus. They were directly used for examination with the rapid strip tests and ELISA BRSV Ag. For RT-PCR, RNA was isolated from them first. To determine diagnostic specificity of the tests we investigated reference strains for BRSV and other paramyxoviruses described above. A further negative control, consisting of transport medium from the UTM-RT Copan system, was included.

Diagnostic specificity, diagnostic sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each of the strip tests. Diagnostic sensitivity was calculated by dividing the total number of true positive results (TP) by the sum of false negatives (FN) and TP, whereas diagnostic specificity was calculated by dividing the total number of true negatives (TN) by the sum of TN and

false positives (FP). PPV was calculated by dividing TP by the sum of TP and FP, and NPV by dividing TN by the sum of FN and TN. The results were multiplied by 100 and expressed as a percentage.

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 the Titan One-Tube RT-PCR System (Roche). Primers specific to gene encoding nucleoprotein N (N-F 5'-GTCAG-CTTAACATCAGAAGTTCAAG-3' and N-R 5'-ACATAGCACTATCATACCACAATCA-3') giving a 114 bp long product were used (Zulauf 2007). Reverse transcription was performed for 1 h at 48ºC,

followed by 2 min denaturation at 94ºC. The amplification was done in 40 cycles according to the following scheme: 45 s denaturation at 94ºC; 45 s of hybrydisation at 51ºC and 1.5 min of elongation at 72ºC. Reaction was finished by final elongation for 7 min at 72ºC. The PCR products were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualized under UV light. The RT-PCR assay was used as the reference test for this study. Samples positive by this method were considered true positives and samples negative were considered true negatives.

Results

All rapid strip tests and RT-PCR reacted positively both with HRSV strain A2 and BRSV reference strains, and negatively with BPIV-3. The ELISA BRSV antigenic test reacted positively only with strains of BRSV. A summary of the results concerning the specificity of evaluated tests is presented in Table 1.

Analytical sensitivity of the tests was determined for the same virus strain of BRSV. The detection limit for RT-PCR was 39.1 $TCID_{50}$. Analytical sensitivity of the strip tests was lower than RT-PCR and each of these rapid tests detected approximately 156 $TCID_{50}$ of the BRSV. The detection limit for ELISA BRSV Ag was 312 $TCID_{50}$.

All three calves vaccinated with the live vaccine Rispoval RS-PI3 were RT-PCR positive when tested using nasal swabs. In total, the vaccine virus was found in 12 swabs out of 30 swabs collected. In calf No. 1 vaccine virus was excreted with nasal discharges continuously for 8 dpi, in calf No. 2 virus shedding lasted from the 2nd to 8th dpi, and in calf No. 3 from the 1st to 8th dpi. Two control calves remained negative in RT-PCR throughout the study (Table 2).

The results concerning virus shedding in vaccinated calves obtained with rapid strip tests are summarized in Table 3. Using the RSV Respi-strip test the BRSV was detected in 7 swabs derived from calves No. 2 (1, 2, 8 and 9 dpi) and 3 (2, 8 and 9 dpi). Swabs collected from calf No. 1 were negative in this test. Four nasal swabs were positive in the TRU RSV test. The virus was detected in calves No. 1 (0, 1 dpi), No. 2 (2 dpi) and No. 3 (1 dpi), respectively. Diagnostic sensitivity of both these tests in detecting BRSV in nasal swabs was 33%.

BinaxNOW RSV test was positive for 9 swabs. The vaccine virus was detected in calf No. 1 (0, 1 dpi), in calf No. 2 (0, 1, 2 dpi) and calf No. 3 (1, 2, 6 and 9 dpi). Diagnostic sensitivity of the test was 50%.

Only one sample was positive in ELISA BRSV Ag. This was the sample from calf No. 2, collected at day 2 pi. However, a slight increase in the percent of positivity was visible in all vaccinated calves during the first days post infection. Results of this test are shown in Fig. 1.
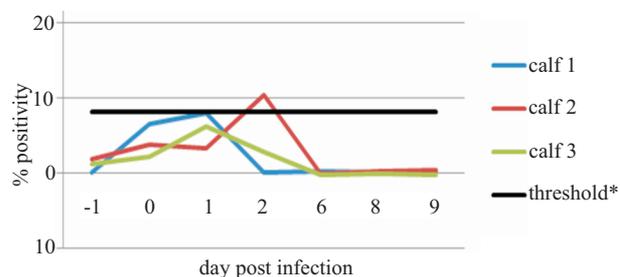

Fig 1. Results of ELISA BRSV Ag for detection of BRSV in nasal swabs.
*Positivity threshold for the test batch used was 8.07%.

Discussion

Most of the HRSV rapid strip tests currently available on the market are designed to detect either glycoprotein F, nucleoprotein N or both. They belong to the most conservative proteins both in HRSV and BRSV, showing 81% identity for glycoprotein F and 93% for nucleoprotein N at the amino acid sequence level (Valarcher and Taylor 2007). It has been shown that monoclonal antibodies to HRSV cross-react with most of the epitopes of F and N proteins of BRSV (Stine et al. 1997, Fogg et al. 2001).

Based on this knowledge and previous successful use of the HRSV enzyme immunoassay for detection of BRSV (Osorio et al. 1989), we assumed that HRSV strip tests could be used in the diagnostics of BRSV as well. In our studies three commercial immunochromatographic strip tests were evaluated for detection of BRSV: BinaxNOW RSV, TRU RSV and RSV Respi-strip. These were chosen based on previous evaluations of various immunochromatographic tests used in the diagnostics of HRSV (Gregson et al. 2005, Selvarangan et al. 2008, Miernyk et al. 2011, Sánchez-Yebra et al. 2012). Although all of these showed generally lower sensitivity compared to the recommended laboratory diagnostic methods, their high specificity, low cost, simplicity and short time required for testing (Gregson et al. 2005, Miernyk et al. 2011, Sánchez-Yebra et al. 2012) mean that these tests can be used as complementary tests in HRSV diagnostics. Moreover, all of these tests were specific to highly conservative proteins of pneumoviruses, which theoretically should also make detection of BRSV possible.

Results of our studies confirmed that rapid immunochromatographic HRSV tests would be able to

detect BRSV. All of the strip tests reacted positively with the reference strains, both HRSV and BRSV, but no reaction with negative controls was recorded. What was particularly important, no reaction with another paramyxovirus e.g. BPIV3 was seen. Positive reaction with HRSV, to which these tests were dedicated, should not be a problem because it is known that this virus has a highly restricted host range and does not have the ability to infect cattle (Bossert and Conzelmann 2002).

All of the strip tests used in this study were previously evaluated in HRSV diagnostics. The specificity of all of these assays was equally high and reached a level of 97-98%, whereas diagnostic sensitivity differed considerably for each of them and amounted to 58% for TRU RSV, 72% for BinaxNOW and 91% for RSV Respi-strip (Wybo et al. 2009, Miernyk et al. 2011, Sánchez-Yebra et al. 2012).

In our study we performed similar calculations for the same three strip tests, which allowed us to observe possible differences in their performance when used for diagnosis of BRSV infections. Compared to RT-PCR, the specificity of strip tests was relatively high, ranging from 87% for RSV Respi-strip and BinaxNOW to 100% for TRU RSV. However, their diagnostic sensitivity was lower, ranging from 33% for TRU RSV and RSV Respi-strip to 50% for Binax-NOW. Although the specificity of strip tests was similarly high for both HRSV and BRSV, their sensitivity was clearly lower when used in the detection of bovine pathogen. This could be explained by existing differences in amino acid sequence of F and N proteins between BRSV and HRSV.

Although it has been shown that some HRSV specific monoclonal antibodies are able to recognize F and N proteins of BRSV, they bind only to selected epitopes which can lead to an overall weaker reaction of the assays (Stine et al. 1997, Fogg et al. 2001). However, it is difficult to fully assess the degree of this effect, due to the limited information provided by the manufacturers about antibodies used in the design of the test.

It is also possible that the lower diagnostic sensitivity detected in our study was not connected with the differences between HRSV and BRSV, but was rather a result of the different type of samples used for testing. Nasopharyngeal washes are described as a recommended specimen for immunchromatographic tests (Selvarangan et al. 2008) and previous evaluations were done based on this type of sample (Wybo et al. 2009, Miernyk et al. 2011, Sánchez-Yebra et al. 2012). However, due to the fact that collection of nasal washes from animals could be complicated, we decided to use nasal swabs as they are much easier to obtain in field conditions.

Variability of the diagnostic sensitivity of the evaluated tests could be surprising when compared with their equal limit of detection. This discrepancy could be due to the fact that diagnostic sensitivity was evaluated on nasal swab samples acquired from vaccinated animals whereas for calculation of analytical sensitivity serial dilutions of infected cell cultures were used. Observed differences in diagnostic sensitivity could therefore represent the different ability of the evaluated assays to cope with contaminations or different titres of the virus in the nasal swabs, which could affect the performance of the tests.

Based on the calculated positive predictive value (PPV) and negative predictive value (NPV) of the strip tests, it can be concluded that the most reliable was TRU RSV. Although its sensitivity was lower compared to BinaxNOW RSV (50%), it was characterized by very high specificity (100%) as well as PPV (100%) and NPV (74%). This means that samples positive by TRU RSV were true positive. TRU RSV could therefore be used as a supportive rapid screen test for BRSV detection on a farm. However, due to the limited sensitivity, negative results would have to be confirmed by more sensitive tests such as RT-PCR. On the other hand, based on the calculated limit of detection and sensitivity, it was shown that TRU RSV outperforms the ELISA BRSV Ag test as a BRSV diagnostic method, even though it was designed for detection of HRSV. Immediate testing using TRU RSV could also reveal some positive samples as negative by cell cultures due to the possible inactivation of the virus during transport. However, due to the fact that the tested group of animals was very small, the results should be treated with caution and regarded as preliminary. Further research on a larger test group is needed before applying the results of the study in veterinary practice.

In the longer perspective, an ideal solution would be strip tests dedicated to BRSV, which would offer not only high specificity like TRU RSV, but also much higher sensitivity.

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