

Comparison of four RT-PCR assays for detection of bovine respiratory syncytial virus

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Abstract

RT-PCR assays for detection of BRSV, based on four different sets of primers were optimized and evaluated for their sensitivity and specificity. Primers used in this study were specific for genes encoding three BRSV proteins, nucleoprotein N and glycoproteins F and G. Our results indicated that RT-PCR with primers B7:B8 for G protein was the most efficient in detecting BRSV. Starters B7:B8 reacted specifically only with BRSV strains, no cross-reaction with other closely related viruses to BRSV was observed. RT-PCR sensitivity was also high and amounted to $10^{1.66}$ TCID₅₀. Starters for F and N genes of BRSV were not sufficiently specific and cross-reacted with RNA of HRSV. RT-PCR with primers for the genes F and N of BRSV was characterized by a lower sensitivity than RT-PCR with primers B7:B8. In conclusion, RT-PCR specific to a sequence of glycoprotein G gene, seemed to be the most useful for BRSV detection.

Key words: cattle, BRSV, RT-PCR, diagnosis

Introduction

Bovine respiratory syncytial virus (BRSV) is an enveloped, negative stranded RNA virus belonging to the subfamily *Pneumovirinae* of the *Paramyxoviridae* family. It is responsible for serious economic losses worldwide, due to the high morbidity, treatment expenses and slower growth of affected animals (Valarcher et al. 2007).

Among currently used methods of diagnosing BRSV infection RT-PCR and its modifications are regarded as the most specific and sensitive (Larsen et al 1999, Valarcher et al. 1999). RT-PCR tests are based either on primers specific to genes encoding glycoproteins F and G (Vilcek et al. 1994) or nucleoprotein N (Valarcher et al. 1999, Zulauf 2007). High

differences in variability of these regions exist with sequences encoding glycoprotein G being the most variable gene of pneumoviruses and nucleoprotein N the most conserved one (Valarcher et al. 2007). It has been suggested that this kind of difference could have a potential effect on specificity of assays (Vilcek et al. 1994). However till now, no study has compared three RT-PCR assays specific to genes of F, G and N protein in parallel.

The aim of this study was to evaluate specificity and sensitivity of RT-PCR assays based on the primers specific to genes of G, F and N proteins, in order to assess if any significant differences exists in their characteristics. An ideal assay for a routine diagnostic test, should offer maximum sensitivity together with the specificity to various BRSV strains, but not to other closely related viruses.

Materials and Methods

RT-PCR was performed using the Titan One-Tube RT-PCR System (Roche). Four pairs of BRSV specific primers were used in the assays: two pairs specific to gene encoding N protein N-F:N-R designed by Zulauf (2007) giving a 136 bp long product, N4F 5'-GTTGCTGCTTTGGTTAT-3': N4R 5'-AGACTTGTATGATGCTGC-3' giving a 562 bp long product; one specific to gene encoding glycoprotein F designed by Vilcek et al. (1994) with 711 bp of expected product length and one specific to glycoprotein G designed by Vilcek et al. (1994) giving a product of 371 bp length. 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 using conditions as follows: 45 s denaturation at 94°C; 45 s of hybridisation and 1.5 min of elongation at 72°C. Reaction was finished by final elongation for 7 min at 72°C. Hybridisation temperatures were set as follows: for B1:B2 primers – 49°C, for N-F:N-R – 51°C, for N4F:N4R – 54°C and for pair of primers B7:B8 – 51°C. Optimal concentrations of reagents for each assay were previously set.

The specificity of each pair of primers was evaluated with the following viruses: 2 positive controls – strains 375 (ATCC-VR1339) and A51908 (ATCC-VR794) of BRSV and 3 negative controls: HRSV strain A2 (ATCC VR-1540D), strain SB BPIV3 (ATCC-VR 739), Non Template Control (water). Apart from HRSV, the RNA of which was ordered as a suspension, all of the viral RNA samples were isolated from viruses that were previously grown in cell cultures. RNA from cell cultures was extracted using TRI reagent (Sigma) according to the producer's instructions. A sensitivity test was performed for each pair of primers using serial 10-fold dilutions of RNA isolated from BRSV strain 375. TCID₅₀ of the strain has been previously calculated as 10^{4.66}/ml.

Results

Specificity study

RT-PCR with primers B7:B8 reacted only to reference strains of BRSV. For primer pairs NF:NR and B1:B2 there was a clear cross reaction with HRSV. N4F:N4R primers reacted specifically with BRSV, however a non-specific band was visible in reaction with HRSV. Results of the RT-PCR specificity test were shown in Fig 1.

Sensitivity study

The highest sensitivity of the test, 10^{1.66} TCID₅₀, was achieved using primer pairs B7:B8 and N-F:N-R. The detection limit for B1:B2 was 10^{2.66} TCID₅₀ and 10^{3.66} TCID₅₀ for N4F:N4R. The results of sensitivity of the test are shown in Fig. 2.

Discussion

Detection of HRSV A2 strain using B1:B2 primers is in accordance with the previous results of a PCR test using the same pair of primers (Vilcek et al. 1994). This could not always be avoided for primers specific to the F gene because of its high level of sequence conservation among pneumoviruses (78% between HRSV and BRSV) (Valarcher et al. 2007). A similar reason could explain the reaction of primers N-F:N-R and N4F:N4R specific to the N protein with HRSV. None of the tested primers reacted with RNA of Bovine Parainfluenza Virus 3 (BPIV3), related to BRSV. Three of 4 pairs of primers were previously used, either in nested PCR (Vilcek et al. 1994) or real time RT-PCR (Zulauf 2007), but classical RT-PCR was done only with primers B1:B2 and B7:B8. Sensitivity of the test for these two pairs of primers was estimated as 1.0 TCID₅₀. This was over 2 orders of magnitude higher sensitivity for B1:B2 and 1 order higher for B7:B8 primers, compared to the results obtained in our studies, in which calculated sensitivities were 10^{2.66} TCID₅₀ for B1:B2 and 10^{1.66} TCID₅₀ for B7:B8. This inconsistency could be explained by the use of a different RNA extraction protocol, a different PCR kit, and local laboratory conditions. Nevertheless, even with this decrease of sensitivity, the RT-PCR reaction based on the detection of G protein using B7:B8 primers seems to be a reliable diagnostic method for diagnosis of BRSV infections.

Primers specific to nucleoprotein N differed considerably in their sensitivity with N-F:N-R showing 100 times higher sensitivity compared to N4F:N4R. This indicates that the choice of the particular region of the virus genome can have great effect not only on their specificity but also sensitivity.

In summary, among the tested primers only one pair, specific to G glycoprotein was characterized by both high sensitivity and specificity in relation to BRSV strains. A highly sensitive N-F:N-R set of primers that reacted with HRSV could still be useful in veterinary diagnostics. Both respiratory viruses have strict species specificity, therefore HRSV could appear in collected samples only by contamination (Schlender et al. 2003), which is improbable in a veterinary diagnostic laboratory where human samples are not tested.

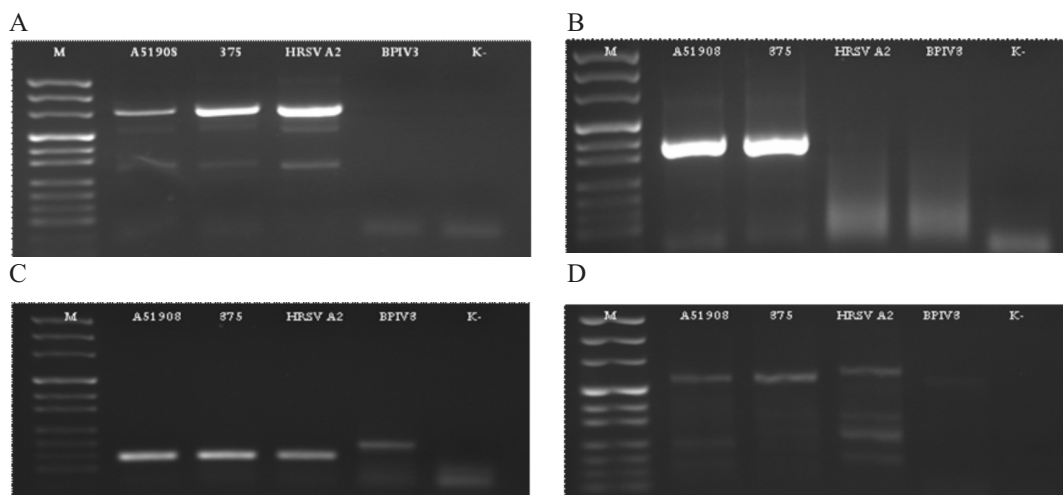


Fig. 1. Results of specificity test: B1:B2 (A), B7:B8 (B), N-F:N-R (C), N4F:N4R (D) pUC Mix Marker 8 (Fermentas) was used as a marker (M).

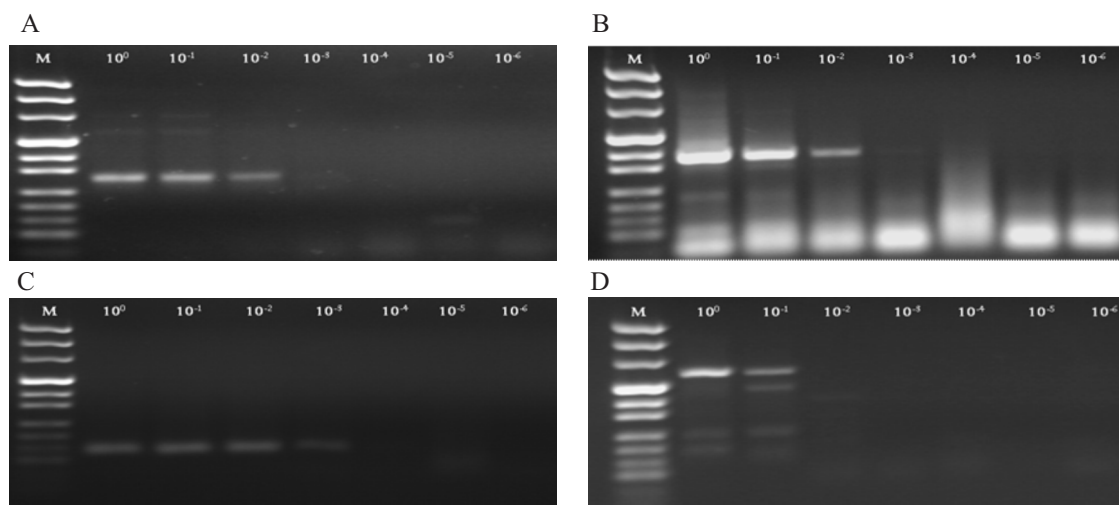


Fig. 2. Results of sensitivity test: B1:B2 (A), B7:B8 (B) N-F:N-R (C), N4F:N4R (D) pUC Mix Marker 8 (Fermentas) was used as a marker (M).

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