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# PARTIAL NUCLEOTIDE SEQUENCE OF THE G GLYCOPROTEIN OF RESPIRATORY SYNCYTIAL VIRUS ISOLATED FROM WILD BIGHORN SHEEP MAY PROVE THAT IT IS NEARLY IDENTICAL TO THAT OF OVINE RESPIRATORY SYNCYTIAL VIRUS OF DOMESTIC SHEEP

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## SUMMARY

The G glycoprotein is the most variable gene among different strains of human and bovine respiratory syncytial viruses (RSV). One isolate of ovine RSV isolated from domestic sheep has been molecularly characterized before. In this study, partial nucleotide sequence (65%) of the G glycoprotein gene of RSV isolate of wild bighorn sheep and its predicted protein are reported. Very high level of identity at the nucleotide and amino acid levels between the two RSV isolates of domestic sheep and wild Bighorn sheep suggests that Bighorn sheep RSV may be considered as a member of the ovine RSV subgroup. This information will help in understanding RSV epidemiology and vaccine development in cattle and sheep.

## INTRODUCTION

Bovine respiratory syncytial virus (BRSV) is a major cause of severe respiratory tract disease in calves and adult cattle (Castleman et al., 1985; Pirie et al., 1981). Ovine respiratory syncytial virus (ORSV) was isolated from domestic and wild bighorn sheep with respiratory symptoms (Leamaster et al., 1983; Evermann et al., 1985; Spraker et al., 1986). It has been suggested that ORSV infects cattle (Bryson et al., 1988; Grubbs et al., 2001). It has been proposed by some investigators that ungulate RSVs be divided into 2 subgroups; one representing BRSV and the other representing ORSV (Alansari and Potgieter, 1993; Mallipeddi and Samal, 1993 a, Alansari et al., 1999; Eleraky et al., 2001). The G (attachment glycoprotein) is one of the two major surface glycoproteins encoded by RSVs. The G glycoprotein is the most vari-

able gene among different strains of human respiratory syncytial virus (HRSV) and BRSV (Johnson et al., 1987; Mallipeddi and Samal, 1993 b). RSV strain (WSU 87- 6750) isolated from wild bighorn sheep has not been molecularly characterized before. In this study, partial nucleotide sequence (65%) of the G glycoprotein gene and its predicted protein are reported. The purpose is to give an idea about the level of identity between ORSV strain isolated from domestic sheep and the other strain isolated from wild bighorn sheep in respect to G protein gene. This will be valuable for subgrouping of ruminant RSVs and complete understanding of RSV epidemiology and vaccine development in cattle and sheep.

## MATERIAL AND METHODS

RT- PCR assay targeting the G glycoprotein gene (Eleraky et al., 2003) was used to amplify part (65%) of the G glycoprotein gene of bighorn RSV. Bighorn sheep respiratory syncytial virus isolate WSU 87-6750 (provided by Dr. Jim Evermann, Washington Animal Disease Diagnostic Laboratory, Pullman, WA, USA) was propagated in Madin Darby Bovine Kidney (MDBK) cells. RNA was extracted with Trizol reagent (Gibco-BRL, Gaithersburg, MD) according to the protocol of the manufacturer. A set of primers described previously (Eleraky et al., 2003) was used for amplification of 542 bp G gene fragment of bighorn sheep RSV. One primer [G164 (5' AGCCCTAGCAATGATAAC 3')] representing bases 147-164 of ovine RSV (WSU 83-1578) G gene was used for cDNA synthesis. The second primer [G672 (5' GACTGGTTCTGTGGTGG 3')] represents the complementary sequence of bases 688-672 of the ovine RSV (WSU 83-1578) G gene. Synthesis of cDNA was done using superscript II Rnase H- reverse transcriptase (Invitrogen Life Technologies) according to the manufacturer's protocol. Amplification is performed using Taq DNA polymerase (Invitrogen Life Technologies) as described previously (Eleraky et al., 2003). PCR amplified product was electrophoresed in 2 % agarose gel (Sigma Chemicals Co., St. Louis, MO). in 1x TAE buffer. Sequencing of the PCR product was done at the University of Tennessee (USA) Molecular Biology Research Facility by using an ABI prism dye terminator cycle sequencing reaction kit and an ABI 373 DNA sequencer, Perkin Elmer Inc., Foster City, CA, USA.

## RESULTS

Comparison of the nucleotide sequence of the PCR product representing 65% of the G glycoprotein gene of the bighorn sheep RSV with the corresponding sequence of ovine RSV isolate WSU 83-1578 showed 99.39% identity (fig. 1).

The alignment of the deduced amino acid sequence of the amplified fragment of bighorn sheep RSV G glycoprotein gene with the corresponding published sequence of ORSV showed

98.17 % identity (fig. 2). Amino acids were substituted in 3 positions (amino acids 80, 87 and 136 of ORSV G protein).

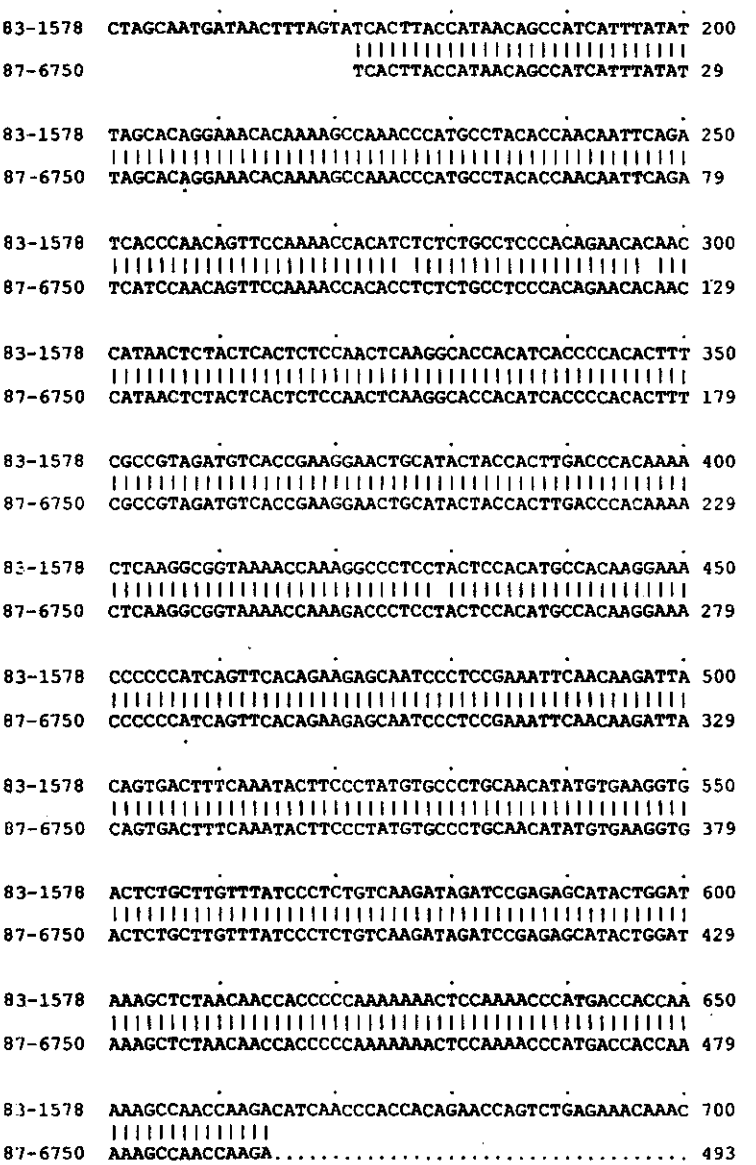


Fig. 1: Comparison of the nucleotide sequence of part of the G glycoprotein of Bighorn sheep RSV isolate WSU 87-6750 with the G glycprotein of ovine RSV isolate WSU 83-1578 (Gene Bank accession number L08470). The dots above the sequence are spaced every 10 nucleotide and the number of the last nucleotide for each line is given on the right end of the line.

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83-1578     msnhthhfefktlkkawkaskyfivglsclyklnlkslvqmalsalamitlvsltitaii  60
                                                                 ||||||||
87-6750                                                          sltitaii   8

83-1578     yistgntkakpmptptiqitqqfqnhislpptehnhnsthsptqgttsphtfavdvtegt  120
            |||||||||||||||||||| |||||| ||||||||||||||||||||||||||||||||
87-6750     yistgntkakpmptptiqiiqqfqnhtslpptehnhnsthsptqgttsphtfavdvtegt   68

83-1578     ayyhlthktqggktkgpptphatrkppissqksnpseiqqdysdfqilpyvpcnicegds  180
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
87-6750     ayyhlthktqggktkdpptphatrkppissqksnpseiqqdysdfqilpyvpcnicegds  128

83-1578     aclslcqdrsesildkaltttpkktpkpmttkkptktsthhrtslrnklyiktnmttpph  240
            |||||||||||||||||||||||||||||||||
87-6750     aclslcqdrsesildkaltttpkktpkpmttkkptk                          164

83-1578     glistakhnknqstvqnprhtla  263
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Fig. 2: Comparison of the predicted amino acid sequence of the amplified fragment of G glycoprotein gene of Bighorn sheep RSV isolate WSU 87-6750 with the G glycprotein of ovine RSV WSU 83-1578 . The dots above the sequence are spaced every 10 nucleotide and the number of the last amino acid for each line is given on the right end of the line.

## DISCUSSION

The very high level of identity at the nucleotide and amino acid levels between RSV isolate of domestic sheep (WSU 83-1578) and RSV isolate of wild Bighorn sheep (WSU 87-6750) suggests that Bighorn sheep RSV may be considered as a member of the ovine RSV subgroup. It has been suggested that the region corresponding to the conserved 13 amino acid region (164 -176) of human RSV G protein is a putative receptor binding site. (Johnson et al., 1987; Mallipeddi and samal , 1993 a) This region is not conserved in ORSV and BRSV (Alansari and Potgieter, 1993). This region is exactly conserved in the two isolates of RSV of domestic and wild Bighorn sheep (fig. 2).

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