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# Analysis of the ovine respiratory syncytial virus (RSV) G glycoprotein gene defines a subgroup of ungulate RSV

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Respiratory syncytial virus (RSV) has been isolated from sheep suffering from respiratory tract disease. Since the greatest differences between bovine RSV and human RSV are found on the attachment G protein, we have determined the nucleotide and deduced amino acid sequences of the G gene of ovine RSV. The latter contained 838 nucleotides and had a major open reading frame encoding a protein of 263 residues, and shared 73% nucleotide sequence identity with that of bovine RSV. The deduced amino acid sequence of the ovine RSV G protein showed only 60% amino acid identity with the G protein of bovine RSV. Despite the low level of identity, there were similarities in the predicted hydropathy profiles of the G proteins of ovine and bovine RSV. The intergenic sequences for the SH–G and G–F gene junctions of ovine RSV showed 64 and 57% identity respectively with the corresponding regions of the bovine RSV. Our results indicate that ovine and bovine RSV might be classified as two subgroups of an ungulate RSV.

Respiratory syncytial virus (RSV) infections are a major cause of respiratory tract disease in humans, cattle, sheep and goats (Stott & Taylor, 1985). Serological studies indicate that RSV infections are widespread in sheep populations (Berthiaume *et al.*, 1973). Experimental infection of ovine RSV caused a mild conjunctivitis in 3-month-old lambs. Although clinical signs of pneumonia were not observed, there was gross and microscopic evidence of pulmonary inflammation in the lungs of lambs inoculated with ovine RSV (Evermann *et al.*, 1985). Although sheep can be experimentally infected with bovine RSV (Cutlip & Lehmkuhl, 1979), there are also reports of natural infections in sheep (Evermann *et al.*, 1985; Spraker & Collins, 1986). As ovine and bovine RSV strains were found to be antigenically related by indirect immunofluorescence and virus neutralization tests, the potential for interspecies transmission of the virus between cattle, sheep and goats could not be ruled out (Lehmkuhl *et al.*, 1980).

Several analyses on the molecular organization of the bovine RSV have been performed (Lerch *et al.*, 1989; Walravens *et al.*, 1990; Samal & Zamora, 1991; Mallipeddi & Samal, 1992). Sequence comparison between proteins from bovine and human RSV showed that the G and small hydrophobic (SH) proteins are least conserved (30 and 38% identity, respectively; Lerch *et al.*, 1990; Samal & Zamora, 1991). Human RSV isolates are divided into two antigenic subgroups, A and B, based on their reactions with monoclonal antibodies (MAbs) to the G protein (Anderson *et al.*, 1985; Mufson *et al.*, 1985; Gimenez *et al.*, 1986). Sequencing studies also have shown that between strains of the same or different subgroups, the G protein of human RSV is less conserved than other proteins (Johnson *et al.*, 1987; Sullender *et al.*, 1991; Cane *et al.*, 1991). Recently, it was shown that the G gene sequences among four bovine RSV strains were highly conserved (90 to 97%; Mallipeddi & Samal, 1993).

The ovine strain of RSV has not been described in molecular detail. The genetic relationship of the ovine RSV strain with the bovine or human RSV strains is also not known. Since the G protein of RSV has been characterized as the viral attachment protein (Levine *et al.*, 1987), and since the G gene is the most variable gene between human and bovine RSV strains, it is possible that sequence differences might exist between the ovine and bovine RSV or between ovine and human RSV strains.

Ovine RSV strains WSU 83-1578 and WSU 87-6750 (provided by J. Evermann, Washington State University at Pullman, Wash., U.S.A.) were grown in bovine nasal turbinate cells. Ovine RSV was partially purified using the protocol described by Huang *et al.* (1985). Virion RNA (vRNA) or ovine RSV, strain WSU 83-1578 was obtained after proteinase K digestion and phenol extraction. Oligonucleotide primers were synthesized based on the sequence data of the bovine RSV strain A51908 genes. The positive-strand primer corresponding to the nucleotides at the 3′ end of the matrix (M) gene (5′TAC-ATT-AAA-CCA-CAA-AGT-CAA-TTT-ATA-

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(*a*)

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Ovine  RSV   GGGGCAAATA CAAGCATGTC CAACCACACT CACCATTTTG AATTCAAGAC TCTTAAGAAA GCTTGGAAGG    70
Bovine RSV                                  C   T   CC A            AT A    GG         A     70

Ovine  RSV   CTTCAAAGTA TTTCATAGTG GGATTGTCTT GTCTATATAA ACTT-AATCT TAAA-TCCCT TGTCCAAATG   138
Bovine RSV    C     A   C        A      A  A    T        -G  C   -      G              C    138

Ovine  RSV   GCCTTATCAG CCCTAGCAAT GATAACTTTA GTATCACTTA CCATAACAGC CATCATTTAT ATTAGCACAG   208
Bovine RSV     T  GA CT   T              C  G AC      CG T             T     C      TGTG    208

Ovine  RSV   GAAACACAAA AGCCAAACCC ATGCCTACAC CAACAATTCA GATCACCCAA CAGTTCCAAA ACCACACCTC   278
Bovine RSV       TG T         G     CAT C A      C CC   ACAA  A       CC               C    278

Ovine  RSV   TCTGCCTCCC ACAGAACACA ACCATAACTC TACTCACTCT CCAACTCAAG GCACCACATC ACCCCACACT   348
Bovine RSV   A  A T          G         C  A  A      A A T    C   A         CT GT    AC A   348

Ovine  RSV   TTCGCCGTAG ATGTCACCGA AGGAACTCGA TACTACCACT TGACCCTCAA AACTCAAGGC GGTAAAACCA   418
Bovine RSV   CCAAA A     CAC   TAG T      AC     GGT   C CA T AA  G    C   AA  A A    T     418

Ovine  RSV   AAGGCCCTCC TACTCCACAT GCCACAAGGA AACCCCCCAT CAGTTCACAG AAGAGCAATC CCTCCGAAAT   488
Bovine RSV     A   AAT          T       CC A     TA  A     ACC   T  G A     C     C      A   488

Ovine  RSV   TCAACAAGAT TACAGTGACT TTCAAATACT TCCCTATGTG CCCTGCAACA TATGTGAAGG TGACTCTGCT   558
Bovine RSV   C  C     C C   ACA    CC    C    C  TC            G     C   C      CA TC    C   558

Ovine  RSV   TGTTTATCCC TCTGTCAAGA TAGATCCG-A GAGCATACTG GATAAAGCTC TAACAACCAC CCCCAAAAAA   627
Bovine RSV       C C A      C   -   C GG T G     AGC  CA AGC G      CC    T       T      G   627

Ovine  RSV   ACTCCAAAAC CCATGACCAC CAAAAAGCCA ACCAAGACAT CAACCCACCA CAGAACCAGT CTGAGAAACA   697
Bovine RSV   G             AA            A            A    T T                C  CTGA GC     697

Ovine  RSV   AACTATACAT CAAAACAAAC ATGACAACTC CACCACATGG CCTCATCTCC ACAGCAAAAC ACAACAAAAA   767
Bovine RSV       GC A C      A      C G          A   A    A  C    T T  C  G       C A C      767

Ovine  RSV   TCAATCCACC GTACAGAATC CAAGACACAC CCTTGCATAA TATCTGTTAT CT-CATATAT AGTTATTTAAAA 838
Bovine RSV         T  T AC     TCT   CA          TCCAT          AA     G T     G                839
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(*b*)

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                           Gene-end                                       Gene-start
Ovine  RSV   SH AATAATTAGTTATTTAAAA-TTAAATTTATAAATAATGTATAAC-ACATACATATATCGGGGCAAATACAAGCATGT  G
Bovine RSV   SH GT                 A    CC   A    GG T   GGTT       G  G T                    G

                           Gene-end                                       Gene-start
Ovine  RSV   G  CATATATAGTTATTTAAAAGAGACAAGGGTGTATATAGTAAACTAATTAAGATTGGGGCAAATAAAAATGGCAACAA  F
Bovine RSV   G       G             -   ------ A G    A TC       T A C            GG     G      F
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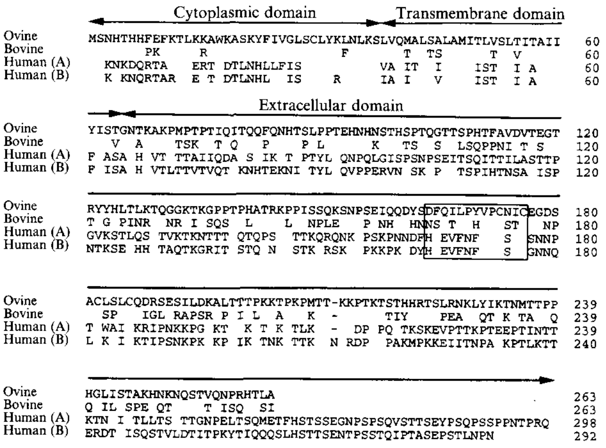
Fig. 1. (*a*) Alignment of the nucleotide sequence of the G gene of ovine RSV strain WSU 83-1578 with that of bovine RSV strain A51908. Alignment was done by the method of Myers & Miller (1988). Gaps introduced during alignment are represented by dashes. Only the non-identical nucleotides are shown in the bovine RSV G gene. The consensus gene-start and gene-stop sequences are overlined, and consensus initiation and stop codons are boxed. (*b*) Alignment of the SH–G and G–F intergenic sequences of WSU 83-1578 with the corresponding sequences of A51908 (Zamora & Samal, 1992). The gene-start and gene-end consensus sequences are overlined, the consensus initiation codons are in bold. Only the non-identical nucleotides are shown in bovine RSV. Nucleotide deletions are represented by dashes.

GTA-GAT-CTT-GG 3′; 652 to 689), and the negative-strand primer corresponding to the nucleotides at the 5′ end of the fusion glycoprotein (F) gene (5′ CCT-CTA-CTA-ACT-GCA-CTG-CAT-G 3′; 120 to 141) were synthesized. The M and F gene sequences of bovine RSV were chosen for the synthesis of primers for PCR amplification of the ovine RSV vRNA because these genes are highly conserved between bovine and human RSV strains and probably are also conserved between ovine and bovine RSV strains.

These primers were used to amplify a region of the vRNA corresponding to the M–SH–G–F genes, by reverse transcription (RT)–PCR (Perkin-Elmer) as suggested by the manufacturer. Since the PCR product was difficult to clone directly due to its large size, we digested it with *Nla*III (four-base restriction endonuclease), and cloned the fragments into the pGEM-7Z(+) vector (Promega) at the *Sph*I site, which has compatible cohesive ends. By sequencing cDNA clones corresponding to position 358 of the SH gene to position 18 of the G gene, and position 733 of the G gene to position 120 of the F gene (positions are in relation to the bovine RSV strain A51908), we obtained the sequence data corresponding to the SH–G and G–F intergenic regions, and the 5′ and 3′ ends of the G gene of ovine RSV. Based on these sequences, specific oligonucleotides corresponding to the 5′ and 3′ ends of the G gene of ovine RSV were made and used to amplify the G gene of ovine RSV by RT–PCR. The amplified product was directly cloned into the PCR II cloning vector (Invitrogen). The nucleotide sequence of the G gene of ovine RSV was derived from two cDNA clones, using the dideoxynucleotide chain termination method (Sanger *et al.*, 1977).

The G gene of ovine RSV contained 838 nucleotides (Fig. 1 *a*). The ovine RSV G gene sequence was compared with that of the bovine RSV strain A51908 (Mallipeddi & Samal, 1993). The ovine RSV G gene shared only 73%



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Ovine      MSNHTHHFEFKTLKKAWKASKYFIVGLSCLYKLNLKSLVQMALSALAMITLVSLTITAII  60
Bovine            PK     R                 F       T  TS      T  V       60
Human (A)    KNKDQRTA   ERT DTLNHLLFIS          VA IT  I    IST  I A     60
Human (B)    K KNQRTAR  E T DTLNHL  IS    R     IA I   V    IST  I A     60

Ovine      YISTGNTKAKPMPTPTIQITQQFQNHTSLPPTEHNHNSTHSPTQGTTSPHTFAVDVTEGT 120
Bovine        V  A    TSK  T Q   P    P L      K   TS  S  LSQPPNI T S   120
Human (A)  F ASA H VT TTAIIQDA S IK T PTYL QNPQLGISPSNPSEITSQITTILASTTP 120
Human (B)  F ISA H VTLTTVTVQT KNHTEKNI TYL QVPPERVN SK P  TSPIHTNSA ISP 120

Ovine      RYYHLTLKTQGGKTKGPPTPHATRKPPISSQKSNPSEIQQDYSDFQILPYVPCNICEGDS 180
Bovine     T G PINR  NR I SQS  L    L  NPLE   P NH  HNNS T  H   ST   NP 180
Human (A)  GVKSTLQS TVKTKNTTT QTQPS  TTKQRQNK PSKPNNDFH EVFNF   S  SNNP 180
Human (B)  NTKSE HH TAQTKGRIT STQ N  STK RSK  PKKPK DYH EVFNF   S  GNNQ 180

Ovine      ACLSLCQDRSESILDKALTTTPKKTPKPMTT-KKPTKTSTHHRTSLRNKLYIKTNMTTPP 239
Bovine       SP   IGL RAPSR P I L  A   K  -      TIY    PEA  QT K TA  Q 239
Human (A)  T WAI KRIPNKKPG KT  K T K TLK  -  DP PQ TKSKEVPTTKPTEEPTINTT 239
Human (B)  L K I KTIPSNKPK KP IK TNK TTK  N RDP  PAKMPKKEIITNPA KPTLKTT 240

Ovine      HGLISTAKHNKNQSTVQNPRHTLA                                     263
Bovine     Q IL SPE QT    T ISQ  SI                                     263
Human (A)  KTN I TLLTS TTGNPELTSQMETFHSTSSEGNPSPSQVSTTSEYPSQPSSPPNTPRQ  298
Human (B)  ERDT ISQSTVLDTITPKYTIQQQSLHSTTSENTPSSTQIPTASEPSTLNPN         292
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Fig. 2. Alignment of the predicted amino acid sequence of the G protein of ovine RSV with those of bovine RSV strain A51908, human RSV subgroup A strain A2 and subgroup B strain 18537 (Wertz *et al.*, 1985; Johnson *et al.*, 1987; Mallipeddi & Samal, 1993). Alignment was done by the method of Myers & Miller (1988). Only the non-identical amino acid residues are shown in bovine and human RSV strains. The proposed domains are indicated above the sequences. The region corresponding to the conserved 13 amino acid region of human RSV (Johnson *et al.*, 1987) is boxed. A gap in the strain A2 G protein sequence compared with that of strain 18537 as described by Johnson *et al.* (1987) is indicated by a dash.

nucleotide identity with that of A51908. Comparison of the coding region of the G genes of ovine and bovine RSV showed single nucleotide insertions at positions 111, 118 and 577 in the ovine RSV G gene. However, the reading frame was maintained by compensatory deletions after the third, fifth and ninth nucleotides downstream from the insertion site positions, respectively. In the non-coding region the base at position 820 in the bovine RSV G gene was absent in the ovine RSV G gene. The gene-start signals of the F and G genes of human, bovine and ovine strains of RSV were perfectly conserved. The gene-end signals of the SH and G genes of ovine and bovine RSV were identical, but differed by one base when compared to that of human RSV. The gene-end signals of bovine and human RSV are followed by an A residue which was absent in ovine RSV.

The G gene of ovine RSV had an open reading frame of 263 amino acids (Fig. 2). The predicted $M_r$ of this polypeptide was 29K. The deduced G protein of ovine RSV was of the same length as that of bovine RSV strain A51908, but had an additional six amino acids at the carboxyl terminus compared to that of bovine RSV strain 391-2 (Lerch *et al.*, 1990).

To examine the sequence divergence between the G genes of two strains of ovine RSV, the G gene of ovine RSV strain WSU 87-6750, isolated from big horn sheep, was synthesized by PCR amplification. Partial sequence analysis showed a high level of conservation at the nucleotide and amino acid levels between the two ovine RSV strains (data not shown). In human RSV, the G protein showed 20% divergence among subgroup A strains, and up to 9% divergence among subgroup B strains (Cane *et al.*, 1991; Sullender *et al.*, 1991). Similarly, there was only 5 to 16% divergence among the G proteins of bovine RSV strains (Mallipeddi & Samal, 1993). Between subgroups A and B of human RSV there was 47% divergence (Johnson *et al.*, 1987). Thus, within a subgroup the G proteins show less diversity than they do between subgroups. In keeping with the division of RSV into subgroups on the basis of G gene sequence relatedness, the ovine RSV G protein is clearly different from that of bovine RSV (40% divergence). Since this difference is somewhat less than the 47% divergence observed between the human subgroup, ovine and bovine RSV might be considered as two subgroups of ungulate RSV rather than as two distinct viruses. However, more ovine isolates need to be similarly analysed to ascertain whether the genetic subgroups of ungulate RSV continue to remain with the respective hosts.

The G protein of RSV is proposed to have three domains: an internal or cytoplasmic domain, a transmembrane domain and an extracellular domain, the latter of which forms three-quarters of the polypeptide (Satake *et al.*, 1985; Wertz *et al.*, 1985). Comparison of amino acid identity within each of the domains of the G proteins showed that the cytoplasmic and transmembrane domains were highly conserved (89 and 78% identity respectively) between ovine and bovine RSV, but the identity between the extracellular domain of the ovine and bovine RSV G proteins was lower than the overall amino acid identity (53% versus 60%). Similar observations were also made between subgroups of human RSV, where cytoplasmic and transmembrane domains exhibited high amino acid similarity (81% and 86% identity, respectively) compared to only 44% amino acid identity in the extracellular domain (Johnson *et al.*, 1987). The divergence of the extracellular domain between ovine and bovine RSV strains could be due to selective immune pressure, as proposed by Johnson *et al.* (1987).

Immunofluorescence and virus neutralization tests using antibodies to bovine RSV suggest that ovine RSV is antigenically related to bovine RSV (Evermann *et al.*, 1985). Polyclonal antibodies to bovine RSV immunoprecipitated the N, P, M, F, G, 22K, NS1 and NS2 proteins of ovine RSV strains WSU 83-1578 and WSU 87-6750 (data not shown). Reaction of the G protein of ovine RSV with polyclonal antiserum to bovine RSV suggested that at least one epitope is common to the G proteins of ovine and bovine RSV strains. Further studies using monoclonal antibodies to the G protein are necessary to examine antigenic differences between ovine and bovine RSV.

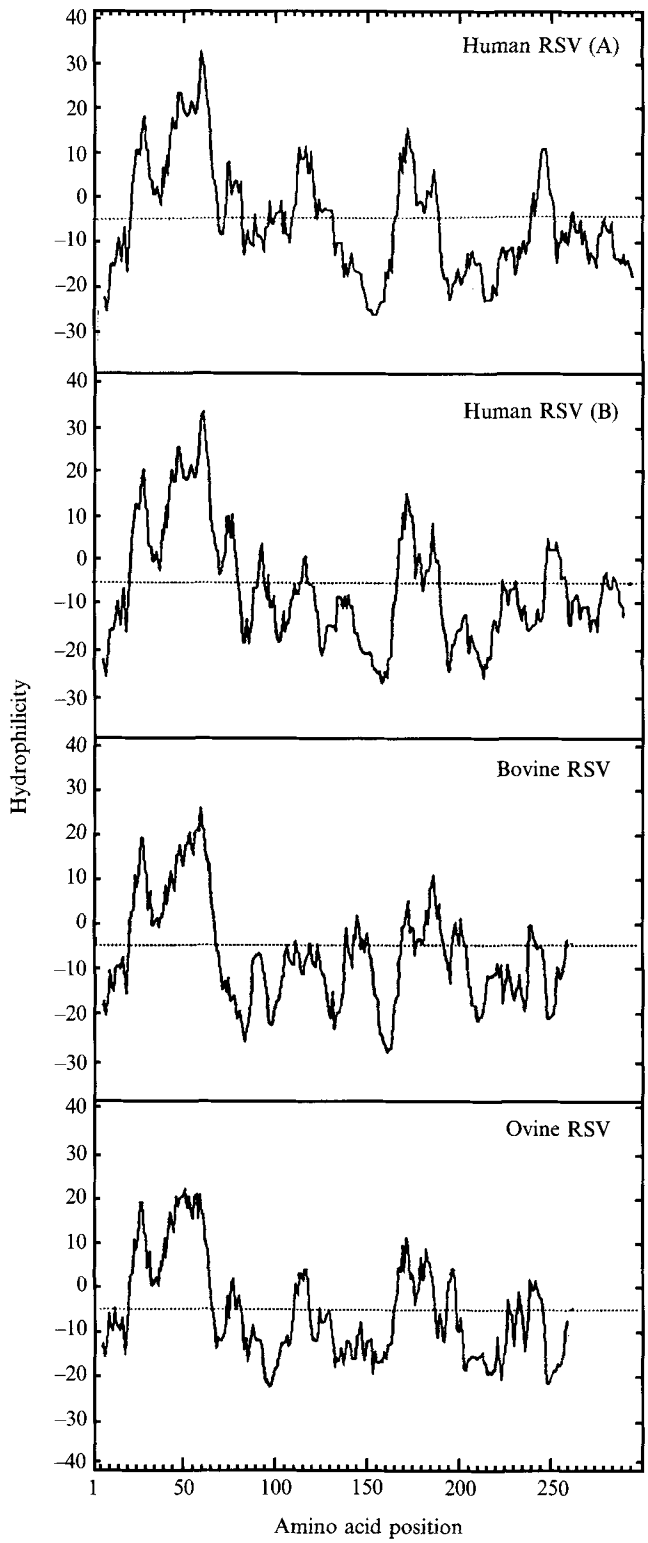

Fig. 3. Hydrophilicity profiles of the G protein of RSV strains. The distribution of the hydrophobic (above the dashed line) and hydrophilic (below the dashed line) regions along the predicted amino acid sequences was determined using the algorithm of Kyte & Doolittle (1982), with a window size of nine amino acid residues.

Although the overall identity of the ovine RSV G protein to that of either bovine or human RSV was low, there were similarities in the hydropathy profiles of the different G proteins (Fig. 3). The ovine RSV G protein had a high content of proline, serine and threonine, similar to that found in the G proteins of bovine and human RSV (Lerch *et al.*, 1990; Wertz *et al.*, 1985). Five cysteine residues present in the G protein of ovine RSV were conserved exactly with respect to position in ovine, bovine and human RSV strains, indicating similar structural features among G proteins.

The SH–G and G–F intergenic regions of ovine RSV consisted of 37 and 35 nucleotides, respectively. Those regions were two nucleotides shorter and seven nucleotides longer than the corresponding bovine RSV sequences (Fig. 1*b*). The nucleotide sequences of the SH–G and G–F intergenic regions of the ovine RSV had only 64 and 57% similarity with the corresponding sequences of bovine RSV (Zamora & Samal, 1992). This is in agreement with the observation that the intergenic sequences between any two genes are relatively non-conserved between viruses of the two human RSV subgroups (Johnson & Collins, 1988).

In conclusion, the work presented here describes the nucleotide and deduced amino acid sequences of the G gene and the SH–G and G–F intergenic regions of ovine RSV. The amino acid sequence of the ovine RSV G protein showed a low level of overall identity with the bovine RSV G protein. There was only 53% identity between the extracellular domains of the G proteins of ovine and bovine RSV. Antigenic relatedness between the G proteins of ovine and bovine RSV was observed. Our results indicate that the G gene sequences of ovine and bovine RSV are sufficiently different to suggest two subgroups of an ungulate RSV.

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