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PCR detection of the sheep-associated agent of malignant catarrhal fever

S. I. F. Baxter, I. Pow, A. Bridgen*, and H. W. Reid

Moredun Research Institute, Edinburgh, U.K.

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Summary. From a genomic library previously constructed from a lymphoblastoid cell line (LCL) propagated from a bovine case of sheep-associated malignant catarrhal fever (SA-MCF), caused by ovine herpesvirus-2 (OHV-2), several OHV-2 clones were identified and characterised by hybridisation using probes from the unique region of the Alcelaphine herpesvirus-1 (AVH-1) genome. Nucleotide sequence from one clone was generated and the predicted amino acid sequence was found to contain regions of homology with the 140 and 160 kDa tegument proteins of Epstein-Barr virus and herpesvirus saimiri respectively. Oligonucleotide primers were constructed and a polymerase chain reaction (PCR) test was developed for the detection of OHV-2 viral DNA. Amplified product was identified by restriction with *Rsa* I and *Bmy* I. The primers were highly specific for OHV-2 DNA with a limit of detection of 6.4 pg of genomic DNA derived from the parent LCL. This was estimated to correspond to one diploid bovine cell. The PCR was successfully applied to detect OHV-2 DNA in peripheral blood leucocytes (pbl) from clinical cases of SA-MCF and normal sheep.

Introduction

Malignant catarrhal fever (MCF), a fatal virus disease of cattle, deer and other large ruminants, is characterised by lymphoproliferation and lymphoid cell infiltration with necrosis in many tissues. There are two very similar forms of the disease, one of which, is caused by Alcelaphine herpesvirus-1 (AHV-1) which infects inapparently the natural hosts wildebeest (*Connochaetes species*) [25, 28]. AHV-1 has been isolated and classified in the subfamily *Gammaherpesvirinae* [32, 33]. Sheep are generally accepted as the reservoir of infection of

* Present address: Institute of Virology, University of Glasgow, U.K.

the other form of the disease, sheep-associated MCF (SA-MCF). The agent of SA-MCF is closely related antigenically [12, 35, 36] to and shares DNA homology [5] with AHV-1. Recently the agent has been classified as ovine herpesvirus-2 (OHV-2) on the basis of its DNA homology with AHV-1 [33]. However, OHV-2 has never been isolated.

The pathogenesis of MCF is thought to result from virus-induced proliferation and dysfunction of T-lymphocytes [29]. Lymphoblastoid cell lines (LCL) have been propagated from animals affected with SA-MCF and some of them transmit MCF to susceptible animals [30, 31]. However, detection methods hitherto available have been unable to identify virus in proliferating lymphocytes or tissues from clinically affected animals. Hybridisation probes have been developed for AHV-1 DNA [4, 41] but so far problems associated with sensitivity and specificity of the probes have limited their use as detection tools.

The high specificity and sensitivity afforded by the polymerase chain reaction (PCR) makes this technique particularly suitable for detection of bacteria and viruses and has been extensively applied since its introduction [37, 38]. The PCR has been applied to the detection of other herpesviruses and a PCR test has been described for the detection of AHV-1 [13, 14]. A 2-stage nested amplification reaction, utilising different primer pairs from the same AHV-1 cloned sequence, subsequently was shown to amplify both AHV-1 and AHV-2 isolates [14].

This paper describes the development of a PCR-based test for the specific detection of an OHV-2 DNA sequence in naturally occurring and experimentally induced cases of SA-MCF and normal sheep.

Materials and methods

Viral and LCL DNA

DNA was prepared, as described previously [3], from the virulent C 500 isolate of AHV-1 [26, 28], bovine herpesvirus-1 (isolate 6660) and bovine herpesvirus-4 (strain Movar 33/63) (which were generously supplied by Dr. P. F. Nettleton and Dr. S. Edwards respectively) and the OHV-2 LCL MF 629, BJ 576, MF 816, and BJ 393 [30]. Negative control DNA was prepared from cultured bovine embryo kidney (BEK) cells, the rabbit kidney cell line RK 13 and from primary deer testis cells. Herring sperm DNA (Sigma) was used as a carrier in DNA dilution series.

Clinical SA-MCF cases

From four naturally occurring, clinically affected animals in Scotland and Eire, all of which were subsequently confirmed histopathologically as cases of MCF, pbl were collected. In addition, pbl from three animals apparently recovered from clinical MCF [19] were examined (Table 1).

The pbl from three normal cattle and from a further six confirmed to have mucosal disease, caused by bovine viral diarrhoea virus (Dr. P. Nettleton, pers. comm.), served as a source of negative control DNA. Six 12 month-old normal ewes, bred at the Institute, were used as the source of normal sheep pbl.

Table 1. Source of clinical SA-MCF cases

Sample number	Species	Origin	DNA source
BJ 793	bovine	recovered natural SA-MCF, Eire	pbl
BJ 773	bovine	recovered natural SA-MCF, Eire	pbl
BJ 784	bovine	natural SA-MCF, Scotland	pbl
BJ 802	bovine	natural SA-MCF, Scotland	pbl
BJ 797	bovine	natural SA-MCF, Eire	pbl
BJ 777	cervine	natural SA-MCF, Scotland	pbl
BJ 716	lapine	experimental SA-MCF derived from bovine case	spleen
BJ 676	bovine	recovered natural SA-MCF, Scotland, 5/9/89	pbl
BJ 796	bovine	above case sampled before slaughter, 12/11/91	pbl

The spleen of one rabbit, displaying clinical MCF following inoculation with the C 2 line of SA-MCF [30], also was tested by the OHV-2 PCR.

DNA preparation and Southern blot hybridisation analysis

Genomic DNA was extracted from pbl and fresh tissues as described by Sambrook et al. [39]. DNA was dissolved in sterile distilled water and quantified by UV absorbance at 260 nm.

DNA was labeled by random hexanucleotide priming using DNA polymerase Klenow fragment and [^{35}S]-dATP [9, 10] and transferred to nylon membrane (Hybond-N, Amersham International) by capillary transfer [42]. Filters were hybridised in the presence of 50% formamide at 42 °C, washed in 0.1 × standard saline citrate (SSC) at 42 °C and X-ray film exposed using standard techniques [17, 39].

Sub-cloning and sequencing strategy

The cloning and characterisation of a genomic OHV-2 DNA fragment from the LCL MF 629 was described previously [5]. A 5.2 kb *Hin*dIII insert from λ 8a was sub-cloned to produce p 8a, using pUC 13, shown in Fig. 1 A. An AHV-1 unique region clone, p 6A, was shown to hybridise to the internal 1.5 kb *Sma*I fragment of OHV-2 clone p 8a [5]. A 2.6 kb *Bam* HI fragment covering this region was excised utilising the *Bam* HI sites contained in the insert and the multiple cloning site of the vector and sub-cloned into M 13 mp 18 and mp 19 and BlueScribe M 13+ (Stratagene) using *Escherichia coli* host strain JM 101 [18], to produce clones p4 a1, p5 a1, and Bp4 a1 respectively. Construction of BlueScribe

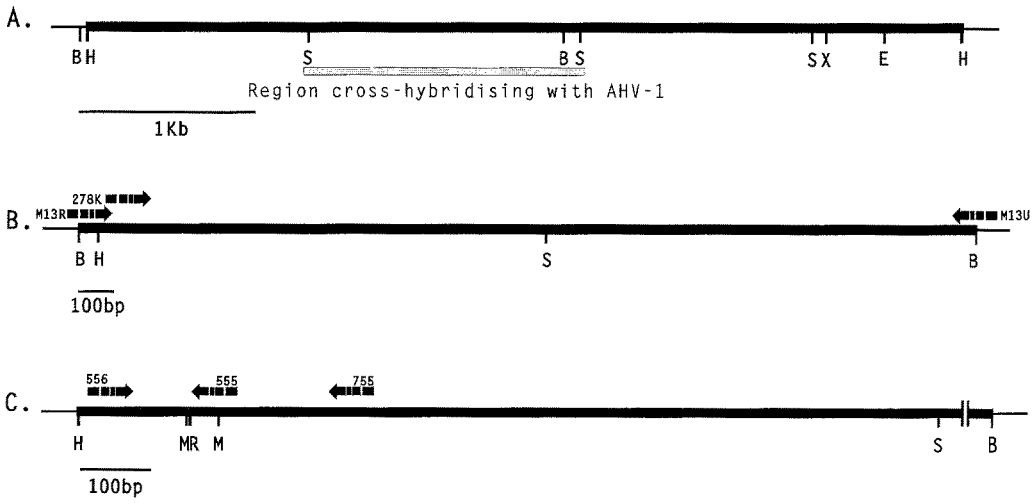

Fig. 1. Development of the OHV-2 PCR. **A** MF 629 lambda clone 8 a showing region of homology shared with AHV-1. **B** Sequencing strategy from BlueScribe + clone Bp4 a1. **C** Position of PCR primers. *B Bam*HI, *H Hin*dIII, *S Sma*I, *X Xho*I, *R Rsa*I, *E Eco*RI, *M Bmy*I

clone Bp4 a1 is shown in Fig. 1 B. The left-hand end of clone Bp4 a1, delimited by the *Hin*dIII site, was sequenced by progressive chain termination reactions using [^{35}S] as the label. This region of non-homology with AHV-1 was chosen in order to provide suitable nucleotide sequence for the design of OHV-2-specific PCR primers.

Nucleotide sequence determination

Nucleotide sequencing was performed using the dideoxynucleotide chain termination technique [40] and direct incorporation of [^{35}S]-dATP (Amersham International) using T 7 DNA polymerase (Pharmacia) and *Taq* DNA polymerase (Promega). Single cycle sequencing reactions were performed with T 7 DNA polymerase (T 7 sequencing kit, Pharmacia). Cycled sequencing reactions were performed with *Taq* DNA polymerase (*fmol* DNA sequencing system, Promega). Sequences and relative positions of oligonucleotide primers M 13 reverse (Pharmacia), M 13 universal (Pharmacia), and 278 are shown in Fig. 1 B. Oligonucleotide primers were supplied by the above manufacturers or synthesised by Oswel DNA services, University of Edinburgh, Scotland.

Chain-terminated fragments were resolved by 6% PAGE, containing 46% w/v urea using standard procedures [39].

Nucleotide sequence analysis

The DNA sequence analysis software packages of the University of Wisconsin Genetics Computer Group [8] and the Protein Identification Resource Foundation were used for the analysis and comparison of DNA and amino acid sequences. The GCG group of programs was used for database homology searches, pairwise nucleotide and amino acid sequence alignments, multiple sequence alignments and structural analysis of translated amino acid sequences. Homology searches were performed using the FASTA program [24]. Amino acid and nucleotide alignments were made using the GAP option [20] in GCG. The packages were available through the User Interface Group, Daresbury Laboratory, England.

Polymerase chain reaction

Amplification reactions were performed in a 50 µl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.01% v/v gelatin, 10% v/v DMSO, 200 µM dATP, dCTP, dGTP, and dTTP (Pharmacia), 1.0 µM each primer and 2 units *Taq* DNA polymerase.

The following cycling program was used: pre-cycle 99 °C for 3 min after which dNTP and enzyme mix was added: cycles 1–25; 94 °C for 20 sec, 60 °C for 30 sec, 72 °C for 30 sec. An aliquot (2%) of the primary amplification product, specified by primer pair 556/755, was transferred directly to a new reaction mixture and amplified, using primer pair 556/555, under identical conditions for a further 25 cycles. The secondary amplification cycles were concluded with a final extension at 72 °C for 5 min.

Amplification product (5% of total) was analysed directly by 1.8% agarose gel electrophoresis and ethidium bromide fluorescence. Restriction fragments derived from amplified fragments were resolved by 12% continuously buffered PAGE [16] and visualised by silver staining [11].

Results

Nucleotide sequence analysis

The deduced nucleotide sequence of 549 nucleotides and predicted amino acid sequence derived from the cloned OHV-2 *Bam* HI fragment are shown in Fig. 2. The nucleotide sequence is shown from the *Hin* dIII site which delimited the original p 8a clone [5]. The OHV-2 nucleotide sequence was found to be relatively A + T rich (47.5%) and translation of the nucleotide sequence in the three forward frames identified an open reading frame (ORF) sequence from nucleotide position 1 to the stop codon sequence TAA at nucleotide position 460. A consensus polyadenylation signal AATAAA was identified at nucleotide position 540, 67 nucleotides downstream of the termination codon TAA. However, no N-terminal methionine was identified which indicated that the cloned OHV-2 sequence consisted of the 3′ terminus of a larger ORF.

A FASTA search of the GENBANK and EMBL data bases revealed a significant level of homology between the OHV-2 sequence and the BNRF 1 [1, 7] and EILF 1 [7, 21] ORFs of Epstein-Barr virus (EBV) and *herpesvirus saimiri* (HVS) respectively. Nucleotide identity of 53% and 50% was observed over a 460 nucleotide region between the OHV-2 sequence and BNRF 1 and EILF 1 respectively (Fig. 3 A). A similar level of nucleotide identity (50%) was observed between BNRF 1 and EILF 1 over the same region (data not shown).

The translated amino acid sequence consisted of 153 residues which began with a lysine residue and terminated with a serine residue. Comparison of BNRF 1 and EILF 1 which encode the non or poorly glycosylated 140 kDa and 160 kDa tegument proteins of EBV and HVS respectively and the predicted amino acid sequence of the OHV-2 ORF is shown in Fig. 3 B. The translated amino acid sequence derived from the OHV-2 sequence shared 43% and 47% identity with the 140 kDa and 160 kDa of EBV and HVS respectively. An amino acid identity of 43% was observed between the 140 kDa and 160 kDa proteins over this C-terminal region (data not shown). This was significantly greater

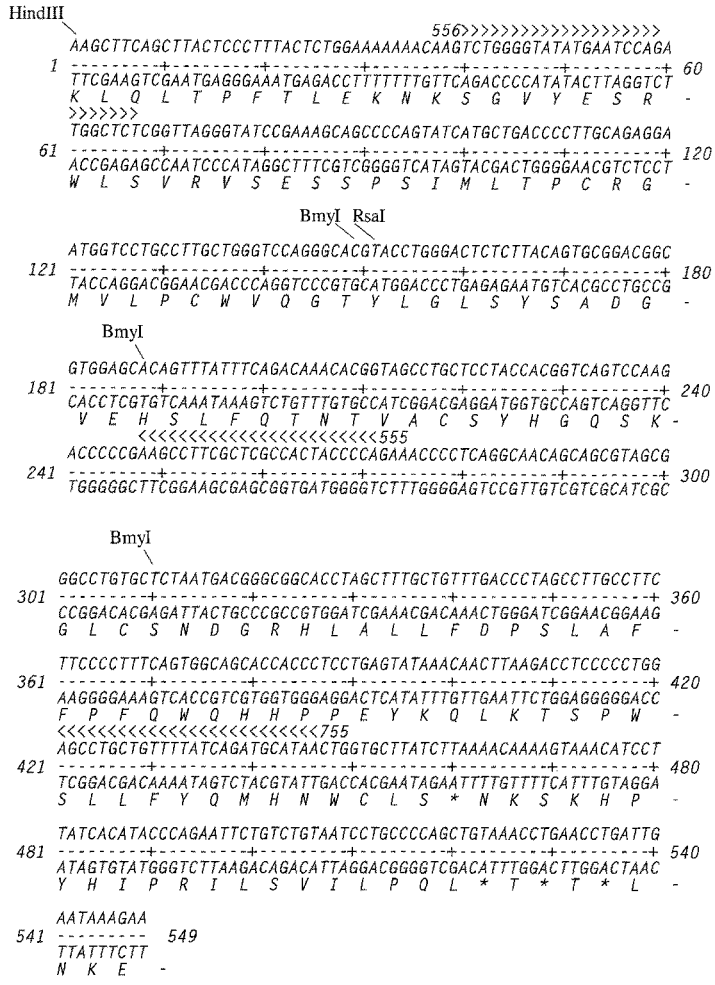

Fig. 2. The derived nucleotide and predicted amino acid sequence of the OHV-2 clone Bp4 a1. Restriction sites for *Hin* dIII, *Rsa* I, and *Bmy* I and the positions of the PCR primers are indicated

than the observed 29% identity over the entire translated protein sequences of BNRF 1 and EILF 1 (data not shown).

Consensus protein motifs were identified in the OHV-2 translated protein sequence using the MOTIFS option and PROSITE data base in the GCG package. Putative N-glycosylation sites, NKSG, NSSV, and NKSK, consensus pattern N-<P>-[ST]-<P> were identified at amino acid positions 12, 96, and 155 respectively. The N-glycosylation site at amino acid position 12 was conserved in both EBV and HVS with the recognition sites NASG and NASK respectively. The N-glycosylation site at position 96 was also conserved as NSSV in HVS but was absent from the EBV sequence. The terminal N-glycosylation site at OHV-2 position 155 was not conserved in either EBV or HVS. One putative protein kinase C phosphorylation site SVR, consensus pattern [ST]-

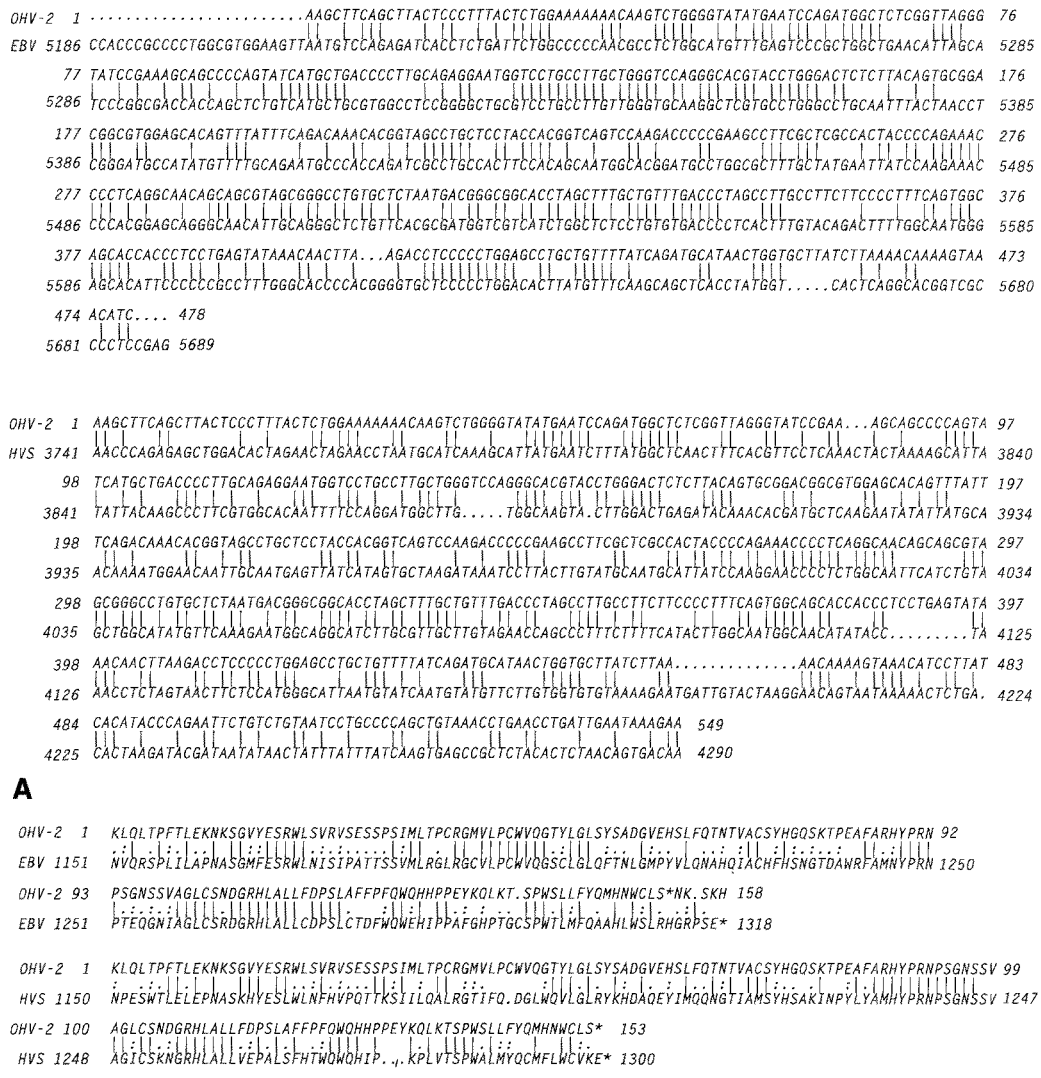

Fig. 3. **A** Comparison of the OHV-2 nucleotide sequence with the BNRF1 and EILF1 open reading frame sequences of EBV (upper) and HVS (lower) respectively. Gaps have been added to the sequence to maximise the alignment. **B** Comparison of the OHV-2 predicted amino acid sequence with those of the 140 kDa and 160 kDa tegument proteins of EBV and HVS respectively. Amino acid identical to all three sequences is indicated by dashes and related amino acids are indicated by dots (:)

x-[RK], was identified at amino acid position 23 in the OHV-2 sequence but was not conserved in either EBV or HVS. The N-myristoylation consensus pattern G-<EDRPKHPFYW>-x-(2)-[STAGCN]-<P>, was identified as amino acid patterns GVYESR, GTYLGL, GLSYSA, GVEHSL, and GLCSND located at amino acid positions 15, 49, 53, 60, and 101 respectively. The consensus patterns located at positions 49 and 53 were conserved as GSCLGL and

GLQFTN in HVS. However, EBV shared no N-myristoylation consensus patterns with the OHV-2 sequence.

PCR primers

Three sequences were selected for use as PCR primers. The specific locations of the three primers are shown in Fig. 1 C and 2 and summarised in Table 2. Amplification using primer pair 556/755 specified a 422 bp fragment. Primer 555 was located internally within this fragment and amplification using primer pair 556/555 specified a fragment of 238 bp. Both amplified-fragments contained *Rsa* I and *Bmy* I restriction sites (Figs. 1 C and 2), providing confirmation of the specificity of the amplified fragments. *Rsa* I restriction analysis of OHV-2-amplified fragments, specified by primer pairs 556/755 and 556/555 produced profiles of 310 bp, 112 bp and 112 bp, 126 bp respectively (Fig. 4).

Table 2. OHV-2 PCR primers. Nucleotide sequence, positions and fragment size specified in conjunction with primer 556

Primer	Sequence	Nucleotide position 5′–3′	Product size (bp)
556	5′-AGTCTGGGTATATGAATCCAGATGGCTCTC-3′	38–68	NA
555	5′-TTCTGGGGTAGTGGCGAGCGAAGGCTTC-3′	275–247	238
755	5′-AAGATAAGCACCAGTTATGCATCTGATAAA-3′	460–431	422

NA Not applicable

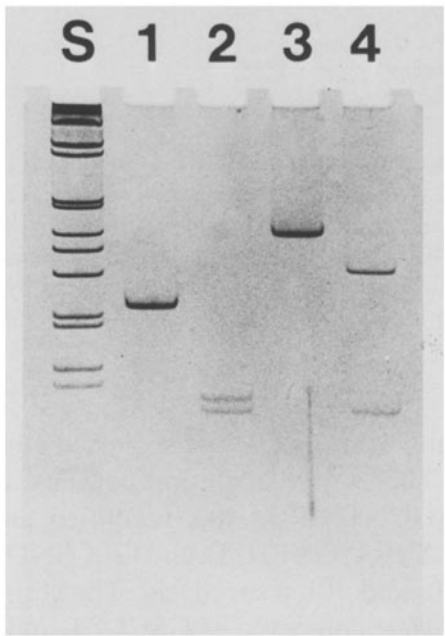

Fig. 4. *Rsa* I restriction analysis of OHV-2 PCR amplified gene fragments specified by primer pairs 556/555 and 556/755. Silver stained continuous PAGE (12%). *1* 556/555 uncut, *2* 556/555 *Rsa* I, *3* 556/755 uncut, *4* 556/755 *Rsa* I. Molecular weight standards are 1 kb ladder (*S*)

Sensitivity of OHV-2 PCR

The limit of detection of the OHV-2 PCR was assessed by agarose gel electrophoresis of amplified product from a dilution series of genomic DNA derived from the parent LCL MF 629. Five-fold serial dilutions were performed in sterile distilled water containing herring sperm DNA (1 μg/μl) as carrier and amplification reactions performed on 10 μl aliquots. The OHV-2 DNA fragment of 238 bp was detected down to the level of 6.4 pg of target DNA. Amplified fragment was not detected in the reagent control, bovine embryonic kidney or herring sperm DNA.

The detection of 6.4 pg genomic DNA was estimated to correspond to 1 bovine cell equivalent on the basis of an average genomic DNA content per nucleus as determined by flow cytofluorometry from various mammalian species [15]. The corresponding viral genome copy number of LCL MF 629 was estimated by densitometry of comparative slot blot analysis performed on DNA from MF 629 and derived plasmid clone Bp4 a1. Extinction points were observed at levels of 10 pg and 312 ng of DNA derived from OHV-2 plasmid clone Bp4 a1 and LCL MF 629 respectively (Fig. 5 A, B). This corresponds to 1.6×10^6 molecules of the 5.84 kb plasmid Bp4 a1. The number of target molecules contained

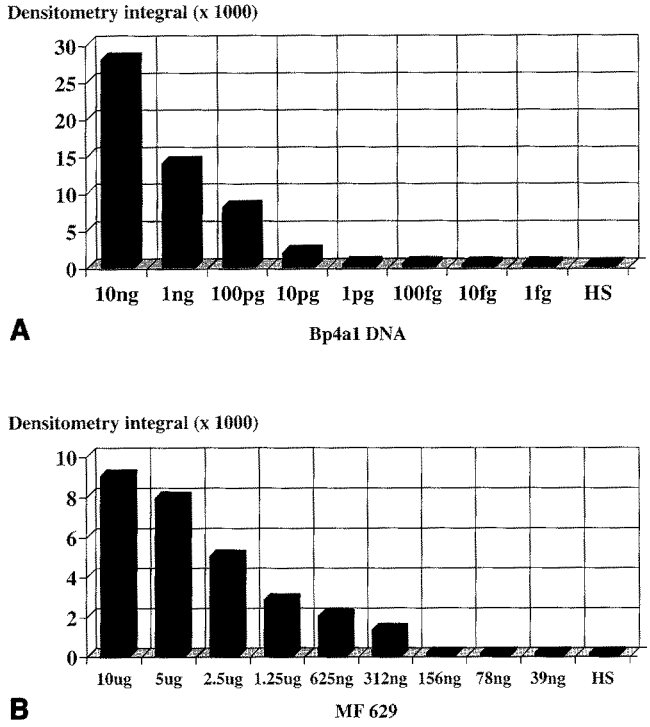

Fig. 5. Comparative slot blot hybridisation of Bp4 a1 OHV-2 and LCL MF 629. **A** Scanning densitometry integral plot of OHV-2 clone Bp4 a1 DNA hybridised with homologous probe. **B** Scanning densitometry integral plot of LCL MF 629 DNA hybridised with OHV-2 clone Bp4 a1 probe

in 1 cell equivalent of MF 629 was estimated to be 35, assuming 1 bovine cell nucleus contained 6.8 pg DNA.

Primer specificity

The specificity of the PCR was assessed by testing 10 µg of target DNA dervied from related herpesviruses. Amplified product was not detected from BHV-1, BHV-4, and AHV-1 (Fig. 6).

Detection of OHV-2 DNA in clinical SA-MCF cases and normal sheep

OHV-2 DNA was amplified from a panel of DNA derived from naturally occurring and experimentally induced cases of SA-MCF (Table 1), the pbl of 6 normal sheep and LCL derived from cervine and bovine cases of SA-MCF. A single amplified fragment of 238 bp was detected by agarose gel electrophoresis in all the clinical SA-MCF cases, the LCLs and in all the normal sheep pbl specimens tested. The amplified fragments were transferred to a nylon membrane and hybridised with [^{35}S]-dATP labeled Bp4 a1 insert which had been excised by *Bam* HI restriction (Fig. 6). A single hybridising fragment of 238 bp was detected in all the DNA samples. Amplified fragment was not detected in the control DNAs derived from AHV-1, BHV-1, BHV-2, and BHV-4; BEK, RK 13, and deer testis cells or from reagent control containing no DNA.

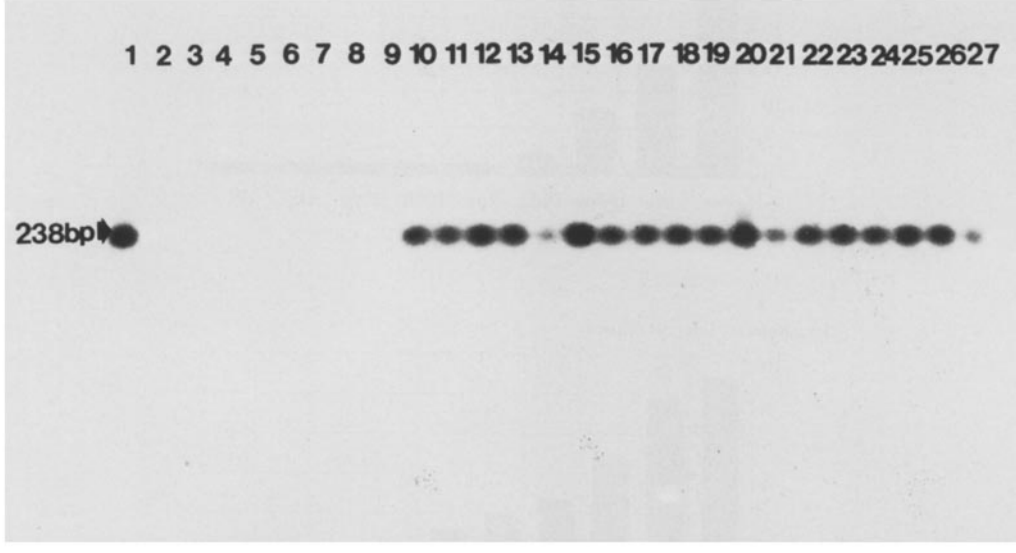

Fig. 6. Confirmation of OHV-2 PCR amplified product. Southern blot hybridisation using [^{35}S]-dATP labeled *Bam* HI excised insert DNA derived from OHV-2 plasmid clone Bp4 a1. *1* MF 629, *2* H_2O, *3* AHV-1, *4* BHV-1, *5* BHV-4, *6* bovine embryonic kidney, *7* fetal lamb muscle, *8* RK 13, *9* deer testis, *10* BJ 576, *11* MF 816, *12* BJ 393, *13* BJ 793, *14* BJ 773, *15* BJ 784, *16* BJ 802, *17* BJ 797, *18* BJ 777, *19* BJ 716, *20* BJ 676, *21* BJ 796, *22* S_1, *23* S_2, *24* S_3, *25* S_4, *26* S_5, *27* S_6. Molecular weight standards are 1 kb ladder (S). Cases of SA-MCF are described in Table 1. S_1–S_6 Normal, control sheep

Amplified fragment was not detected in the 3 normal cattle or the 6 cattle suffering from BVD tested by the OHV-2 PCR (data not shown).

The specificity of the amplified fragments was further confirmed by *Rsa* I and *Bmy* I restriction analysis. All the amplified fragments gave rise to *Rsa* I and *Bmy* I profiles identical to the parent LCL MF 629 (data not shown).

Discussion

The nucleotide sequence data presented comprises the first sequence derived from OHV-2. Database searches of nucleotide and derived amino acid sequences identified the greatest homology with EBV and HVS.

Analysis of the OHV-2 clone from which the sequence employed was derived was identified with the λ 8a clone of AHV-1 DNA which maps to the leftward end of the unique region [3, 6]. Similarly the BNRF 1 and EILF 1 ORF of EBV and HVS respectively which were found to share homology with the OHV-2 sequence are located terminally. This data lends supports to the classification of OHV-2 as a gammaherpesvirus and indicates that the position and regulatory class of this gene, not found in the alpha- and beta-herpesviruses, may be conserved among the gammaherpesviruses [6, 7, 22].

The application of the derived OHV-2 nucleotide sequence for the development of an OHV-2-specific PCR test has allowed the direct detection of OHV-2 in clinical cases of SA-MCF and in the implicated natural host, the sheep. This has provided, for the first time, a sensitive and specific detection technique which can be used to investigate the pathogenesis and epidemiology of SA-MCF.

The OHV-2 PCR primers were shown to be highly specific under the stringent annealing temperature used in the test and amplification of target DNA derived from the other bovine herpesviruses was not detected. Further, amplified product was not detected from target DNA derived from BHV-4 and AHV-1 despite the DNA homology shown to exist between AHV-1 and OHV-2 [5] and thought to be conserved amongst the gammaherpesviruses at this gene locus [22]. Degenerate PCR priming should allow the amplification and analysis of the analogous gene loci in AHV-1.

Previous attempts to detect AHV-1 directly in tissue from MCF-affected animals by immunofluorescence [22, 23, 34] and by in situ hybridisation [4] have indicated that very few cells contained a detectable level of viral antigen or DNA. The high degree of sensitivity afforded by the PCR has provided the technology for the detection of rare DNA sequences and has been used extensively for viral detection and diagnosis. A PCR for the detection of AHV-1 DNA sequence [41], has been reported for the diagnosis of AHV infections [13, 14]. The sensitivity of this nested PCR was reported to be 0.01 $TCID_{50}$ of infective AHV-1 virus [14]. The apparent high sensitivity of detection most probably was due to the presence of virus particles which failed to replicate in tissue culture. However, the amplified fragments derived from 5 AHV-1 and 2 AHV-2 isolates were not differentiated by restriction profile analysis using *Pvu* II

and *Stu* I. The sensitivity of the OHV-2 PCR was determined by titration of genomic DNA derived from the parent LCL and was therefore quantified more accurately. The absolute level of sensitivity was estimated to be 35 viral genome equivalents. This figure was the genome copy number derived for MF 629 on the assumption that no duplication of the target gene sequence had taken place through genetic rearrangements during culture of the LCL. However, comparison of SA-MCF cell lines by Southern blot hybridisation [5] has indicated that such genetic rearrangement may have occurred in cell lines such as MF 629.

The OHV-2 PCR was applied directly for the detection of OHV-2 DNA in the pbl fraction (and spleen from one rabbit) from naturally occurring and experimentally induced cases of SA-MCF. The development and application of the OHV-2 PCR has provided the means by which suspected cases of SA-MCF can now be diagnosed in live animals. In addition, the detection of OHV-2 DNA in the pbl of apparently recovered animals confirms the observation that some cattle affected with SA-MCF can recover from the OHV-2 infection. This indicates that as with cattle recovered from AHV-1-induced MCF a persistent infection results [27].

More significantly, the detection of OHV-2 DNA in the pbl fraction of sheep has confirmed the presence of the viral agent of SA-MCF in the suspected natural host. This directly confirms previous work which has detected in normal sheep sera cross-reactive antibodies to some AHV-1 polypeptides by immunoblotting analysis [12] and indirect immunofluorescent antibody test [35].

Analysis by restriction endonuclease profiles of amplified OHV-2 gene fragments from clinically affected animals, normal sheep and SA-MCF LCLs has confirmed the aetiological role of the SA-MCF agent and has further supported the observation that this specific gene locus is well conserved within the gammaherpesviruses. Direct sequence determination and analysis of the amplified gene fragments derived from the clinical cases and normal sheep will be required to examine for sequence heterogeneity in viruses associated with single and multiple case outbreaks of SA-MCF in cattle.

The OHV-2 PCR has provided the necessary technology to allow the detection of OHV-2 in both clinically affected animals and sheep. This provides the means, for the first time, to monitor the infection in both MCF-susceptible animals and in the normal host and will enable the study of virus replication and shedding in the sheep and give an insight into the pathogenesis of MCF. The authors are currently investigating virus shedding in peri-natal lambs, the specific lymphocyte population infected in the pbl fraction of sheep and susceptible animals and the tissue types where productive viral replication occurs in the sheep.

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References

1. Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ, Hatfull G, Hudson GS, Satchwell SC, Seguin C, Tuffnell PS, Barrell BG (1984) DNA sequence and expression of the B 95-8 Epstein-Barr virus genome. Nature 310: 207–211
2. Bridgen A (1991) The derivation of a restriction endonuclease map for *Alcelaphine herpesvirus 1* DNA. Arch Virol 117: 183–192
3. Bridgen A, Herring AJ, Inglis NF, Reid HW (1989) Preliminary characterization of the Alcelaphine herpesvirus-1 genome. J Gen Virol 70: 1141–1150
4. Bridgen A, Munro R, Reid HW (1992) The detection of Alcelaphine herpesvirus-1 DNA by in situ hybridization of tissues from rabbits affected with malignant catarrhal fever. J Comp Pathol 106: 351–359
5. Bridgen A, Reid HW (1991) Derivation of a DNA clone corresponding to the viral agent of sheep-associated malignant catarrhal fever. Res Vet Sci 50: 38–44
6. Bublot M, Lamonte P, Lequarre A-S, Albrecht J-C, Nicholas J, Fleckenstein B, Pastoret P-P, Thiry E (1992) Genetic relationships between bovine herpesvirus 4 and the gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. Virology 190: 654–665
7. Cameron KR, Stamminger T, Craxton M, Bodemer W, Honess RW, Fleckenstein B (1987) The 160,000-M_r virion protein encoded at the right end of the herpesvirus saimiri genome is homologous to the 140,000-M_r membrane antigen encoded at the left end of the Epstein-Barr virus genome. J Virol 61: 2063–2070
8. Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12: 387–395
9. Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13
10. Feinberg AP, Vogelstein B (1984) Addendum. Anal Biochem 137: 226–227
11. Herring AJ, Inglis NF, Ojeh CK, Snodgrass DR, Menzies JD (1982) Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. J Clin Microbiol 16: 473–477
12. Herring A, Reid H, Inglis N, Pow I (1989) Immunoblotting analysis of the reaction of wildebeest, sheep and cattle sera with the structural antigens of Alcelaphine herpesvirus-1 (malignant catarrhal fever virus). Vet Microbiol 19: 205–215
13. Hsu D, Shih LM, Castro AE, Zee YC (1990) A diagnostic method to detect Alcelaphine herpesvirus-1 of malignant catarrhal fever using the polymerase chain reaction. Arch Virol 114: 259–263
14. Katz J, Seal B, Ridpath J (1991) Molecular diagnosis of Alcelaphine herpesvirus (malignant catarrhal fever) infections by nested amplification of viral DNA in bovine blood buffy coat specimens. J Vet Diagn Invest 3: 193–198
15. Lee GM, Thornthwiate JT, Rasch EM (1984) Picogram per cell determination of DNA by flow cytofluorometry. Anal Biochem 137: 221–226
16. Löening UE (1969) The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem J 113: 131–138
17. Meinkoth J, Wahl G (1984) Hybridization of nucleic acids immobilized on solid supports. Anal Biochem 138: 267–284
18. Messing J (1983) New M 13 vectors for cloning. Methods Enzymol 101: 20–78
19. Milne EM, Reid HW (1990) Recovery of a cow from malignant catarrhal fever. Vet Rec 126: 640–641
20. Needleman SB, Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequences of two proteins. J Mol Biol 48: 443–453
21. Nicholas J, Cameron KR, Coleman H, Newman C, Honess RW (1992) Analysis of nucleotide sequence of the rightmost 43 kbp of herpesvirus saimiri (HVS) 1-DNA:

general conservation of genetic organization between HVS and Epstein-Barr virus. Virology 188: 296–310
22. Patel JR, Edington N (1980) The detection of the herpesvirus of bovine malignant catarrhal fever in rabbit lymphocytes *in vivo* and *in vitro*. J Gen Virol 48: 437–444
23. Patel JR, Edington N (1981) The detection and behaviour of the herpesvirus of malignant catarrhal fever in bovine lymphocytes. Arch Virol 68: 321–326
24. Pearson WR, Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85: 2444–2448
25. Plowright W, Ferris RD, Scott GR (1960) Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. Nature 188: 1167–1169
26. Plowright W, Herniman KAJ, Jesset DM, Kalunda M, Rampton CS (1975) Immunisation of cattle against the herpesvirus of malignant catarrhal fever: failure of inactivated vaccines with adjuvant. Res Vet Sci 19: 159–166
27. Plowright W, Kalunda M, Jessett DM, Herniman KAJ (1972) Congenital infection of cattle with the herpesvirus causing malignant catarrhal fever. Res Vet Sci 13: 37–45
28. Plowright W, Macadam RF, Armstrong JA (1963) Growth and characterization of the virus of bovine malignant catarrhal fever in East Africa. J Gen Microbiol 39: 253–266
29. Reid HW, Buxton D (1984) Malignant catarrhal fever of deer. Proc R Soc Edinburgh 82B: 261–273
30. Reid HW, Buxton D, Pow I, Finlayson J (1989) Isolation and characterisation of lymphoblastoid cells from cattle and deer affected with "sheep-associated" malignant catarrhal fever. Res Vet Sci 47: 90–96
31. Reid HW, Buxton D, Pow I, Finlayson J, Berrie EL (1983) A cytotoxic T-lymphocyte line propagated from a rabbit infected with sheep-associated malignant catarrhal fever. Res Vet Sci 34: 109–113
32. Roizman B (ed) (1982) The herpesviruses, vol 1. Plenum Press, New York London, pp 1–23
33. Roizman B, Desrosiers RC, Fleckenstein B, Lopez C, Minson AC, Studdert MJ (1992) The family *Herpesviridae*: an update. Arch Virol 123: 425–448
34. Rossiter PB (1980) A lack of readily demonstrable virus antigens in the tissues of rabbits and cattle affected with malignant catarrhal fever virus. Br Vet J 136: 478–483
35. Rossiter PB (1981) Antibodies to malignant catarrhal fever virus in sheep sera. J Comp Pathol 91: 303–311
36. Rossiter PB (1983) Antibodies to malignant catarrhal fever virus in cattle with non-wildebeest associated malignant catarrhal fever. J Comp Pathol 93: 93–97
37. Saiki RK, Gelfand DH, Stoffel S, Scharf ST, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487–491
38. Saiki RK, Scharf S, Faloona F, Mullis KS, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic DNA sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230: 1350–1354
39. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York
40. Sanger FS, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467
41. Shi L-M, Irving JM, Zee YC, Pritchett RF (1988) Cloning and characterization of a genomic probe for malignant catarrhal fever virus. Am J Vet Res 10: 1665–1668
42. Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98: 503–517

Authors' address: Dr. H. W. Reid, Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, U.K.

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