CHARACTERIZATION OF RKZ ISOLATE OF OVINE HERPESVIRUS 1

J. KOPÁČEK[1], O. REJHOLCOVÁ[2], D. KOPTIDESOVÁ[1], F. ČIAMPOR[1], F. M. RIJSEWIJK[3], H. W. REID[4], J. PASTOREK[1], V. ZELNÍK[1*]

[1]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 45 Bratislava, Slovak Republic; [2]Pharmagal, Nitra, Slovak Republic; [3]ID-DLO, Lelystad, The Netherlands; [4]Moredun Research Institute, Edinburgh, United Kingdom

Received May 12, 2000; accepted September 21, 2000

Summary. – Cytopathic effect (CPE) characterized mainly by foci of rounded cells was observed in cultures of primary *plexus choroideus* cells from healthy lamb following cryopreservation. It was possible to transmit the infectious agent to other primary cells of ovine origin by co-cultivation with infected cells. By indirect immunofluorescence microscopy it was found that high percentage of sheep (65–80% in 3 different herds from Slovakia) are infected with this infectious agent. Electron microscopy of cells with CPE revealed the presence of herpesvirus particles. Viral DNA was isolated from infected cells using pulse-field gel electrophoresis and further used as probe in Southern blot analysis. The probe reacted specifically only with DNA from cells infected with Ovine herpesvirus 1 (OvHV-1) but not with DNA of other ruminant herpesviruses. Some of the *Hind*III restriction fragments of DNA of the obtained OvHV-1 isolate denominated RKZ were cloned. Part of the H9 clone was sequenced identifying a gene that encoded a polypeptide homologous to conserved herpesvirus VP23 structural protein. From comparison of the sequence of this clone with VP23 sequences of other herpesviruses it was deduced that OvHV-1 might be classified within the *Rhadinovirus* genus of the *Gammaherpesvirinae* subfamily. The sequencing of the H9 clone of DNA of RKZ isolate enabled establishment of sensitive and highly specific polymerase chain reaction (PCR) assay for detection of OvHV-1.

Key words: Ovine herpesvirus 1; genetic analysis; VP23 capsid protein; virus classification; genus Rhadinovirus; subfamily Gammaherpesvirinae; family Herpesviridae

Introduction

Infection of sheep with OvHV-1 was believed for a long time to have some involvement in sheep pulmonary adenomatosis (SPA) (MacKay, 1969a; De Villiers *et al.*, 1975; Sharp, 1987). During the seventies and early eighties primary experiments on isolation and biological characterization of OvHV-1 have been performed. In most cases, OvHV-1 isolates were obtained from sheep affected by SPA (Sharp, 1987). The virus can induce full productive infection in sheep alveolar macrophages (SAM) (MacKay, 1969b) and can spread to other tissues possibly by infiltration of infected peripheral blood mononuclear cells (PBMC) to healthy organs. Presence of neutralizing antibodies to and isolation of OvHV-1 has been reported from sheep herds from different parts of the world (Scott, 1984; Verwoerd *et al.*, 1979). Experimental infection of specific pathogen-free lambs with OvHV-1 results in development of interstitial changes in the lungs ranging from focal cellular infiltration to a widespread proliferative pneumonia but not in SPA (Scott *et al.*, 1984). In addition, studies on the etiological agent of SPA have identified retrovirus particles in lungs of affected sheep (Perk *et al.*, 1974) and further experiments have clearly confirmed that the agent of SPA is the jaagsiekte

*E-mail: viruzelo@savba.sk; fax: +421-7-5477 4284.

Abbreviations: AlHV = *Alcelaphine herpesvirus 1*; BSS = basal salt solution; CPC = cells of *plexus choroideus;* CPE = cytopathic effect; MCF = malignant catarrhal fever; FCS = fetal calf serum; MEM = Minimum Eagle's Medium; OvHV-1 = Ovine herpesvirus 1; PBMC = peripheral blood mononuclear cells; PCR = polymerase chain reaction; SAM = sheep alveoral macrophages; SPA = sheep pulmonary adenomatosis

retrovirus (Martin *et al.*, 1976; Palmarini *et al.*, 1999), genetically related to sheep endogenous retrovirus (York *et al.*, 1992; Hecht *et al.*, 1994). The presence of OvHV-1 in lungs of sheep affected by SPA can be explained by its persistence in latent infection from which it is reactivated following the development of SPA (Scott *et al.*, 1984).

In the presently valid taxonomy of viruses, OvHV-1 is listed as an unassigned virus in the *Herpesviridae* family (Van Regenmortel *et al.*, 2000). However, in the past, on the basis of its lymphotropism it has been proposed a member of the *Gammaherpesvirinae* subfamily (Verwoerd *et al.*, 1979; Roizman *et al.*, 1992). However, its precise assignment to *Lymphocryptovirus* or *Rhadinovirus* genus is not clear due to the lack of detailed information on the genetic and molecular biological characterization of OvHV-1. Someof the studies were complicated by the fact that OvHV-1 is strongly cell-associated during cell culture propagation (Mackay, 1969b). Nevertheless, it was possible to isolate OvHV-1 and perform analysis indicating that its DNA has a buoyant density of 1.697–1.706 g/ml and a G/C content of 37–47%. Reports on the calculated size of DNA differ more significantly, with the anticipated size of the genome of Scottish isolates of 71.6–83.8 kbp (restriction endonuclease analysis; Scott (1984)) and that of a South-African isolate of approximately 101 kbp according to sedimentation analysis (De Villiers, 1979).

In the presented paper we report on obtaining the RKZ isolate of OvHV-1 from tissue of a healthy lamb and on its further characterization. Analysis of sera from different herds from Slovakia revealed high prevalence of infection with OvHV-1 under natural conditions. Identification of the isolate as an OvHV-1 was possible on the basis of genetic analysis. Furthermore, for the first time reported sequencing of the part of the genome of RKZ isolate enabled development of sensitive PCR detection of OvHV-1 genome and proposal of its classification within the *Rhadinovirus* genus, the *Gammaherpesvirinae* subfamily, the *Herpesviridae* family on the basis of phylogenetic comparative analysis.

Materials and Methods

Cells and viruses. Explant CPC cultures were prepared according to commonly used procedure. Briefly, the tissue was repeatedly rinsed in basal salt solution (BSS), then finely minced with scissors, rinsed 3 times with BSS and twice with a growth medium (Minimum Eagle's Medium (MEM)) supplemented with 10% of fetal calf serum (FCS). Tissue fragments were dispersed in a small volume of the growth medium and transferred into 6-cm Petri dishes. They were incubated without moving for 7–10 days at 37°C in 5.2% CO_2. Attached cells were passaged 3 times in total and then used either for other experiments or were cryopreserved with DMSO in liquid nitrogen. During subsequent subcultures the amount of serum in the growth medium was reduced to 5%.

Other primary cell cultures and cell lines used in the study were prepared and cultivated according to standard protocols. For determination of the susceptibility to RKZ isolate cell cultures were co-cultivated with RKZ-infected CPC. After three subsequent subcultures the occurrence and intensity of CPE was evaluated.

Some of other ruminant herpesviruses, namely the Cooper strain of *Bovine herpesvirus 1* (BoHV-1), the TV strain of *Bovine herpesvirus 2* (BoHV-2), the MOVAR strain of *Bovine herpesvirus 4* (BoHV-4) and the WC11 strain of *Alcelaphine herpesvirus 1* (AlHV-1) were obtained from the Collection of Zoopathogenic Microorganisms of the Institute of Veterinary Medicine, Brno, Czech Republic. DNA from cells infected with the South African FSN strain of OvHV-1 and the MF/629 isolate of OvHV-2 were from Moredun Research Institute, Edinburgh, UK. DNAs of the N569 isolate of *Bovine herpesvirus 5* (BoHV-5) and of the E/CH isolate of *Caprine herpesvirus 1* (CpHV-1) were kindly provided by Dr. M. Engels, University of Zürich, Zürich, Switzerland. Cells infected with Scottish OvHV-1 isolates 3V49, AB199, J9, and 532 were kindly provided by Dr. F.M.M. Scott, Moredun Research Institute, Edinburgh, UK.

Indirect immunofluorescence microscopy. CPC were co-cultivated with RKZ-infected cells on coverslips in a ratio of 4:1 for 72–96 hrs. Then the cultures were rinsed with serum-free medium, air-dried and fixed with cold acetone. Serum samples diluted 1:10 in BSS were applied on the fixed coverslip cultures and incubated in a moist chamber at 37°C for 1 hr. Following BSS washing the cultures were stained with 1:50 diluted FITC-labeled antibodies directed against sheep IgG (Sigma). After BSS washing the coverslip cultures were mounted on slides with buffered glycerol and examined by fluorescent microscope.

Electron microscopy. CPC were fixed in 2.5% glutaraldehyde in 0.2 mol/l sodium cacodylate buffer pH 7.2 for 30 mins at 4°C, washed in the same buffer and post-fixed with 1% OsO_4 prepared in the same buffer for 60 mins at room temperature. The samples were then dehydrated at increasing acetone concentrations and embedded into LR Gold embedding medium. Ultrathin sections were prepared on an Ultracut ultramicrotome and stained with 2% uranyl acetate and lead citrate (Venable and Coggeshall, 1965). The samples were examined by Philips EM 300 electron microscope at 80 kV.

DNA analyses. Pulse-field electrophoresis of DNA was performed using the Gene Navigator Pulsed Field System (Pharmacia Biotech) according to the recommendations of the manufacturer. Viral DNA was extracted from infected cells according to Hirt (1967). Total DNA isolation from infected cells, digestion with restriction enzymes, Southern blot analysis, cloning of OvHV-1 fragments and other standard procedures of DNA analysis were performed according to Sambrook *et al.* (1989).

DNA fragments were labeled with ^{32}P using the Megaprime Labeling Kit (Amersham Biotech).

Templates for partial sequencing of the RKZ H9 fragment were prepared using the Nested Deletion Kit and nucleotide sequencing was performed using the T7 Sequencing Kit (both from Pharmacia Biotech). For PCR the primers OHV-PR1 (5′-TGT GCA ATA TCC GTA GGC ACC ACA) and OHV-PR2 (5′-GCT GTT TTA GAA GAA GTG AAT GCA GAC A) were used; the mapping of the primers is detailed in Fig. 4. PCR conditions were: 30 cycles, 94°C for 30 secs, 58°C for 30 secs, and 72°C for 1 min.

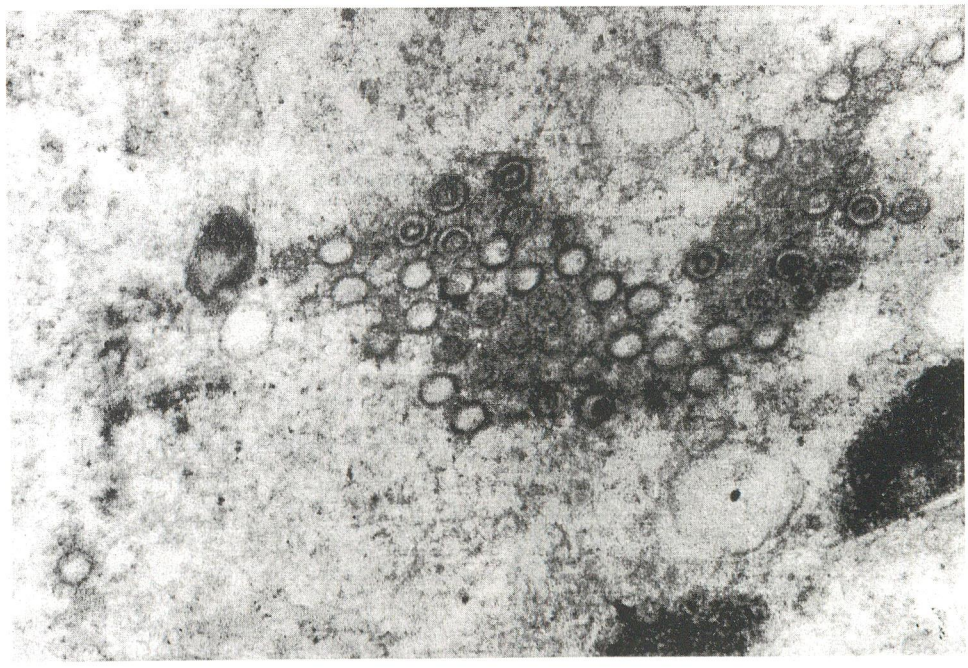

Fig. 1
Electron microscopy of OvHV-1 infected CPC
Aggregates of viral nucleocapsids are present in the nucleus in the vicinity of nuclear membrane. Magnification 66,000x.

The sequence data reported in this paper will appear in the EMBL Nucleotide Sequence Database under Acc. No. AJ276702.

Computer analysis. The obtained sequencing data were analyzed using DNASIS (Hitachi, Japan) and DNASTAR (DNAStar, Inc.) softwares and BLAST search program available on internet (page http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

Virus isolation and characterization

After culturing one batch of cryopreserved CPC a CPE characterized mainly by foci of rounded cells was observed. It was possible to transmit infection to "healthy" CPC derived from other batches (different animals), however, only by direct cell-to-cell contact. Other primary cell cultures and/or cell lines from different animal species were also tested for their susceptibility to the infectious agent. Only in cells of lamb/sheep origin was transmission and resulting CPE observed. These included primary lamb kidney, lung, testicle, and CPC and a stabilized ovine kidney cell line.

For determination of prevalence of natural infection with the isolated infectious agent, sheep sera from four different farms in Slovak Republic were tested for their reaction with the agent by the indirect immunofluorescence microscopy. Fifty-six sera were analyzed in total, identifying 40 (71.4%) positive sera with a range from 64.7% to 80%. It should be mentioned that the collecting of sera from sheep was random in herds and the animals had no signs of sickness or pathogenic disorders.

To characterize more closely the identified infectious agent, electron microscopy studies were performed on infected CPC. As shown in Fig. 1, several intranuclear capsids and associated structures were observed. Nucleocapsids frequently aggregated in the vicinity of the nuclear membrane. Nucleocapsids appeared to become enveloped by membranes formed *de novo* in the nucleus. Empty capsids were rarely enveloped, which differed from more common envelopment of empty capsids of the virus associated with Burkitt's lymphoma (Toplin and Schidlovsky, 1966).

From electron microscopy studies it was clear that the infectious agent identified was a herpesvirus. To date there are reports of two ovine herpesviruses, both belonging to *Gammaherpesvirinae* subfamily. OvHV-1 is rather better characterized, its isolation has been reported from different parts of the world mainly from SPA cases (see a review by Sharp (1987)). OvHV-2 is non-pathogenic for sheep but causes sheep-associated malignant catarrhal fever (MCF) in other ruminants (Plowright, 1990). The OvHV-2 has not yet been isolated, but its genetic and antigenic relationship

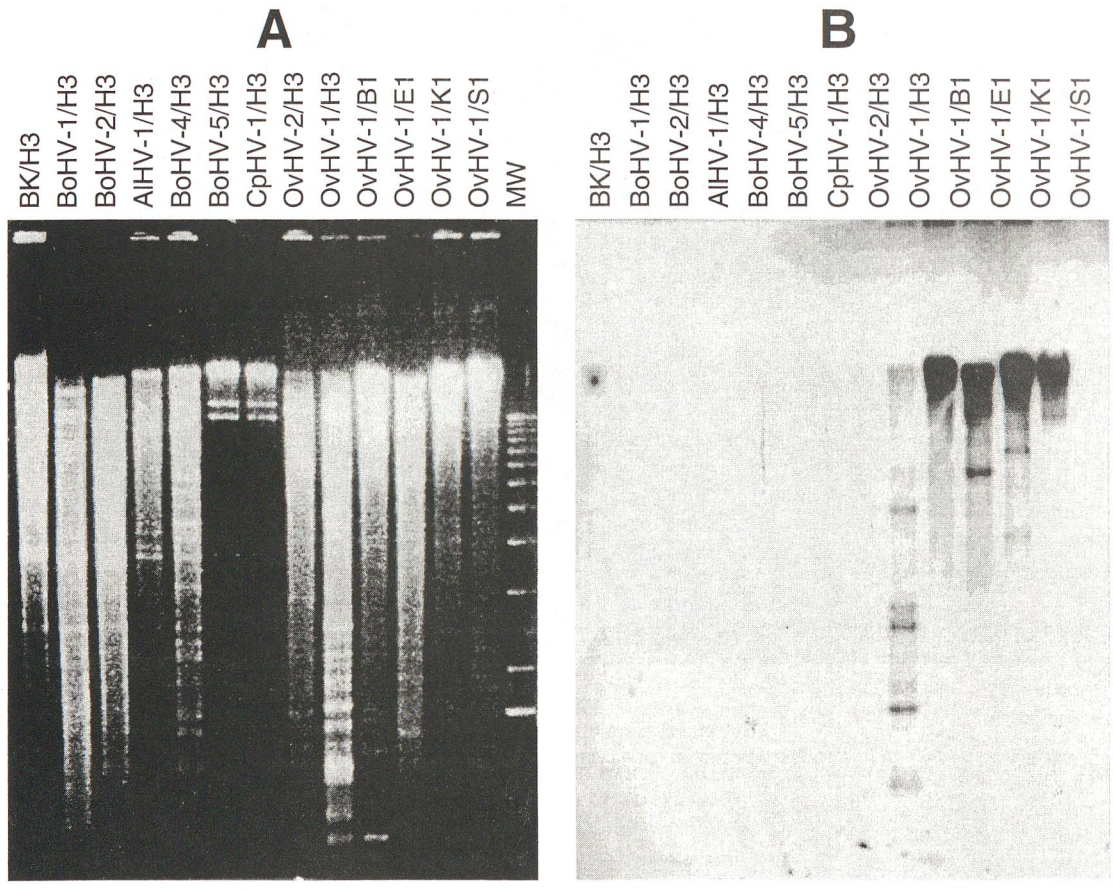

Fig. 2
Southern blot analysis of ruminant herpesvirus DNAs using the labeled RKZ DNA probe
A. Ethidium bromide-stained agarose gel with separated restrictionion fragments of DNAs tested. B. Autoradiograph of the blot with DNA fragments specified in (A) and detected using the ^{32}P-labeled RKZ probe. Abbreviations of herpesvirus names are indicated in the text, restriction endonucleases used for DNA digestion were: *Hind*III (H3), *Bam*HI (BI), *Eco*RI (EI), *Sma*I (SI), and *Kpn*I (KI). A 1-kbp ladder (Life Technologies) (lane MW). Mock-infected bovine kidney cells (lane BK).

to AlHV-1 has been demonstrated (Herring *et al.*, 1989; Bridgen and Reid, 1991). As there are no reports on successful *in vitro* transmission and propagation of OvHV-2, we assume that our RKZ isolate is OvHV-1.

Isolation of OvHV-1 from healthy animal is rather unusual. It is not possible to identify the source of the OvHV-1 prior to its identification in cultured CPC after cryopreservation. However, we can speculate that the virus was transferred from infected PBMCs to *plexus choroideus*, a tissue forming the blood-brain barrier, and entered latent stage of infection in it. The virus was then reactivated after re-culturing following cryopreservation. Reactivation of herpesviruses from latent infection from cryopreserved cells has been observed in other cases. Ability of RKZ isolate to replicate in different sheep cell types indicates that it has broader tropism than the Scottish Grimm strain of OvHV-1, the replication of which has only been demonstrated in SAM (Scott, 1984).

High prevalence of antibodies directed against OvHV-1 in sera from sheep obtained from different farms in Slovak Republic was at first instance rather surprising. On the other hand, the published prevalence of neutralizing OvHV-1 antibodies in sera from different countries was also relatively high (Scott, 1984), 22% (35/157) in Great Britain, 58% (57/98) in USA, 36% (69/193) in Australia, and 13% (23/182) in New Zealand. Interestingly, all sera tested (52) from Iceland were negative indicating that this country might be free of OvHV-1 natural infection (Scott, 1984). In addition, this serological survey showed that a high proportion of sheep affected by SPA in Scotland (85%) have antibodies to OvHV-1. Similar results have been obtained by Verwoerd *et al.* (1979), who have identified approximately 70% of sera of sheep with SPA in South Africa as positive for OvHV-1 and have confirmed Iceland to be free of OvHV-1.

Genetic analysis of RKZ isolate

To characterize more closely RKZ isolate molecular biological techniques were used. Viral DNA was separated from CPC DNA using pulse-field gel electrophoresis and subsequently extracted from gel. The isolated RKZ DNA was then used as a probe in Southern blot analysis of *Hind*III-digested DNAs of other ruminant herpesviruses. As it can be seen from Fig. 2B, the RKZ probe hybridized specifically only with the digested DNA of the South African FSN strain of OvHV-1. Hybridization with DNAs of other ruminant herpesviruses was not observed even under more relaxed stringency allowing approximately a 15% mismatch (data not shown).

Some of the RKZ *Hind*III restriction fragments were cloned in the pUC19 vector. End-proximal parts of the

Table 1. Summary of results of comparative OvHV-1 VP23 sequence analysis

Herpesvirus[a]	Acc. No.	Score	Identity (%)	Positivity (%)
AlHV-1	gi|2337989	213	68	80
SaHV-2	sp|Q01008	180	57	75
AtHV-2	gi|4019252	174	55	73
BoHV-4	Gb|AAD33779.1	166	54	69
RRV	Gb|AAD21352.2	162	54	70
HHV-4	sp|P25214	162	53	69
EHV-2	pir|S55620, gi|1718278	161	51	73
HHV-8	gi|2246498, gi|3282761	158	50	70
MuHV-4	Emb|CAA70344	132	42	63

[a]Abbreviations of herpesvirus names are detailed in the text and in the legend to Fig. 4.

```
VP5-> V   F   L   E   Q   P   C   I   F   L   Q   E   T   F   P   T   L   V   R   S
  1  GTG TTT TTA GAA CAG CCT TGC ATC TTT TTG CAA GAA ACT TTT CCT ACT TTG GTG CGT TCG

       H   K   A   L   L   D   G   Y   M   S   S   Q   V   T   H   A   P   V   H   M
 61  CAT AAG GCG CTA CTG GAC GGA TAT ATG TCC TCG CAA GTT ACA CAT GCT CCA GTG CAT ATG

       G   Q   Y   L   I   E   E   T   A   P   I   K   R   V   F   K   I   G   N   K
121  GGC CAG TAT CTT ATT GAA GAA ACT GCT CCA ATT AAA AGA GTA TTT AAG ATT GGC AAC AAA

       L   A   N   *                                                   VP23-> M   F   V   D
181  CTG GCA AAT TAA GCT CAG TTA TAT TTT TTG CTT ATT TTT TAA AGC AAC ATG TTT GTA GAC

       R   K   I   L   V   S   C   T   S   R   L   Y   A   D   E   I   S   K   L   Q
241  AGA AAA ATT TTA GTT TCT TGC ACT TCT CGC CTT TAC GCG GAT GAA ATA TCT AAG CTG CAA

       E   K   I   G   A   I   V   P   L   I   D   S   H   A   S   Q   N   L   H   S
301  GAA AAA ATT GGC GCT ATA GTG CCC CTT ATA GAC TCA CAT GCT AGC CAA AAC TTA CAC TCT

       I   G   L   S   P   N   T   H   T   D   I   T   S   D   Y   V   L   M   F   N
361  ATA GGC CTC AGT CCT AAT ACT CAC ACA GAC ATT ACT TCT GAC TAC GTT CTC ATG TTT AAC

       Y   L   S   K   C   T   L   A   V   L   E   E   V   N   A   D   S   L   V   L
421  TAC TTA TCT AAG TGC ACT TTA GCT GTT TTA GAA GAA GTG AAT GCA GAC AGC TTA GTG CTG
                                 OHV-H9PR1

       T   K   I   Q   K   N   Q   A   Y   Q   I   K   N   I   Y   Q   P   F   F   H
481  ACA AAA ATT CAA AAG AAC CAA GCA TAT CAA ATT AAA AAC ATT TAC CAG CCA TTT TTT CAT

       W   D   N   H   I   Q   L   C   V   I   P   P   L   F   G   R   E   S   N   T
541  TGG GAC AAT CAC ATA CAG CTT TGT GTG ATT CCT CCT CTG TTT GGC AGA GAA AGC AAC ACT

       V   V   L   E   S   N   N   F   T   L   L   L   P   S   V   V   P   T   D   I
601  GTA GTG CTA GAG TCT AAC AAC TTT ACT CTC TTG CTC CCC TCT GTG GTG CCT ACG GAT ATT
                                                             OHV-H9PR2

       A   H   E   A
661  GCA CAT GAA GCT T
          HindIII
```

Fig. 3
Sequence of a part of the RKZ H9 clone
Mapping of the PCR primers OHV-PR1 and OHV-PR2 is indicated by underlined corresponding sequences.

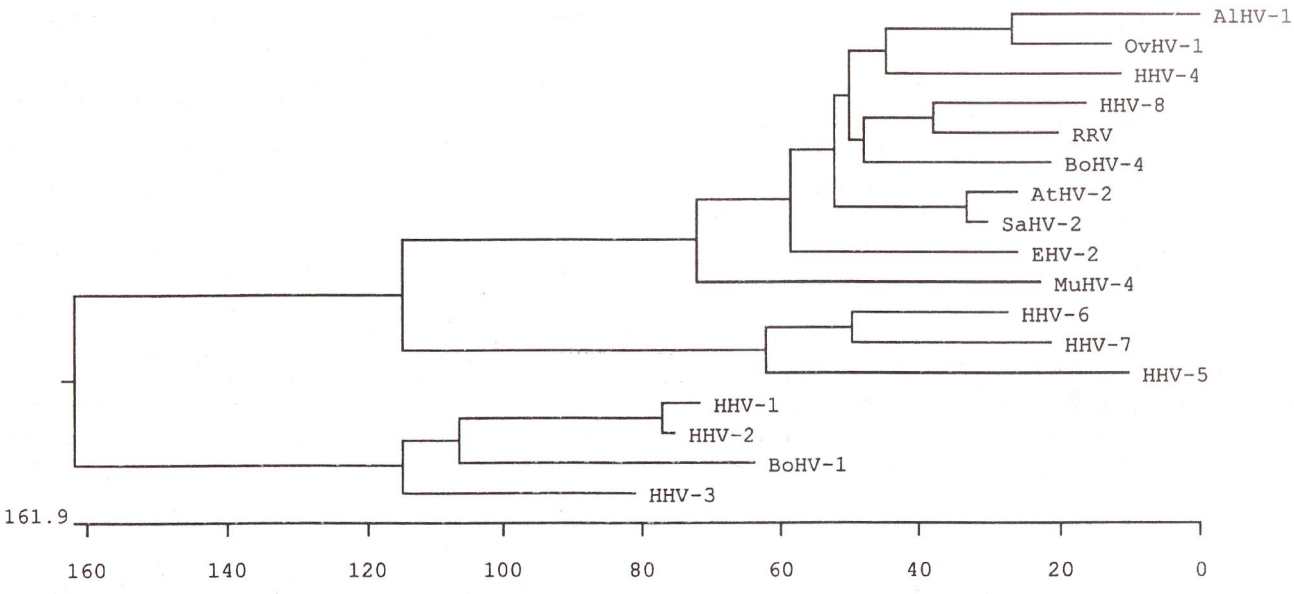

Fig. 4

Phylogenetic relationships of OvHV-1 to other herpesviruses on the basis of partial sequence of minor capsid protein VP23

The phylogenetic tree was constructed by the MegAlign Program (DNAStar) using the Clustal method with the PAM250 residue weight table. If not defined in the text, abbreviations of herpesvirus names are: HHV-4 = *Human herpesvirus 4* (Epstein-Barr virus), HHV-8 = *Human herpesvirus 8* (Kaposi's sarcoma-associated herpesvirus), RRV = Rhesus rhadinovirus (*Cercopithecine herpesvirus* 17), SaHV-2 = *Saimiriine herpesvirus 2* (Herpesvirus saimiri), AtHV-2 = *Ateline herpesvirus 2*, EHV-2 = *Equid herpesvirus 2*, MuHV-4 = *Murid herpesvirus 4*, HHV-6 = *Human herpesvirus 6*, HHV-7 = *Human herpesvirus 7*, HHV-5 = *Human herpesvirus 5* (Human cytomegalovirus), HHV-1 = *Human herpesvirus 1* (Herpes simplex virus 1), HHV-2 = *Human herpesvirus 2* (Herpes simplex virus 2), HHV-3 = *Human herpesvirus 3* (Varicella-zoster virus).

cloned fragments were analyzed by sequencing and the obtained data were compared to sequences of some OvHV-1 fragments encoding polypeptides homologous to other herpesvirus proteins from databases. By these means it was found that the H9 clone carries part of the gene encoding conserved herpesvirus minor capsid protein VP23. Sequence data of this fragment were expanded by preparation of nested deletion templates and their subsequent analysis. Finally we obtained a sequence of 673 nucleotides that was further characterized. By comparative analysis using BLAST-X program we found that this part of the H9 clone encodes the 63 C-terminal amino acids of the OvHV-1-homologous major capsid VP5 protein and 148 N-terminal amino acids of the minor capsid VP23 protein (Fig. 3). The outcome of the database homology search clearly showed that the obtained sequences of OvHV-1 capsid proteins display more similarities to homologous proteins of viruses of the *Rhadinovirus* genus than to other herpesviruses.

The results of the OvHV-1 VP23 homology search are summarized in Table 1. The obtained data were further used for phylogenetic analysis comparing the OvHV-1 VP23 sequence to those of other ruminant herpesviruses and also to those of human herpesviruses that represent individual herpesvirus subfamilies and genera. As it can be seen from Fig. 4, the OvHV-1 coding sequences phylogenetically resemble mostly those of the viruses of the *Rhadinovirus* genus.

The obtained sequence data enabled to design a primer set that can be used for highly specific and sensitive detection of OvHV-1 and to distinguish it from other ruminant herpesviruses. The mapping of the designed primers within the OvHV-1 VP23 locus is indicated in Fig. 3. The primer set used in this study is able to amplify DNA fragments specific only for OvHV-1 but not for other ruminant herpesviruses (Fig. 5). In addition, this specific primer set can detect OvHV-1 DNA from different isolates, including Scottish isolates, South African FSN isolate and Slovak RKZ isolate.

DNA hybridization techniques are highly specific and sensitive methods for identification of herpesvirus genomes during infection of cultured cells *in vitro* and also during natural infection *in vivo*. By modification of hybridization and washing conditions, conserved homologous genetic regions of phylogenetically related organisms can be also detected. E.g., Engels *et al.* (1987) found that the restriction map of CpHV-1 DNA differs significantly from that of BoHV-1 DNA, however, DNAs of both viruses share a high degree of similarity as identified by hybridization. Similarly Fukuchi *et al.* (1985) showed that Marek's disease virus (MDV) and its serologically related vaccine, herpesvirus of

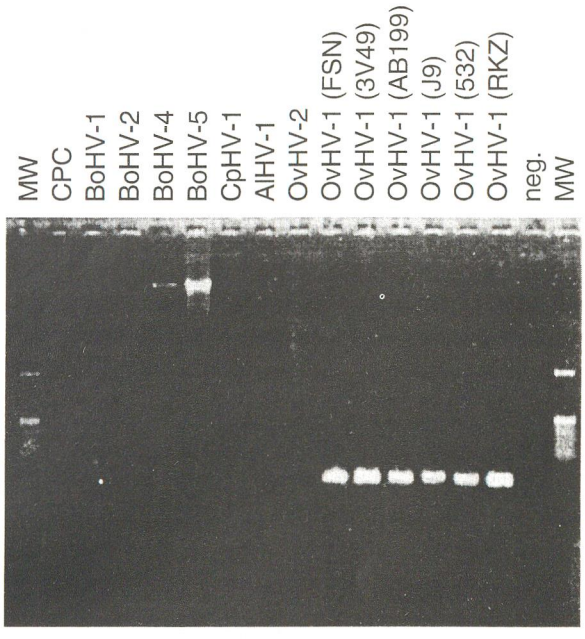

Fig. 5
PCR analysis of ruminant herpesvirus DNAs using OvHV-1-specific primers
Ethidium bromide-stained agarose gel with separated PCR products. Abbreviations of herpesvirus names are detailed in the text. A 100-bp ladder (Life Technologies) (lane MW). Mock-infected CPC (lane CPC). No DNA in PCR (lane neg.).

turkeys (HVT) contain genome parts that can cross-hybridize under relaxed conditions.

In our study, we employed Southern blot analysis to define the relationship of RKZ isolate to other ruminant herpesviruses. The results of this analysis confirmed our assumption that RKZ isolate is indeed OvHV-1 as it can hybridize specifically only to DNA of the South-African strain of OvHV-1. In addition, the hybridization under relaxed stringency indicated that OvHV-1 DNA does not share highly conserved genetic regions with other ruminant herpesviruses.

Despite the fact that the analyzed sequence of RKZ isolate is rather short and contains only parts of the VP5 and VP23 open reading frames (ORFs), it enabled to perform a phylogenetic analysis. It showed that OvHV-1 VP23 amino acid sequence is most closely related to those represented within the *Rhadinovirus* genus, e.g. AlHV-1 and AtHV-2. Further analysis of other RKZ (OvHV-1) genes is required to confirm this finding and to enable precise assignment of RKZ (OvHV-1) within the *Herpesviridae* family.

Characterization of the part of the OvHV-1 genome provided opportunity to develop PCR-based method for detection of OvHV-1. PCR is even more sensitive than hybridization analysis, it enables identification of limited copies of herpesvirus genome, e.g. in clinical samples containing a latent virus. Although VP23 capsid protein is highly conserved in all herpesviruses analyzed, the developed PCR protocol is highly specific for OvHV-1. Finally, the performed PCR experiments revealed that this genetic region is also highly conserved in all OvHV-1 isolates tested as all were positive regardless of their origin (Scotland, South Africa, and Slovakia). The described PCR detection method can be also used to monitor natural or experimental infection of other species with OvHV-1. In the case of OvHV-2 the virus can spread to other ruminants and cause fatal sheep-associated MCF.

A PCR protocol for OvHV-2 detection, identifying part of the tegument protein homologous gene was described earlier by Baxter *et al.* (1993). The availability of detection methods for both viruses can be used for studies concerning the question whether both viruses can co-infect single animal.

In this study we report for the first time data on OvHV-1 sequence and their further use for phylogenetic analysis and PCR detection method exploitation. Additional genetic analyses are required to confirm our assumption that OvHV-1 can be assigned to the *Rhadinovirus* genus of the *Herpesviridae* family. Furthermore, these studies might contribute to the understanding of molecular pathogenesis of OvHV-1 and other rhadinoviruses.

Acknowledgements. This study was supported by the Contract No. IC15-CT96-0917 of the EC INCO-Copernicus Program and partially by the grant No. 2/6075/20 from the Scientific Grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences. The research stay of V.Z. at the Moredun Research Institute, Edinburgh, UK was supported by the British Council.

References

Baxter SI, Pow I, Bridgen A, Reid HW (1993): PCR detection of the sheep-associated agent of malignant catarrhal fever. *Arch. Virol.* **132,** 145–159.

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.

De Villiers EM, Els HJ, Verwoerd DW (1975): Characteristics of an ovine herpesvirus associated with pulmonary adenomatosis (jaagsiekte) in sheep. *S. Afr. J. Med. Sci.* **40,** 165-170.

De Villiers EM (1979): Purification of the JS-3 isolate of *herpesvirus ovis* (bovid herpesvirus 4) and some properties of its DNA. *J. Virol.* **32,** 705–709.

Engels M, Loepfe E, Wild P, Schraner E, Wyler R (1987): The genome of caprine herpesvirus 1: genome structure and relatedness to bovine herpesvirus 1. *J. Gen. Virol.* **68,** 2019–2023.

Fukuchi K, Sudo M, Tanaka A, Nonoyama M (1985): Map location of homologous regions between Marek's disease virus and herpesvirus of turkey and the absence of detectable homology in the putative tumor-inducing gene. *J. Virol.* **53,** 994–997.

Hecht SJ, Carlson JO, DeMartini JC (1994): Analysis of a type D retroviral capsid gene expressed in ovine pulmonary carcinoma and present in both affected and unaffected sheep genomes. *Virology* **202,** 480–484.

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.

Hirt B (1967): Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* **26,** 365–369.

MacKay JMK (1969a): Tissue culture studies of sheep pulmonary adenomatosis (jaagsiekte). I. Direct culture of affected lungs. *J. Comp. Pathol.* **79,** 141–146.

MacKay JMK (1969b): Tissue culture studies of sheep pulmonary adenomatosis (jaagsiekte). II. Transmission of cytopathic effects to normal cultures. *J. Comp. Pathol.* **79,** 147–154.

Martin WB, Scott FMM, Sharp JM, Angus KW, Norval M (1976): Experimental production of sheep pulmonary adenomatosis (Jaagsiekte). *Nature* **264,** 183–185.

Minson AC, Davison A, Eberle R, Desrosiers RC, Fleckenstein B, McGeoch DJ, Pellet PE, Roizman B, Studdert DMJ (2000): Family Herpesviridae. In van Regenmortel MHV, Fauquet CM, Bishop DHL (Eds): *Virus Taxonomy.* Academic Press, San Diego, USA, pp. 203–225.

Palmarini M, Sharp JM, De Las Heras M, Fan H (1999): Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *J. Virol.* **73,** 6964–6972.

Perk K, Michalides R, Spiegelman S, Schlom J (1974): Biochemical and morphologic evidence for the presence of an RNA tumor virus in pulmonary carcinoma of sheep (Jaagsiekte). *J. Natl. Cancer Inst.* **53,** 131–135.

Plowright W (1990): Malignant catarrhal fever virus. In Dinter Z, Morein B (Ed.): *Virus Infections of Ruminants.* Elsevier Science Publishers B.V., Amsterdam, Oxford, New York, Tokyo, pp. 123–150.

Roizman B, Desrosiers RC, Fleckenstein B, Lopez C, Minson AC, Studdert MJ (1992): The family *Herpesviridae*: an update. *Arch. Virol.* **123,** 425–449.

Sambrook J, Fritsch EF, Maniatis T (1989): *Molecular Cloning: A Laboratory Manual.* 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Scott FMM (1984): Studies on sheep herpesvirus and its role in jaagsiekte (sheep pulmonary adenomatosis). *Ph.D. Thesis*, Napier College, Edinburgh, Moredun Research Institute, Edinburgh.

Scott FM, Sharp JM, Angus KW, Gray EW (1984): Infection of specific-pathogen free lambs with a herpesvirus isolated from pulmonary adenomatosis. *Arch. Virol.* **80,** 147–162.

Sharp JM (1987): Sheep pulmonary adenomatosis: a contagious tumour and its cause. *Cancer Surv.* **6,** 73–83.

Toplin I, Schidlovsky G (1966): Partial purification and electron microscopy of virus in the EB-3 cell line derived from a Burkitt lymphoma. *Science* **152,** 1084–1085.

Van Regenmortel MHV, Fauquet CM, Bishop DHL (2000): *Virus Taxonomy. Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego.

Venable JH, Coggeshall R (1965): A simplified lead citrate stain for use in electron microscopy. *J. Cell. Biol.* **25,** 407–408.

Verwoerd DW, Meyer-Scharrer E, Broekman J, de Villiers EM (1979): The serological relationship of herpesvirus ovis to other herpesviruses and its possible involvement in the aetiology of jaagsiekte. *Onderstepoort J. Vet. Res.* **46,** 61–63.

York DF, Vigne R, Verwoerd DW, Querat G (1992): Nucleotide sequence of the jaagsiekte retrovirus, and exogenous and endogenous type D and B retrovirus of sheep and goats. *J. Virol.* **66,** 4930–4939.