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# Ovine herpesvirus 1 (OVHV-1) thymidine kinase locus sequence analysis: evidence that OVHV-1 belongs to the *Macavirus* genus of the *Gammaherpesvirinae* subfamily

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**Summary. –** The *Hind*III-*Hinc*II fragment of the 5.5 kbp H11 *Hind*III clone of ovine herpesvirus 1 (OvHV-1) was cloned and its primary structure was determined by preparation of nested deletion subclones and their sequencing. Sequence analysis of the overlapping clones revealed that 3239 bp OvHV-1 fragment contains complete thymidine kinase (TK) gene, a partial open reading frame of ORF20 and that encoding glycoprotein H (gH). The conserved OvHV-1 TK displayed the highest similarity to homologous TK proteins encoded by members of the *Macavirus* genus of the *Gammaherpesvirinae* subfamily. These data including our previous analysis of the partial sequence of VP23 homologue might serve as further evidence that OvHV-1 should be categorized within the genus *Macavirus* of the *Herpesviridae* family.

**Keywords:** ovine herpesvirus 1; thymidine kinase; Macavirus; Gammaherpesvirinae

## Introduction

Ovine herpesvirus 1 (OvHV-1) was initially identified as a virus associated with sheep pulmonary adenomatosis, SPA (Smith and Mackay, 1969; De Villiers *et al.*, 1975). However, the Jaagsiekte sheep retrovirus, JSRV, was later identified as a causative agent of this disease, even though OvHV-1 has been often isolated from SPA tissues (Martin *et al.*, 1976; Sharp, 1987; Palmarini *et al.*, 1999). It was proposed that OvHV-1 reactivates from this and/or other tissues, where it persists in latent state, upon stress conditions such as superinfection with other viruses (Scott *et al.*, 1984).

Prevalence of neutralizing antibodies against OvHV-1 has been reported surprisingly at rather high levels in sheep herds in number of different countries (Verwoerd *et al.,* 1979; Scott, 1984; Kopáček *et al.*, 2000), with exception of Iceland that has appeared to be free from OvHV-1 infection.

There have been presented only a few data on further analysis and characterization of OvHV-1 and its genome (Kúdelová *et al.*, 2013). De Villiers (1979) described some properties of the JS-3 OvHV-1 isolate, at that time identified as bovid herpesvirus 4. The buoyant density of OvHV-1 DNA was determined to be $1.706 \pm 0.001$ g/cm$^3$ and molecular weight (Mr) was estimated to be in the range of $67.3 \pm 5.4 \times 10^6$.

In our previous studies, which included analysis of partial sequences of VP5 and VP23 herpesvirus homologues, we provided basic information on characterization of the RKZ isolate of OvHV-1, described OvHV-1-specific PCR for diagnostic purposes and proposed its classification as a member of the *Gammaherpesvirinae* subfamily (Ovečková *et al.*, 1998; Kopáček *et al.*, 2000).

In this paper we report on cloning of *Hinc*II-*Hind*III DNA fragment of OvHV-1 bearing complete thymidine kinase (TK) gene, nucleotide sequencing and detailed phylogenetic analysis of TK gene. The obtained data have clearly indicated that OvHV-1 is a member of the *Gammaherpesvirinae Macavirus* genus.

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**Abbreviation:** CPE = cytopathic effect; FUDR = 5′-fluoro-2′-deoxyuridine; HSV-1 = herpes simplex virus 1; JSRV = Jaagsiekte sheep retrovirus; MuHV-4 = murid herpesvirus 4; OvHV-1 = ovine herpesvirus 1; SPA = sheep pulmonary adenomatosis; TK = thymidine kinase

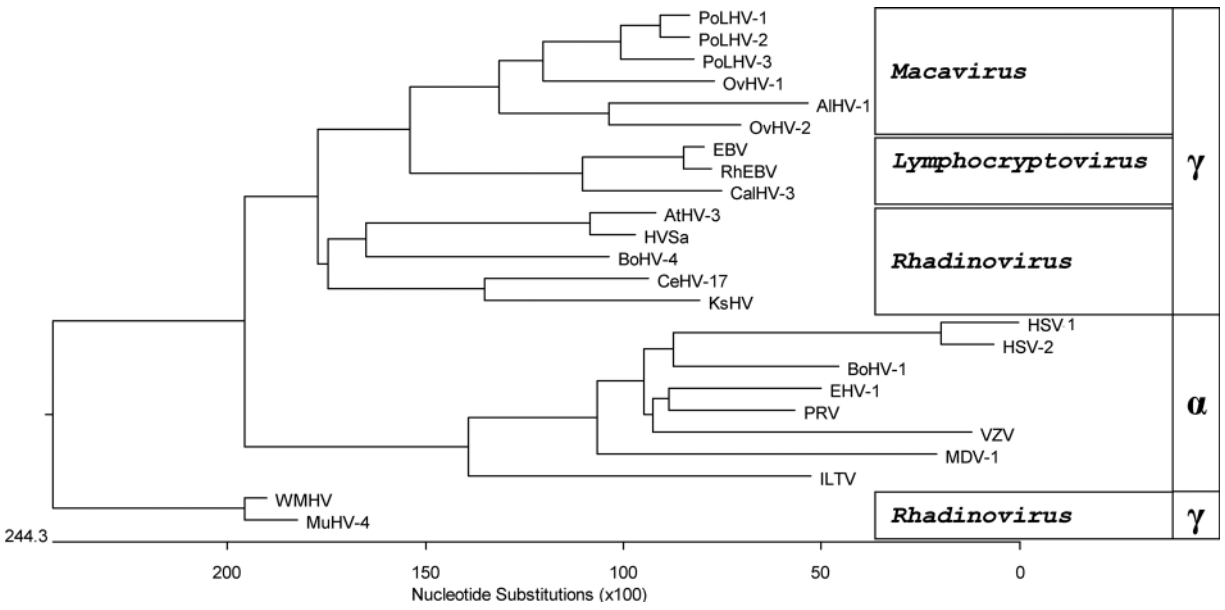


**Fig. 1**
**Dendrogram of phylogenetic relationships of herpesvirus thymidine kinases**
The dendrogram was created from multiple alignment using program ClustalW. α and γ refer to *Alphaherpesvirinae* or *Gammaherpesvirinae* subfamilies. Virus names abbreviations: AlHV-1 = alcelaphine herpesvirus 1; AtHV-3 = ateline herpesvirus 3; BoHV-1, -4 = bovine herpesvirus 1 or 4; CalHV-3 = callitrichine herpesvirus 3; CeHV-17 = cercopithecine herpesvirus 17; EBV = Epstein-Barr virus; EHV-1 = equine herpesvirus 1; HSV-1, -2 = herpes simplex virus 1 or 2; HVSa = herpesvirus saimiri; ILTV = infectious laryngotracheitis virus; KsHV = Kaposi´s sarcoma-associated herpesvirus; MDV-1 = Marek's disease virus 1; MuH-4 = murid herpesvirus 4; OvHV-1, -2 = ovine herpesvirus 1 or 2, PoLH-1, -2, -3 = porcine lymphotropic herpesvirus 1, 2 or 3; PRV = pseudorabies virus; RhEBV = rhesus EBV-like herpesvirus; VZV = varicella zoster virus; WMHV = wood mouse herpesvirus.

## Materials and Methods

*Cloning of OvHV-1 fragments.* OvHV-1 *Hind*III DNA fragments cloning and their end-encoding sequences were characterized previously (Ovečková *et al*., 1998). A *Hind*III-*Hinc*II fragment (H3H2) was subcloned from the approximately 5.5 kbp H11 *Hind*III clone in pBluescriptII KS plasmid vector, yielding clone 31. Templates for sequencing of H3H2 clone 31 were prepared by nested deletion method (Pharmacia Biotech).

*Sequence analysis of the OvHV-1 TK locus fragment.* Nucleotide sequencing of nested deletion subclones of the H3H2 clone 31 was performed using T7 Sequencing Kit (Pharmacia Biotech) according to manufacturer´s instructions.

*Computer analyses.* The obtained sequencing data were analyzed using DNASTAR software (SeqMan and MegAlign; DNAStar, Inc., Ver 5.01) and BLAST search programs available on web site http://www.ncbi.nlm.nih.gov/BLAST/. The sequence data reported in this paper were released in GenBank Acc. No. KC683534.

## Results and Discussion

During our earlier experiments we cloned several random OvHV-1 *Hind*III fragments in the vector pUC18 and also prepared λ-GEM11 OvHV-1 genomic library (Ovečková *et al.*, 1998). By sequencing of the end-parts of these *Hind*III fragments, we were able to predict their mapping within the OvHV-1 genome based on expected genes co-linearity with genomes of other *Gammaherpesvirinae* subfamily members as described e.g. for the H9 *Hind*III clone that carries sequences encoding parts of VP5 and VP23 homologues (Kopáček *et al.*, 2000).

By similar means there was identified approx. 5.5 kbp long H11 *Hind*III fragment of OvHV predicted to contain the complete TK gene sequence. After the restriction fragments analysis of H11 clone (data not shown), we subcloned approximately 3.2 kbp H3H2 *Hind*III-*Hinc*II fragment (clone 31) to the pBluescriptII KS vector. Subclones used for sequencing were prepared by nested deletions as described in the Materials and Methods.

By assembling of overlapping subclones strings we finally obtained 3239 bp long complete sequence of the OvHV-1 H3H2 clone 31. Three potential coding regions were identified within this sequence: a partial sequence of UL20 ORF homologue on the complementary strand starting at the nucleotide 167, the complete gene of OvHV-1 TK spanning nucleotides 500–2191 and a partial sequence of glycoprotein gH homologue with the initiation codon starting at the nucleotide 2229.

OvHV-1 TK is 563 aa long protein with predicted $M_r$ of 64936, exhibiting approximately average aa length and $M_r$

**Table 1. Conserved regions in herpesvirus thymidine kinases**

| Site[a] | Consensus motif[b] | Sequence in OvHV-1 TK | Proposed function |
|---|---|---|---|
| 1 | **G**XX(G/A/D)X**GK**(T/S/V) | 246-GGMAVGKS-253 | ATP binding |
| 2 | **E**(P/A)(M/L/I/V)X(Y/F/A)**W** | 273-EPMYYW-278 | |
| 3 | **DRH** | 348-DRH-350 | Nucleoside substrate recognition |
| 4 | (C/V/I/L)(F/Y)**P** | 357-VFP-359 | |
| 5 | **R**(L/V/I)XX**R**X**R** | 401-RLKRRAR-407 | |

[a]Conserved sites as defined by Balasubramaniam *et al.* (1990). [b]Amino acids conserved in sequences of all herpesviral TKs identified up to date are highlighted in bold.

among gammaherpesvirus TKs homologues varying from 445 to 662 aa and from 50945 to 74578, respectively. It should be noted that TKs of alphaherpesviruses are smaller, e.g. HSV-1 TK is 376 aa long (Mc Knight, 1980).

Search for proteins homologous to OvHV-1 TK performed by BLAST tool identified the highest scores of homology for gammaherpesvirus TKs. Subsequently, we performed multiple alignment using MegAlign DNASTAR software and identified several conserved domains within family of herpesvirus TKs as they were described by Balasubramaniam *et al.* (1990), Champness *et al.* (1998), and Evans *et al.* (1998). The comparative analysis provided in addition the evidence that OvHV-1 TK has the highest homology to its counterparts encoded by members of the *Macavirus* genus. The dendrogram resulting from the phylogenetic analysis is shown in Fig. 1. Interestingly, TK homologues of murid herpesvirus 4 (MuHV-4) and wood mouse herpesvirus (WMHV) branch separately from their counterparts encoded by alphaherpesviruses and even gammaherpesviruses, although MuHV-4 is generally accepted as an animal model for Epstein-Barr virus or Kaposi's sarcoma-associated herpesvirus infection (Rajčáni and Kúdelová, 2007). We observed similar results while analysing phylogenetic relationships of a partial sequence of OvHV-1 gH identified here (data not shown).

TKs catalyse phosphorylation of thymidine monophosphate and have a key function in the synthesis of DNA as they are part of reaction chain to incorporate thymidine into the DNA. Herpesvirus TKs are products of early genes and are involved in herpesviral DNA synthesis. Conserved regions in sequences of herpesviral TKs were identified, which have important function in ATP binding or nucleoside substrate recognition as it is summarized in Table 1.

Unlike host TKs, herpesvirus TKs display affinity to some nucleoside analogues, such as aciclovir and ganciclovir, as well as other recently developed analogues, e.g. 5′-fluoro-2′-deoxyuridine (FUDR), enabling their potential use as a selection marker and/or therapeutics (Kudelova and Matis, 2001). These prodrugs are not toxic to the host cell, but are converted to toxic substances by viral TK phosphorylation, thus prodrug-treated cells expressing herpesviral TK produce highly toxic metabolites that lead to cell death. In contrast, host cell TKs have more narrow specificity and are unable to phosphorylate and activate these alternative substrates. Utilizing these properties of herpesviral TKs made it possible to use them as negative selection marker for the production of TK-negative viruses and also to construct recombinant herpesviruses expressing heterologous gene(s) from deleted TK locus (Lowe *et al.*, 1987).

It should be noted that different herpesviruses display different susceptibility to individual nucleoside analogues, e.g. MuHV-4 TK is significantly more sensitive to FUDR than its HSV-1 counterpart (Rašlová *et al.*, 2000). Alternative capability of individual herpesviral TKs to recognize diverse substrates can be explained by structural variations in their nucleoside recognition site. In the above mentioned examples of MuHV-4 and HSV-1 there can be found changes within the conserved site 4 (refer to Table 1), where most herpesviral TKs have cysteine (C) or valine (V) in the first position, whereas MuHV-4 carries isoleucine (I) and WMHV leucine (L). In addition, most herpesviral TKs carry in the second position of the conserved site 4 a phenylalanine (F), while HSV-1, HSV-2 or BoHV-1 have a tyrosine (Y).

Identification and primary structure analysis of OvHV-1 TK enables its further characterization and potential construction of TK-negative, safe live OvHV-1 vector(s) for the expression of heterologous antigens, as OvHV-1 appears to be non-pathogenic for its natural host, the sheep.

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