

Biological and Genetic Differences Between Lung- and Brain-Derived Isolates of Maedi-Visna Virus

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Abstract. During the epidemic caused by maedi-visna virus (MVV) of sheep in Iceland, the pulmonary affection, maedi, was the predominant clinical manifestation. In some flocks, however, a central nervous system (CNS) affection, visna, was the main cause of morbidity and mortality. As there is only one breed of sheep in the country, host factors did apparently not play an important role in the different clinical manifestations. To obtain some information on possible viral genetic determinants of neurotropism and neurovirulence we studied both phenotypic and genotypic properties of two maedi-visna virus strains; a strain that was originally isolated from the brain of sheep with encephalitis (visna), and another strain isolated from the lungs of a sheep suffering from pneumonia (maedi). The brain isolate was found to grow faster in sheep choroid plexus cells than the lung isolate, whereas the growth rate in macrophages was similar for the maedi and visna virus strains. Intracerebral inoculation indicated that the visna virus isolate induced more severe brain lesions than the maedi isolate. In addition, a pathogenic molecular clone derived from a visna strain (KV1772kv72/67) was tested for growth in sheep choroid plexus cells and macrophages. The molecularly cloned virus retained the fast growth rate in choroid plexus cells. The nucleotide sequence of the env gene and the U3 of the LTR was determined for the maedi strain and compared to that of the visna strains. There was an 11.7% difference in deduced amino acid sequence in the Env protein and a 6% difference in the LTR. The molecular clone KV1772kv72/67 will be a useful reagent for characterization of viral determinants of cell tropism in vitro and possibly neurovirulence in vivo.

Key words: lentivirus, maedi-visna virus, neurovirulence, cell tropism

Introduction

Maedi-visna virus (MVV), one of the lentivirus subgroup of retroviruses (1) has a broad range of organ tropism, i.e. it causes encephalitis (visna), pneumonia (maedi), mastitis and arthritis in sheep (reviewed in 2). Maedi-visna virus was introduced to Icelandic sheep with the importation of apparently healthy sheep of the Karakul breed in 1933. During the epidemic in Iceland the main clinical manifestation was maedi. However, in some sheep flocks in a restricted area of the country, visna was the main cause of morbidity and mortality (3). The occurrence of severe CNS disease caused by MVV in a restricted area in Iceland has been a puzzle. Host factors apparently do not play a major role as there is only one breed of sheep in Iceland that has lived in almost total isolation for 1100 years (3). Epidemiological and experimental data indicate that differences in organ tropism may be due to viral genetic determinants. Results of earlier *in vitro* experiments support this

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view. Thus virus isolates from visna-affected brains were found to grow nearly twice as fast as isolates from maedi-affected lungs in choroid plexus cells (4).

The primary target cells of MVV *in vivo* are considered to be of the monocyte lineage (5–7) although viral RNA and DNA (8) as well as viral proteins (9) have been detected in other cell types including neuroglia, endothelial cells and lymphocytes. In infections with related lentiviruses, such as HIV-1 and SIV, brain derived virus isolates have been shown to be macrophage-tropic by a number of laboratories (10–12). Viral genetic determinants in the *env* gene seem to play an important role in the cellular tropism of the virus (13–18) and the long terminal repeats have also been shown to vary in the efficiency of directing replication in various cell types (19,20).

In this study we examined a number of biological properties of two closely related brain isolates, one of which is molecularly cloned, and a lung isolate of MVV in order to find out whether the brain isolates retain a neurovirulent property on transmission *in vivo*, and whether there is a difference in cell tropism *in vitro*. The *env* gene and the U3 of the LTR of the maedi strain KM1071 were sequenced and compared with the published sequence of the visna strain KV1772 (21) to obtain information on possible viral genetic determinants of neurovirulence and cell tropism.

Materials and Methods

Virus Strains and Cells

Maedi virus strain KM1071 was isolated in 1962 from the lungs of a sheep suffering from maedi, and has been through relatively few passages in tissue culture. Visna virus strain KV1514 is a descendant of a virus that was isolated from the brain of a sheep suffering from visna (22). This strain has been passaged extensively in sheep and tissue culture. Visna virus strain KV1772 is of the same lineage as KV1514 and they are 99% identical in nucleotide sequence (21). Strain KV1772kv72/67 is a pathogenic molecular clone derived from strain KV1772. Virus was propagated in monolayers of sheep choroid plexus cells (SCP) (1) and macrophages. Choroid plexus cells were grown at 37°C in a humidified atmosphere of 5% CO2 in Dulbecco's modified Eagles medium (DMEM) supplemented with 200 units/ml penicillin,

100 units/ml streptomycin, 2 mM glutamine and either 10% lamb serum (growth medium) or 1% lamb serum (maintenance medium).

Leukocytes were isolated from heparinized peripheral blood of healthy, uninfected sheep on a density gradient, Histopaque—1077 (Sigma Diagnostics). They were suspended in growth medium with 10% lamb serum and 5×10^{-5} M mercaptoethanol added, and counted and distributed at a density of 12×10^{6} / ml into 4×1 ml chamber with 18 mm² tissue culture slides (Permanox, Nunc, Inc). Cells were allowed to settle at 37°C in a humidified atmosphere of 5% CO₂ for 24 h. Then the supernatant and unattached cells were removed and the slide washed twice energetically with PBS before adding 1 ml of new growth medium to each chamber. Adherent cells were further incubated for at least 7 days before they were infected.

Virus Titration

The viruses were assayed by endpoint titration in roller tube cultures or 96-well flatbottomed tissue culture plates. Final readings of cytopathic effects were made after 2–4 weeks. The infectivity titres were calculated by the Reed-Muench method (23). Alternatively virus growth was monitored by assaying the supernatants of infected cells for RT activity. Viral particles from 0.5 ml of cell-free supernatants from infected cells were pelleted at 70,000 rpm for 10 min in a Beckman TLA 100.4 rotor.

Reverse Transcriptase Assay

Reverse transcriptase (RT) was determined as described previously (24). Briefly, 0.5 ml of cell-free supernatants from infected cells were pelleted at 70,000 rpm and 4°C for 10 min in a Beckman TLA 100.4 rotor. The pelleted virus was resuspended in 10 µl of NTE (100 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA) containing 0.1% Triton X-100. This disrupted virus was incubated at 37°C for 1 h in a 50-µl reaction mixture containing 50 mM Tris-HCl (pH 7.8), 20 mM MgCl₂, 2 mM dithiothreitol, 20 mM KCl, 0.25 optical density unit each of poly (rA) and oligo (dT_{12-18}) per ml, and 2.5 µCi of $[^{3}H]TTP$ (10–25 Ci/mmol; NEN). The reaction mixture (50 µl) was applied to DE81 filter paper discs (Whatman) and air dryed. The filters were washed three times in 5% Na₂HPO₄, once in H₂O, and at last in 70% ethanol. After drying, the filters were placed in vials with

Ultima Gold (Packard) and radioactivity was measured using a Packard Tri-Carb liquid scintillation counter.

Experimental Animals and Infection

Four outbred female Icelandic sheep, 7–12 months old, were infected with 0.3 ml of visna virus strain KV1514, titer $10^{6.5}$ TCID_{50/}^{ml} and three with maedi strain KM1071, titer $10^{6.4}$ TCID_{50/}^{ml}. The inoculations were done intracerebrally as described previously (25). The sheep were sacrificed three months post infection. The intracerebral inoculations were done in Vetenarcol anaesthesia.

Virus Isolation

Blood was drawn at regular intervals during the experimental period and at sacrifice. Buffy coat cells were tested for virus by inoculation onto SCP monolayers. Spinal taps were done repeatedly during the experimental period and at sacrifice. Cells were counted and virus isolated from the spinal fluid by inoculations onto SCP monolayers. At sacrifice the following organs were tested for virus by explantation: CNS (3 samples from the brain, and 3 from different levels of the spinal cord), lungs (1 sample from each lobe), spleen, bone marrow, cervical, mediastinal and mesenterial lymph nodes.

Histology

The following organs were sampled and processed for histological examination: Brain (left half), spinal cord (cervical, midthoracic and midlumbar), lungs (1 block from each lobe), spleen, bone marrow, lymph nodes (cervical, mediastinal and mesenterial), thymus, heart, kidneys, liver, adrenal gland, mammary gland, and synovia. The samples were fixed in 10% phosphate buffered formalin, embedded in paraffin and sections cut at 3–5 microns. The brain was cut at 9 standard levels. The sections were routinely stained with haematoxylin-eosin but in addition lymphoid tissues were stained with Giemsa, and selected sections from the CNS with Klüver-Barrera stain for myelin.

The brain lesions were graded on a scale 0-6 as described previously (Pétursson et al., 1976) with a slight modification: 1, definite minimal lesions (2–3 of the following histological changes: periventricular inflammation, perivascular cuffs in white and/or gray

matter, glial nodules, leptomeningitis, inflammation of the choroid plexus); 2, similar to 1 but more intense or widespread; 3, subconfluent but extensive periventricular lesions with some involvement of the white matter; 4, extensive confluent periventricular inflammation with some confluent involvement of the white matter; 5, as in 4 plus small necrotic lesions; 6, most severe cases of visna with more extensive necrosis. The lesions were assigned an intermediate grade, i.e. 0.5, if lesions were not observed in all main areas.

Cloning and Sequencing

Hirt DNA from strain KM1071 was isolated (26) from SCP cells after 3 days of infection. The DNA was digested with *SacI* and cloned into lambda gt11. The library was screened with ³²P-labeled 8.6 kb *SacI* fragment of visna virus. Of 48 positive clones, only 3 had the 3' end of the genome intact. One of these was subcloned into pUC 18 and M13 for DNA sequencing. Sequencing was performed by the dideoxy-nucleotide chain termination method (27) using Sequenase 2.0 as specified by the manufacturer (United States Biochemicals). Every base was sequenced at least three times.

Amplification of Sequences in the env Gene and LTR

For PCR amplification in the LTR the following primer set was used: 5'-GATTATCATGGGAATT-GTAGG-3' and 5'-BioCTTTCCTTCGAGCTCTCCA for subsequent purification of single strands using strepavidin-coated magnetic beads (Dynal), or 5'-GATTATCATGGGAATTGTAGG-3' and 5'-CCA-GGCAAGCTCAGATATCA-3' for subsequent cutting with SacI and HindIII and cloning into M13 phage. Amplification of the region containing the premature termination in the env gene was carried out using the following primer set: 5'-GCAAAATACAGTTGTG-AGAGTA-3' and 5'-TGGTCTCGGTGTCGCAAA-3'. The PCR was performed by using Taq DNA polymerase and subjecting the samples to 30 cycles of denaturing at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 1 min.

The region between the bases 7736 and 7979 was cut with *XbaI* and *PstI*, cloned into M13 and sequenced.

The error rate of *Taq* DNA polymerase has been estimated at 2×10^{-4} nucleotides/cycle. This error rate is not likely to affect our results, since we are

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looking for specific changes, i.e. a specific amino acid change in the *env* gene and a duplication in the LTR, and the PCR products were sequenced directly as well as cloned in M13.

Results

Growth Properties of Maedi Strain KM1071 and Visna Strains KV1514 and KV1772kv72/67 in SCP Cells and Sheep Macrophages

SCP cells were infected with the virus strains at various multiplicities of infection (m.o.i.), ranging from 0.5–10. Samples were taken at regular intervals for titration and RT assay. Fig. 1a shows that there is an initial lag in the growth of the maedi strain KM1071, it grows slower and to a slightly lower titer than the visna strains KV1514 and KV1772kv72/67. The average time for the visna strains to reach a titer of $10^{6.5}$ /ml was 100 h when inoculated at m.o.i. 0.5 whereas the maedi strain reached the same titer in

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180 h. At an m.o.i. of 10 the average time for the visna strains to reach a titre of $10^{6.5}$ /ml was 28 h and 36 h for the maedi strain (data not shown). This difference between the visna strains on the one hand and the maedi strain on the other was consistent regardless of whether the inoculum was determined by RT units or end-point titration. We were not able to find any consistent difference in the growth rates of the three strains in macrophages (Fig. 1b,d).

Intracerebral Inoculation

Virus isolations. Four sheep were infected intracerebrally with visna virus strain KV1514 and three sheep were infected with maedi strain KM1071. At sacrifice three months post infection virus was recovered from several organs, including brain, lungs, spleen, lymph nodes and bone marrow. The total frequency of virus isolation was comparable in sheep infected with visna strain KV1514 and those infected with maedi strain KM1071 except that virus was recovered more



Fig. 1. Growth curves of visna and maedi strains in sheep choroid plexus cells (a,c) and in macrophages (b,d) measured by titration (a,b) and reverse transcriptase (c,d). The cells were infected at an m.o.i. of 0.5.



Hours p.i.



B



Fig. 1. (Continued)



Fig. 1. (Continued)

frequently from the CNS in the visna-infected group (Table 1).

Histopathology. Definite visna lesions were found in the brain of all 4 sheep infected with visna virus KV1514. The lesion grades varied from 2 to 5, the average grade of lesions was 3.5 (Table 1). In those infected with the maedi strain KM1071 definite visna lesions were found in 1 out of 3. The average lesion grade was 0.7 (Table 1). Furthermore CSF cell counts were significantly higher in the visna infected group both one month p.i. and at sacrifice (Table 1). Fig. 2 shows the most severe brain lesions found in the sheep infected with maedi strain KM 1071 (a-d) and with visna virus strain KV1514 (e-h). The brain lesions showed the typical location and character of visna lesions (Fig.2). They consisted mainly of periventricular inflammation, sometimes extending into the adjacent white matter and leading in the most severe cases to confluent inflammation accompanied by myelin-breakdown. Leptomeningitis of a mild to severe degree was present and in the most severe

cases occasional glial nodules and/or inflammatory foci were observed in the cortex (Fig. 2). Inflammatory infiltration of the choroid plexus was commonly observed, occasionally very prominent with formation of active lymphoid follicles.

Other organs did not show any specific changes that could be related to the infection with maedi or visna virus.

Nucleotide Sequence of the env Gene

The nucleotide sequence of the *env* gene in maedi strain KM1071 is shown in Fig. 3 and compared to the sequence of visna strain KV1772kv72/67 (Andrésson et al., 1993). This comparison revealed an overall difference of 8% in nucleotides and 11.7% in amino acids. There are 26 potential glycosylation sites in the Env protein of maedi KM1071, 21 in the surface glycoprotein and 5 in the transmembrane protein, whereas there are 29 potential glycosylation sites in visna KV1772kv72/67. Twenty-three of these sites are common to the two virus strains, and one is shifted by

			Virus Isolation				
Sheep No.	Blood	Lymph.	Lungs	CSF	CNS	CSF Cells	CNS Lesions Grade ²
1532	0/2	2/2	_	0/2	2/3	434 ³ -97 ⁴	3
1533	0/2	1/2	0/1	0/2	1/2	198-113	2
1534	0/2	2/2	0/2	0/2	2/2		4
1535	0/2	1/2	0/2	1/2	1/2	393-57	5
Total	0/8	6/8	0/5	1/8	6/9		
average	0%	75%	0%	12.5%	66.7%	341-94	3.5
1878	1/4	2/2	1/2	0/2	1/3	63-12	\pm
1879	0/4	2/2	0/2	0/2	1/3	11-22	±
1880	0/4	2/2	0/2	1/2	1/3	173-14	2
Total	1/12	6/6	1/6	1/6	3/9		
average	8.3%	100%	16.7%	16.7%	33.3%	82-16	0.7

Table 1. Comparison of intracerebral inoculation of visna virus KV1514 and maedi virus strain KM1071. Three months post infection

Sheep no. 1532-1535 were infected with visna strain KV1514.

Sheep no. 1878-1880 were infected with maedi strain KM1071.

¹Number positive/number tested. ²Lesions graded on a scale 0–6. ³One month post infection. ⁴Three months post infection.

one amino acid (Fig. 3). Constant and variable regions were identified as described previously (28). A highly variable region at nt 7565–7717 near the carboxyl end of the extracellular glycoprotein carries two deletions of five and one amino acids. The regions coinciding with *rev* are also very variable (Fig. 4). A transition at nt position 7790 creating a premature termination codon was found in the clone of maedi KM1071 that was sequenced. Direct sequencing of a PCR product of this region in the Hirt supernatant revealed glutamine at this site as in visna KV1772.

Nucleotide Sequence of U3 of the Long Terminal Repeats (LTR)

U3 of the LTR of the maedi strain KM1071 was 6% divergent from visna KV1772. In the clone that was sequenced there was only one copy of a 43 bp repeat which is present in visna KV1772 and there was a 9 bp deletion in this region as well (Fig. 5). PCR products of the Hirt supernatant of maedi virus 1071 revealed that about half of the viral population had molecules of a size corresponding to two copies and the other half one copy of the 43 bp element.

Discussion

In this study we addressed the question whether strains of the ovine lentivirus MVV isolated from lungs (a maedi strain) and brain (a visna strain) are different with respect to neurovirulence and cell tropism and whether a molecular clone of a brain isolate can be used to map these phenotypic differences. Furthermore we have sequenced parts of the viral genome of the maedi strain and compared it to known sequences of the visna virus strain KV1772 (21).

Our findings *in vivo* indicate that there is a difference in neurovirulence between the maedi strain KM1071 and the visna strain KV1514. The visna virus strain KV1514 induced more severe CNS lesions than the maedi strain KM1071 and the CSF cell counts, another indicator of lesion activity in the brain, were higher in the visna virus infected group. There was, however, no qualitative difference in the lesion pattern between those infected with the maedi strain and those infected with the visna strain. The lesions were identical to those already described as characteristic for visna (2). Since the neurovirulence does not seem to be an all or none phenomenon, the number of sheep (4 for visna and 3 for maedi) are too low to draw statistically significant conclusions about



Fig. 2. Comparison of brain lesions in sheep infected by i.c. route with maedi strain KM1071 (a–d), lesion grade 2 and with visna virus strain KV1514 (e–h), lesion grade 5. a) Mild subconfluent periventricular inflammation; e) Severe confluent periventricular inflammation extending into adjacent white matter; b) Discrete infiltration of meninges; f) Severe meningitis; c) Normal white matter; g) Severe inflammation of white matter with myelin breakdown; d) Normal cortex; h) Perivascular infiltrates and glial nodule in cortex. H.E., bar 50 μ m.

Comparison of Lung- and Brain-Derived MVV

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Fig. 3. Nucleotide sequence of the env gene in the maedi KM1071 strain compared to the visna KV1772 strain. The fully printed sequence (and numbering) is that of KV1772. Sequence identity is represented by dashes, and nucleotide changes and deduced amino acids are indicated. Potential glycosylation sites are underlined. A stop codon created by a transition at nt position 7790 is indicated (*).

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CAAAATAGCAO	CATAACZ	ACCAATZ	ATOOGA	ACGAA	CACIA	ATIG	-		0104	ACATCI	ALCAA	AAT 1G	ÇIÇAA	GAIGI	AAC	AALA	JINGO	111111	CAC-AA	AUUA	GIQ	GACC	7615
Gln <u>AsnSerTh</u>	<u>r</u> IleIn	GlyIle	MetGly	ThrAs	nThr <u>A</u>	snTm	<u> Thr</u> Th	hrMe	tTrp#	snile	fyrGln	AsnCy	<u>/sSer</u> A	rgCys	s <u>Asn</u>	AsnS	<u>erSer</u>	LeuAs	pArgI '	hrGLyS	erGl	lyIhr	
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LeuGlyThrVa	lAsnAsr	Leuly	sCysSer	LeuPn	oHisA	rgAsr	GluSe	<u>er</u> As	nLys'I	rpIhr	Ys			Lys	sSer	GlnA	rgAsp	Ser	TyrI.	leAlaG	lyAı	gAsp	
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PheTrpGlyLy	sValLys	AlaLy	TyrSer	CysGl	uSerA	snLeu	GlyGl	lyLe	uAsp9	erMetN	1etHis I	GlnGl	nMetL	euLeu	Gln	ArgT	/rGln	/alIle	eArgVa I	alArgA	laTy	rThr	
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TyrGlyValVa	lGluMet	ProGl	SerTyr	MetGl	uAlaG	InGly	GluAs	snLy	sArgS	erArg/	rgAsn	LeuGl	nArgL	ysLys	Arg	GlyI	leGly	euVa	lilev	alLeuA	laI	leMet	
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GCAATAATCOC	TGCTGC/	AGGAGC	IGGICIC Clui au	GGIGN		ACGCC	GIGC/ Walc	AGCA 1nG1	NTCCI nSer1	ATACC2 VrThri	AGGACG ArroThr	GCIGI AlaVa	CCAGI CCAGI	CICFI erl <i>e</i> i	IGCI 1Ala	AACO	CAACIN LaThr	CIGO AlaAl	ocage aGluG	ACCAAC Inclut	NGL Valle	DAGAA ≃uGlu	8077
AIdIIEIIEAI	antanto	agi yan	agranen	oryva		1	avaro.	11 231		.yr mur	rgur	niave		CLINC	4110	2 501 2 7	101111					Jubic	
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GCATOGIAIGO	CATOGIN	ACAGCA	IATAGCC	AAAGG	ААТАА	GAAIC	CTAC	AGOC	AAGAG		AGOGIG	GAAGC	XCIGG	TAGAI	ICGA	ATGA	IGGTA	IACCA	AGAAT	TGGATI	IGCIT	TACOG	8197
AlaSerTyrAl	aMetVa	lGlnHi:	sIleAla	LysCl	yIleA	rgIle	LeuG	luAl	aArgi	alAla	ArgVal	GluAl	aLeuV	alAsr	Arg	MetM	etVal	TyrGl	nGluL	euAspC	.ysTi	pHis	
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TATCAACACTA TyrGlnHisTy	CIGIGI rCysVa	ACCIC 1ThrSe	AACAAGA rThrArg	AGIGA (SerGl	AGTAG uValA	CCAA1	riaig. nyrVa	TAAA al <u>As</u>	CIGG ²	CAAGA hragi	rttaaa Phelys	GATAA Asp <u>As</u>	CIGIA EnCysI	CCTGC) CAA pGlr	CAGT GlnT	Ile 30040 rpGlu	Hi: GAAGA GluGl	s AATAG uIleG	-A AACAAC	'ACG Hisg.	1933A luGly	8317
TATCAACACTA TyrGlnHisfy	CIGIGI rCysVa	AACCIC lThrSe	AACAAGA rThrArg 	AGIGA SerGl	AGTAG uValA	CCAAI AlaAsi	TAIG TyrVa	TAAA al <u>As</u>	CTGG4 n[1n2]	CAAGA: <u>hr</u> Argi	ritaaa Phelys	GATAA Asp <u>As</u>	LCIGIA SnCysI	CCTGC hrTn	3CAA oGlr	CAGI GlnI 	Ile 30GAG mpGlu	Hi: GAAGA GluGl	s AATAG uIleG	-A AACAAC luGlnF	ACG HisG	NGGGA LUGIY	8317
TATCAACACTA TyrGlnHisTy	CIGIGIX rCysVa	NACCIC lThrSe	AACAAGA rThrArg A- I vesting	AGIGA SerGl	AGTAG uValA G	CCAAI AlaAsi T	rTAIG ATyrVa	TAAA al <u>As</u>	CTGG# n/Tng/ T	CAAGA <u>hr</u> Argi G	PTTAAA PheLys	GATAA Asp <u>As</u>	CICIA EnCysI	CCTG hrTn	3CAA pGlr	CAGT GlnT A-	Ile 39349 rpGlu A	Hi: GAAGA GluGl	s AATAG uIleG G	-A AACAAC luGlnF	74037 Hisgi T	NGGGA NuGly C- Ala	8317
TATCAACACTA TyrGlnHisTy 	CIGIGI TCysVa	ACCTC IThrSe	AACAAGA rThrArg A Lys	AGIGA SerGl	AGTAG uValA G	CCAAI AlaAsi T	rTATG ATyrVa	TAAA al <u>As</u>	CTGG n[Trp] T	CAAGA <u>hr</u> Argi G	Phelys	GATAA As <u>pAs</u> 	CIGIA anCysI	CCTGC <u>hr</u> Trr	CAA cGlr	CAGI GlnT A-	Ile 30040 rpGlu A	Hi: GAAGA GluGl	s AATAG ulleG G	-A AACAAC IuGlnF 	74037 Hisgi T	NGOGA NuGly C- Ala	831
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCysVa 	AACCTC	AACAAGA rThrArg A- Lys AGCAGCA	AGIGA Sergi TTACA	AGIAG uValA G AGIAC	CCAAI LaAst T	riaig. nTyrVa	TAAA al <u>As</u> AAAG	CTGG n[1p] T	CAACAI InrArgi -G CTACCI	TTTAAA PheLys	GATAA Asp <u>As</u> 	ACIGIA INCVI INCVI INCVI	CCTGC InrTrg 	Glr	CAGI GINT A- ATAC	Ile 300AG npGlu A	Hi: GAAGA GluGl GluGl	s AATAG ulleG G TAACT		ACC Hisci T	1003GA 100Gly 1 C- Ala 00FTTC	831 [.] 843 [.]
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCysVa AITACIX RULeuLe	AACCTC IThrSe CAGAGA JArgGlu	AACAAGA rThrArg A- Lys AGCAGCA UAlaAla	AGIGA (Serg) (TTACA Leug)	AGTAG UValA G AGTAC nValH	CCAAT \laAs T ACAT HisIle	riaig niyrva Agcici eAlag	al <u>As</u> AAAG	CTGG n(Ing) T AGATG gAsp/	CAACA: hrArgi -G CTACC: ilaArgi	TTTAAA PheLys 	GATAA Asp <u>As</u> CCAG A ProAs	CIGIA EnCysI VIGCII 5pAlai	hrTr	CAA Colr AGCA	CAGT GlnT A- ATAC illeG	Ile 303AG mpGlu A	Hi: SAAGA GluGl GluGl SCATT AlaPh	s AATAG UILEG G TAACT IEASMI	-A AACAAC luGlnH GGICCI MDSer:	ACG Lisg: -T T TCTTC SerT	NGCA NGLY Ala SETTC npPhe	8317 8437
TATCAACACTA TyrGlnHisTy AACTTGAGTCT AsnLeuSerLee A	CIGIGIX TCysVa ATTACIX ULeuLea	AACCTC IThrSe CACACA JArgGlu	AACAACA rThrArg Lys AQCAQCA UAlaAla 3	AGIGA SerGl , ITIACA LeuGl	AGIAG UValA G AGIAC nValH	CCAAI LaAs T ACAI LisIle	rivits nTyrVa AGCTCI eAlaG	TAAA al <u>As</u>	CTGG n[112] T AGATO gASp	CAAGA ImArgi -G CIACC LIACC LIAArgi T-	TTTAAA PheLys YCAATC ArgIle	GAIAA As <u>pAs</u> CCAGA ProAs	CIGIA nCysi nCysi NGCII spAlai j T	ccTGa hrTn GGAA/ npLy: 00	SCAA oGlr SGlr SAla SAla	CAGI GINI A- ATAC IIIeG	Ile 3334G npGlu A	Hi: SAAGA GluGl GluGl SCATT AlaPh	AATAG UILEG G TAACT IEASMI	-A AACAAC luGlni- GGICCI InSers	ACG Hisci T ICTIC SerTi	AGGGA luGly Ala corric npPhe	8317 8437
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCYSVa ATTACIX ALLEULA	AACCTC IThrSe JACACA JACACA	AACAACA rThrArg A Lys AGCAGCA UAlaAla G	AGIGA (SerGl:)))))))))))))))))))	AGFAG UValA G AGFAC nValH	CCAAI \laAs T ACAII HisIle	riaig niyrVa Agcito eAlag	IAAA AAAG InAr	CIGG n[11] T AGATO gAsp/	CAAGA hrArgi -G CTAGG llaArgi T- Sei	TTTAAA PheLys 	GATAA Asp <u>As</u>	CIGIA mCysI	<u>in</u> Trp	CAA cGlr AGCA sAla 3G	CAGT GlnT A- ATAC ulleG C-G- Leu	Ile 300AG npGlu A AAGAA InGlu	Hi: GAAGA GluGl	AATAG ulleG G TAACT MAACT MAACT MAACT MAACT ASp	-A	ACG Lisc: T TCTIC SerT:	WOGA luGly Ala OSTIC rpPhe	8317 8437
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCysVa ATTACIX NLeuLeu 	AACCIC IthrSe CAGAGA JArgGlu	AACAACA rThrArg Lys AGCAGCA UAlaAla G	AGIGA (Sergl) TTACA Leugl	AGIAG UValA G AGIAC nValH 	CCAAN T CACAN Hislle	TATG. MyrVa AGCTC eAlaG	TAAA al <u>As</u> AAAG lnAr	CTGG nThy I T GASP gASP	CAAGA ImrArgi G CIAGG LIAArgi 	TTTAAA PheLys AGAATC Argile	GATAA Asp <u>As</u>	CIGIA <u>mCysI</u> vigciti spAlai T Val	thrTh 	acaa ogir Agca sala JG r	CAGIT GlnI A- ATAC AILEG C-G- Leu TIGC	Ile BOGAG IpGlu A AAGAA InGlu	Hi GAAGA GluGl GCATT AlaPh	S AATAG UILEG G TAACT IAACT II CG Asp GCAAG		ACC lisc: -T CTTC SerT	AGOGA luGly Ala AGTTC cpPhe	831 [°] 843 [°] 855
TATCAACACTA TyrGlnHisTy 	CTGIGIY TCysVa ATTACTO ALEULA ATTATAT STyrIlo	AACCTC IThrSe CAGAGA JArgGli ACCCTG ProTrj	AACAACA rThrArg A- Lys AQCAQCA UAlaAla GATTAIC pIleIle	AGIGA SerGl MITACA LeuGl AIGGG MetGl	AGIAG UValA G AGIAC nValH yIleV	CCAAI \laAsr T ACAII HisIle HisIle 	TATS. MyrVa AGCTC2 AlaG HTAA /LeuM	IAAA alAs AAAAG InAr IGIG	CTGG n[11] T GASD GASD TTTT SPhe/	CAAGA ImrArgi G CIACG LIAArgi LIAArgi Ser	PTTAAA PheLys XGAATC ArgIle CTAATG	GATAA Asp <u>As</u> CCAG A ProAs ITGIGI	CIGIA <u>mCysI</u> spAlaT T Val IILeS	the Tra the Tra the Tra the Tra GGAAZ GGAAZ Tra GGAAZ Tra CAATC er Met	AGCAA oglir AGCAA sAla JG r SIGI	CAGT GlmI A- ATAC UILEG C-G- Leu TTGC Leu LeuG	Ile 303AG rpGlu A InGlu AG3CT InAla	Hi: SAAGA SluGl OCAIT AlaPh IACAA IyrLy	S AATAG UILEG G TAACT EASTI II CG ASP GCAAG SGInV	-A	ACC Hisc: T CTTC SerT: SerT: SerT:	luGly Ala corric cpPhe rCAGA leArg	831 [.] 843 [.] 855 [.]
TATCAACACTA TyrGlnHisTy 	CTGTGTY rCysVa ATTACT alleule 	ACCTC IThrSe AccCIG	AACAACA rThrArg Lys AQCASCA UAIAAIA GATTAIC pIleIle	AGIGA SerGl TTACA LeuGl AIGOG MetGl	AGIAG UVALA G nValH AGIAC nValH 	CCAAT laAs T CACAT tisIle 	TATS. MyrV AGCTC ALAG	IAAA AAAG InAr IGIG etCy	CTGG mTro T T AGATO gAsp TTTT sPhez	CAAGA hrArgi -G CTACC laArgi laArgi Ser	TTTAAA PheLys AGAATC ArgIle CTAATG	GAITAA Asp <u>As</u>	CIGIA anCysI anCysI algeria apAlaT b yal yal yal yal	· furTry · · · · · · · · · · · · ·	CAA cGlr sAla sAla 	GlnT A- ATAC ulleG C-G- Leu Leu Leu	AA Ile mpGlu A InGlu A InGlu A	Hi: GAAGA GluGl GCAITT AlaPh IACAA IyrLy	S AATAG UILEG G TAACT IAACT IAACT IAACT ASP GCAAG SGInV	-A	ACG HisG T CTTC SerT SerT	AGGGA luGly] C- Ala	8317 8437 8557
TATCAACACTA TyrGlnHisTy 	CIGICIY TCysVa ATTACIN ALLeuLeu ALLeuLeu ALLeuLeu ALLeuLeu ATATATIY STyrII G	ACCTC IThrSe CACACA JArgGlu ACCCTG ProTrg	AACAACA rThrArg Lys AGCACA AGCACA AACAACA SATTAIC pIleIle	AGIGA SerGl TTACA LeuGl AIGGG MetGl	AGIAG UValA G AGIAC nValF yIleV J L	CCCAAI LaAst T CACAIF HisIle MAGOC /alGly /alGly 	TAIG MyrV AGCTC ALaG ALaG ALaM ILeI ILeI	TAAA alAs AAACC lINAr IIGIC etCy -A le	CTICCF mTmp1 T AGATC gASp1 mTTTTF SPheF	CAAGAI ImArgi G CIAGGI IlaArgi T. Ser	Argile CTAAIG	. GAITAA As <u>pA</u> . . CCCAG4 ProAs	CIGIA mCvsI ancvsI ancvsI pAlaI j T Val canat allles	CCTOX hrrm 	acaa cGlr sAla sAla 	CAGIT GlmI A- ATAC UILEG C-G- Leu TTICC LeuG	Ile Ile ICC ICC ICC ICC ICC ICC ICC IC	Hi: GAGA GluGl GCATT AlaPh IACAA IyrLy	S AATAG UILEG G TAACT I I AASP GCAAG SGINV A	-A Plugini Concri Inser: Inse: In	ACC HisG —T- CTTC SerT SerT Slu	, AGGA luGly Ala CAGTIC AGTICACA ICAGA leArg	831 ⁻ 843 ⁻ 855 ⁻
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCYSVa ATTACIX MLeuLeu LeuLeuLeu LeuLeuLeu LeuLeuLeu LeuLeu LeuLeu LeuLeu LeuLeu LeuLeu LeuL	ACCTC IThrSe CAGAGA DArgGlu ACCCTG ProTrg	AACAACA rThrArg A- ys AQCACA JALAALA JALAALA JALAALA , GATTAIC pIleIle	AGIGA [SerGl 	ASTAC UVALA G nValH yIleV I I	CCCAAI LaAsr - - - - - - - - - - - - -	TAIG TYTAG AGCTCI ALAG JITAA A-AM ILA	· TAAA alas · · AAAAG InAr · IGIG etCy -A le ·	CTGGP nTup] T gAsp7 gAsp7 TTTTP sPhe7	CAAGAI ImArgi G LiaArgi T- Ser CAATAA rgIlel	Phelys AGAATC Argile CTAATG	CGATAA Asp <u>A</u> sp <u>A</u> s CCCAG P ProAs	ACIGIA ACVSI ACVSI ALICIT SpAlaT J J Val CATAT ALILES	CCTQC hrTn 	AGCA AGCA SAla JG T SIGI	CAGIT Ghrii A- ATACC IIleG C-G- Leu TTICC LeuG	Ile 33346 pGlu pGlu pGlu pGlu AAGAA InGlu AAGAA InGlu InAla InAla	Hi: GAACA GluGl GCATT AlaPh TACAA IACAA	AATAG ulleG G ITAACTI IRACTI Asp GCAAG sGInV A	-A AACAAC ClucInf Concern Con	ACCI HisG —T CCTIC SerT SerT SacA	1033A 1033A	831 [°] 843 [°] 855 [°]
TATCAACACTA TyrGlnHisTy 	CIGIGIY TCYSVa ATTACIN MICULAN ATTACIN MICULAN STYRIIO G AACAGIN	ACCTC ThrSe ACAGA ArgGlu ACCCTG ProTry CortCAT	AACAACA rThrArg A- ys AQCACA JALAALA 3	AGIGA SerGl ITTACA LeuGl ATGGG MetGl	AGTAG UVALA G AGTAC NVALH AGTAC VILEV JICON	CCCAAN	TARGANA	· TAAA alAs · · · · · · · · · · · · · · · · · · ·	CTGG2 nTh2J T AGATC gASpJ SPhe/ AAAAA	CTACA InrArgi G CTACC LIAArgi T- Ser T- Ser 	NTIAAA PheLys VGAATCO Argile CIAATG CIAATG CAMPE	GATAA GATAA CCCAG2 ProAs IIGIGI UCCYSV2	ACTORIA ACTORITORIA AC	CCTGC hrTrn	CCAA cGlr AGCA sAla I I I I I I I I	CAGIN GlmI A- ATACC Leu Leu Leu Leu AGCT	Ile 33346 pGlu pGlu pGlu AAGAA InGlu A AGGCT InAla InAla C	Hi: GAAGA GluGl GCATT AlaPh IACAA Iyrly	AATAG MATAG UILeG G ITAACTI IIAACTI ReASTI ASp CCAAG SGINV A GAGAA		ACCT isc CTTC SerT: CASA Salari Lass	ACCA	831 ⁻ 843 ⁻ 855 ⁻ 867 ⁻
TATCAACACTA TyrGlnHisTy 	CTGTGT TCYSVa ATTACIN ATTACIN ATTACIN U ATTATATI STYTIL G G AACAGIU LIThrVa	ACCTC InhrSe ACAGA ArgGlu AccCTG aProTry CorteAn IvalII	AACAACA rThrArg A- Lys A- Lys A- 	AGIGA (SerGl) (TIACA LeuGl MetGl MetGl (CC33I ProVa	AGTAG UVALA G nValH	T 	TTAIG MYAV HOCTCI PACTCI PALAC ILE ILE ILE ILE ILE ILE ILE ILE ILE ILE	· IAAA · · · · · · · · · · · · · · · · ·	CTGGF n[]m] T gAGAIC gAGAIC gAGAIC sPhe/ AAAAA/ nLys/	CAAGA InrArgi G CTAGG LIAArgi CTAGG Sei	ITTAAA PheLys VGAATCO CTAATGIle CTAATGIL CTAATGILE CTAAT	CCAG2 ProAs CCAG2 ProAs	ATTOCH ATCOM ATCOM ATTOCH ATTO		CAA CGIr AGCA SAla JG r SIGI CCys CCys CCys CCys SAla	CAGT Glm A- ATAC UILEG C-G- Leu TTGC Leu	Ile CCAG TPClu IpClu IpClu IpClu InClu InClu InClu InAla InClu InAla	Hi: SAAGA GluGl GCATT AlaPh IACAA IJYIJY CCCCA ArgGl	AATAG MITAG G TRACTI TRACTI TRACTI CG Asp CCAAG SGINV A GAGAA WArgA	-A AACAAC IluGIni GGTCCTI IDSERS - ITAAAGC - - - - - - - - - - - - -	ACCF Hisci T Certi Serti CAGA Slu Serti	LUGIY Ala 30TTC	831 ⁻ 843 ⁻ 855 ⁻ 867 ⁻
TATCAACACTA TyrGlnHisTy 	CTGTGT TCYSVa ATTACIN ATTACIN ATTACIN U ATTACIN STYTIL G G ACAGIN LIThrVa	AACCIC IThrSe CACAGA AACCTG ProTry COCCTG ProTry COCCTG ProTry COCCTG	AACAACA rThrArg A- Lys A- Lys A- 	AGIGA (SerGl) (TIACA LeuCl AIGGG MetGl (CCG3I ProVa	AGTAG UVALA G AGTAC AGTAC NVALH I L CGAAT I GGAAT I GGAAT I GGAAT I GGAAT I GGAAT I AC	COCAAN lass T T T	TAIG MyrV PCCTC2 PAIAG MTTAA MACM IleI STAAA SIUI? A A	· IAAAA · AAAAC InAr · IGIC etCy -A le · AACA ysGl -G	CTGG2 mTmm T gAsp2 mTTTTP sPhe2 AAAA2	CAACAO ImArgi ImArgi I CTACGC I I CAACAO CAATAA CAATAA CAATAA CAAACAO CAAACAO	ITTAAA PheLys VGAATCO ArgIle CIPAATG CIPAATG CAUNET CIPAATG CIPAATG CIPAATG CIPAATG CIPAATG CIPAATG CIPAATG CIPAAA	GATIAA Asp <u>A</u> sp <u>A</u> CCCAG 2 CCCAG 2	ATOCH ATOCHA	CCTGC hrTrr GGAAI TrLyz Thr CAATG	AGCA AGCA SAla SAla G-G Cys SIGI SIGI SIGI SIGI SIGI SIGI SIGI SIG	CAGITI GlmI A- ATACC C-G- Leu TTICC Leu	Ile Ile Ile Ile Ile Ile Ile Ile	Hii SAAQA SluGl SCATT AlaPh IACAA IYALy CCCCA ArgGl I -A	S AATAG UILEG G TAACTI TAACTI IAACTI CG Asp CCAAG SGINV A GAGAA A GAGAA	A-CAAC lucini gestoon inceri MIAAACC allyso allyso catomic catomic allyso catomic catom	ACCI HisCI —T- TCTTC SerT: SlnI SlnI Slu FCCCZ SerH	AGGAA JuGly C- Ala GOTTC Ala TCAGA IcArg LeArg	831 ⁻¹ 843 ⁻¹ 855 ⁻¹ 867 ⁻¹
TATCAACACTA TyrGlnHisTy 	CIGIGIE TCYSVa ATTACIN ALEULA ATATATA STATIL G AACAGIN IJIInVa 	AACCIC IThrSe CACACA AACCIG 2ProTr 301CAT IVal11	AACAACA rThrArg A- Lys A- Lys A- 	AGTGA SerGl MITIACA LevGl ATG3G MetGl 	AGTAG UVALA G nVAL+ 	CCCAAT lass T T T	HTANG MyrVi AGCTCI Alex Martak Alex Martak Ileli SGAAA SGUI; A	ITAAA alAs AAAAG InAr IGIG ectCy -A le AAAA ! I aAAAA !	CTCCP nTrol T gASpl TTTTQ sPhe/ AAAAA nLys/	CAACAO IntArgi I 	YTTAAA Phelys Argale CTAATC CTAATC CTAATC CTAATC CTAATC CTAATC CTAATC CTAATC	GAITAA Asp <u>A</u>	ATOCIT TEXT ATOCIT ATOCIT ATOCIT ATOCIT ATOCIT A ATOCIT A ATOCIT A ATOCIT A ATOCIT A A ATOCIT A A A A A A A A A A A A A A A A A A A	CCTCC hrrTrr GGAAV TrpLy:	AGCA SAla SAla JG SIGI CCys SALa	CAGITI GlmI A- ATACC Lau Lau Lau Lau Lau Lau Lau Lau Lau Lau	Ile Ile Ile Ile Ile Ile Ile Ile	Hii SAAGA SluGl SCAITT AlaPh IACAA IYrly CCCCAA ArgGl I -A His	S AATAG UILEG G ITAACTT ITAACTT II CGG ASP CGAAG SGInV A GAGAA A L	A	ACCF HisCI I TT SerT SlnI SlnI SlnI Slu CCCF SerH G	AGGAA JuGly C- Ala GTPhe	8311 8437 8557
TATCAACACTA TyrGlnHisTy 	CIGIGIE TCysVa MITACIU ALEULA ALEU	AACCIC IthrSe CACACA ACCCIG 2ProTh 2010AII 1001111	AACAACA	AGTGA Sergi ITTACA LevGl ATG3G MetGl ProVa	AGTAG WValA AAGTAC MValH - - - - - - - - - - - - - - -	CCAAI AlaAs T ACAI HAGO ALGI 	TTAIG: flyrVi GCTC: GTTAA A A IleI: Glui; I Clui; I Clui; Cl	IAAAG INAY IIGIC etCy -A le AACA ysGl -G	CTIGGY ITTID T AGATO GAGATO TTTTIZ SPhe? AAAAA ATTID SPhe? AAAAA ATTID SPhe? AAAAA CAGATO GAGATO	CAACAA	YTTAAA Phelys Argale CTAAIC CT	GAITAA AS <u>pAs</u> CC2AG4 ProAs	ACIGIA ACCIGIA ACCONT ACCON	CCTGC hrTry CGGAAA rpLyr CGGAAT CAATC	ACCA ACCA SAla J JG r SIGI CCys SALa SALa	CAGIMI GlmI A- C-G- Leu TIGC Leu SerL	Ile Ile Ile Ile Inglu Ing	Hii SAAGA SluGl SCAITT AlaPh INCAA Iyrly CCCCA ArgGl I -A His AIATT	s AATAG UILeG G TRACTI TRACTI TRACTI TRACTI CG Asp CGAAG GAAAG CAAAG SGINV L CGAGAA L CGAGAA	AACAAC lugini moser aliyes ali	ACCI Hisci ————— Cerri Serri Serri Serri Silu CCCC SerH G	AGGA LuGly agric cpPhe compression leArg compression leArg compression compression leArg compression	831 ⁻ 843 ⁻ 855 ⁻ 867 ⁻ 879 ⁻
TATCAACACTA TyrGlnHisTy 	CIGIGIE TCysVa ATTACIN alcule alcule antarian astyrild G AACAGIN lThrVa e SATAIGS nileIng	AACCIC IThrSe 22GAGA AARGGI AACCIG 22F0Th 23GGAT IVal II. 23GGAT 23GGAT 23GGAT 23GGAT 23GGAT 23GGAT	AACAACA rThrArg A- Lys A- Lys A- 	ASTGA SerGI ITTACA LeuGI ATGGG MetGI ProVa ITGGGC ITrpAI	AGTAG WalA AGTAC NAIH - - - - - - - - - - - - - - -	CCAAI AlaAs 	PTAIG MyrVa AGCTC: eAlaG ATTAM /LeaM IleI: SGAAA A CTCAO rSerP	· ITAAA alas · · · ITGIC · · · · · · · · · · · · · · · · · · ·	CTIGGP ITTTT T AGATO GAGA TTTTTP SPhe? AAAAA AAAAA AAAAA AAAAAA AAAAAA AAAAAA	CAACAA hrangi - - - - - - - - - - - - -	ITTAAA PheLys 	GAIAA Asp <u>As</u> CCAG4 ProAs	ACIGIA ACIGIA ACIGIA ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGCIT ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGIA ACIGCIT ACIG	CCTOC hrTry CGGAAA ipLyy CAATC CAATC	CCAA cGlr SAGCA SAla SAla 	CAGIT GlmI A- ATAC UILEG C-G- Leu TTCC LeuG	Ile 33349 IpGlu IpGlu IpGlu InGlu InGlu InGlu InAla InGlu InAla InGlu InAcorr IACACI ICACI ICACCI IACACI ICACCI IACACI ICACI ICACI	Hi. SAACA SluGl SCATT Alaph IALAPH IALAP	s AATAG UulleG G TRACTI TRACTI TRACTI TRACTI CG Asp CGAAG GAAAG CAAAG SGINV I L CGAQAA L CACAAA CACAAA CACAAA L CGGIGA UVAII	AACAAC Jucini GGROCI InSer JINAACC Allyss GAACAI rgHrs A ys JIAACC GAACAI A ys	ACCI Hisci ————— CETTI SerTi SerTi SerHi SerHi GetHi Gatta	AGGA LuGly C- Ala 30TTC cpPhe trCAGA LeArg La MTCGC cisArg La MTCGC galaction gala	831 ⁻ 843 ⁻ 855 ⁻ 867 ⁻ 879 ⁻
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Fig. 3. (Continued)



Fig. 4. Differences between maedi KM1071 and visna KV1772 virus envelope genes. The number of nucleic acid changes in 30 nucleotides were counted. The shaded area represents silent mutations and the black area represents nonsynonymous changes. Deletions are not counted.

the difference in neurovirulence. However, cumulative results with 10 intracerebral inoculations with the visna strains KV1514 and KV1772 showed that the average lesion grade was 3.5 and never below 2. This agrees with the results for visna virus presented in this paper and strengthens the indication that there is a real difference in neurovirulence between the visna and maedi strains. Infections with the molecularly cloned visna virus result in brain lesions of similar severity as with the uncloned visna virus (21).

Our findings of a higher growth rate of the visna virus strain KV1514 than the maedi strain KM1071 in SCP cells is in accord with earlier reports on *in vitro* behavior of other maedi and visna strains (4) and is

reflected in the fact that end-point titration of maedi virus strains in SCP cells usually takes three weeks whereas it takes two weeks with visna virus strains. The possibility cannot be excluded that this difference is due to adaptation of the visna virus strains to SCP cells. It is, however, of interest in this context, that KV796, a common ancestor of the visna virus strains in this study, was originally isolated from choroid plexus (1). The choroid plexus may be the initial site of entry for the neurotropic strains of MVV as has been suggested for infection with HIV-1 (29). This is supported by a high frequency of virus isolations from the choroid plexus in a large group of sheep infected with KV1514 (25), the density of antigen positive





cells in choroid plexus (9) and the initial CNS lesions, i.e. inflammation of the choroid plexus and of the subependymal region (2).

The molecularly cloned visna virus KV1772 kv72/ 67 retained the high growth rate of the visna strains in SCP cells and will therefore be a useful tool to map viral genetic determinants of the different cell tropism of maedi- and visna virus strains.

In macrophages, however, the growth rate was similar for the three strains. Thus the difference in neurovirulence in the MVV strains that we describe in this paper, which is quantitative rather than qualitative, is apparently not a special feature of macrophage-tropic MVV strains, in contrast to findings in other lentiviruses, i.e. HIV-1 (30) and SIV (31,32).

Sequencing of the *env* and LTR genes of the maedi strain KM1071 revealed an 11.7% difference in the Env protein when compared with known sequences of the visna strains KV1514 and KV1772 (33,34,21). A highly variable region was identified near the carboxyl end of the outer glycoprotein. This region includes a stretch of approximately 25 amino acids which lies between conserved cysteine residues and is largely hydrophilic (35) and may be analogous to the V3 loop of HIV (36).

Stop codons in the env gene seem to appear at a higher frequency in MVV than would be expected by chance. The first published sequence of MVV contains a stop codon in the env gene (33), and of four clones that have been sequenced of MVV strain KV1772, two contained stop codons in the env gene, and in one of the clones the stop codon occurred at the same site as we found in the maedi strain KM1071. These termination codons occur either in the last part of the outer glycoprotein or in the transmembrane protein. Sequencing of PCR products directly from tissue samples from infected sheep indicate that these premature stop codons are not as common in vivo as in vitro (data not shown). Stop codons have also been found in the env genes in SIV and HIV-2 (37,38). In these cases the stop codons occurred in the cytoplasmic domain of the transmembrane glycoprotein and were believed to arise in in vitro cultures.

We found considerable heterogeneity in the U3 of the LTR in the MVV strains as has been reported by others as well (39). There are several potential AP-1 binding sites in the MVV U3 region. The AP1 site most proximal to the TATA box has been shown to be active in transcription in the visna strain KV1514 (40). In maedi strain KM1071 this AP1 motif is imperfect, and this holds true also for other MVV strains that have been sequenced (28,41). In the visna strains, however, either of the two potential AP1 sites proximal to the TATA box contain a perfect AP1 motif. This may result in a higher rate of replication in the visna strains than in the maedi strains. Sargan et al. examined a number of LTR variants from the EV1 isolate for transcriptional activity in SCP cells and included the visna strain KV1514 described here. A higher rate of transcription was consistently observed from the KV1514 LTR than from the LTR of the different EV1 isolates (39).

We cannot deduce from the nucleotide sequence where in the genome the difference in neurovirulence and cell tropism resides, but further studies involving construction of chimeric viruses using the pathogenic molecular clone of visna virus are in progress.

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