



**HAL**  
open science

## Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes

M.L. Carrozza, M. Mazzei, D. Lacerenza, L. del Chiaro, M. Giammarioli, C.  
Marini, D. Rutili, S. Rosati, F. Tolari

### ► To cite this version:

M.L. Carrozza, M. Mazzei, D. Lacerenza, L. del Chiaro, M. Giammarioli, et al.. Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes. *Veterinary Microbiology*, 2009, 137 (3-4), pp.369. 10.1016/j.vetmic.2009.01.032 . hal-00485539

**HAL Id: hal-00485539**

**<https://hal.science/hal-00485539>**

Submitted on 21 May 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes

Authors: M.L. Carrozza, M. Mazzei, D. Lacerenza, L. Del Chiaro, M. Giammarioli, C. Marini, D. Rutili, S. Rosati, F. Tolari



PII: S0378-1135(09)00053-4  
DOI: doi:10.1016/j.vetmic.2009.01.032  
Reference: VETMIC 4347

To appear in: *VETMIC*

Received date: 11-6-2008  
Revised date: 15-1-2009  
Accepted date: 21-1-2009

Please cite this article as: Carrozza, M.L., Mazzei, M., Lacerenza, D., Del Chiaro, L., Giammarioli, M., Marini, C., Rutili, D., Rosati, S., Tolari, F., Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.01.032

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected**  
2 **with different SRLV genotypes.**

3

4 Carrozza ML.<sup>1</sup>, Mazzei M.<sup>2</sup>, Lacerenza D.<sup>3</sup>, Del Chiaro L.<sup>2</sup>, Giammarioli M.<sup>4</sup>, Marini C.<sup>4</sup>, Rutili  
5 D.<sup>4</sup>, Rosati S.<sup>3</sup>, Tolari F.<sup>2</sup>

6 1- Scuola Normale Superiore, 56126, Pisa, Italy;

7 2- Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, Facoltà di Medicina  
8 Veterinaria, 56124, Pisa, Italy.;

9 3- Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, Facoltà di Medicina  
10 Veterinaria, 10095 Grugliasco, Torino, Italy.;

11 4- Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, 06126 Perugia, Italy.

12

13 Corresponding author: Mazzei M., tel: +39 050 2216964; fax: +39 050 2216941;

14 [mmazzei@vet.unipi.it](mailto:mmazzei@vet.unipi.it)

15 **Abstract**

16 Synthetic peptides were generated, corresponding to SU5 domain of envelope glycoprotein of  
17 Italian SRLV isolates It-561 and It-Pi1, belonging respectively to MVV- and CAEV-like  
18 genotypes. The peptides, encompassing an N-terminal variable and a C-terminal conserved  
19 antibody-binding site, were used in an ELISA assay to analyse the sera of two groups of sheep  
20 experimentally infected with these isolates. The kinetics and specificity of the humoral response  
21 to the homologous and heterologous antigen and the affinity maturation of the sera were  
22 evaluated. Seroconversion occurred between week 3 and 8. The response to SU5 antigen was  
23 mostly type-specific. The few broadly reacting sera may reflect the production of antibodies

24 directed to the SU5 constant antibody-binding site. All sera underwent with time avidity  
25 maturation, resulting in the appearance of high affinity antibodies. This study suggests constant  
26 monitoring of the circulating viral variants to develop a panel of diagnostic peptides  
27 representative of local genotypes.

28

## 29 **Keywords**

30 Small Ruminant Lentiviruses, envelope glycoprotein, SU5 peptides, type-specific  
31 seroconversion.

## 32 **Introduction**

33 Maedi Visna Virus (MVV) and Caprine Arthritis Encephalitis Virus (CAEV) are non oncogenic  
34 lentiviruses of the Retroviridae family, presently referred to as Small Ruminant Lentiviruses  
35 (SRLVs) (Zanoni, 1998). A recent phylogenetic classification of these viruses defines two main  
36 groups, A and B, including respectively MVV-like and CAEV-like genotypes (Shah et al., 2004).  
37 SRLV infections occur almost worldwide and cause relevant economic losses. Therapy and  
38 effective vaccine are not yet available (Gonzalez et al., 2005; Petursson et al., 2005;  
39 Torsteindottir et al., 2007; Niesalla et al., 2008; Reina et al., 2008), therefore eradication and  
40 prevention of the infection largely depend on early, efficient and correct identification of infected  
41 animals (de Andrés et al., 2005). This is routinely done by serological analysis, the ELISA  
42 assay being the most sensitive and suitable technique both for large scale screening and  
43 individual examination. Several ELISA protocols have been developed so far, mostly based on  
44 antigens from a single viral strain. The specificity of these assays is generally high, but the  
45 sensitivity shows extensive variability (de Andrés et al., 2005) due to antigenic heterogeneity of

46 SRLVs. Recent analysis of two Italian SRLV isolates, MVV-like It-561 and CAEV-like It-Pi1,  
47 demonstrated that gag-encoded capsid (CA) and matrix (MA) proteins carry type-specific  
48 epitopes and most sheep and goat sera reacted to these antigens in a type-specific manner,  
49 irrespective of the species of origin (Grego et al., 2002 ; Grego et al., 2005). Furthermore,  
50 homologous CA-MA fusion protein was able to detect seroconversion at an earlier stage  
51 compared to the heterologous protein (Lacerenza et al., 2006). The present study extends the  
52 analysis of the humoral response induced by It-561 and It-Pi1 to SU5, one of the major epitopes  
53 of the envelope (Env) protein (Bertoni et al., 2000; Valas et al., 2000). Two SU5 synthetic  
54 peptides were generated, corresponding to It-561 and It-Pi1 sequences. The peptides were  
55 used as antigens to assay sera from two groups of sheep experimentally infected with these  
56 viruses, in order to investigate the kinetics and specificity of the humoral response to the  
57 homologous and heterologous antigen.

## 58 **Materials and Methods**

### 59 *Viral strains and experimental infection*

60 SRLV strains It-561 (genotype A) and It-Pi1 (genotype B) (Grego et al., 2002) were used in the  
61 present study. Infection of sheep with these strains has been described by Lacerenza et al.  
62 (2006). Detection of viral sequences in blood of experimental sheep was done by *LTR* PCR  
63 (Extramiana et al., 2002) and *PoI* PCR (Grego et al., 2002).

### 64 *Cloning, sequencing and sequence analysis of env region coding for SU5*

65 An *env* gene fragment encompassing the 75bp "SU5 total" domain (Mordasini et al., 2006)  
66 coding sequence (nt 7800-7874) was amplified from DNA of foetal ovine lung fibroblasts  
67 infected respectively with It-561 and It-Pi1 viral stocks. Primer sequences (Bertoni et al., 2000)  
68 were as follows: 563F: GAYATGRYRGARCAYATGAC (nt.7272-7291); 567F:  
69 GGIACIAAIACWAATTGGAC (nt. 7482-7501); 564R:GCYAYATGCTGIACCATGGCATA (nt.

70 8089- 8067). The nucleotide positions refer to the CAEV sequence published by Saltarelli et al.  
71 (1990; Genbank accession number M33677). Amplified fragments were cloned and sequenced.  
72 Genetic distances were calculated with MEGA 3 (Kumar et al., 2001) and used to construct a  
73 neighbor-joining tree with the Tamura-Nei two-parameter distance option (Tamura et al., 1993).

#### 74 *Peptide ELISA*

75 Peptides corresponding to the 25aa “SU5 total” domain of both viruses, were resuspended in  
76 0.1M carbonate buffer pH 9.6 at 5 µg/mL. ELISA plates were coated overnight at 37°C  
77 (50µL/well), incubated for 1 hour at 37°C with sera diluted 1/20 and for 1 hour at room  
78 temperature with a 1:8000 dilution of peroxidase-labelled anti-sheep/goat IgG monoclonal  
79 antibody (Sigma). Optical density was measured at 405 nm wavelength after 40 min incubation  
80 with ABTS (Sigma).

#### 81 *Cut-off*

82 A panel of sera collected before experimental infection as well as sequential sera from two  
83 mock-infected sheep, were repeatedly assayed: no significant interplate variation of the  
84 absorbance was observed. The cut-off was calculated as mean absorbance of negative sera +  
85 3x Standard Deviation. Samples with absorbance higher than 0.3 were considered positive with  
86 a 99% confidence level. A serum collected from a sheep belonging to a long term seronegative  
87 flock was loaded on each plate.

#### 88 *Antibody avidity measurements*

89 The avidity index values of SU5-specific antibodies were measured by testing the stability of the  
90 antigen-antibody complexes following a wash in 8 M urea (Mordasini et al., 2006). Antibodies  
91 with avidity indexes <30% were considered to be of low affinity; those with values between 30%  
92 and 50% of intermediate avidity and those with values >50% of high avidity.

## 93 Results

### 94 *Cloning and sequencing of SU5-encoding env region*

95 The *env* sequences encompassing respectively the SU5 domain of It-561 and It-Pi1 strains  
96 (Genbank accession numbers: It-561: EU702487; It-Pi1: EU709743) were aligned with MVV  
97 and CAEV prototypic *env* sequences of worldwide origin. It-561 clusters with representatives of  
98 group A genotypes and It-Pi1 with B genotypes (fig.1 a), in agreement with the results of a  
99 phylogenetic analysis of *gag* and *pol* sequences (Grego et al., 2002; 2005). The SU5 aminoacid  
100 sequences of It-561 and It-Pi1 share homology in the N-terminal region, which is well  
101 conserved among MVV and CAEV strains, and diverge in the C-terminal, more variable portion  
102 (fig.1 b).

### 103 *Peptide ELISA*

104 Peptide ELISA assays were performed on a panel of sera obtained over a period of up to 92  
105 weeks p.i. from three groups of experimentally infected sheep: group A, sheep 1 to 5, infected  
106 with It-561; group B, sheep 6 to 9, infected with It-Pi1 (fig.2 a, b) and group C, four mock-  
107 infected sheep. Group C sheep remained seronegative throughout the study (data not shown).  
108 Group A sheep 1 to 4 seroconverted to the homologous antigen between week 4 and 8 and  
109 remained positive throughout the study. Sheep 1 to 3 were completely negative to the  
110 heterologous It-Pi1 peptide, while sheep 4 raised a weak humoral response to It-Pi1, from week  
111 77 onwards. Sheep 5 did not seroconvert and was the only one negative to both *LTR* and *Pol*  
112 PCR assays performed throughout the study to confirm the presence of virus in the infected  
113 animals (data not shown). Group B sheep seroconverted to the homologous antigen between  
114 week 3 and 8; all sheep remained positive until the end of the experiment, with high absorbance  
115 values. Only sheep 9 responded to the heterologous antigen from week 8 onwards. The

116 absorbance, which was about two- to threefold lower than for the homologous antigen, reached  
117 a peak at week 22 and gradually decreased thereafter, to values just above the cut-off.

118

#### 119 *Kinetics of antibody avidity maturation*

120 The avidity maturation of sera was assayed at week 8 p.i., corresponding to the first antibody  
121 peak, at week 35 and at week 78. The results obtained for group A sheep are depicted in fig.3  
122 a. Sheep 2, 3 and 4 underwent a marked increase of antibody avidity over the period analyzed.  
123 All avidity indexes of sheep 1 were low; therefore additional time points were analyzed.  
124 Maturation to high avidity was detected at week 56, followed by a gradual decrease to low  
125 avidity by the end of the experiment (data not shown. Group B sheep underwent a marked  
126 avidity maturation, reaching the highest values at week 35 p.i (fig.3b).

#### 127 **Discussion**

128 Most ovine isolates collected in Italy belong to the CAEV-like group (Grego E. et al., 2002) and  
129 coinfection of goats with both MVV- and CAEV-like strains in an Italian flock has recently been  
130 reported (Pisoni et al., 2007). In the last years serological assays based on Gag antigens  
131 derived from Italian isolates belonging to both phylogenetic groups were developed and applied  
132 in field conditions: the response obtained was largely type-specific, indicating the opportunity to  
133 use antigens representative of both genotypes to improve the sensitivity of the diagnostic  
134 assays (Grego et al., 2002; 2005). Furthermore, when sequential sera collected from sheep  
135 experimentally infected with either It-561 or with It-Pi1, were analyzed by ELISA with both  
136 homologous and heterologous CA-MA fusion proteins, the homologous antigen detected  
137 infected animals up to months before the heterologous antigen (Lacerenza et al., 2006). In this

138 study the same panel of sequential sera was analyzed with an Env ELISA based on It-561 and  
139 It-Pi1 SU5 peptides. Five epitopes have been identified in the SU subunit of Env (Bertoni et al.,  
140 2000; Valas et al., 2000). SU5 in particular, a 25 aminoacid sequence located at the C terminus  
141 of SU, is immunodominant and partly type-specific: it carries at least three antibody-binding  
142 sites, one in the N-terminal conserved region, one within the C-terminal most variable domain,  
143 and the third encompassing the junction between the variable and conserved regions  
144 (Mordasini et al., 2006). In this study, two *env* gene fragments encoding respectively It-561 and  
145 It-Pi1 SU5, were sequenced. Alignment of these fragments with sequences of MVV and CAEV  
146 prototypic strains of worldwide origin showed that It-561 clusters with the MVV strains and It-Pi1  
147 is phylogenetically related to classical CAEV-like prototypes. The SU5 N-terminal aminoacid  
148 sequences of the two strains share extensive homology; the C-terminal region is more  
149 divergent, confirming the results of Mordasini et al. (2006). Seroconversion kinetics of group A  
150 and B sheep were similar, with antibodies being mostly type-specific. The few broadly reacting  
151 sera may reflect the production of antibodies directed to the constant antibody-binding site. The  
152 antibody levels varied between animals and for the same animal during the time course of the  
153 experiment. In group A, the first antibody peak was followed by a slight decrease of reactivity  
154 and then by a rise to three to fourfold higher values. One sheep of this group did not  
155 seroconvert. This result was most likely caused by a technical failure during the experimental  
156 infection, since the sheep was repeatedly negative to both *LTR* and *pol* PCR assays which  
157 were performed on blood samples of the experimental animals to confirm the presence of viral  
158 genomes. Group B sheep developed immediately an antibody response well over the cut-off. All  
159 sera underwent with time avidity maturation, resulting in the appearance of high affinity  
160 antibodies, although with different kinetics. It-Pi1 sera, which developed higher antibody levels  
161 from the beginning, underwent high avidity maturation at an earlier time point than It-561 sera.

162 Sheep 1 showed a peculiar pattern of avidity maturation, with indexes decreasing from week  
163 56 onwards, to return to low values by the end of the experiment, in spite of a persisting high  
164 antibody level. The reason for sheep 1 behaviour is unclear; the genetic variability of SU5 could  
165 possibly explain the disappearance of the high affinity antibody-producing clone(s).  
166 Alternatively, this sheep may present some failure in the mechanisms that underly the ability to  
167 maintain the production of high affinity antibodies. A failure of T helper function may be  
168 responsible for the loss of high affinity antibodies as well as the associated decay of the  
169 antibody titer, that have been reported in individuals who later developed AIDS (Chargelegue et  
170 al., 1993; 1995; Thomas et al., 1996). SRLV infection, however, does not involve the  
171 lymphocyte compartment, and sheep 1 antibody titer does not decrease, therefore a different  
172 mechanism may be responsible. A comparison of the results obtained in this study and in  
173 Lacerenza et al. (2006) shows that both CA-MA fusion antigens and SU5 peptides detect  
174 infection at early stages, from week three onwards. Furthermore, while It-561 drives type-  
175 specific antibody response towards both Gag and Env antigens, It-Pi1 seems to drive  
176 production of type-specific antibody only against SU5. In fact, all group B sheep reacted against  
177 the heterologous Gag subunits as well (Lacerenza et al., 2006). Since the humoral response to  
178 SU5 is mainly directed towards the variable antibody-binding site, a constant monitoring of the  
179 circulating viral variants will be necessary to develop a panel of SU5 peptides representative of  
180 most local genotypes.

### 181 **Acknowledgements**

182 This work was supported by the Italian MIUR.

### 183 **Conflict of interest statement**

184 *All authors deny any financial and personal relationships with other people or organizations that*  
185 *could inappropriately influence this work.*

## 186 **References**

187 Bertoni G, Hertig C, Zahno ML, Vogt HR, Dufour S, Cordano P, Peterhans E, Cheevers WP,  
188 Sonigo P, Pancino G., 2000. B-cell epitopes of the envelope glycoprotein of caprine  
189 arthritis–encephalitis virus and antibody response in infected goats. *Journal of General*  
190 *Virology.*; 81, 2929–2940.

191 Chargelegue, D., Colvin, B.T., O’Toole, C.M., 1993. A 7 year analysis of anti-gag (p17 and p24)  
192 antibodies in HIV-1 seropositive patients with haemophilia: immunoglobulin titre and  
193 avidity are early predictors of clinical course. *AIDS.* 7, S87–S90.

194 Chargelegue, D., Stanley, C.M., O’Toole C.M., Colvin, B.T., Steward, M.W., 1995. The affinity  
195 of IgG antibodies to gag p24 and p17 in HIV-1 infected patients correlates with disease  
196 progression. *Clin. Exp. Immunol.* 99, 175–81.

197 de Andrés, D., Klein, D., Watt, N.J., Berriatua, E., Torsteinsdottir, S., Blacklaws, B.A., Harkiss,  
198 G.D., 2005. Diagnostic tests for small ruminant lentiviruses. *Vet. Microbiol.* 107, 49–62.

199 Extramiana, A.B., González, L., Cortabarría, N., García, M., Juste, R.A., 2002. Evaluation of a  
200 LTR-PCR technique for the detection of Maedi-Visna proviral DNA in blood, milk and  
201 tissue samples of naturally infected sheep. *Small Ruminant Research* 44, 109-117.

202 Gonzalez, B., Reina, R., Garcia, I., Andres, S., Galaria, I., Alzueta, M., Mora, M.I., Jugo, B.M.,  
203 Arrieta-Aguirre, I., de la Lastra, J.M., Rodriguez, D., Rodriguez, J.R., Esteban, M., Grillo,  
204 M.J., Blacklaws, B.A., Harkiss, G.D., Chebloune, Y., Lujan, L., de Andres, D., Amorena,  
205 B., 2005. Mucosal immunization of sheep with a Maedi-Visna virus (MVV) env DNA  
206 vaccine protects against early MVV productive infection. *Vaccine.* 23, 4342-4352.

- 207 Grego, E., Profiti, M., Giammarioli, M., Giannino, L., Rutili, D., Woodall, C., Rosati, S., 2002.  
208 Genetic heterogeneity of Small Ruminant Lentiviruses involves immunodominant epitope  
209 of capsid antigen and affects sensitivity of single-strain-based immunoassay. *Clin. and*  
210 *Diagn. Lab. Immunol.* 9, 828–832.
- 211 Grego, E., Bertolotti, L., Carrozza, M.L., Profiti, M., Mazzei, M., Tolari, F., Rosati, S., 2005.  
212 Genetic and antigenic characterization of the matrix protein of two genetically distinct  
213 ovine lentiviruses. *Vet. Microbiol.* 106, 179-185.
- 214 Kumar, S., Tamura, K., Jakobsen, I. B., Nei, M., 2001. MEGA2: molecular evolutionary genetics  
215 analysis software. *Bioinformatics.* 17,1244–1245.
- 216 Lacerenza, D., Giammarioli, M., Grego, E., Marini, C., Profiti, M., Rutili, D., Rosati, S., 2006.  
217 Antibody response in sheep experimentally infected with different small ruminant lentivirus  
218 genotypes. *Vet. Immunol. Immunopathol.* 112, 264-271.
- 219 Mordasini, F., Vogt, H.R., Zahno, M.L., Maeschli, A., Nenci, C., Zanoni, R., Peterhans, E.,  
220 Bertoni, G., 2006. Analysis of the antibody response to an immunodominant epitope of the  
221 Envelope glycoprotein of a lentivirus and its diagnostic potential. *J. Clin. Microbiol.* 44,  
222 981-991.
- 223 Niesalla, H., de Andrés, X., Barbezange, C., Fraiser, C., Reina, R., Arnarson, H., Biescas, E.,  
224 Mazzei, M., McNeilly, T.N., Liu, C., Watkins, C., Perez, M., Carrozza, M.L., Bandecchi, P.,  
225 Solano, C., Crespo, H., Glaria, I., Huard, C., Shaw, D.J., de Blas, I., de Andrés, D., Tolari,  
226 F., Rosati, S., Suzan-Monti, M., Andrésdottir, V., Torsteinsdottir, S., Petursson, G.,  
227 Badiola, J., Lujan, L., Pepin, M., Amorena, B., Blacklaws, B., Harkiss, G.D., 2009.  
228 Systemic DNA immunization against ovine lentivirus using particle-mediated epidermal  
229 delivery and modified vaccinia Ankara encoding the gag and/or env genes. *Vaccine* 7,  
230 260-269.

- 231 Petursson, G., Matthiasdottir, S., Svansson, V., Andresdottir, V., Georgsson, G., Martin, A.H.,  
232 Agnarsdottir, G., Gisladdottir, E., Arnadottir, S., Hognadottir, S., Jonsson, S.R., Andresson,  
233 O.S., Torsteinsdottir, S., 2005. Mucosal vaccination with an attenuated maedi-visna virus  
234 clone. *Vaccine*. 23, 3223-3228.
- 235 Pisoni, G., Bertoni, G., Puricelli, M., Maccalli, M., Moroni, P., 2007. Demonstration of coinfection  
236 with and recombination by caprine Arthritis-Encephalitis Virus and Maedi-Visna Virus in  
237 naturally infected sheep. *J. of Virol.* 81, 4948-4955.
- 238 Reina, R., Barbezange, C., Niesalla, H., de Andrés, X., Arnarson, H., Biescas, E., Mazzei, M.,  
239 Fraiser, C., McNeilly, T.N., Liu, C., Perez, M., Carrozza, M.L., Bandecchi, P., Solano, C.,  
240 Crespo, H., Glaria, I., Huard, C., Shaw, D.J., de Blas, I., de Andrés, D., Tolari, F., Rosati,  
241 S., Suzan-Monti, M., Andrésdottir, V., Torsteinsdottir, S., Petursson, G., Lujan, L., Pepin,  
242 M., Amorena, B., Blacklaws, B., Harkiss, G.D., 2008. Mucosal immunization against ovine  
243 lentivirus using PEI-DNA complexes and modified vaccinia Ankara encoding the gag  
244 and/or env genes. *Vaccine*. 26, 4494-4505.
- 245 Saltarelli, M., Querat, G., Konings, D.A., Vigne, R., Clements, J.E., 1990. Nucleotide sequence  
246 and transcriptional analysis of molecular clones of CAEV which generate infectious virus.  
247 *Virology*. 179, 347-364.
- 248 Shah, C., Boni, J., Huder, J.B., Vogt, H.R., Muhlherr, J., Zanoni, R.G., Miserez, R., Lutz, H.,  
249 Schupbach, J., 2004. Phylogenetic analysis and reclassification of caprine and ovine  
250 lentiviruses based on 104 new isolates: evidence for regular sheep-to-goat transmission  
251 and worldwide propagation through livestock trade. *Virology*. 319, 12-26.
- 252 Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control  
253 region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512-526.
- 254 Thomas, H.I.J., Wilson, S., O'Toole, C.M., Lister, C.M., Saeed, A.M., Watkins, R.P.F., Morgan-

- 255 Capner, P., 1996. Differential maturation of avidity of IgG antibodies to gp41, p24 and p17  
256 following infection with HIV-1. *Clin. Exp. Immunol.* 103, 185–191.
- 257 Torsteinsdottir, S., Carlsdottir, H.M., Svansson, V., Matthiasdottir, S., Martin, A.H., Petursson,  
258 G., 2007. Vaccination of sheep with Maedi-visna virus gag gene and protein, beneficial or  
259 harmful?. *Vaccine.* 25, 6713-6720.
- 260 Valas, S., Benoit, C., Baudry, C., Perrin, G., Mamoun, R.Z., 2000. Variability and  
261 immunogenicity of caprine arthritis-encephalitis virus surface glycoprotein. *J. Virol.* 74,  
262 6178-6185.
- 263 Zanoni, R.G., 1998. Phylogenetic analysis of small ruminant lentiviruses. *J. Gen. Virol.* 79,  
264 1951-1961.

265 Fig.1:

266 a: Phylogenetic analysis of It-561 and It-Pi1 *env* fragments encompassing SU5 coding region.

267 The neighbor-joining tree was constructed using MEGA. Bootstrap values are based on 5000

268 repetitions. The accession numbers refer to the following strains: AF338227 (MVV K1514,

269 Iceland); S51392 (MVV EV1, Great Britain); M31646 (SA-OMVV, South Africa); EF154467

270 (SRLV 12-5, Italy); U64439 (OLV 85/34, USA); EF154474 (SRLV 12-20, Italy); AY900630

271 (CAEV Gansu, China); M33677 (CAEV-Co, USA), AF322109 (CAEV 1GA, Norway); NC001482

272 (FIV). b: Alignment of SU5 aminoacid sequences of It-561 and It-Pi1 with MVV and CAEV

273 strains.

274

275 Fig. 2:

276 Kinetics of humoral responses of experimentally infected sheep to homologous (white bars) and

277 heterologous (black bars) antigen. The cut-off was set at 0.3 OD. \*: not available. a: It-561

278 infected sheep. b: It-Pi1 infected sheep.

279

280 Fig. 3:

281 Avidity maturation of anti SU5 antibody taken at 8, 35, 78 weeks p.i. a: sheep infected with It-

282 561; b: sheep infected with It-Pi1. Serum antibodies with avidity index values lower than 30%

283 are designated low avidity antibody, those with avidity index values between 30 and 50% are

284 intermediate-avidity antibodies, and those with values higher than 50% are considered high

285 avidity antibodies.

286

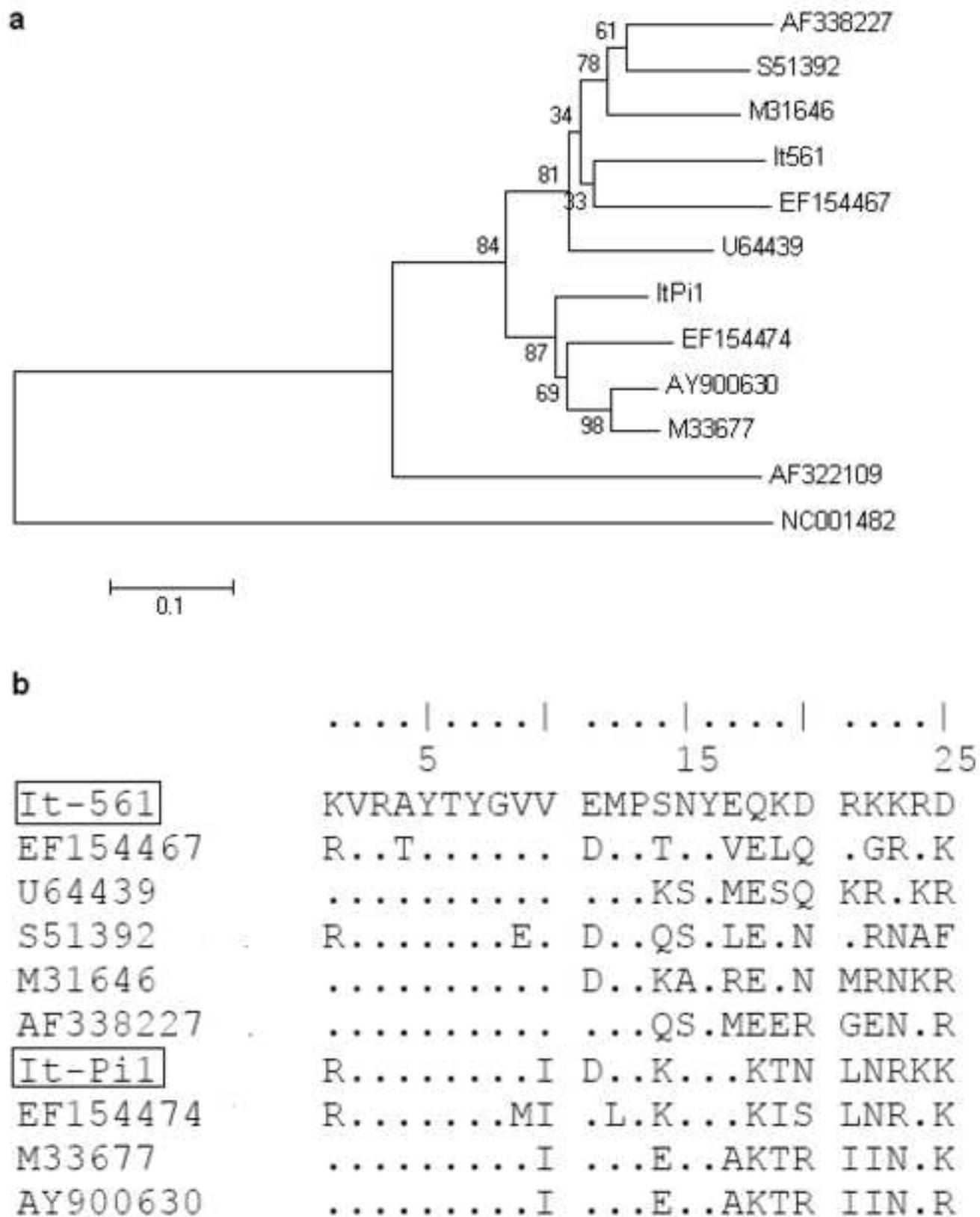


Fig. 1

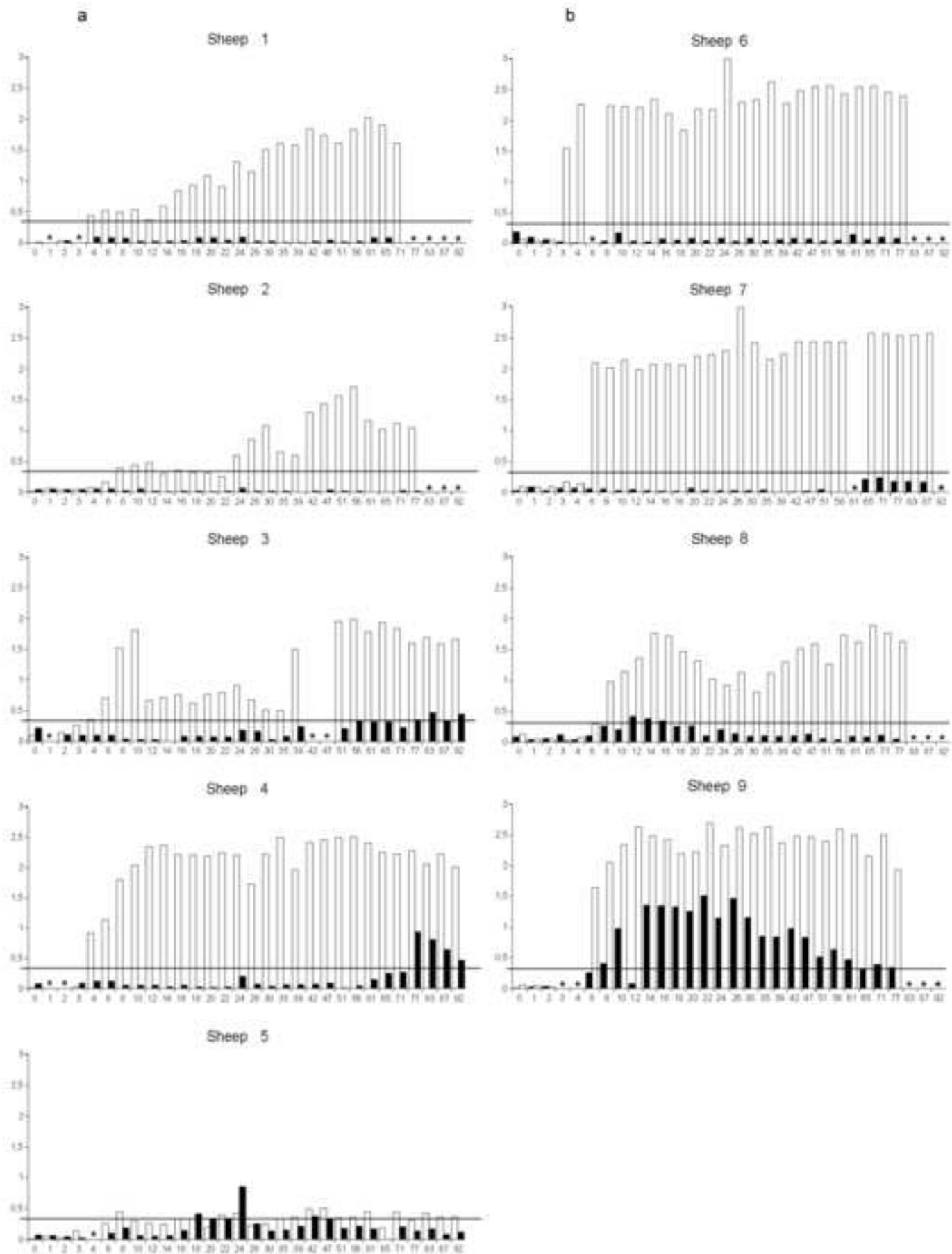


Fig 2

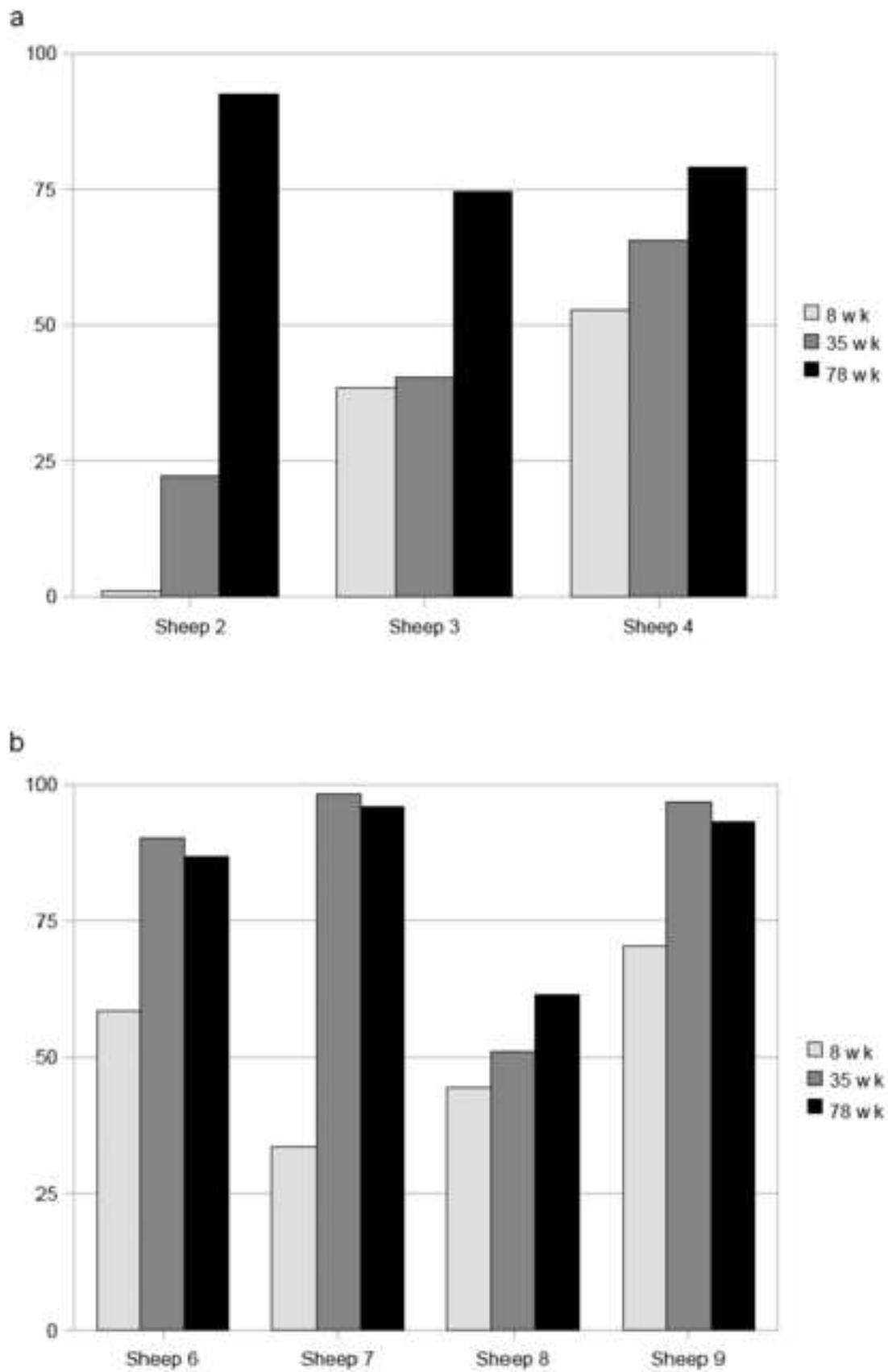


Fig. 3