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Diverse host–virus interactions following caprine arthritis-encephalitis virus infection in sheep and goats

Antoine Rachid 1, Benoit Croisé 1, Pierre Russo², Michel Vignoni 2, Daniela Lacerenza 3, Sergio Rosati 3, Jacek Kuźmak 4 and Stephen Valas 1

+ Author Affiliations

¹Anses, Niort Laboratory, Ruminant Retrovirus Unit, F-79012 Niort, France

²Anses, Sophia-Antipolis Laboratory, Ruminant Pathology Unit, 06902 Sophia-Antipolis, France

³Department of Animal Production, Epidemiology and Ecology, University of Turin, Italy

⁴Department of Biochemistry, National Veterinary Research Institute, 24-100 Pulawy, Poland

Correspondence: Stephen Valas stephen.valas@anses.fr

Abstract

Interspecies transmissions substantially contribute to the epidemiology of small ruminant lentiviruses (SRLVs), including caprine arthritis encephalitis virus (CAEV) and visna-maëdi virus. However, comprehensive studies of host–virus interactions during SRLV adaptation to the new host are lacking. In this study, virological and serological features were analysed over a 6 month period in five sheep and three goats experimentally infected with a CAEV strain. Provirus load at the early stage of infection was significantly higher in sheep than in goats. A broad antibody reactivity against the matrix and capsid proteins was detected in goats, whereas the response to these antigens was mostly type-specific in sheep. The humoral response to the major immunodominant domain of the surface unit glycoprotein was type-specific, regardless of the host species. These species-specific immune responses were then confirmed in naturally infected sheep and goats using sera from mixed flocks in which interspecies transmissions were reported. Taken together, these results provide evidence that SRLV infections evolve in a host-dependent manner, with distinct host–virus interactions in sheep and goats, and highlight the need to consider both SRLV genotypes in diagnosis, particularly in sheep.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are JX878404 and JX878405.

Introduction

Small ruminant lentiviruses (SRLVs), comprising caprine arthritis-encephalitis virus (CAEV) and ovine visna-maëdi virus (VMV), have been assigned to five distinct genetic groups (A to E) that are subdivided into numerous subtypes (Reina et al., 2010; Shah et al., 2004a). Groups A and B are predominant and refer to VMV-like and CAEV-like strains, respectively. To date, group A includes 13 subtypes (A1–A13) and group B contains only three subtypes (B1–B3). This high genetic diversity is associated with changes in the cytopathic phenotype, cellular tropism and pathogenicity of SRLV strains (Angelopoulou et al., 2008; Hötzel & Cheevers, 2001; Lairmore et al., 1987; Oskarsson et al., 2007; Quérat et al., 1984). In addition, the

antigenic cross-reactivity between SRLV groups is limited (Grego et al., 2002, 2005; Mordasini et al., 2006; Olech et al., 2012), which represents a diagnostic drawback (Herrmann-Hoesing et al., 2010; Lacerenza et al., 2006; Reina et al., 2009).

SRLV strains have long been considered to be species-specific pathogens, with groups A and B associated with sheep and goats, respectively. It is presently acknowledged that interspecies transmissions regularly occur in both directions (sheep to goat and goat to sheep) under natural conditions, and most subtypes have been found in both species (Germain et al., 2008; Gjerset et al., 2007; Grego et al., 2007; Pisoni et al., 2005; Shah et al., 2004a, b; Zaroni, 1998). However, the underlying mechanisms and consequences associated with SRLV adaptation to new hosts following interspecies transmission are poorly explored and understood. Because SRLV strains differ in their biological properties, the outcome of the infection could vary widely. For instance, some group A viruses are highly neurovirulent, while group B viruses seem to have a propensity to induce arthritis (Glaria et al., 2009; Oskarsson et al., 2007). Recently, a significantly higher rate of lactogenic transmission has been reported for VMV-like viruses in goats co-infected with SRLV groups A and B (Pisoni et al., 2010). Several lines of evidence indicate that host determinants may also influence the outcome of SRLV infection. Epidemiological studies have shown that the seroprevalence and frequency of PCR detection of provirus from blood among seropositive animals vary between sheep and goats in mixed flocks infected by a single viral strain (Germain et al., 2008; Gjerset et al., 2009). Moreover, analysis of the humoral immune response in naturally infected animals has revealed that the spectrum of antibody reactivity is higher in goats than in sheep (Grego et al., 2002), while neutralizing activity is more frequently detected in sheep (Narayan et al., 1984; Torsteinsdottir et al., 2007). These observations indicate that the virus is exposed to different selective pressures following interspecies transmission. In this respect, it has been suggested that adaptation of CAEV-like viruses to the new ovine host is associated with a shift of the viral genetic and phenotypic properties towards those of VMV-like viruses (Glaria et al., 2009; Karr et al., 1996). These findings provide evidence that interspecies transmissions may influence many aspects of the biology of SRLV, highlighting the need for a direct comparative analysis of SRLV infection in sheep and goats to gain a better understanding of the discrete mechanisms of viral adaptation to the new host.

In this study, for the first time, serological and virological parameters during the early stages of infection were compared in sheep and goats experimentally infected with a CAEV variant. Analysis of the blood proviral load and B-cell humoral response towards the viral structural proteins revealed specific host–virus interactions. The differences in antibody reactivity between sheep and goats were confirmed in naturally infected animals from mixed flocks.

Results

To investigate differences in host–virus interactions between sheep and goats infected with SRLV, the French strain Agh283 was used as infecting virus in cross-species infections. Proviral sequences of strain Agh283 were originally detected in both sheep and goats from a mixed flock (Germain et al., 2008), indicating that this isolate could infect and propagate in both species. The virus was isolated from a seropositive sheep by co-cultivation of blood monocyte-derived macrophages with goat synovial membrane (GSM) cells. It was assigned to subtype B2, according to the phylogenetic classification inferred from sequences coding for the nucleoprotein and the V1V2 region of SU protein. To further characterize this SRLV strain, two fragments encompassing the nearly complete MA/CA coding sequence and the V5 region of SU protein were amplified from infected GSM cells, cloned and sequenced. As shown in Table 1, the assignment of strain Agh283 to genotype B was also strongly supported by the similarity of the Gag and

SU immunodominant regions of strain Agh283 with those of CAEV-like viruses, although the SU5 domain exhibited substantial diversity among isolates within each genotype, supporting the type-specific antibody response towards this immunodominant region (Carrozza et al., 2009; Mordasini et al., 2006). Additionally, the phenotype of strain Agh283 was examined in different caprine cells; this strain established persistent infections and replicated to moderate levels in macrophages and GSM cells with formation of large syncytia (>15 nuclei), whereas neither cytopathic effect nor reverse transcriptase (RT) activity was detected in choroid plexus (CP) cells (data not shown). These growth properties led to the classification of strain Agh283 as a slow/low virus.

Strain*	Genotype	MA	CA	SU5
Agh283	B2	KGLTPEESNKKDFMSL	KLNEEAERWRRNNPPPPA	RVRAYTYGVIDMPKNEYEKTINRKK
1217	B2	.L.....TH.GLRNG....
It-Pi1	B2	.L.....NL....
It-561	A	.N....TS.RE.A..	...D....V.Q...G.N	K.....VE..S...QKDRKKRD
0016	A13	.Q....DTS.RE.A..V.Q...G.N	K.....VE..RS.IEKQQK..Q

*The strain Agh283 was used as infecting virus. Antigens of strains It-Pi1 and It-561 were used in ELISAs based on the P16-P25 recombinant proteins and SU5 synthetic peptides. Antigens of strains 1217 and 0016 were used in ELISAs based on the multi-epitope SU1/Gag/SU5 and SU1/SU5 recombinant proteins.

Table 1. Sequence homology of Gag and SU immunodominant regions between the infecting virus and SRLV strains used as antigens in ELISA tests

Kinetics of seroconversion and proviral detection in sheep and goats following experimental infection

Three goats (g47, g05 and g62) and five sheep (s91, s31, s36, s88 and s32) were infected by intratracheal inoculation of cell culture supernatant containing 4×10^2 TCID₅₀ of strain Agh283. Three goats and three sheep were mock-infected with supernatant from a non-infected cell culture and used as controls. All animals were bled at regular intervals over a 6 month period. Infection was first assessed by detecting seroconversion using a whole virus ELISA test (Chekit CAEV/MVV; Idexx Laboratories) and provirus in blood by PCR. As shown in Fig. 1, the three infected goats seroconverted between weeks 3 and 7 post-infection (p.i.) and remained seropositive during the investigated period, except one goat (g05), which was transiently negative at weeks 5–7 p.i. The five infected sheep seroconverted between weeks 4 and 10 p.i., and remained positive during the whole study. The time to seroconversion did not significantly differ between sheep and goats. Infection was confirmed by PCR detection of provirus from blood in all seropositive sheep and goats (Table 2). All animals became PCR positive between weeks 2 and 4 p.i., except one goat (g47) that turned PCR positive at week 7 p.i. While the time to become PCR positive did not significantly differ between sheep and goats, the frequency of both provirus detection and proviral load were significantly higher in sheep than in goats ($P < 0.0003$). All control animals remained seronegative and PCR negative throughout the experiment (data not shown).

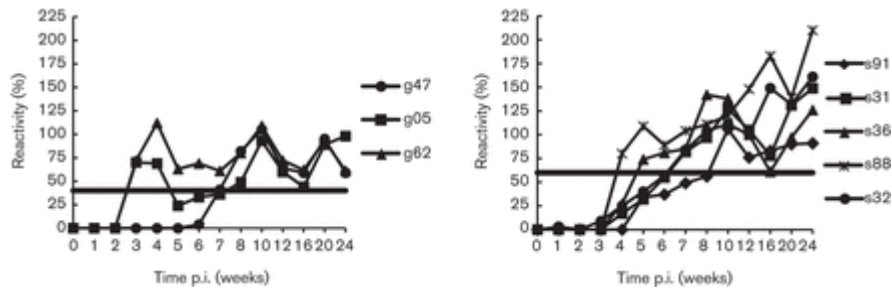


Fig. 1.

Kinetics of seroconversion in a whole virus ELISA of goats and sheep experimentally infected with CAEV strain Agh283. Animals were intratracheally infected with strain Agh283 and sera were tested at different time points using the Chekit ELISA test. The bold line represents the cut-off value of the test. g, Goat; s, sheep.

Species	Animal no.	Weeks p.i.													
		1	2	3	4	5	6	7	8	10	12	16	20	24	
Goat	g47	-	-	-	-	-	-	<10	<10	<10	-	<10	-	<10	
	g05	-	<10	20±7	-	-	-	-	-	<10	<10	-	<10	-	
	g62	-	-	19±11	<10	<10	<10	<10	-	<10	-	<10	-	<10	
Sheep	s91	-	-	-	60±13	18±13	27±20	<10	<10	<10	<10	<10	52±7	19±12	
	s31	-	<10	89±49	15±2	62±25	59±23	39±18	57±4	48±22	NA	<10	20±4	84±34	
	s36	-	-	37±21	32±18	25±12	25±1	17±5	26±11	<10	22±14	13±6	-	<10	
	s88	-	<10	85±11	153±44	90±54	68±26	69±18	70±4	60±23	32±16	25±11	NA	<10	
	s32	-	-	14±7	54±21	17±9	11±3	22±8	11±14	12±11	<10	<10	<10	NA	

Table 2. Blood proviral load (copies μg^{-1} of genomic DNA) in sheep and goats experimentally infected with CAEV strain Agh283 <10, Samples tested in triplicate consistently positive by real-time PCR but with Ct values above 40, not permitting a precise quantification; -, undetectable; NA, not available.

Antibody responses to SRLV structural proteins in experimentally infected animals

The kinetics of antibody production towards the matrix (MA/P16) and capsid (CA/P25) proteins was analysed by ELISA using the previously described P16-P25 recombinant antigens derived from either the It-Pi1 (CAEV-like) or It-561 (VMV-like) strains (Lacerenza et al., 2006). As shown in Fig. 2, all infected goats seroconverted to the CAEV P16-P25 antigen between weeks 3 and 6 p.i., and to the VMV P16-P25 antigen between weeks 3 and 8 p.i. Seroconversion of all infected sheep to the CAEV P16-P25 antigen occurred between weeks 4 and 5 p.i. Four sheep seroconverted to the VMV P16-P25 antigen between weeks 6 and 10 p.i., whereas one sheep (s32) remained negative versus VMV antigen throughout the study. While all sera of sheep and goats showed increased reactivity levels that stably settled into the positive range in the CAEV P16-P25 ELISA, they displayed more fluctuating kinetics of reactivity in the VMV P16-P25 ELISA, reaching the weak positive range (two sheep) or falling into the negative range (one sheep and two goats). These results suggested that antibody reactivity against the Gag proteins was more type-specific in sheep than in goats during the early stages of infection.

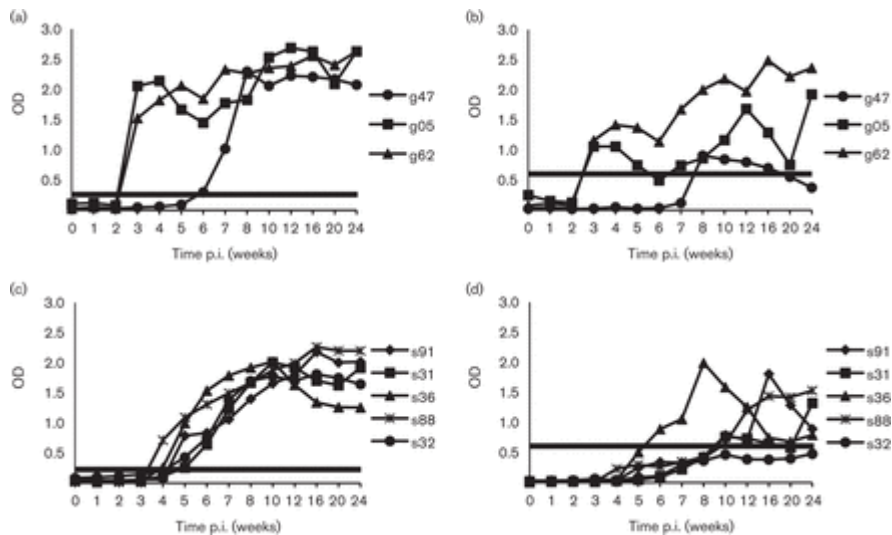


Fig. 2.

Kinetics and patterns of antibody reactivity against Gag epitopes in goats and sheep experimentally infected with CAEV strain Agh283. Panels (a) and (b) show the ELISA reactivity of goat sera against CAEV (strain It-Pi1) and VMV (strain It-561) P16/P25 antigens, respectively. Panels (c) and (d) represent the ELISA reactivity of sheep sera against CAEV (strain It-Pi1) and VMV (strain It-561) P16/P25 antigens, respectively. Bold lines are the cut-off values for each test. g, Goat; s, sheep. OD, Optical density.

The kinetics of antibody production towards the major antigenic site (SU5) of the surface glycoprotein was analysed by ELISA using synthetic peptides corresponding to the SU5 sequence of the aforementioned SRLV strains ItPi1 and It561. As shown in Fig. 3, infected goats seroconverted to the CAEV SU5 peptide between weeks 3 and 8 p.i., concomitantly with the production of antibodies directed against the Gag antigens, and remained clearly positive during the whole study. All infected sheep produced a high and steady humoral immune response against the CAEV SU5 peptide as soon as 2–4 weeks p.i., thus preceding seroconversion to the Gag antigens. Conversely, only two animals showed weak and transient positive responses in the VMV SU5 ELISA, one goat (g05) and one sheep (s32) being positive at weeks 1 and 4 p.i. and weeks 7–12 p.i., respectively. Taken together, these results indicated that antibody response against the SU5 domain was mostly type-specific in both sheep and goats during the early stages of infection.

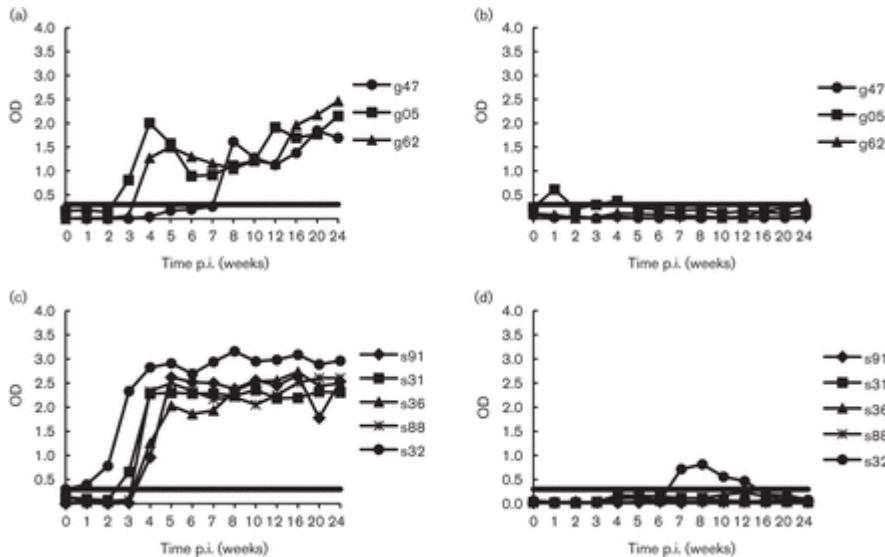


Fig. 3.

Kinetics and patterns of antibody reactivity against the SU5 immunodominant domain in goats and sheep experimentally infected with CAEV strain Agh283. Panels (a) and (b) show the ELISA reactivity of goat sera against CAEV (strain It-Pi1) and VMV (strain It-561) SU5 peptides, respectively. Panels (c) and (d) represent the ELISA reactivity of sheep sera against CAEV (strain It-Pi1) and VMV (strain It-561) SU5 peptides, respectively. Bold lines are the cut-off values for each test. OD, Optical density; g, goat; s, sheep.

Spectrum of antibody responses to SRLV structural proteins during natural CAEV infection

To confirm the relationships between the host species and the range of antibody reactivity towards the immunodominant epitopes of the MA/CA and SU proteins during natural infection, we tested a well-defined panel of 75 sera from mixed flocks in which sheep and goats were found to be infected with the same CAEV variants (Germain et al., 2008). These sera, including 45 sheep sera and 30 goat sera, were positive in the commercially available Chekit ELISA. The spectrum of reactivity of these sera was assessed in ELISA using two sets of multi-epitope recombinant antigens. The SU1/Gag/SU5 antigens contained the P16-P25 precursor flanked at both its terminal ends by the two immunodominant domains (SU1 and SU5) of the SU protein. The SU1/SU5 antigens only contained the SU epitopes and were derived from the previous ones by removing the Gag domain. Each set of antigens was established from either CAEV (strain 1217) or VMV (strain 0016) sequences belonging to subtypes B2 and A13, respectively (Olech et al., 2012). The patterns of reactivity of sera from experimentally infected animals towards these antigens were consistent with those obtained using P16-P25 recombinant antigens and SU5 peptides (Table 3). Indeed, sheep sera reacted in a type-specific manner against both the Gag and SU immunodominant regions, while goat sera exhibited a broad reactivity against the Gag determinants and a type-specific response against the SU domains. Analysis of antibody responses in naturally infected animals confirmed these species-specific features of the B-cell immune response following CAEV infection. As shown in Fig. 4(a), all goat sera reacted to the CAEV SU1/Gag/SU5 antigen and most of them (90%) also reacted to the homologous VMV-like antigen. In contrast, while 100% of sheep sera reacted to the CAEV SU1/Gag/SU5 antigen, only a few of them (27%) were also reactive to the corresponding VMV antigen. The reactivity to the SU1/SU5 antigens is shown in Fig. 4(b). While most goat and sheep sera (97 and 73%, respectively) reacted to the CAEV-like antigen, only very few sera reacted to the VMV-like antigen, with 13 and 7% of positive sera in goats and sheep, respectively. The reactivity of sera against both recombinant antigens was significantly higher in goats than in sheep ($P < 0.0003$). These results corroborated those obtained upon experimental infection, showing a

large spectrum of antibody reactivity against the Gag proteins in goats, a narrow range of reactivity against these viral proteins in sheep, and a mostly type-specific immune response against the SU protein in both sheep and goats.

Animal*	SU1/Gag/SU5		SU1/SU5	
	CAEV	VMV	CAEV	VMV
Goats	3/3	2/3	3/3	0/3
Sheep	5/5	0/5	4/5	0/5

Table 3. Seroconversion of experimentally infected animals in ELISA using Gag/Env multi-epitope recombinant antigens

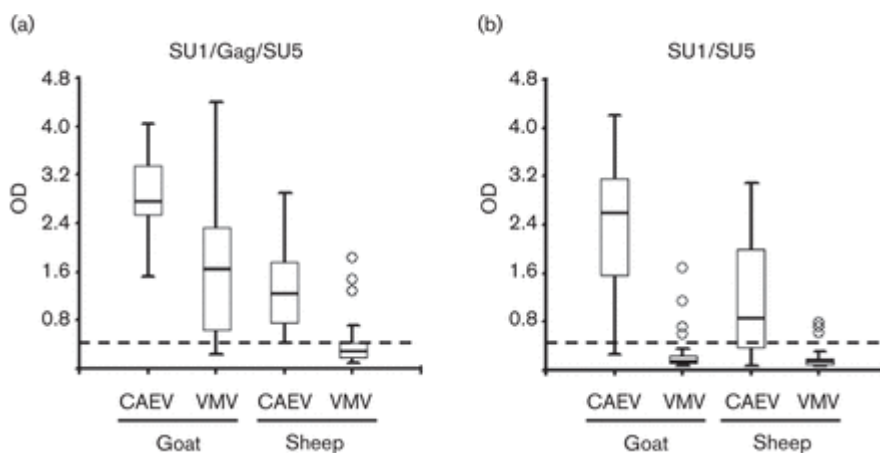


Fig. 4.

Patterns of antibody reactivity against the MA/CA and/or SU epitopes in naturally infected sheep and goats. Sera from sheep and goats from mixed flocks infected with single CAEV strains were tested in ELISA against multi-epitope recombinant antigens containing either the MA/CA and SU1/SU5 epitopes (a) or only the SU-derived epitopes (b). Both sets of antigens were established from either CAEV (strain 1217) or VMV (strain 0016) sequences, as indicated below the panels. Values within the second and third quartiles are boxed and medians are indicated. Whiskers (vertical bars) indicate values up to 1.5 box lengths beyond the box. Values outside the box plots are indicated by open circles. Dashed lines are the cut-off values for each test. OD, Optical density.

Discussion

In this study, we compared serological and virological features in sheep and goats experimentally or naturally infected with the same CAEV variant. The early stages of experimental CAEV infection in sheep were characterized by a sustained peak of virus replication in peripheral blood and a strong and steady production of anti-SU antibodies which preceded the antibody response to the MA and CA proteins. In contrast, experimental CAEV infection in goats was associated with a low provirus load in peripheral blood and concomitant production of antibodies towards the immunodominant epitopes of the viral structural proteins which gradually increased during the first 6 months following infection.

Experimental infection with SRLV was characterized by an initial burst of viral replication within a few weeks associated with a vigorous host humoral response, followed by an extended period of restricted replication or latency (Brahic et al., 1981; Staskus et al., 1991; Vigne et al., 1987). The underlying mechanisms responsible for the striking difference in provirus load between sheep and goats during the early stage of infection are unclear. It is unlikely that these differences result from the route of infection.

Intratracheal injection is known to be a highly efficient method of experimental infection in sheep (Torsteinsdóttir et al., 2003), and alveolar macrophages are permissive cells that represent an important viral reservoir in vivo (Brodie et al., 1995; Gelmetti et al., 2000; Luján et al., 1994). A recent study demonstrated that intratracheal injection of SRLV group E at a 5×10^5 TCID₅₀ infectious dose led to productive infections in all inoculated goats (Reina et al., 2011). In this study, the fact that antibody responses to viral structural proteins in goats were detected as early as those observed in sheep and increased over time following inoculation with a low viral dose (4×10^2 TCID₅₀) confirmed that the tracheal route for infection is efficient in goats. The infecting virus used in this study belonged to SRLV subtype B2, which predominates in sheep flocks from France and Italy (Germain & Valas, 2006; Grego et al., 2002). Although this subtype can productively infect goats, its prevalence remains higher in sheep than in goats from mixed flocks (Germain et al., 2008). In contrast, analysis of infection with CAEV strains representative of SRLV group C in mixed flocks revealed a higher prevalence in goats, a lower frequency of provirus detection in seropositive sheep, and a tendency of goat isolates to replicate more efficiently in cells of caprine origin (Gjerset et al., 2007, 2009). These results strongly suggested that CAEV subtypes differ in their propensity to spread in sheep and goats.

In addition to the different provirus loads in blood, the kinetics of antibody responses to the viral structural proteins differed in sheep and goats following experimental infection with subtype B2. While antibody responses to the Gag (MA and CA) and SU proteins were detected at the same time in infected goats, in all infected sheep anti-SU antibodies were produced at a high titre and earlier than those directed against the MA and CA proteins. The pattern of the immune response in sheep contrasted with those previously reported in sheep naturally and experimentally infected with VMV strains, where the Gag proteins are considered to be the first antigens recognized by the host humoral response, preceding the SU protein (Houwens & Nauta, 1989; Kajikawa et al., 1990; Singh et al., 2006; Torfason et al., 1992). This discrepancy of results may be explained by virus-specific properties together with the genetic background of animals which strongly modulates the immune response elicited after SRLV infection (Fluri et al., 2006). Interestingly, it has been shown that high titres of anti-SU antibodies associate with and predict the severity of clinical signs in chronically infected animals, and that viral load in blood also correlates with and predicts the extent of histological lesions in sheep (Herrmann-Hoesing et al., 2009; Knowles et al., 1990). In the case of primate lentivirus infections, early viral and host immune events can predict subsequent disease progression (Lifson et al., 1997; Pantaleo et al., 1997; Verhofstede et al., 1994). Since most of subtype B2 strains are pathogenic in naturally infected sheep (Grego et al., 2002), the contrasting virological and serological features observed in sheep and goats in this study could reflect the process of SRLV adaptation in their natural hosts. Therefore, the relationships between either the provirus level or titre of anti-SU antibodies during the early stages of infection and the development of the different disease forms are important questions to address in further in vivo experiments.

Our comparative analysis of SRLV infection in sheep and goats also revealed species-specific patterns of antibody reactivity to the immunodominant domains of viral structural proteins. We showed that both sheep and goats produced a type-specific humoral response to the SU5 epitope during the early stage of infection. We expanded these results to natural infection, demonstrating a restricted pattern of antibody reactivity to the SU1 and SU5 epitopes during all stages of infection. These results were consistent with other studies and confirmed the potential use of the major SU immunodominant epitopes as serotyping tools to provide information on the genotype classification of the infecting viruses (Bertoni et al., 2000; Carrozza et al., 2009; Mordasini et al., 2006). In contrast, the spectrum of antibody reactivity to the Gag proteins clearly differed between the two species. Following experimental infection, cross-reactive antibodies recognizing Gag antigens representative of SRLV group A appeared as soon as did those elicited

towards the infecting group B virus in all goats, while a delayed onset of such cross-reactive antibodies was observed in all sheep except for one which produced only type-specific antibodies. Moreover, the kinetics of humoral responses to the heterologous antigens was more fluctuating during the 6 month period of observation, suggesting that these species-specific patterns of anti-Gag antibodies may also be found in long-term infected animals. Analysis of sera from mixed flocks in which sheep and goats were infected with the same virus confirmed that most sheep reacted in a type-specific manner, while goats produced a broad antibody reactivity to these Gag antigens. Several studies aimed to determine the antigenic cross-reactivity between CAEV and VMV structural proteins, leading to discordant results. Indeed, earlier studies reported that CAEV and VMV were antigenically closely related, with immunologically related epitopes within all of the major structural proteins, including Gag antigens (Gogolewski et al., 1985; Rosati et al., 1999). These results were obtained using either sera from naturally infected goats or antisera produced in rabbits, goats and sheep. However, sheep antisera were less reactive against heterologous antigens than goat antisera. This observation was confirmed by another study showing that, in field conditions, sheep sera reacted in a type-specific manner against a major CA epitope, while most of the goat sera recognized the analogous CA epitope derived from both SRLV groups A and B (Grego et al., 2002). Finally, experimental infections of sheep with either group A or B viruses clearly revealed a type-specific pattern of antibody reactivity to the MA and CA proteins, at least during the early stages of infection (Lacerenza et al., 2006). Here, our results provide evidence that the spectrum of humoral responses to the SRLV structural proteins also depends on the host species.

In conclusion, even though the study was carried out on a small number of animals and requires further experiments, our results demonstrated that specific host–virus interactions involving both viral and host determinants take place during SRLV infection in sheep and goats. Thus, this study indicates that interspecies transmissions may influence the biology of SLRV infection, including pathogenicity, viral transmission and diagnosis. Notably, it highlights that molecular and serological tools for diagnosis of SRLV infections may have different sensitivity in sheep and goats, independently of viral diversity.

Methods

Virus and cells.

The infecting virus corresponded to the French ovine strain Agh283 (Germain et al., 2008). The virus was isolated from a seropositive sheep by co-cultivation of blood monocyte-derived macrophages (BMDM) with goat synovial membrane (GSM) cells, passaged once on GSM cells and stored at -80°C until used. Virus infectivity titre was determined by limited dilution assay and formation of syncytia on GSM cells, and expressed as TCID₅₀. The growth properties of strain Agh283 were examined in different caprine cells, including BMDM, GSM and choroid plexus (CP) cells. GSM and CP cells were maintained in Dulbecco's minimal essential medium supplemented with l-glutamine (2 mM), gentamicin (50 $\mu\text{g ml}^{-1}$) and 5% FBS. BMDM were maintained in Roswell Park Memorial Institute 1640 medium (RPMI 1640) supplemented with l-glutamine (2 mM), HEPES buffer (10 mM), 2-mercaptoethanol (50 μM), gentamicin (50 $\mu\text{g ml}^{-1}$) and 10% newborn calf serum. Infection was performed onto 1-week-old macrophages and 80% confluent monolayers of GSM and CP cells, and virus growth was monitored by the observation of lysis and formation of giant multinucleated cells, the typical cytopathic effects of SRLV infection, and by measuring the RT activity in the supernatant from infected cells cultures (Lenti-RT Activity kit; Cavid), according to the manufacturer's protocol.

PCR amplification of proviral DNA.

Two fragments spanning either the nearly complete MA/CA coding sequence (990 bp) or the V5 region of SU protein (608 bp) were amplified by PCR from GSM cells infected by strain Agh283 using primer pairs MA3f/NC3r and 567/564, respectively. The primers and PCR conditions used were as described previously (Mordasini et al., 2006; Olech et al., 2012). PCR amplicons were cloned with the Qiagen PCR cloning kit according to the manufacturer's instructions and then sequenced.

Experimental infection.

Eight PréAlpes cross sheep and six Alpine goats were selected from certified SRLV-free flocks and housed in level 2 containment facilities at the experimental station of the French Agency for Food, Environmental and Occupational Health and Safety (Anses). Animals were utilized in compliance with the relevant national legislation on experimental animals and animal welfare, upon authorization by the French competent authority. At 6 months of age, five sheep and three goats were inoculated intratracheally with 4×10^2 TCID₅₀ of strain Agh283. The remaining animals (three sheep and three goats) were inoculated with uninfected cell culture supernatant and used as negative controls. Blood samples were collected before inoculation, weekly during the first 2 months p.i., and then at weeks 10, 12, 16, 20 and 24 p.i. Infection was assessed by detecting SRLV-specific antibodies in ELISA and provirus in blood by PCR.

Serological diagnosis.

Several ELISA tests were applied to detect seroconversion and production of antibodies directed against the immunodominant epitopes of the matrix (MA/P16), capsid (CA/P25) and surface unit (SU/gp135) proteins in experimentally infected animals. Seroconversion was detected using a commercially available whole virus ELISA (w-ELISA) (Chekit; Idexx Laboratories), according to the manufacturer's instructions. The production of antibodies directed against MA and CA proteins was monitored using a previously described ELISA test based on P16-P25 recombinant antigens carrying the MA protein and the immunodominant subunit of CA (Lacerenza et al., 2006). Antibodies directed against the major antigenic site (SU5) of the SU protein were detected using synthetic peptides corresponding to the 25 aa, complete SU5 domain, as previously described (Carrozza et al., 2009). Each set of antigens (P16-P25 recombinant protein and SU5 peptides) was established from sequences derived from either It-Pi1 (CAEV-like) or It-561 (VMV-like) strains (Grego et al., 2002). Finally, sera from either experimentally or naturally infected animals were tested in an indirect ELISA based on Gag and/or Env multi-epitope recombinant antigens (Olech et al., 2012). Briefly, two sets of antigens were used, the SU1/GAG/SU5 antigens containing the nearly complete MA and CA domains fused to the SU1 and SU5 antigenic sites of the SU, and the SU1/SU5 antigens in which the Gag domain was removed. Once again, each set of antigens was derived from either CAEV-like (strain 1217) or VMV-like (strain 0016) sequences. The three in-house ELISA tests were carried out as described previously, except that the same peroxidase-conjugated anti-goat/sheep IgG mAb (Sigma) was used as secondary antibody in all tests. The reactivity of each serum was calculated by subtracting the absorbance against negative control antigen (water). The cut-off values of P16-P25 and SU5 ELISA tests were determined as the mean absorbance of negative sera plus threefold sd. The cut-off value of SU1/GAG/SU5 and SU1/SU5 ELISA tests was set at an OD of 0.4, as previously determined (Olech et al., 2012).

Proviral load quantification.

Blood samples were drawn into EDTA-coated tubes and genomic DNA was isolated using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions. Proviral load was determined with a quantitative

real-time PCR assay. Quantification was based on amplification of a 96 bp fragment located in the CA coding region using specific primers AghGag5rt: 5'-GCTAACATGGATCAAGCAAGACA-3' and AghGag3rt: 5'-CATAGGGTTTCCTGGTCTATGAGA-3'. All real-time PCRs were performed with a CFX system (Bio-Rad) and contained 9 µl extracted DNA and 300 nM of each primer in 20 µl of 1× buffer SsoAdvanced SYBR Green Supermix (Bio-Rad). The amplification protocol consisted of an initial denaturation step at 95 °C for 3 min to activate the HotStart Taq DNA polymerase followed by 45 cycles of 95 °C for 5 s and 57 °C for 30 s. To confirm the specificity of each PCR product, a melting curve analysis was carried out immediately after the amplification from 55 to 95 °C in 0.5 °C increments. All samples were analysed in triplicate. The provirus copy number was determined by comparison with an external standard curve and expressed as copies µg⁻¹ of genomic DNA.

The DNA standards corresponded to 10-fold serial dilutions (107–100 copies) of linearized plasmid pDrive Cloning vector (Qiagen) containing the 990 bp gag fragment of strain Agh283. The PCR efficiency and correlation coefficient were 96.8 and 0.996, respectively, and the assay was able to amplify at least one replica sample of the low copy standards (>10 copies).

Statistical analysis.

Comparison of virological and serological parameters between sheep and goats was performed using a non-parametric Mann–Whitney U-test with Statistica software (StatSoft France, version 10).

Nucleotide sequence accession numbers.

The nucleotide sequences of the gag and env sequences of CAEV strain Agh283 used as infecting virus were deposited in the GenBank database under accession numbers JX878404 and JX878405, respectively.

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References

Angelopoulou, K., Poutahidis, T., Brellou, G. D., Greenland, T. & Vlemmas, I. (2008). A deletion in the R region of long terminal repeats in small ruminant lentiviruses is associated with decreased pathology in the lung. *Vet J* 175 , 346–355.

Bertoni, G., Hertig, C., Zahno, M. L., Vogt, H. R., Dufour, S., Cordano, P., Peterhans, E., Cheevers, W. P., Sonigo, P. & Pancino, G. (2000). B-cell epitopes of the envelope glycoprotein of caprine arthritis-encephalitis virus and antibody response in infected goats. *J Gen Virol* 81 , 2929–2940.

Brahic, M., Stowring, L., Ventura, P. & Haase, A. T. (1981). Gene expression in visna virus infection in sheep.

Nature 292, 240–242.

- Brodie, S. J., Pearson, L. D., Zink, M. C., Bickle, H. M., Anderson, B. C., Marcom, K. A. & DeMartini, J. C. (1995). Ovine lentivirus expression and disease. Virus replication, but not entry, is restricted to macrophages of specific tissues. *Am J Pathol* 146, 250–263.
- Carrozza, M. L., Mazzei, M., Lacerenza, D., Del Chiaro, L., Giammarioli, M., Marini, C., Rutili, D., Rosati, S. & Tolari, F. (2009). Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes. *Vet Microbiol* 137, 369–374.
- Fluri, A., Nenci, C., Zahno, M. L., Vogt, H. R., Charan, S., Busato, A., Pancino, G., Peterhans, E., Obexer-Ruff, G. & Bertoni, G. (2006). The MHC-haplotype influences primary, but not memory, immune responses to an immunodominant peptide containing T- and B-cell epitopes of the caprine arthritis encephalitis virus Gag protein. *Vaccine* 24, 597–606.
- Gelmetti, D., Gibelli, L., Brocchi, E. & Cammarata, G. (2000). Using a panel of monoclonal antibodies to detect Maedi virus (MV) in chronic pulmonary distress of sheep. *J Virol Methods* 88, 9–14.
- Germain, K. & Valas, S. (2006). Distribution and heterogeneity of small ruminant lentivirus envelope subtypes in naturally infected French sheep. *Virus Res* 120, 156–162.
- Germain, K., Croise, B. & Valas, S. (2008). Field evaluation of a gag/env heteroduplex mobility assay for genetic subtyping of small-ruminant lentiviruses. *J Gen Virol* 89, 2020–2028.
- Gjerset, B., Jonassen, C. M. & Rimstad, E. (2007). Natural transmission and comparative analysis of small ruminant lentiviruses in the Norwegian sheep and goat populations. *Virus Res* 125, 153–161.
- Gjerset, B., Rimstad, E., Teige, J., Soetaert, K. & Jonassen, C. M. (2009). Impact of natural sheep-goat transmission on detection and control of small ruminant lentivirus group C infections. *Vet Microbiol* 135, 231–238.
- Glaria, I., Reina, R., Crespo, H., de Andrés, X., Ramirez, H., Biescas, E., Perez, M. M., Badiola, J., Lujan, L. & other authors (2009). Phylogenetic analysis of SRLV sequences from an arthritic sheep outbreak demonstrates the introduction of CAEV-like viruses among Spanish sheep. *Vet Microbiol* 138, 156–162.
- Gogolewski, R. P., Adams, D. S., McGuire, T. C., Banks, K. L. & Cheevers, W. P. (1985). Antigenic cross-reactivity between caprine arthritis-encephalitis, visna and progressive pneumonia viruses involves all virion-associated proteins and glycoproteins. *J Gen Virol* 66, 1233–1240.
- Grego, E., Profiti, M., Giammarioli, M., Giannino, L., Rutili, D., Woodall, C. & Rosati, S. (2002). Genetic heterogeneity of small ruminant lentiviruses involves immunodominant epitope of capsid antigen and affects sensitivity of single-strain-based immunoassay. *Clin Diagn Lab Immunol* 9, 828–832.
- Grego, E., Bertolotti, L., Carrozza, M. L., Profiti, M., Mazzei, M., Tolari, F. & Rosati, S. (2005). Genetic and antigenic characterization of the matrix protein of two genetically distinct ovine lentiviruses. *Vet Microbiol* 106, 179–185.
- Grego, E., Bertolotti, L., Quasso, A., Profiti, M., Lacerenza, D., Muz, D. & Rosati, S. (2007). Genetic characterization of small ruminant lentivirus in Italian mixed flocks: evidence for a novel genotype circulating in a local goat population. *J Gen Virol* 88, 3423–3427.

- Herrmann-Hoesing, L. M., Noh, S. M., White, S. N., Snekvik, K. R., Truscott, T. & Knowles, D. P. (2009). Peripheral ovine progressive pneumonia provirus levels correlate with and predict histological tissue lesion severity in naturally infected sheep. *Clin Vaccine Immunol* 16, 551–557.
- Herrmann-Hoesing, L. M., Broughton-Neiswanger, L. E., Gouine, K. C., White, S. N., Mousel, M. R., Lewis, G. S., Marshall, K. L. & Knowles, D. P. (2010). Evaluation of a caprine arthritis-encephalitis virus/maedi-visna virus indirect enzyme-linked immunosorbent assay in the serological diagnosis of ovine progressive pneumonia virus in U.S. sheep. *Clin Vaccine Immunol* 17, 307–310.
- Hotzel, I. & Cheevers, W. P. (2001). Host range of small-ruminant lentivirus cytopathic variants determined with a selectable caprine arthritis-encephalitis virus pseudotype system. *J Virol* 75, 7384–7391.
- Houwers, D. J. & Nauta, I. M. (1989). Immunoblot analysis of the antibody response to ovine lentivirus infections. *Vet Microbiol* 19, 127–139.
- Kajikawa, O., Lairmore, M. D. & DeMartini, J. C. (1990). Analysis of antibody responses to phenotypically distinct lentiviruses. *J Clin Microbiol* 28, 764–770.
- Karr, B. M., Chebloune, Y., Leung, K. & Narayan, O. (1996). Genetic characterization of two phenotypically distinct North American ovine lentiviruses and their possible origin from caprine arthritis-encephalitis virus. *Virology* 225, 1–10.
- Knowles, D., Jr, Cheevers, W., McGuire, T., Stem, T. & Gorham, J. (1990). Severity of arthritis is predicted by antibody response to gp135 in chronic infection with caprine arthritis-encephalitis virus. *J Virol* 64, 2396–2398.
- Lacerenza, D., Giammarioli, M., Grego, E., Marini, C., Profiti, M., Rutili, D. & Rosati, S. (2006). Antibody response in sheep experimentally infected with different small ruminant lentivirus genotypes. *Vet Immunol Immunopathol* 112, 264–271.
- Lairmore, M. D., Akita, G. Y., Russell, H. I. & DeMartini, J. C. (1987). Replication and cytopathic effects of ovine lentivirus strains in alveolar macrophages correlate with in vivo pathogenicity. *J Virol* 61, 4038–4042.
- Lifson, J. D., Nowak, M. A., Goldstein, S., Rossio, J. L., Kinter, A., Vasquez, G., Wiltrout, T. A., Brown, C., Schneider, D. & other authors (1997). The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection. *J Virol* 71, 9508–9514.
- Lujan, L., Begara, I., Collie, D. & Watt, N. J. (1994). Ovine lentivirus (maedi-visna virus) protein expression in sheep alveolar macrophages. *Vet Pathol* 31, 695–703.
- Mordasini, F., Vogt, H. R., Zahno, M. L., Maeschli, A., Nenci, C., Zanoni, R., Peterhans, E. & Bertoni, G. (2006). Analysis of the antibody response to an immunodominant epitope of the envelope glycoprotein of a lentivirus and its diagnostic potential. *J Clin Microbiol* 44, 981–991.
- Narayan, O., Sheffer, D., Griffin, D. E., Clements, J. & Hess, J. (1984). Lack of neutralizing antibodies to caprine arthritis-encephalitis lentivirus in persistently infected goats can be overcome by immunization with inactivated *Mycobacterium tuberculosis*. *J Virol* 49, 349–355.
- Olech, M., Rachid, A., Croise', B., Kuz'mak, J. & Valas, S. (2012). Genetic and antigenic characterization of small ruminant lentiviruses circulating in Poland. *Virus Res* 163, 528–536.

- Oskarsson, T., Hreggvidsdottir, H. S., Agnarsdottir, G., Matthíasdottir, S., Ogmundsdottir, M. H., Jonsson, S. R., Georgsson, G., Ingvarsson, S., Andresson, O. S. & Andresdottir, V. (2007). Duplicated sequence motif in the long terminal repeat of maedi-visna virus extends cell tropism and is associated with neurovirulence. *J Virol* 81, 4052–4057.
- Pantaleo, G., Demarest, J. F., Schacker, T., Vaccarezza, M., Cohen, O. J., Daucher, M., Graziosi, C., Schnittman, S. S., Quinn, T. C. & other authors (1997). The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia. *Proc Natl Acad Sci U S A* 94, 254–258.
- Pisoni, G., Quasso, A. & Moroni, P. (2005). Phylogenetic analysis of small-ruminant lentivirus subtype B1 in mixed flocks: evidence for natural transmission from goats to sheep. *Virology* 339, 147–152.
- Pisoni, G., Bertoni, G., Manarolla, G., Vogt, H. R., Scaccabarozzi, L., Locatelli, C. & Moroni, P. (2010). Genetic analysis of small ruminant lentiviruses following lactogenic transmission. *Virology* 407, 91–99.
- Que´rat, G., Barban, V., Sauze, N., Filippi, P., Vigne, R., Russo, P. & Vitu, C. (1984). Highly lytic and persistent lentiviruses naturally present in sheep with progressive pneumonia are genetically distinct. *J Virol* 52, 672–679.
- Reina, R., Grego, E., Profiti, M., Glaria, I., Robino, P., Quasso, A., Amorena, B. & Rosati, S. (2009). Development of specific diagnostic test for small ruminant lentivirus genotype E. *Vet Microbiol* 138, 251–257.
- Reina, R., Bertolotti, L., Dei Giudici, S., Puggioni, G., Ponti, N., Profiti, M., Patta, C. & Rosati, S. (2010). Small ruminant lentivirus genotype E is widespread in Sarda goat. *Vet Microbiol* 144, 24–31.
- Reina, R., Juganaru, M. M., Profiti, M., Cascio, P., Cerruti, F., Bertolotti, L., De Meneghi, D., Amorena, B. & Rosati, S. (2011). Immunological parameters in goats experimentally infected with SRLV genotype E, strain Roccaverano. *Vet Immunol Immunopathol* 139, 237–244.
- Rosati, S., Mannelli, A., Merlo, T. & Ponti, N. (1999). Characterization of the immunodominant cross-reacting epitope of visna maedi virus and caprine arthritis-encephalitis virus capsid antigen. *Virus Res* 61, 177–183.
- Shah, C., Bo¨ni, J., Huder, J. B., Vogt, H. R., Mu¨hlherr, J., Zanoni, R., Miserez, R., Lutz, H. & Schu¨pbach, J. (2004a). Phylogenetic analysis and reclassification of caprine and ovine lentiviruses based on 104 new isolates: evidence for regular sheep-to-goat transmission and worldwide propagation through livestock trade. *Virology* 319, 12–26.
- Shah, C., Huder, J. B., Bo¨ni, J., Scho¨nmann, M., Mu¨hlherr, J., Lutz, H. & Schu¨pbach, J. (2004b). Direct evidence for natural transmission of small-ruminant lentiviruses of subtype A4 from goats to sheep and vice versa. *J Virol* 78, 7518–7522.
- Singh, I., McConnell, I. & Blacklaws, B. (2006). Immune response to individual maedi-visna virus gag antigens. *J Virol* 80, 912–919.
- Staskus, K. A., Couch, L., Bitterman, P., Retzel, E. F., Zupancic, M., List, J. & Haase, A. T. (1991). In situ amplification of visna virus DNA in tissue sections reveals a reservoir of latently infected cells. *Microb Pathog* 11, 67–76.

Torfason, E. G., Guðnadóttir, M. & Lóve, A. (1992). Comparison of immunoblots with neutralizing and complement fixing antibodies in experimental and natural cases of visna-maedi. *Arch Virol* 123, 47–58.

Torsteinsdóttir, S., Matthíasdóttir, S., Vidarsdóttir, N., Svansson, V. & Pétursson, G. (2003). Intratracheal inoculation as an efficient route of experimental infection with maedi-visna virus. *Res Vet Sci* 75, 245–247.

Torsteinsdóttir, S., Andrésdóttir, V., Arnarson, H. & Pétursson, G. (2007). Immune response to maedi-visna virus. *Front Biosci* 12, 1532–1543.

Verhofstede, C., Reniers, S., Van Wanseele, F. & Plum, J. (1994). Evaluation of proviral copy number and plasma RNA level as early indicators of progression in HIV-1 infection: correlation with virological and immunological markers of disease. *AIDS* 8, 1421–1427.

Vigne, R., Barban, V., Quérat, G., Mazarin, V., Gourdou, I. & Sauze, N. (1987). Transcription of visna virus during its lytic cycle: evidence for a sequential early and late gene expression. *Virology* 161, 218–227.

Zanoni, R. G. (1998). Phylogenetic analysis of small ruminant lentiviruses. *J Gen Virol* 79, 1951–1961.