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REVIEW ARTICLE

The genetic variability of small-ruminant lentiviruses and its impact on tropism, the development of diagnostic tests and vaccines and the effectiveness of control programmes

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Abstract

Introduction: Maedi-visna virus and caprine arthritis encephalitis virus are two closely related lentiviruses which cause multisystemic, progressive and persistent infection in goats and sheep. Because these viruses frequently cross the species barrier, they are considered to be one genetic group called small-ruminant lentiviruses (SRLV). They have *in vivo* tropism mainly for monocytes and macrophages and organ tropism with unknown mechanisms. Typical clinical signs are pneumonia in sheep, arthritis in goats, and mastitis in both species. Infection with SRLV cannot currently be treated or prevented, and control programmes are the only approaches to avoiding its spread. These programmes rely mainly on annual serological testing and elimination of positive animals. However, the high genetic and antigenic variability of SRLV complicate their early and definitive diagnosis. The objective of this review is to summarise the current knowledge of SRLV genetic variation and its implications for tropism, the development of diagnostic tests and vaccines and the effectiveness of control and eradication programmes. **Material and Methods:** Subject literature was selected from the PubMed and the Google Scholar databases. **Results:** The high genetic diversity of SRLV affects the performance of diagnostic tools and therefore control programmes. For the early and definitive diagnosis of SRLV infection, a combination of serological and molecular tests is suggested. Testing by PCR can also be considered for sub-yearling animals. There are still significant gaps in our knowledge of the epidemiology, immunology and biology of SRLV and their impact on animal production and welfare. **Conclusion:** This information may aid selection of the most effective SRLV spread reduction measures.

Keywords: SRLV, control, diagnosis, pathogenesis, variability.

Introduction

Maedi-visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are two closely related lentiviruses considered at present as a single genetic group called small-ruminant lentiviruses (SRLV); however, this terminology has not been approved by the International Committee on Taxonomy of viruses (ICTV). Small-ruminant lentiviruses mainly infect sheep and goats, but can infect other closely related wild small ruminants such as red deer, roe deer and mouflons. The viruses show an affinity mainly for monocyte-macrophage lineage cells. Clinical and subclinical SRLV disease is associated with progressive and persistent inflammatory lesions in the lungs, udder, joints and central nervous system (19, 101). Four corresponding main clinical signs present: pneumonia, arthritis, mastitis and encephalitis. Encephalitis is rarer than the other clinical signs, mastitis is common in both host species, pneumonia is the main clinical sign in sheep, and arthritis is the prominent feature in clinically affected goats. The mechanisms of organ tropism involved in the development of the various forms of the disease are unknown. Small-ruminant lentiviruses cause latent infections and most infected animals are asymptomatic. However, both asymptomatic and symptomatic animals carry the virus throughout their lives, and virus present

in their secretions and milk or colostrum is the source of infection for progeny and other animals in the flock and herd (65, 149).

The viral genome comprises two linear molecules of single-stranded RNA which are transformed into double stranded DNA by the viral reverse transcriptase (RT), and then the viral genome is integrated into the host genomic DNA as a provirus. The genome is comprised of three genes encoding for structural proteins and glycoproteins. The *gag* gene encodes three group-specific antigens (capsid, matrix and nucleocapsid), the *pol* gene encodes the enzymes engaged in viral replication and DNA integration (RT, protease and integrase) and the *env* gene encodes the surface protein (SU) gp135 which recognises the putative cell receptor and transmembrane protein (TM) gp46 which mediates fusion between the viral envelope and the host membrane. Other accessory genes which have information for the synthesis of proteins that regulate viral replication are co-located with *pol* and *env*. The proviral DNA is surrounded by non-coding sequences known as long terminal repeats (LTRs), which contain components that activate DNA transcription (101).

Small-ruminant lentiviruses cause economic losses, but the significance and extent of these losses has not yet been fully assessed (77, 88). There are no effective drugs or vaccines available to treat or prevent SRLV infection, and control programmes remain the only approach to preventing the spread of SRLV infection. This control of the disease caused by SRLV is based on identifying and eliminating infected animals. Therefore, accurate and relatively inexpensive laboratory diagnosis is of particular importance for identifying positive animals. However, diagnosis of SRLV is complicated because of the high genetic variability of the viruses. There is no test that can detect all SRLV strains, and many infected animals remain undiagnosed carriers of the virus. The high genetic variability of SRLV manifests itself in the presence of multiple virus subtypes with variable pathogenic properties. Moreover, new viral variants capable of compartmentalising form, as SRLV derives mechanisms to evade and counter virus replication interference by innate host immunity. The purpose of this review is to summarise the current knowledge on SRLV genetic variability and its implications for tropism, the development of diagnostic tests and vaccines and the effectiveness of control and eradication programmes. This information may be helpful in selecting the most effective measures to use to reduce the spread of SRLV.

Genetic diversity

Genetic variability is a main feature of SRLV. Lentiviruses have one of the fastest-evolving genomes and it has significant variability. These viruses occur even in single animals as a population of genetic variants which may be termed quasi-species, one of which will be dominant. The quasi-species are characterised by less than 5% nucleotide divergence and they are consistently generated by mutation, recombination and selection pressure from the host immune system (101). The mutation of SRLV, which takes place at 0.2–2 mutations per genome per cycle, is caused by a lack of proofreading ability in the RT enzyme and by cytosine deamination by APBOEC3 proteins in reverse-transcribed single-stranded DNA, which leads to G-to-A mutation in the plus-stranded DNA. Furthermore, macrophages, the main SRLV target cells, contain excess amount of intracellular dUTPs that can be incorporated into DNA and also cause mutations (149).

The phylogenetic classification of SRLV has evolved over time. In 1998, Zanoni (178) classified the SRLV into six clades based on LTR, *gag*, *pol* and *env* fragments, without clearly separating isolates from goats and sheep. Then, in 2004 Shah *et al.* (162) revised this phylogenetic classification using the 1.8 kilobase pair (kb) *gag-pol* and 1.2 kb *pol* sequences or the 279 base pair (bp) RT region, and SRLV were divided into four major groups (A–D), which differ from each other in 25–37% of their nucleotide sequences. These groups were further subdivided into different subtypes, varying in 15–27% of their nucleotide sequence. Nevertheless, the high genetic variability of SRLV hampered detection of these fragments and classification based on these fragments could not be consistently achieved. Therefore, over the years, many other strains have been identified using different genetic fragments and regions. Nowadays, classification of SRLV is mainly performed on a conservative ~400 bp *gag* fragment for which sequences representing almost all subtypes are available. Currently, SRLV are divided into five genotypes (groups A–E) and at least 34 subtypes (A1–A27, B1–B5, and E1 and E2) (Table 1) (122). Some strains have been classified as A2/A3 because the differences between A2 and A3 were not enough to exclude these strains from either group (58, 162). Some subtypes have been classified on the basis of one region only, and others on the basis of two or more different regions. As more strains are analysed, new subtypes are constantly emerging.

Group A is a heterogeneous group which contains MVV strains while group B contain CAEV strains. Both groups are widespread in goats and sheep and have been reported in countries around the world, whereas the other three genotypes are less common. Genotypes C and E seem to be restricted to Norway and Italy, respectively, while genotype D is restricted to Switzerland and Spain (149). However, it is not known whether the occurrence of these genotypes is truly limited to specific countries, because in many parts of the world where molecular testing for SRLV has not been carried out and knowledge on the existing

genotypes is limited. Therefore, we cannot exclude the presence also in other regions of genotypes with apparent specificity to one or two countries. Information about SRLV subtypes circulating in each country is important for monitoring antigenic variability, since this phenomenon might be responsible for the misdiagnosis of highly different strains (27).

Table 1. Overview of SRLV subtypes

Subtype	Country of origin	Species	Genomic fragments used for classification	References
A1	Mexico	Goats	*gag* (578 bp)	2
	Italy	Goats	*gag* (800 bp)	63
	Belgium	Sheep and goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	97
	Poland	Goats	*gag* (625 bp), *env* (~400 bp)	125
	Brazil	Goats	*pol* (239 bp)	68
	Iceland	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb), RT (~300bp)	162
A2	Mexico	Sheep	*gag* (578 bp)	2
	China	Sheep	Whole genome	179
	Brazil	Sheep	LTR (460 bp)	30
	Turkey	Sheep	*gag* (800 bp)	111
	Canada	Sheep and goats	*gag* (1200 bp)	159
	China	Sheep	whole genome	177
	North America	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb), RT (~300bp)	162
A3	Turkey	Sheep	*gag* (800 bp)	111
	Italy	Sheep	*gag-pol* (800 bp)	15
	Switzerland	Sheep and goats	*gag-pol* (1.8 kb), *pol* (1.2 kb), RT (~300bp)	162
A2/A3	Czech Republic	Sheep and goats	*gag-pol* (800 bp)	14
	Spain	Sheep	*gag-pol* (800 bp)	52
A4	Switzerland	Sheep and goats	*gag-pol* (1.8 kb), *pol* (1.2 kb), RT (~300bp)	162
	Germany	Sheep	*gag* (~400 bp)	104
A5	Slovenia	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb)	80
	Turkey	Sheep	*gag* (800 bp)	111
	Italy	Goats	*gag-pol* (800 bp)	15
	Poland	Goats	*gag* (625 bp)	118
	Germany	Sheep	*gag* (~400 bp)	102
	Switzerland	Goats	*gag-pol* (1.8 kb), *p*ol (1.2 kb), RT (~300bp)	162
A6	France	Sheep and goats	RT (~300 bp)	162
A7	Switzerland	Goats	*pol* (1.2 kb)	162
A8	Italy	Goats	*gag* (800 bp)	17
A9	Italy	Sheep and goats	*gag-pol* (800 bp)	15
	Turkey	Sheep	*gag* (800 bp)	111
A10	Italy	Goats	*pol* (1.2 bp)	144
A11	Turkey	Sheep	*gag* (800 bp)	111
	Italy	Sheep and goats	*gag-pol* (800 bp)	54
	Germany	Sheep	*gag* (~400 bp)	102
A12	Poland	Sheep and goats	*gag* (625 bp), *env* (610 bp)	119
A13	Poland	Sheep	*gag* (625 bp), *env* (~400 bp)	125
A14	Slovenia	Goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	80
A15	Slovenia	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb)	80
A16	Poland	Goats	*gag* (625 bp), *env* (~400 bp)	128
	Germany	Sheep	*gag* (~400 bp)	102
A17	Poland	Goats	*gag* (625 bp), *env* (~400 bp)	128
A18	Poland	Sheep	*gag* (625 bp)	128
A19*	Italy	Goats	Full genome, *gag-pol* (800 bp)	15, 34
A20*	Italy	Sheep and goats	*gag-pol* (800 bp)	15, 34
A21	Germany	Sheep	*gag* (~400 bp)	102
A22	Iran	Sheep	*gag* (~400 bp)	102
A23	Italy	Sheep and goats	*gag-pol* (800 bp)	15
A24	Italy	Sheep	*gag-pol* (800 bp)	15
A25*	Poland	Sheep	*gag* (625 bp)	119
A26*	Poland	Sheep	*gag* (625 bp), *env* (610 bp)	119

A27	Poland	Goats	*gag* (625 bp), *env* (610 bp)	122
	Japan	Goats	*gag* (~529 bp)	79
	Mexico	Sheep and goats	LTR (300 bp), *gag* (578 bp)	2, 96
	Italy	Sheep and goats	*gag-pol* (800 bp)	101
	Slovenia	Goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	80
	Canada	Goats	*gag* (1200 bp)	159
B1	Belgium	Goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	97
	Philippines	Goats	partial gag	133
	U.S.	Sheep and goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	162
	Brazil	Sheep and goats	*env* (~400bp), *gag* (625 bp)	22
	Poland	Goats	*gag* (625 bp)	128
	France	Goats	RT (~300 bp)	162
	Spain	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb)	57
	Italy	Sheep	*gag-pol* (800 bp)	15
B2	Czech Republic	Sheep	*gag-pol* (800 bp)	14
	Poland	Sheep and goats	*gag* (625 bp)	119
	Switzerland	Sheep	*gag-pol* (1.8 kb), *pol* (1.2 kb)	162
	France	Sheep and goats	RT (~300 bp)	162
B3	Italy	Sheep and goats	*gag-pol* (800 bp)	15
B4	Canada	Goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	90
B5	Belgium	Goats	*gag-pol* (1.8 kb), *pol* (1.2 kb)	97
C	Norway	Sheep and goats	*pol* (710 bp), *tat-env* (2.1–2.4 kp)	55
D	Switzerland	Sheep and goats	*pol* (1.2 kb)	162
E1	Italy	Goats	*gag-pol* (800 bp)	63
E2	Italy	Goats	*gag-pol* (800 bp)	63

bp – base pairs; kp – kilobase pairs; RT – reverse transcriptase; LTR – long terminal repeats; *– two subtypes A18 were published by two research groups. The A18 found by Colitti *et al.* (34) was renamed to A19, and similarly the A19 identified by them was renamed to A20. Furthermore, subtypes A23 and A24 were also defined by two research groups at the same time. The subtypes found by Olech *et al.* (128) were renamed from A23 to A25 and from A24 to A26

Currently, phylogenetic analyses are performed based on *gag*, *pol*, *env* and LTR sequences (15, 96). Affiliation founded on different fragments is sometimes problematic, as it can lead to inconsistent results. Such a situation was observed for the A19, A20, B4 and B5 subtypes (90, 97, 119). Strain It38.2017 affiliated to subtype A19 on the basis of a partial *gag* sequence, but belonged to subtype B2 on the basis of its *env* sequence (119). Strain It009.2017 was defined as subtype A20 on the basis of its *gag* sequence, while on the basis of the *pol* sequence this strain belonged to subtype A1. In addition, the LTR sequence of this strain showed closest similarity to strains belonging to subtype B1 (121). Strains were identified as subtype B5 based on analysis of the *pol* region, while on the basis of the *gag-pol* sequences these strains belonged to the B1 subtype. Furthermore, subtype B4 identified by Santry *et al.* (159) appeared to be a recombinant strain (90), while genotype D turned out to be genotype A, showing a variation in the *pol* gene (149). Some authors made other discordant observations, for example that the Norwegian strain 1GA belonging to group C according to Shah *et al.* (162) was classified as a genotype-B strain based on the *gag-pol* and LTR sequences (96, 97, 119, 122, 121). Comparing different fragments (*gag*, *env* and LTR) of the same strains, Olech *et al.* (121) also noted some discrepancies, mainly between the LTR and *gag/pol* sequences. It was noted that most of such strains originated from mixed sheep and goat flocks, in which more than one subtype circulated. Therefore, these discrepancies may be related to the occurrence of cross-species transmission and adaptation to a new host (97, 119, 121, 159).

In addition, subtype A18, which was discovered first by Olech *et al.* (123), was not included in the study by Colitti *et al.* (34), who defined a different new subtype as SRLV A18, also contributing a new A19 subtype. Therefore, in future studies the subtypes found by Colitti *et al.* (34) were changed from A18 and A19 to A19 and A20, respectively (15, 102, 119). Recently, two different research groups (from Poland and Italy) revealed the occurrence of new A23 and A24 subtypes at the same time (15, 119). Phylogenetic analysis revealed that these subtypes were different, and Olech *et al.* (122) suggested renaming the subtypes found by that group of researchers from A23 and A24 to A25 and A26 (121). Therefore, current SRLV classification should be definitely updated.

SRLV easily cross the species barrier between sheep and goats, and there is no clear evidence that particular genotypes occur only in sheep or only in goats (100, 116, 153). Mixed flocks are places of continuous interspecies transmission. This is facilitated by management practices, such as feeding lambs with combined goat and sheep milk, allowing the two species close contact, and using the same feeding equipment for both (56). Circulation of different subtypes in mixed flocks was identified by many

authors, indicating that the co-existence of different SRLV subtypes is a normal feature when sheep and goats are farmed together (63, 119). Co-infection with more than one SRLV subtype, which gives rise to recombination of the virus, was also detected by many authors (15, 50, 119, 128, 145, 148). Recombination was evidenced between SRLV genotypes A and B, as well as between subtypes belonging to the same group. The *env* gene is a privileged recombination site; however, recombination events were also detected in the *gag* gene (8, 44, 90, 148, 159). Interestingly, more recombinations have been reported in goats than in sheep. There are still limited complete genome sequences of SRLV. Therefore, it would be desirable to fully sequence the genomes using the recently developed third-generation sequencing technique to investigate recombination frequencies among SRLV (179).

Tropism and pathology

The major tropism of SRLV is for monocytes, macrophages and dendritic cells. Small-ruminant lentiviruses may also infect other cell types, where they act as reservoirs of virus. Replication of SRLV in circulating monocytes is absent until the monocytes mature into macrophages in target organs. Thus, monocytes and immature macrophages remain latent and act as "Trojan horses", allowing the virus to spread throughout the body (136). A study performed by Illius *et al.* (74) suggested that a window lasting at least a few months, and possibly approximately as long as a year, is the latent period following infection when animals are not infectious. The differentiation of monocytes into macrophages increases the expression of various transcription factors, which triggers the transcription of proviral DNA and leads to the production of new particles (149). However, the precise events that trigger SRLV replication are still not understood completely. It seems that virus expression may be correlated with hormone levels (60). Therefore, the effects of various hormones on SRLV replication deserve further study.

Originally SRLV strains from sheep were classified phenotypically as rapid to yield virus for isolation and high in their pathogenicity while strains isolated from goats were classified as slow to yield virus for isolation and low in their pathogenicity, but many viruses isolated from both sheep and goats have intermediate phenotypes (101). It was also shown that strains isolated from animals with low and high proviral loads showed opposite *in vitro* phenotypes. Virus isolation was fast from blood-derived macrophages originating from animals with high proviral loads, while no isolation was obtained from blood-derived macrophages or fibroblast cell lines of animals with low proviral loads (35). In addition, animals with higher loads of proviral DNA were found to have more severe histological changes in different affected tissues, demonstrating that proviral load is positively related to the presence and intensity of disease symptoms (72, 151). Because SRLV strains differ in their biological features, infection outcomes vary between SRLV strains; however, the host–virus interaction and other factors on which outcomes depend are not fully understood (43, 101).

The tissues primarily infected by SRLV are the lungs, mammary glands, joints and the central nervous system. In general, MVV strains are more pathogenic in sheep while CAEV strains are more pathogenic in goats. The most prevalent form of disease in sheep infected with MVV (genotype A) is respiratory; however, genotype-C viruses can also cause lung lesions in sheep (30). Maedi-visna virus–like strains can occasionally cause arthritis, as described by some authors (119). This syndrome mainly occurs in adult goats infected with CAEV-like strains; Perez *et al.* (138) showed nevertheless that the B2 CAEV-like strain caused arthritis in sheep. Encephalomyelitis was occasionally seen in young goats with CAEV. However, strain 697 belonging to the A2 and A3 (MVV-like) subtypes was more prone to produce encephalitis in sheep than strain 496 belonging to subtype B2 (141). Mastitis has been reported in goats and sheep infected with MVV, CAEV and genotype-C SRLV (56). A single animal can also show multiple organ infection, the severity of the lesions varying over the set of organs affected. Genotype E has been isolated from Italian goats which showed no clinical signs associated with SRLV. This finding, along with the natural deletion of the dUTPase subunit of the *pol* gene and the *vpr*-like gene, led to the designation of these strains as low pathogenic (154).

It was suggested that sequence variation in the promoter/enhancer in the LTR may affect the interaction with cellular factors and modify viral gene expression and replication, affecting tissue tropism and disease outcome (9). However, the exact role of the LTR is still unclear (3, 59, 110, 122, 130). A correlation has been suggested between a deletion in the R region of the LTR and the presence of clinical symptoms, but many authors did not confirm this assumption, as this deletion was found in animals with and without clinical signs (9, 57, 124). Deletions and insertions have been also identified in the U3 region of the LTR in strains from many parts of the world, and they do not appear to be associated with specific host tropism (59, 110). Gayo *et al*. (52) also showed no significant correlation between grades of mastitis in sheep and alteration in the LTR sequences. Neurovirulence has been previously linked with duplication of CAAAT sequences in the viral LTR. Deletion of the CAAAT sequence reduced replication in sheep choroid plexus cells, but replication in macrophages was not affected (130). This may suggest that different transcription factors control the expression of SRLV in different cell types. However,

many authors showed that LTR promoter sequences from different tissues have no unique tissue-specific sequences controlling SRLV gene expression (3, 59, 110, 122).

The relation between SRLV subtypes and their biological features is not clear, although several subtype-specific markers in the LTR of SRLV have been identified by Olech *et al.* (120), who also revealed different levels of promoter activity for strains representing different subtypes. Therefore, differences between subtypes may be responsible for different transcriptional activity and replication rates, which may result in biological differences between SRLV subtypes. For example, low-pathogenic SRLV strains were detected in goats and sheep infected by subtype A4 strains, and it was suggested that mutations and deletions in the promoter sequence of the LTR of these strains may explain their attenuated phenotype (13, 20). Recently, a specific signature pattern associated with different SRLV subtypes and different clinical status in goats and sheep was identified in the V4 region of the *env* gene. The signature pattern was identified at position 54, where residues N, T and G occurred only in arthritic animals infected with genotype B, asymptomatic animals infected with genotype A and asymptomatic animals infected with genotype B, respectively (62). However, further studies should be performed to confirm these results. Additionally, it has been shown that two simultaneous mutations in the capsid gene and the *vif* gene caused attenuated SRLV replication in macrophages and decreased infectivity *in vivo* (66).

The genetic background of the host and compartmentalisation of SRLV strains have also been suggested as determining the outcome of infection (43, 101, 145). Within a single animal, SRLV can vary widely from tissue to tissue or cell type to cell type, forming viral compartments. As demonstrated by Pisoni *et al.* (145), the V4–V5 sequence of SRLV from blood differed to this sequence from colostrum cells. Compartmentalisation of SRLV based on the *env* sequence was also observed in the central nervous system, mammary glands and lungs of sheep infected with genotype A (150). Olech and Kuźmak (117) showed that compartmentalisation of A17 strains in goats is not strictly specific to the *env* gene, since the LTR and *gag* sequences also compartmentalised between colostrum and blood. Most recently, Echeverria *et al.* (46) detected SRLV in cultured macrophages from vaccine-derived granulomas and demonstrated the coexistence of different SRLV quasispecies in granulomas and peripheral blood mononuclear cells (PBMC). The exact mechanisms leading to the development of SRLV compartmentalisation have not been sufficiently investigated, but probably include the route of infection, varied immune selection pressure and cell type-specific changes in replication or gene expression of individual SRLV strains.

It is well known that SRLV are capable of crossing the interspecies barrier and can infect both goats and sheep. However, SRLV can also infect some wild small ruminants. Serological evidence of SRLV infection has been reported in some species including ibex, mouflons, chamois, and roe and red deer. Viruses of genotype B have been detected in wild ibex and Rocky Mountain goats. Descriptions of clinical signs of SRLV infection are rather uncommon in wild ruminants. It is only known that Rocky Mountain goats infected with CAEV developed pneumonia and wasting, and that neurological and joint signs were observed in some animals (135). Preliminary evidence also suggests that SRLV in wild small ruminants may be distinct from MVV and CAEV (139). Other animals are not thought to be hosts for SRLV. Experimental infection of cattle calves by a CAEV strain showed that this strain caused productive but not persistent infections (107). However, this experiment suggested that repeated host–virus interactions may, in the future, lead to emergence of a virus that will be able to adapt to a new host and cause disease. There is also no evidence that humans are susceptible to any SRLV strains. However, Tesoro-Cruz *et al.* (164) showed that 18 out 30 serum samples from children who had contact with CAEV-infected goats and consumed milk from those goats reacted with the CAEV gp135 protein. Unfortunately, it is not known whether the virus replicated in the children or whether there was only passive antigenic recognition. Moreover, Mselli-Lakhal *et al.* (109) showed that the inability of the virus to enter the cell is the only obstacle to CAEV replication in human cells, and it would be able to jump the species barrier to humans by gaining a new receptor or by collaborating with a helper virus. Therefore, it is still possible for SRLV to acquire a capability which would sustain its infection of human cells. Additional studies are needed to determine the epidemiological risk of infection from SRLV that crossed the species barrier, as the viruses could acquire new biological properties, broadening their host-range tropism and generating potentially zoonotic viruses (100, 107, 135). The implication of SRLV chimeric recombinants on disease pathogenesis and progression has not been determined and requires further research.

There is still limited information on the mechanisms that underlie the differences in pathogenicity between SRLV strains following infections by homologous (MVV-like strains in sheep and CAEV-like strains in goats) and heterologous (CAEV-like strains in sheep and MVV-like strains in goats) virus varieties. Michiels *et al.* (98) showed that heterologous infections with genotype A and B strains were less likely to lead to virus replication and virus persistence in target organs. Strains belonging to genotypes A and B were more able to replicate and induce changes in target organs after a homologous infection than heterologous one. Therefore, SRLV replication in target organs after

heterologous infection appears to be limited (98). Gjerset *et al.* (55) showed that caprine SRLV of genotype C induced higher viral loads and replicated more efficiently in goat cells. Furthermore, an A4 SRLV strain had higher viral loads in sheep than in goats and was described as attenuated for goats. On the other hand, experimental infection with strains 496 (subtype B2) and 697 (subtype A2/A3) indicated that strain 496 was more virulent for lambs than strain 697 (143). Also, Glaria *et al.* (57) revealed that strain B2 detected in Rasa Aragonesa sheep in Spain changed its phenotype during adaptation to the new host and caused an outbreak of arthritis in these sheep. This strain has MVV-like integrase in its genome, which represents an adaptation of caprine virus to sheep. No overall pattern is discernible for an infection's potential to propagate more strongly because it is homologous or its potential to adapt to a heterologous host, and the factors responsible for the differences in disease outcomes following infection of goats and sheep with a particular strain are unknown.

The receptor or receptors for SRLV have not yet been definitively identified. They may include a membrane-associated proteoglycan, major histocompatibility complex (MHC) class II molecules, CD4 and CXC chemokine receptor type 4, three membrane proteins defined as MVV binding proteins, and mannose receptor (149). However, none of these particles has been defined as a major receptor. It has been suggested that the receptor for SRLV is a common molecule because SRLV can enter different target cells, which does not dictate cell tropism (83). It has also been suggested that MVV and CAEV do not use the same receptors (73). Infection triggers the activation of adaptive and innate immune responses that initiate host restriction. Thus, productive infection failing to occur is not only because the functional receptors are lacking, as both factors of post-entry restriction may also be responsible. There is evidence that the host's genetic background plays an important role in determining susceptibility or resistance to SRLV infection and pathogenesis. Some breeds (Texel, Border Leicester, Finnish Landrace, Biellese, Churra and Assaf) are more susceptible to SRLV infection than others (Rabouillet, Île de France, Suffolk Columbia, Rambo, Polipay, Delle Langhe, Bergamasca, Raza Navarra and Aragonesa) (69, 83, 106, 147). Furthermore, many authors reported the presence of loci linked to SRLV susceptibility or resistance, such as *ZNF389* (174), *DRB1* (85), *TLR9* (160), *DPPA2/DPPA4* and *SYTL3* (173), *TMEM154* (69) and *CCR5* (175).

The most studied gene pertinent to SRLV infection is the transmembrane protein gene *TMEM154*. Allelic differences at the TMEM154 locus have been associated with susceptibility or resistance to SRLV infection and have been proposed as a locus for genetic marker-based selection (5, 69). It was shown that German, North American and Turkish sheep breeds with haplotypes encoding glutamate (E) at position 35 of *TMEM154* were highly susceptible to SRLV infection, while those with haplotypes encoding lysine (K) at the same position had a decreased risk of infection (69, 89, 104). However, Molaee *et al.* (103) and Moretti *et al.* (106) suggested that the relationship between the single nucleotide polymorphism (SNP) *TMEM154_E35K* and susceptibility or resistance must be treated with caution, since in the German Merinoland breed and the Italian Biellese, Delle Langhe and Bergamasca breeds, a large number of sheep with the less susceptible genotype were seropositive. Ramirez *et al.* (147) also suggested that the relationship between *TMEM154_E35K* genotyping and SRLV susceptibility is not clear. Furthermore, Moretti *et al.* (106) indicated that the SNP in the *TMEM154* gene is potentially protective only against SRLV representing genotype A. There is little information regarding the association of *TMEM154* haplotypes with SRLV susceptibility in a continent other than North America and countries other than Germany, Turkey, Italy and Iran; therefore, it is unclear whether the same association exists in different environments and breeds and for exposure to different SRLV. Studies on *CC5R* have also yielded divergent results. White *et al.* (175) indicated that a deletion in the *CCR5* promoter was linked with reduction of SRLV proviral load, with a 3.9-fold differential transcription in heterozygous animals. However, Molaee *et al.* (103) and Alshanbari *et al.* (5) did not show any association between a deletion in the *CCR5* promoter and the serological status of sheep or the SRLV proviral load. In a genome-wide associated study, the *ZNF389* deletion variant was associated with higher levels of MVV provirus in Rambouillet, Polypay and Colombia sheep (173). Polymorphic variants in *MHC* class I and II genes have been associated with SRLV proviral loads and disease progression; however, the highly polymorphic nature of many loci in *MHC* hinders investigation of the region's contribution to SRLV pathogenesis (83).

Toll-like receptors (TLRs) are host pattern recognition receptors that have an important function in the innate immune system. During SRLV infection, TLRs are activated, triggering the induction of cytokines and expression of antiviral proteins (19). Larruskain *et al.* (83) found that *TLR7* and *TLR8* were upregulated in the lungs of animals that had lesions compared to the lungs of control animals. Studies performed by Abendano *et al.* (1) also revealed significant upregulation of *TLR8* in infected animals as well in animals with lung lesions. Similar results were reported by Olech *et al.* (127), who showed that genes belonging to the toll-like family (*TLR2*, *TLR4*, *TLR7* and *TLR8*) were significantly upregulated in infected Carpathian breed goats compared to uninfected animals. Furthermore, another study conducted by Olech *et al.* (126) showed that some polymorphisms identified in the *TLR7* and *TLR8* genes were

significantly linked to SRLV proviral load in goats, which may indicate that SNPs in these genes can induce a differential innate immune response to SRLV affecting proviral load and thus disease pathogenesis and progression. Further investigations are needed to confirm the role of TLRs and their mutations in SRLV infection. Interferon (IFN)-stimulated genes have received increasing interest recently because of their effective inhibition of viral replication. However, there is little research on the role of internal restriction factors in SRLV infection. Jauregui *et al.* (75) showed that cells transduced with *TRIM5α* restricted SRLV DNA synthesis. In addition, Crespo *et al.* (35) showed that higher expression of *APOBEC3*, *TRIM5α* and *BST-2* (Tetherin) may be related to lower proviral loads of SRLV, which may provide protection against viral shedding and initiation of disease. The immune response to SRLV infection is very complex. A number of genes associated with SRLV infection and disease have been identified to date, but more studies are required to understand the host–virus interaction. Identifying the loci responsible for disease resistance is of fundamental importance, as it could assist selective breeding for naturally resistant animals.

Transmission

There are many possible routes of SRLV transmission. Precise knowledge of the routes of SRLV transmission is critical to designing efficient disease control programmes. For many years, lactogenic transmission was thought to be the prime route of SRLV infection. This route is more significant in small ruminants than in primates, because the digestive tract of new-born small ruminants is more permeable during the first 24 h after birth and viruses and infected cells can be absorbed through the intestine (144). However, recent publications suggested that lactogenic transmission is not effective under natural conditions. Alvarez *et al.* (7) and Broughton-Neiswanger *et al.* (26) showed that only 10–15% of lambs that were born to and fed by seropositive ewes were infected, while Hermann-Hoesing *et al.* (72) showed that no such lambs developed persistent infection or seroconverted. This inefficient transmission may be partially due to the presence of high amounts of maternal anti-SRLV antibodies in the colostrum/milk of infected ewes (72). The predominant route is currently unclear, and there is a growing agreement that SRLV transmission is primarily by the horizontal route through inhalation of respiratory secretions in prolonged close contact. Broughton-Neiswanger *et al.* (26) calculated that horizontal transmission accounted for 85–90% of all transmission in a ewe flock which was not included in any control programmes. However this transmission is efficient only in adult animals. Alvarez *et al.* (7) showed that transmission between infected lambs was restricted, perhaps due to latency. The pulmonary fluid, which is the source of infection if an animal coughs, contains both cell-associated and cell-free virus. The virus can be detected in exhaled air; however, airborne transmission has not been proved, indicating close and continuous exposure to the virus would be needed in order for airborne transmission to occur (171). Compared to the intratracheal route, intranasal inoculation is considered ineffective (168). The main site of SRLV entry by respiratory transmission is the lower respiratory tract rather than the nasal cavity or nasopharynx; therefore, sheep are unlikely to be infected with SRLV through pure nose-to-nose contact (26).

The effectiveness of horizontal transmission may differ according to the management practices applied to flocks, which vary between countries and regions. Berriatua *et al.* (16) showed that in a semi-intensively farmed sheep flock, horizontal transmission was relatively more important than transmission from seropositive dams to offspring. This is because the time when animals are exposed to horizontal transmission is much longer than the time of exposure to colostral transmission, and colostrum does not necessarily contain a sufficient dose of virus to cause persistent infection. Many studies have shown a relationship between housing time and SRLV seroprevalence (16, 87, 137). The spread of SRLV is more easily observed in intensively farmed sheep constantly housed in poorly ventilated crowded sheds, among animals that are suffering from respiratory failure and have increased nasal secretions (101). Illius *et al.* (74) revealed that the average latency to seroconversion was significantly reduced in sheep with weak body condition and parasitic infections. Therefore, bad husbandry conditions and occurrence of secondary infections favour the spread of SRLV.

The route of transmission may vary depending on the host species. It has been indicated that in goats, the humoral immune response does not seem to reduce the shedding of provirus in milk as efficiently as in sheep (12); therefore, the effectiveness of SRLV transmission from mothers to offspring is considered more significant in goats than in sheep, while horizontal transmission is believed to be more important in sheep than in goats (7, 26, 149). Moreover, animals with low proviral load did not shed virus, in contrast to animals with high proviral load (35). Such animals, known as long-term non-progressors, demonstrate a positive serological response but no viral replication, which likely reduces viral shedding and ultimately poses no real threat of the spread of the virus in the flock. The route of infection also depends on the type of virus. The mammary gland has been shown to be the only target organ that effectively allows transmission of attenuated SRLV belonging to subtypes A4 and E1. Consequently, a high proviral load was found only in the mammary gland, while the proviral load was low in other tissues (43). Moreover, goats infected with SRLV of genotypes A10 and B1 tended to transmit A10

genotype viruses to their offspring more effectively than B1 genotype viruses. This may suggest that SRLV genotype A is particularly effective in lactogenic transmission (144).

Intrauterine transmission is possible, because proviral DNA has been detected in uterus, oviduct and ovary tissue (49). De Sousa Rodrigues *et al.* (42) indicated that there is a possibility of low-level (1.4%) transplacental transmission of SRLV. Furthermore, Alvarez *et al.* (7), Hasegawa *et al.* (67) and Furtado Araújo *et al.* (51) revealed that a significant proportion of newborns taken from their mothers immediately after birth were SRLV positive, suggesting the occurrence of intrauterine transmission. However, opinions regarding intrauterine transmission are still divided and the exact significance of this route remains unknown.

The role of semen in SRLV transmission has not been completely investigated. The presence of SRLV in male genitals and in semen has been shown, but it is unknown whether provirus is transmitted to future progeny and ewes through respective parental and sexual transmission (19, 140). Several studies also reported gender differences in the seroprevalence of SRLV and higher prevalence in females. However, there are no biological reasons for this phenomenon; most likely it reflects differences in the management of females and males in flocks (10). Fomites are also suspected as a means of infection with SRLV (27), but their importance in the transmission of the viruses has not been well studied. Climate change and the spread of potential vectors raise the importance of this route. The transmission of SRLV through contaminated barns and feeding and drinking equipment has not been fully elucidated (139), but SRLV has been detected in drinkers inside pens and in faeces, so the role of contaminated water and faeces in SRLV transmission needs further attention (170). The potential risk linked to the iatrogenic spread *via* shearing equipment and reused needles has also not been assessed.

The results of the transmission pathway investigations may be unreliable, because the use of different tests and the fluctuation of viral loads lead to results being discrepant. The virus load is also controlled by certain genetic components of the host, which are components raising the effectiveness of the immune system. Sheep and goats respond differently to infection, which affects the results of serological and molecular diagnostics.

Diagnostic testing

There are currently no SRLV tests on the World Organisation for Animal Health (WOAH, formerly OIE) List of Diagnostic Tests, but the Terrestrial Code specifies test methodologies. The diagnosis of SRLV infections is based on clinical signs, pathological changes and laboratory tests. However, the clinical symptoms of SRLV infections can be similar to those of other diseases and infections may be asymptomatic. The infections are identified using indirect techniques that detect antibodies against SRLV or direct techniques that detect the virus itself. A gold standard test not as yet having been developed, the methods most commonly used to diagnose SRLV are presently agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

Agar gel immunodiffusion and ELISA tests. Serological diagnosis is currently the best option for detecting SRLV in livestock. The two most commonly used tests for detecting specific antibodies to SRLV are AGID and ELISA, and both of them are recommended by the WOAH (176). The most frequent antigens used in AGID tests are the capsid antigen (CAEV p28 or MVV p25) and the envelope glycoprotein gp135, which are extracted from cell culture. Commercial AGID tests are available, but the antigens used in these tests are derived from only one strain, mainly CAEV-63 or MVV WLC1 (176). Michiels *et al*. (99) revealed that combining the results of two AGID tests, one based on CAEV p28 and the second based on MVV p135, resulted in 100% specificity and sensitivity for detecting SRLV in Belgian goats and sheep. Therefore, the antigens used in both tests could be combined in the future in a single AGID test with excellent test characteristics; however, the development of such a test would still leave the market without an AGID test able to differentiate MVV-like from CAEV-like infections. The use of locally circulating strains will also undoubtedly improve the diagnostic performance of the AGID test. These tests are specific but time-consuming, and their outcomes may be influenced by the subjective interpretation of the person reading the results. Furthermore, the sensitivity of AGID tests is low; therefore, the use of AGID is limited and the method has almost completely been replaced by ELISAs (99).

The ELISA is quantitative, economical and can be automated, making it useful for testing large numbers of sera. Many commercial or in-house ELISA tests have been developed for detection of antibodies against SRLV. Unfortunately, only a few are registered for use in veterinary diagnostics rather than laboratory research. Most of these tests are indirect assays using whole virus, recombinant proteins or glycoproteins and synthetic peptide antigens which are produced from the whole or partial *gag* or *env* gene. Only a low number of competitive ELISAs (cELISAs) have been designed. A disadvantage of the indirect ELISA is the need to dilute sera to reduce false positives. Competitive ELISAs are characterised by high sensitivity afforded by undiluted sera samples, but the specificity of these tests is lower than that of indirect ELISAs. Most diagnostic indirect ELISA tests use the gp135SU surface unit envelope and/or gp46TM transmembrane antigens or peptides derived from them, or use the

p25CA capsid protein. In some tests the SU gp135 and TM gp46 antigens are used separately, while in other tests, especially in-house developed tests, these antigens are used in combination with capsid antigens (24, 31). In the commercial ID Screen MVV/CAEV Indirect test (IDvet, Grabels, France), a mix of peptide antigens derived from the MVV/CAEV capsid protein, transmembrane peptides and the surface protein is used as the antigens. The MVV/CAEV p28 Ab Screening Test (IDEXX, Westbrook, ME, USA), ELITEST MVV/CAEV (Hyphen Biomed, Neuville-sur-Oise, France) and Enferplex Goat/Sheep Multi-Disease 5D (Enfer Scientific, Naas, Ireland) use as their antigen a recombinant capsid protein and transmembrane protein derived from a genotype-A isolate (166), while the Eradikit SRLV Screening (In3 Diagnostic, Turin, Italy) uses peptides from transmembrane, matrix and capsid proteins from genotypes A, B and E. The combination of capsid and envelope SU gp135 and TM gp46 antigens results in higher test sensitivity, as antibodies against capsid antigens are detectable early after infection whereas anti-TM and anti-SU antibodies prevail in later stages of infection (20–33 weeks after infection) (24, 31, 82, 99, 155, 176). The combination of both recombinant proteins as antigens resulted in higher sensitivity and specificity compared to tests based on the whole-virus antigen or core protein p25 (24, 31). Therefore, combining capsid- and envelope-derived antigens in a single test is crucial for identifying seropositive animals at all stages of infection. A comparison of five commercial ELISAs showed that the highest sensitivities in sheep and goats were found for the tests using multiple antigens (the Eradikit SRLV Screening, ELITEST MVV/CAEV and ID Screen MVV/CAEV tests). The highest specificity was observed in the MVV/CAEV p28 Ab screening test, which uses only one capsid antigen, significantly reducing the sensitivity of the test. Overall, the sensitivity and specificity of a test are generally in inverse proportion to each other. The higher the sensitivity, the lower the specificity, and *vice versa* (97). These results showed that higher sensitivity is achieved when both *gag*- and *env* gene–derived antigens from more than one genotype are used (2, 155, 166).

Despite progress in diagnostic techniques, the antigenic variability of SRLV, which reflects the high genetic variability of these viruses, and the limits to the antigenic cross-reactivity between SRLV groups definitely impair the diagnostic performance of monostrain ELISAs, and can result in their failure to detect all infections (27, 37, 84). The specificity of monostrain ELISAs is usually high, but the sensitivity is extremely variable. Differences in the sensitivity of ELISA tests lead to contradictory results from these tests (2, 4, 24, 36, 76, 99, 161). The CAEV/MVV Total Ab test (IDEXX Switzerland AG, Liebefeld-Bern, Switzerland) has sensitivity of 98.6% and a specificity of 99.3%; however, this test could not detect animals infected with A4 strains, which reveals some limitations (27). Serological studies conducted with a cELISA that utilises the recombinant SU protein of a CAEV-type strain (Small Ruminant Lentivirus Antibody test kit; VMRD, Pullman, WA, USA) and an ELISA that utilises peptides derived from transmembrane, matrix and capsid proteins from genotypes A and B (Eradikit SRLV Screening) revealed 164 discordant results. A higher number of seropositive animals were found using the cELISA than using the Eradikit, the former identifying 15.3% more goats and 21% more sheep (2).

The use of single strain–specific epitopes dramatically reduces the sensitivity of ELISAs if animals are infected with a strain different from that employed in the test, and leads to misdiagnoses (27, 82, 152). To avoid this, certain commercial ELISA test use antigens derived from more than one SRLV strain. In the INGEZIM Maedi ELISA (Eurofins Ingenasa, Madrid, Spain) and the LSIVet Ruminant Maedi-Visna/CAEV kit (LSI, Thermo Fisher Scientific, Waltham, MA, USA), specific peptides derived from SRLV genotypes A and B are used. However, detailed information about the specific protein(s) used in the tests is not available. In the ID Screen MVV/CAEV Indirect test, a mix of peptide antigens derived from MVV/CAEV TM, gp135 and p25 is used as the antigen while in the Eradikit SRLV Screening, peptides originating from transmembrane, matrix and capsid proteins from genotypes A, B and E are incorporated, thus allowing detection of infection caused by genotype-A, -B and -E viruses. Most of the results revealed that ELISA tests were more specific and sensitive when homologous antigens were used. Consequently, infections with genotype B were best detected by CAEV-derived antigens while genotype-A infections were preferably detected by MVV-derived antigens. However, the commercial VMRD ELISA was effective in detecting SRLV in both goats and sheep infected with A and B genotypes, despite its being based only on the B genotype strain (2, 70). This may be due to dilution of serum not being required in this ELISA, unlike others. Furthermore, the Chekit CAEV/MVV Antibody Elisa Test Kit (IDEXX) detected a relatively low rate of genotype-A infections despite the use by this test of whole virus as its antigen, which should recognise cross-reacting antibodies (158); this test also gave false-positive results in animals vaccinated against bluetongue (169). Echeverria *et al.* (46) showed that caution should be exercised when ELISA tests are considered individually, as combining tests can enhance the detection rate of seropositive animals by up to 50%. Therefore, the homology between the strain used in the test and the strain present in the population or region being tested should always be considered when selecting an ELISA. The perfect example is misdiagnosis of genotype-E infection using commercially available ELISAs due to the low similarity between genotype E and genotype B (CAEV)

or A (MVV) in the major immunodominant regions (63, 153). In this regard, these results underscore the usefulness of applying more than one test, specifically when the antigenic sequences of viruses circulating in the area under investigation are unknown (166). It is crucial to increase the sensitivity of the test and improve the detection of immune responses induced not by one but by several circulating genotypes. The differences in the performance offered by different commercial kits may suggest that updating serological tests by incorporating antigen mixtures is sorely needed. It is important to consider multiplex serology using multisite polypeptide antigens obtained by fusing different multitope regions of proteins to obtain a small number of single polypeptides in the assay. However, covering an ELISA plate with too many proteins can be problematic, and can reduce assay sensitivity because proteins can compete for uptake, leading to a suboptimal amount of each protein attached to the wells (105). Moreover, in order for a multistructured polypeptide to contribute to increasing an immunoassay's affinity, each antigen integrated into it should mimic its native state very closely (131).

Although ELISAs are the most widely used serological tests, most of them have not been validated against standard reference tests, such as radio-immunoprecipitation or Western blot, as recommended by the WOAH in the Terrestrial Manual (Chapter 1.1.6. "Principles and methods for the validation of diagnostic tests for infectious diseases"). To date, only a few ELISA tests have met these standards (71). Since no gold standard for diagnosing SRLVs exists, the performance of an ELISA undergoing validation is assessed either by using other tests as references or by defining each sample as infected or uninfected based on the results from most of the different tests evaluated (39, 99, 161). Ideally, these should be serum samples from animals infected with a known genotype, and for the most rigorous validation, should be reference serum samples. In addition, statistical methods that do not require defining samples as being truly positive or negative can be used to estimate the sensitivity and specificity of ELISA tests. Given the lack of a gold standard, assessing sensitivity and specificity is very difficult, and validation data reported by manufacturers should be interpreted with caution.

Cardinaux *et al.* (27) showed that the Small Ruminant Lentivirus Antibody Test Kit cELISA (VMRD, Pullman, WA, USA) had different sensitivity with sheep and goat sera despite both species having been infected with the same SRLV subtype. Therefore, differences in the sensitivity and specificity of tests may also result from different sensitivity in sheep and goats to the test antigen. This implies that validation of ELISAs should be performed separately on ovine and caprine serum samples. The differences in reactivity can be explained by genetic differences between sheep and goats affecting their ability to form an effective antibody response. Moreover, the virus appears to be subject to different selection pressures after interspecies transmission. Analysis of the humoral immune response after experimental infection showed that the antibody response developed faster in sheep and goats infected with genotype B than in sheep and goats infected with genotype A (98). Goats and sheep seemed similarly susceptible to the genotype-B strain, while goats were less susceptible to infection with the genotype-A strain than sheep (98). In contrast, some authors revealed that genotype-A–derived antigens better detect heterologous infection than genotype-B–derived antigens (37, 158). In both goats and sheep, stronger antibody responses against SU and TM proteins than against CA proteins have been reported (24, 27, 98). The mechanisms and consequences related to SRLV adaptation to a new host after interspecies transmission have been poorly investigated and are still insufficiently understood. More scientific knowledge is needed about antibodies to various viral proteins and the differences between homologous and heterologous infection. Characterisation of the immune response after interspecies transmission may be relevant to identify any differences that may be important to the choice of diagnostic method.

It has been proposed that infection of wild ruminants in close contact with infected domestic small ruminants plays a role in the epidemiology of SRLV. Diagnosis of infectious diseases is more challenging in wild animals than in domestic animals because of difficulties in accessing animals and samples and because of the poor quality of samples. In most serological studies performed to date using wildlife samples, commercial diagnostic kits designed for domestic animals were used, although these kits had not been validated for free-living animals. A number of tests designed for domestic animals do not have the same levels of specificity and sensitivity when used in wild species (156). Consequently, antibodies to SRLV were detected sporadically or not at all (81, 92, 135, 157). When in-house ELISAs were used, higher reactivity was observed for serum from wild animals. For example, using a modified in-house ELISA based on synthetic peptides, Sanjosé *et al.* (157) detected SRLV antibodies in 14 out of 193 (7%) red deer and 1 out of 10 (10%) mouflons, but when the same samples were tested using a commercial assay, none of the animals were seropositive. A similar situation was observed by Olech *et al.* (124), who estimated that the prevalence of SRLV in wild ruminants ranged from 5.3% to 24.6% with in-house multi-epitope antigen ELISAs, while the estimated prevalence using a modified commercial ELISA was 2%. This may indicate that the real number of infected free-ranging animals may be much higher than that detected by commercial tests. Consequently, there is a need to design diagnostic tests suitable for wildlife species. However, there are many challenges to validating diagnostic tests for wildlife samples, such as the lack of

a gold standard and difficulties in obtaining positive and negative controls.

Another drawback to ELISA testing for SRLV is the misdiagnosis which may result from the significant fluctuations in antibody titres over the life of the animal: in some cases, the low points in the fluctuations could cause an ELISA to give intermittent negative results (36, 37, 71, 149). Furthermore, induction of antibodies post infection is slow and in some cases seroconversion occurs several months after or infected animals may never seroconvert at all. The time to seroconversion varies depending on which viral antigens antibodies are directed against, and antibody tests utilise different viral antigens to detect antibodies. The reason for the serological latency is not known for certain, but is thought to be the very low levels of non-cellular virus produced, leaving replication to occur in tissue macrophages rather than circulating monocytes (7). This latency means that the first stage of infection cannot be diagnosed by serology.

Testing milk instead of blood eliminates stress in animals and decreases the costs associated with field sample collection. Some studies have examined the usefulness of milk in detecting antibodies to SRLV, and most have shown fairly good agreement between the results of antibody detection in this matrix and detection in blood (11, 23, 95). Brinkhof *et al.* (23) even indicated that milk is a suitable substitute for serum, but the number of tested samples was low in that experiment. Serological tests of milk are relatively rarely used in veterinary practice with dairy goats, but their use in eradication campaigns should be considered. There are four commercial tests for the identification of antibodies against SRLV in milk: the ID Screen MVV/CAEV Indirect Screening test, Eradikit SRLV Screening test, CAEV/MVV Total Ab test and Enferplex Goat/Sheep Multi-Disease 5D. Other tests have also been used for research studies. The ID Screen MVV/CAEV Indirect Screening test and IDEXX MVV/CAEV p28 Ab Screening test showed sensitivity of 89.3% and 98.3% and specificity of 91.4% and 95.5%, respectively, when milk samples were used. In both indirect ELISAs, the optimal dilution of lactoserum was 0.5, ten times less than the dilution of serum samples recommended by the manufacturer (146). The Small Ruminant Lentivirus Antibody Kit cELISA was significantly less accurate at testing milk samples and showed sensitivity of 71.2% and specificity of 96.6%. A study performed by Potăriche *et al.* (146) revealed that indirect ELISA tests could be used to test an individual goat's milk samples for SRLV infection, although some animals were negative in milk and positive in serum. However, it should be noted that the studies were performed only on goat samples and the test's effectiveness on sheep's milk samples is unknown. Using milk as a matrix for testing also has its drawbacks. The results of a study performed by Barquero *et al.* (11) revealed that the presence of antibodies and proviral DNA in sheep's and goat's milk can change over time. The use of milk samples may render diagnoses less reliable because antibody secretion declines during lactation. In addition, mastitic inflammation and increased milk fat content are potentially positively correlated with an increase in false-positive results. Therefore, the use of milk as a sampling matrix requires additional field verification. The use of bulk milk should also be considered for initial characterisation of a flock as SRLV-free or infected. A very sensitive ELISA should then be used. Brinkhof *et al.* (23) demonstrated that plasma can be used instead of serum. Using plasma can reduce the costs of sampling since blood samples in ethylenediaminetetraacetic acid may be available through their collection in other monitoring programmes. To date, only the ID Screen MVV/CAEV Indirect Screening test, Eradikit SRLV Screening test, CAEV/MVV Total Ab test, Enferplex Goat/Sheep Multi-Disease 5D and MVV/CAEV p28 Ab Screening test have been officially approved for detection of SRLV infection in plasma samples.

Many commercial tests are unable to differentiate between SRLV genotypes. There is only one (the Eradikit SRLV Genotyping) which could differentiate between infecting A, B and E genotypes. Detailed information about the specific protein(s) used in the test is not available. This test is rarely used and has produced indeterminate results in many cases. Acevedo Jimenez *et al.* (2) revealed that this test was unable to classify 34.8% of samples. In addition, in only 13 out of 43 sheep and in 11 out of 49 goats did PCR-sequencing results coincide with the serotyping results. Overall, genotyping and serotyping results coincided in only 25.7% of cases, indicating that this kit is not an adequate or reliable method for predicting the genotype of an infecting virus (2, 113). Many researchers have used in-house ELISAs based on the SU5 protein. This protein is a type-specific B-cell epitope located in the carboxy-terminal region of the Env protein and is a good candidate for the development of a new serotyping tool to provide information on the genotype classification of infecting viruses (29, 105, 125). However, using five ELISAs containing synthetic SU5 peptides of SRLV subtypes A1, A3, A4, B1 and B2, Schaer *et al.* (161) obtained 24 false positives. Consequently, it was only possible to distinguish between MVV-like and CAEV-like viruses. Even at this less fine level, 19 out of the 59 samples previously confirmed as CAEV-like were misclassified as MVV-like. Limitations also emerge of SU5 ELISAs when applied to sera showing broad SU5 reactivity (105).

Polymerase chain reaction. Recently, several PCR methods for detecting SRLV have been developed, and they are now routinely used in many laboratories. Small-ruminant lentiviruses are cell-associated and free virus is rarely found in blood and other fluids, so the opportunity to detect viral RNA by reverse transcription PCR in these specimens is almost

non-existent. Most studies have used PBMC or peripheral blood leukocytes (PBLs) as the target cells for the PCR (39). The use of alternative sample sources for PCR yields variable results. It is mainly from colostrum or milk, or from tissues including brain, lung, mammary gland and udder that DNA is extracted, while DNA extracted from semen or from heart, kidney, liver, synovial membrane, bone marrow, spleen, lymph node, testis, ovary and uterus tissue is less frequently used (134). Blood clots, which are available in tubes used for serum samples, have also proved to be an unsuitable option because of high loss of test sensitivity (99).

Besides conventional PCR, other PCR methods have been developed in order to improve the specificity, sensitivity and accuracy of these reactions. A combination PCR for different genomic regions (multiplex PCR), a (semi-)nested PCR, a real-time PCR and nested real-time PCR have been used, with contradictory results.

The major advantages of PCRs over serological testing methods are the early detection of infected animals before the antibody response and the detection of latent infections (32, 71, 134, 149). They also allow the testing of young animals with maternal colostral antibodies; therefore, PCR is the best method for testing animals under one year old (36). Several authors have shown that in the post-seroconversion phase of infection, viral load is low and at this stage PCR is less sensitive than serology, but before seroconversion PCR is much more sensitive in identifying infected animals. Echeverría *et al.* (46) showed that the true infection rate in a population could not be estimated by a single ELISA or even three distinct commercial ELISAs, because about 10% of infected animals were seronegative in all tests but were PCR positive. Therefore, it has been suggested that for SRLV diagnosis, PCR should not be considered as an alternative to serology but as a complementary test. Therefore, a combination of serological testing and PCR is essential for optimal detection of SRLV-infected animals (36, 46, 47, 101).

Sequence variability, low amounts of provirus during the latency period and low levels of viral load in samples limit the usefulness of PCR as a commercial diagnostic assay (158). Therefore, the market for PCR tests is very limited compared to the market for serological tests. There are only a few commercially available PCR tests for diagnosing SRLV infection, and only one article has been published, describing only one of these tests. Specifically, the article describes a real-time quantitative PCR (qPCR) kit (EXOone Maedi Visna CAEV oneMix; Exopol, Zaragoza, Spain), which exhibited higher sensitivity by detecting more positive animals than did an in-house *gag* PCR (46). The test is available to detect SRLV genotypes A, B and E, but it has not been confirmed that the test can identify all of these genotypes.

Only 1 out every 10^4–10^5 PBMCs contains provirus in SRLV-infected animals (149), so low numbers of infected monocytes or limited viral replication may hamper detection of proviral DNA, causing false-negative results which reduce the sensitivity of PCRs (143). Wagter *et al.* (172) showed that weakly positive serological animals were PCR negative when DNA was prepared from whole blood but PCR positive when DNA was extracted from monocyte fractions. Thus, a higher concentration of monocytes in the starting material improves PCR sensitivity. Although qPCR is able to detect provirus DNA before antibodies can be detected by ELISA, ELISA is recommended because antibodies are detectable through the entire duration of infection, whereas this is not the case for provirus detection by qPCR (98). The variability of PCR sensitivity through an infection is due to proviral levels in blood changing over time, depending on temporary changes in the host immune response.

Developing a single assay that universally detects all SRLV is extremely difficult because of the high level of genetic heterogeneity of SRLV, which are divided into five genotypes and several subtypes. The design of primers with a wide genetic window is prerequisite for the development of PCRs and a key step in improving the diagnosis of SRLV. Therefore, sequence information from a large number of diverse isolates is very valuable. Some authors have indicated that the development of PCR tests to detect local strains circulating in a given geographical region may be a more realistic goal for diagnosis of SRLV in the field (12, 36). Many of the PCR detection methods described to date have been developed to detect a specific SRLV strain, and are not efficient in detecting all SRLV subtypes (2, 21, 28). The difficulty in detecting suitable regions that would effectively amplify SRLVs belonging to different groups has directed some researchers to develop a type-specific approach with type-A primers and type-B primers. Various qPCRs developed by Kuhar *et al.* (80), Michiels *et al.* (99) and Acevedo Jimenez *et al.* (2) targeting the *gag* gene, and an in-house nested qPCR developed by Schaer *et al.* (161) were able to detect and discriminate between SRLV genotype-A and genotype-B strains. However, differentiation between these strains was only possible using two separate reactions, and these tests were not able to detect group E SRLV strains. The only assay that was able to simultaneously detect strains belonging to groups A and B was the semi-nested real-time PCR developed by Chassalevris *et al.* (32). This real-time PCR targeted the *pol* sequence and used degenerate primers, which consist of a mixture of very similar oligonucleotides that allow simultaneous detection of different viral variants or several closely related viruses. The degenerate primers designed by Eltahir *et al.* (47) allowed the amplification of SRLV belonging to subtypes A1, A3, A4, A5, B1 and B2 aswell as isolates from the Netherlands and Greece. Also, LTR and leader-*gag*

real-time PCRs developed by Brinkhof *et al.* (25) were presumably able to detect MVV and CAEV strains; however the phylogenetic classification of the tested strains was not provided. Another successful PCR was a quantitive reverse transcription PCR developed by De Regge *et al.* (41), which was able to detect genotype-A SRLV strains originating from different geographical regions. An alternative test allowing discrimination between SRLV groups is the heteroduplex mobility assay (HMA). In this method, DNA of the reference strain and PCR product from unknown samples are mixed, denatured, and slowly cooled. Then, polyacrylamide gel electrophoresis is performed. The differences in the migration of heteroduplex bands indicate the difference in nucleotide sequence between the reference DNA and the sample DNA. For example, when heteroduplex bands migrate faster with a CAEV-like reference strain than with an MVV-like reference strain, this indicates that the tested sample is more similar to CAEV and belongs to the group B (53, 116). Genotyping of SRLV strains circulating in a specific regions would undoubtedly help in selecting appropriate serological tests and designing specific primers for PCRs.

The high natural genomic variability of SRLV complicates the selection of conservative regions for primer design. Various regions of the virus genome, including the LTR and the *gag*, *pol* and *env* genes, have been selected for primer design. It was assumed that the LTR and *pol* sequences are more conserved than that of *gag*, while the *env* sequence has the highest variability. The polypurine tract and rev response element are also relatively conserved (101). If PCRs based on different genome fragments are used with the same samples, they give divergent results. Carrozza *et al.* (28) showed that a *pol* PCR failed to detect some infected animals that were positive in the *gag* PCR. A PCR targeting LTR sequences developed by Extramiana *et al.* (48) was found to be 100% specific and 98% sensitive; however its sensitivity varied depending on type of samples used (83.5% for PBLs, 66.7% for milk samples and 89.6% for tissue samples including spleen, brain, lymph node, mammary gland and lung). Moreover, Marinho *et al.* (93) showed that a PCR detecting SRLV was significantly more sensitive when using LTR primers than when using *gag* primers. However, other researchers showed that a *gag*-target PCR was more sensitive than an LTR PCR (25, 86). Similar discrepant results were observed when *pol* and LTR PCRs were performed. Some authors found that a *pol* PCR had lower sensitivity compared to an LTR PCR (7, 93), while Barquero *et al.* (12) obtained opposite results, *i.e.* that a *pol* PCR had higher sensitivity than an LTR PCR. A real-time PCR assay based on the leader–*gag* and LTR regions, showed a sensitivity of 82–88% in sheep and goats originating from different geographical areas in Norway, Spain, France and Italy (25). The leader–*gag* test showed superior performance than the LTR PCR. The sequence of the leader–*gag* region is very similar to that of the primer binding site, and is presumably the most conserved part of the genome of SRLV (23, 25). Studies on HIV have also revealed that the most conserved part of the HIV genome is not located in one of the open reading frames, but in the 5′ untranslated leader region (129). Mosa and Zenad (108) revealed that amplification of the *gag*, *pol*, *env* and LTR regions led to different results, suggesting that a multiplex PCR would be an ideal tool for diagnosis of SRLV. Such a test has not yet been developed. Therefore, further development of PCR-based methods to increase diagnostic specificity and sensitivity is needed. Marinho *et al.* (93) developed only a duplex nested PCR using *pol* and LTR primers. This test allowed more accurate diagnosis than single PCRs and was able to detect both MVV and CAEV strains in a single assay.

Some negative PCR results may result from compartmentalisation of SRLV, *i.e.* the presence of different viral sub-populations in different organs or tissues in a single animal (32, 117, 150). The virus may reach different tissues including the lungs, central nervous system and mammary glands. Therefore, an important aspect of SRLV detection is the choice of the appropriate body part and tissue. Olech *et al.* (117) revealed that in goats infected by subtype A17 and presenting with arthritis, the highest proviral load was found in the synovial membrane tissue. A relatively high proviral load was found in DNA extracted from the mammary glands, lungs and PBMCs and the lowest proviral load or no provirus was identified in choroid plexus and brain cells. Deubelbeiss *et al.* (43) showed that the highest proviral load in goats infected by subtype A4 was found in the mammary gland, a moderate-to-low proviral load was observed in synovial membrane and PBMCs, and the lowest proviral load was noted in choroid plexus cells. Udder and mammary proviral load and virus detectability in milk may not be correlated, because Adjadj *et al.* (4) revealed that the sensitivity of SRLV detection by qPCR was lower in milk than in blood. In addition, ELISA-positivity in animals that were always negative by PCR on milk samples may indicate that the viral infection may occur in organs other than the udder. Moreover, infection distribution within this organ has been noted to be uneven: more sheep had positive PCR results in the right mammary gland than in the left (12). Michiels *et al.* (98) showed that the genotype-A strain was identified in almost all organs tested in infected sheep, but the highest viral load was found in the mammary gland and the lowest in the synovial membranes. In contrast, only synovial membrane samples from goats infected with genotype-A were positive by qPCR. A similar situation was found in animals infected with a genotype-B strain. Most organ samples from goats infected with genotype B were qPCR-positive. After heterologous infection of sheep with a strain belonging to genotype B, the virus was not detected in any of the animals' organs. Therefore,

heterologous infections with genotype-A and -B strains are less likely to replicate the virus and persist in target organs (98).

An important aspect in the use of PCR is specificity, because nonspecific products can be amplified. Therefore sequencing or cloning and sequencing of PCR products are the best direct methods to confirm the specificity of PCR results and is recommended by the WOAH. Furthermore, PCR, cloning and sequencing provide knowledge of SRLV strains.

Vaccination

There is no effective vaccine against SRLV, despite extensive research having been undertaken on the development of one. Multiple vaccines have been tried for SRLV without successful results. Most vaccine approaches have been tried: inactivated whole virus, recombinant viral protein subunits, viral vectors, and plasmid DNA with and without boosts. Most of them induced an antibody response but caused more severe lesions, and none of them completely protected against SRLV infection (33, 38, 61, 114, 115, 142, 152, 167). In some cases only partial protection was noted. For example, vaccination of goats with a CAEV *env*-based plasmid DNA vector led to decreased post-challenge virus replication and provirus load and weaker development of arthritis in goats (33). Intratracheal vaccination with an attenuated MVV strain LV1KS1 clone failed to protect against superinfection with the more pathogenic strain KV1772, but led to a reduced virus load and delayed the onset of clinical symptoms (142). A promising approach was gene-gun mucosal vaccination of naturally infected sheep in Spain (61). In this approach, sheep were immunised with a plasmid expressing the *env* gene of MVV and boosted with a plasmid that expressed INF-γ besides the Env protein. Vaccination resulted in a significant early protective effect against MVV infection evident in restriction of virus replication and the absence of neutralising antibodies, which unfortunately disappeared two years after the challenge (61). Vaccination of sheep with inocula including plasmids encoding the MVV *gag* and/or *env* genes together with plasmids encoding the B7 or IFN-γ gene, and booster immunisation with recombinant modified vaccinia Ankara virus, induced immune responses before and after virus challenge and in some cases reduced proviral load in tissues. However, immunisation with the *env* gene significantly increased inflammation in target tissues, while the *gag* gene had no equivalent effect or reduced the lesion score (38, 115, 152). Unfortunately, the efficiency of these partial protection vaccines is expected to be limited upon challenge with heterologous genotypes, since cellular and humoral immune responses are generally genotype specific. Long-term protection is also doubtful as new quasi-species are expected.

Goats infected with the Roccaverano strain representing genotypes E (non-pathogenic) and B (highly pathogenic) had lower proviral loads than goats infected only with genotype B, suggesting that the Roccaverano strain may mediate protection against the pathogenic genotype-B strain. Roccaverano infection may open new approaches to natural immunisation against SRLV. This strain may be utilised for the first naturally attenuated vaccine providing protection against increased viraemia and development of lesions (18). Using a mathematical model, Venturino *et al.* (170) showed that when both genotypes (B and E) are present in a flock, the farmer should not separate the offspring from their dams, but rather should rear them with all the other animals. These researchers also made the case that for a farm affected only by genotype B, serological examinations and separation of mother and offspring should still be considered the best strategy for CAEV control. Such strategies totally reverse the current removal policy and may be very reasonable and inexpensive measures to control SRLV infection (170). It is not known whether Roccaverano infection can protect against other genotype-B strains or even other genotypes, and studies to ascertain this are definitely required.

Despite proof of some protection in vaccinated animals, the immunisation strategies used to date have shown limited effectiveness, urging against their wider use. There is a need for a reliable immunisation method (with defined type, route, dose, booster dose, immunomodulators, *etc*.) which demonstrates a high level of protection against heterologous and homologous infections. The choice of immunisation route and delivery method is critical in vaccine research to improve antigen presentation to the immune system. A possible disadvantage of DNA vaccines is that the encoded immunogen expressed in transfected cells may not get into the MHC class II antigen processing and presentation pathway. The selection of antigen is also a factor in determining vaccine success: immunisation with *env* plasmids gave incomparably better results than immunisation with *gag* plasmids (33, 61, 114, 167).

Since stimulation of adaptive immune response resulted only in partial protection against SRLV of homologous strains, some ongoing research efforts are focused on using Sendai virus (SeV) to control SRLV replication. Sendai virus induces production of type 1 IFN, which drives the induction of other genes in a secondary signalling cascade that amplifies and regulates the cellular antiviral state. De Pablo-Maiso *et al.* (132) used SeV to stimulate innate immune responses in cells originating from SRLV-infected sheep. The results were promising and showed inhibition of SRLV at different stages of viral replication depending on the cell type analysed. The antiviral state was confirmed by the expression of some intrinsic factors (A3Z1, RIG-I and BST2). This approach opens new perspectives on

the development of new therapeutic and prophylactic strategies, as it leads to the induction of an antiviral response in the absence of virus-specific epitope recognition.

Highly active anti-retroviral treatment (HAART) used against HIV could theoretically be used against SRLV. However, analogous drugs for SRLV are not currently available and would not be cost effective even if they were. An mRNA vaccine approach may provide an increase in vaccine efficiency over what has been achieved to date with plasmid DNA vaccines for SRLV.

Control

Infection with SRLV is known to have a negative effect on the production and welfare of goats and sheep, but information on the actual economic impact is still unsubstantiated and the size of the impact is subject to debate. While some authors claim that SRLV infection plays a role in reducing the quantity and quality of animal production (12, 46, 94), others have shown no differences in productiveness between infected and uninfected animals (10, 45, 112). The effect of infection on milk production is also a contentious issue. While many studies have demonstrated a notable negative impact of SRLV infection on milk production, some have shown that milk production remained unchanged or even increased in infected flocks (101). The inconsistent and limited nature of data on the economic impact of SRLV infection points to a knowledge gap, which is important because the lack of this information makes it difficult to convince farmers to take control measures. Therefore, it is very important to evaluate production and economic losses in different production systems and geographic areas.

In the absence of effective vaccines, control programmes remain the only way to avoid the spread of SRLV infection. These programmes are mainly based on annual serological testing and elimination of serologically positive animals or the separation of seropositive animals from negative flockmates, removal of lambs from seropositive mothers and artificial rearing of progeny. However, there is little benefit associated with artificially rearing lambs if horizontal infection cannot be avoided (6). Control strategies must address both vertical and horizontal transmission of SRLV. Separating lambs from their mothers after birth is a stressor that can increase lambs' susceptibility to SRLV infection; moreover, this and the other methods in control programmes are costly and labour-intensive and may not be practical in many commercial flocks. Current control and eradication programmes rely mainly on serological testing to detect infected animals. Consequently, only seropositive animals are believed to be infected and are removed from the flock. Genetic variability and the lack of universal diagnostic tests capable of identifying all possible infectious genotypes and subtypes are significant limitations of the current diagnostic measures for SRLV infections, as many infected animals remain undiagnosed carriers of the virus (64, 71, 149). For example, the serological tests routinely used in the Swiss control programme (the CAEV/MVV Total Ab Screening test, MVV/CAEV p28 Ab Screening test, CAEV Antibody test kit, cELISA and Western blot) afforded efficient detection of goats infected with SRLV B strains but were not efficient when applied to SRLV A4-infected goats (27). The failure of these serological tests was most probably caused by the antigenic divergence between the proteins of subtype A4 virus and those in these tests. Therefore, in the final phase of an eradication programme, it would be also highly advisable to use tests based on antigens from locally circulating strains for efficient detection of carrier animals (27, 36). While carriers which are long-term non-progressors do not disseminate the virus through a flock to an appreciable extent, vertical transmission from these animals is a potential impact of their presence in a flock; nevertheless current control programmes that are based on serological testing and culling of seropositive animals cannot yield accurate data on total carriage in the flock because they do not take into account long-term non-progressive animals (27, 35). Identification of factors which increase or decrease shedding of the virus could be used to help management of infection.

The occurrence of unexpected positive ELISA test results in certified SRLV-free herds, along with the temporary suspension of certification and the time and costs associated with collection and testing, can cause frustration among participants and abandonment of voluntary programmes. Therefore eradication programmes are mainly implemented in countries where governmental assistance is available (4). Only in some European countries in specific regions have SRLV eradication programmes have been implemented on a compulsory basis (163). Voluntary programmes have been established in many European countries and the USA. Beyond these regions, most countries pay little attention to SRLV infections and have no control programme. As a result, trade in live small ruminants from countries where the disease has been reported could be a major cause of SRLV spread.

The first MV disease eradication programme was introduced in Iceland in 1960, where all sheep in flocks with diseased animals were culled. The flocks were then repopulated with new healthy sheep from other parts of the island. Today, Iceland is considered an MVV-free region, but not a CAEV-free one (91). In other countries, such drastic measures were not introduced, and only infected animals were removed. The CAEV eradication programme in Switzerland (running since 2012) has reduced the CAEV seroprevalence from 60–80% to less than 1% and completely eradicated clinical cases from the goat

population (27). A compulsory CAEV eradication programme in South Tyrol (Italy) cut the seroprevalence of this particular SRLV to 0.3% (163). However, after a significant reduction in the incidence of antibodies in both countries, a tail phenomenon occurred with the occurrence of new positive cases (163, 165). The reason for this may be that in these countries, control measures were limited to goats and genotype-B infections only. Sheep were not included in the eradication programmes and may have functioned as a source of infection of SRLV for goats in mixed flocks. In Spain and South Tyrol, goats infected with virulent CAEV strain (B1 subtype) were mandatorily culled, while goats infected with MVV were not. In Italy, sheep were mandatorily subjected to SRLV screening only in mixed flocks where seropositive goats were identified; however, the culling of infected sheep was not compulsory (163). The selective nature of the screening and eradication omitted animals which should have been included and resulted in the spread of SRLV A, which is now believed to be dominant in Swiss and Italian goats (27, 165). The opposite scenario with regard to the specific SRLV species and ruminant species of focus pertained in Ontario, Canada, where the Ontario Maedi Visna Flock Status Program has been implemented, in which goats were not included. The programme's exclusivity to sheep resulted in 58% seroprevalence in goats in the province (159). Since both goats and sheep can serve as reservoirs for SRLV, the success of eradication programmes will be jeopardised if both species are not included. Therefore, the same regulations should apply to MV disease and CAE. Programmes targeting only one species should no longer be considered adequate.

However, there were also problems in completely eliminating SRLV in countries that tested both sheep and goats. For example, the Belgian voluntary programme brought out unexpected positive ELISA results in certified SRLV-free herds, indicating that a small proportion of infected animals remained undetectable (99). In Norway, the surveillance programme initially has only serological examination for SRLV of sheep in its scope. Goat herds were included in the surveillance programme in 2018. Its results for MVV in 2003–2005 showed a positive flock prevalence of less than 0.22%. Maedi-Visna virus was not detected in 2006–2018. In 2019, the prevalence of infected sheep flocks was 0.03%, while in 2020 MVV was not detected in any sheep. There was seropositivity in 1.6% of surveyed goat herds in 2018. Caprine arthritis encephalitis was detected in several hobby goat herds in 2019, as well as in two dairy goat herds in 2020 where CAE had previously been eradicated (78). In the Netherlands many CAEV-accredited goat flocks also lost their accreditation. In the majority of affected flocks, the route of introduction of infection remained unclear. In most cases the purchase and introduction of infected goats was the most likely cause of reinfection (141). Trade in animals is a well-recognised risk factor for spreading SRLV infection. According to the WOAH, the trading of sheep and goats is allowed when the animals show no clinical signs of SRLV, when the animals gave negative results during the 30 days prior to shipment or when SRLV have not been diagnosed clinically or serologically in the flock in the past three years and when no new animals have been introduced into the flock during that period. However, clinical symptoms are an insensitive indicator of infection, since symptoms of the disease progress slowly and most of the infected animals do not develop the obvious clinical signs. This is a significant blind spot as infection can easily be transmitted within and between countries. The introduction to an SRLV B1-free Swiss flock of an SRLV-infected but clinically healthy Belgian goat led to the spread of the infection throughout the flock (40). Furthermore, assessment of animals' safe status for trade based solely on the occurrence of clinical symptoms may pave the way for potential fraudulent abuses. Therefore, it would make sense to only trade in animals from certified-negative flocks.

Conclusions

Small-ruminant lentiviruses are a highly heterologous group of lentiviruses infecting mainly goats and sheep, which lead to different clinical signs depending on incompletely understood factors that involve host–pathogen interactions. It is suggested that the genetic background of the host and the biogenetic properties of specific SRLV strains have a decisive influence on the outcome of infection. Attempts to identify regions of the genome involved in tropism indicate that these may be the LTR and *env* genes. However, there are still significant gaps in our knowledge of the epidemiology, immunology and biology of SRLV and their impact on animal production and welfare. The importance of the various routes of SRLV transmission and conditions that promote the spread of SRLV have yet to be fully explored. For the early and definitive diagnosis of SRLV infection, a combination of serological and molecular tests is suggested, which is challenging because new variants emerge constantly. Molecular testing in the form of PCR can be considered in control programmes for testing animals less than one year old. It is not likely that there will be any significant advances in SRLV vaccines or therapies, considering the scientific challenges and market economics. Nevertheless, advances in the vaccine or therapy areas should be monitored for potential applicability to SRLV. Involving the innate immune response in the natural suppression of SRLV infection may improve control programmes. A number of specific genes associated with susceptibility or resistance to SRLV infection have already been found, providing an alternative strategy for reducing the incidence of

infection in the selection of the most genetically resistant animals. However, future studies in this area are required.

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