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# Phylogenesis of Small Ruminant Lentiviruses: a systematic review protocol V.1

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**We use this protocol and it's working**

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## Abstract

Small ruminant lentiviruses (SRLV) include the closely related Visna-Maedi virus (VMV) and caprine arthritis encephalitis virus (CAEV), which infect sheep and goats. These viruses cause huge economic losses in the small ruminants industry, affecting production and animal welfare. To date, there are five recognized SRLV genotypes (A, B, C, D, and E), based on nucleotide sequences derived from *gag*, *pol*, *env*, and *LTRs* regions and several subgenotypes. Thanks to the improvement of molecular studies, the knowledge on genetic characterization and phylogenesis of SRLVs has increased since the 1990s. However, using different protocols among the various studies produced problems concerning the exact placement of subgenotypes in the phylogenetic tree. This systematic review protocol aims to define strict selection criteria and critical appraisal of published studies to perform a critical synthesis of all knowledge on the phylogenesis of SRLVs following the PRISMA-P guidelines. Eligibility criteria, information sources, search strategy, data management, selection process, data collection process, data extraction, outcomes and prioritization, studies quality assessment, and data synthesis will be performed. Thank the systematic review, an updated overview of the phylogenesis of SRLVs will be performed, and the best reliable molecular approach in diagnosis and research may be highlighted.

## Guidelines

To draft this protocol we followed the PRISMA-P guidelines<sup>1</sup>.

1. Moher D, Shamseer L, Clarke M, Gherzi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.[doi:10.1186/2046-4053-4-1](https://doi.org/10.1186/2046-4053-4-1)

## Troubleshooting

## INTRODUCTION

### 1 **Rationale**

Small ruminant lentiviruses (SRLVs) are members of the Retroviridae family and Lentivirus genus comprising the closely related Visna-Maedi virus (VMV) and caprine arthritis encephalitis virus (CAEV), which infect sheep and goats.<sup>1</sup> These viruses cause huge economic losses in the small ruminants industry, affecting production and animal welfare.<sup>1</sup>

To date, there are five recognized SRLVs genotypes (A, B, C, D, and E), based on nucleotide sequences derived from *gag*, *pol*, *env*, and *LTRs* regions, and several subgenotypes.<sup>1, 2</sup>

Thanks the improvement of molecular studies, the knowledge on genetic characterization and phylogenesis of SRLVs has increased since the 1990s. However, using different protocols among the various studies produced problems concerning the exact placement of subgenotypes in the phylogenetic tree.

### **Objectives**

This systematic review aims to provide an in-depth and comprehensive overview of the phylogenesis of SRLVs in sheep and goats. Thank to the systematic review protocol, we will critically summarize available data on the phylogenetic analysis of SRLVs. The review will include studies performed in various geographical areas and with different types of molecular approaches. In doing so, we will identify possible knowledge gaps and problems related to subgenotypes' phylogenetic position, providing valuable information for future studies on this topic.

## METHODS

### 2 **Eligibility criteria**

Inclusion criteria: Cross-sectional studies, reviews, case reports

Exclusion criteria

- Study design: experimental or intervention studies, editorials, commentaries, notes, conference proceedings, conference abstracts, dissertations
- Population: any animal species other than sheep and goats
- Outcome: studies on clinical, pathological and epidemiological aspects.
- Languages: Studies written in languages other than English
- Type of journal: studies published in non-peer-reviewed journals

### **Information sources**

Comprehensive searches of MEDLINE (via PubMed), Web of Science, Excerpta Medica Database

(Embase), and Scopus without publication date limitation will be performed.

## Search strategy

The search will be done using the following keywords grouped in three blocks:

-maedi OR maedi-visna OR maedi/visna OR caev OR lentivirus OR lentiviruses OR small ruminant lentiviruses OR srlv

-sheep OR goat OR goats OR ovine OR caprine OR small ruminant OR small ruminants

-phylogenetic analysis OR phylogenesis OR genetic analysis OR characterization OR genotyping OR classification OR subtype

## Study records

### *Data management/Selection process/Data collection process*

The study will collect data on the SRLVs genotyping and phylogenetic analysis in sheep and goats. The data will be recorded in EndNote™. Two independent reviewers (PG, SP) conducted a title- and abstract-based screening to exclude researches irrelevant to the review's scope, and any discrepancies were solved by discussion and consulting other review authors (MO, MG). Information collected was related to identified subgenotypes, accession number, genomic regions, and molecular method (conventional PCR or real-time PCR) used for the classification, sample type investigated, species (sheep or goat) in which the subtype was detected, year of publication, country of origin, references, and doi. All data were organized into an Excel document (Microsoft Office, Microsoft, Redmond, WA, United States).

### *Data extraction*

Two review authors will independently extract information on the subgenotypes identified, accession number, genomic regions, molecular method used for the classification, sample type investigated, animal species in which the subtype was detected, year of publication, country of origin, references, and doi. All data will be organized into an Excel document (Microsoft Office, Microsoft, Redmond, WA, United States).

## Outcomes and prioritization

Main outcome 1: data on the methods and genomic region used to perform molecular characterization of SRLV

Main outcome 2: SRLVs genotyping and phylogenetic analysis in sheep and goats and harmonization of their classification.

## Risk of bias (quality) assessment

The methodological quality of the included studies will be assessed by the appropriate checklist. Therefore, modified for the purpose checklist developed by Moola et al. (2020) will be used for the quality assessment of the cross-sectional and case reports included studies.<sup>3</sup> On the other hand, the original checklist developed by Moola et al. (2020) will be applied to the review studies. Each study will receive an overall risk of bias score designed on each type of selected study.<sup>3</sup>

Two investigators will independently assess the study's methodological quality, and any disagreements will be resolved by consulting the other review authors.

### **Data synthesis**

The systematic review will report the data collected in narrative form. The data will be grouped together based on the protocol of molecular characterization used. Studies that used similar analytical procedures and the same genomic region will be synthesized together, and genogroups, genotypes, and individual sequences will be reported. Instead, studies that used different molecular approaches will be highlighted, and a critical analysis of them will be set up.

### **References**

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