

## PRELIMINARY RESULTS OF MVV AND CAEV SEROPREVALENCE IN ROMANIAN SHEEP AND GOATS

Dan Alexandru ENACHE<sup>1</sup>, Stelian BARAITAREANU<sup>1</sup>, Marius DAN<sup>2</sup>,  
Maria Rodica GURAU<sup>1</sup>, Fanel OTELEA<sup>3</sup>, Armenac DOBRE<sup>3</sup>, Doina DANES<sup>1</sup>

<sup>1</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest,  
59 Marasti Blvd, District 1, Bucharest, Romania

<sup>2</sup>Institute for Control of Biological Products and Veterinary Medicines,  
39 Dudului Street, District 6, Bucharest, Romania

<sup>3</sup>Sanitary Veterinary and Food Safety Directorate of Braila County,  
344 Galati Way, Braila, Romania

Corresponding author email: enachedan1990@yahoo.com

### Abstract

*Maedi-Visna (MV) and Caprine Arthritis Encephalitis (CAE) are diseases of sheep and goats. They are caused by lentiviruses which belong to Retroviridae family. The usual way of contamination is the cohabitation of animals. Diseases are widespread in many countries as: Norway, France, Italy, Spain, USA, Panama, Cyprus, Greece, and Japan. The present paper aimed to present the MVV and CAEV antibodies seroprevalence in samples collected in different sheep and goats farms from Romania. There were collected blood samples from the following counties: Cluj-Napoca, Ilfov, Constanta, Galati, Giurgiu, Braila, Arges, Bacau, Dâmbovita, Ialomita, Suceava, Calarasi, Buzau, Vrancea, and Vaslui. In order to determine the presence of antibodies, the samples were analysed by indirect ELISA, using commercial kits. There were registered negative results in only four counties and the possible existence of viruses in farms cannot be excluded. In order to confirm and strengthen the preliminary results, we recommend to analyses the samples by molecular biology techniques. Also, national authorities could establish a program of surveillance and diagnosis at national level, able to provide a more complete picture of the SRLVs prevalence in each county.*

**Key words:** small ruminant's diseases, maedi-visna, caprine arthritis encephalitis, SRLVs.

### INTRODUCTION

The lentiviral pathology of small ruminants is caused by Caprine Arthritis Encephalitis Virus (CAEV) and Maedi-Visna Virus (MVV), viruses which caused persistent infections all over the world (Gufler et al., 2007; Stonos et al., 2014). CAEV and MVV are included in the group of small ruminant lentiviruses (SRLVs) belonging to *Retroviridae* family, *Lentivirus* genera (Junkuszew et al., 2016).

During the last decades, several wild species of ruminants have been introduced in Europe, and therefore new SRLV isolates have been reported. It is already known that wild ruminants could host emerging or re-emerging pathogens and the spread of them to domestic populations of sheep and goats can be done. Recent studies revealed several SRLV strains in Alpine ibexes (*Capra ibex*) from French Alps and in domestic hybrids, Rocky Mountain goats (*Oreamnos americanus*) or Mouflon (*Ovis orientalis*) (Sanjose et al., 2016).

The target cells of SRLVs are lymphocytes, mainly monocytes (Stonos et al., 2014), and the infections can evolve subclinical (latent infection) or clinical (Gufler et al., 2007; Junkuszew et al., 2016) with a long period of incubation (Sigurdsson et al., 1957; Haase, 1986). Bjorn Sigurdsson estimated that the specific viral diseases of small ruminants have spread rapidly in Iceland, through imported sheep from Germany (Sigurdsson et al., 1957; Haase, 1986).

Peterhans et al. (2004) concluded that the specific lentiviruses of small ruminants could affect sheep and goats and the most common routes which are incriminated are the direct contact and the lactogenic.

The presence of viruses as divergent genetic variants called quasispecies, may favor cross-species transmission (Sanjose et al., 2016).

An efficient vaccine is still a concern for science but, the high mutation rate of SRLVs daunt every attempt (Stonos et al., 2014).

The most common way of horizontal transmission is the close contact between small ruminants, even at pasture (Gufler, 2004, Gufler et al., 2007). Vertical transmission is not fully understood (Gufler et al., 2007). According with Straub (2004), the primary mode of infection is by the dam's milk, especially the colostrum.

The morbidity rate among individuals easily rise when infected colostrum is ingested, or if sick animals are in close contact with the susceptible ones (Junkuszew et al., 2016).

Nevertheless, Houwers and Van der Molen, (1987), Arsenault et al., (2003) and Berriatua et al. (2003) agree to consider the direct contact as less important in the economy of the disease.

There are five SRLV genotype (A-E) which have been discovered to date. Up to Gjerset et al. (2009), VMV-like and CAEV-like strains belong to genotypes A and B, while strain variants isolated from sheep and goats in Norway belong to genotype C. The strains isolated in Switzerland and Spain have been genotype D, and the ones isolated in Italian goats genotype E (Reina et al., 2010).

The control of SRLV infection could be realized by several procedures. One approach is based on the early detection of the infected animals (adult animals and their offspring) by serological methods (ELISA or AGID). The clinical picture of those infections register the decrease of the milk production and the poor quality of the milk, as a result of the increased somatic cells and shortened period of lactation. (Turin et al., 2005; Martinez-Navalon et al., 2013; Sanjose et al., 2015). The SRLV diseases are deeply harming the production of wool, milk and lamb. In that sense, the direct losses caused by death or premature culling can be consider on the second plan (Houwers, 1990; Brodie et al., 1998; Benavides et al., 2013).

The most convenient way to diagnose SRLV infections is to perform serology. A variety of laboratory techniques are available for this purpose. These include the agar gel immune-diffusion, enzyme-linked immunosorbent assay (ELISA), radioimmunoprecipitation (RIPA), radioimmunoassay (RIA) and western blotting (WB) (Minguijon et al., 2015; de Andres et al., 2005).

The researches cited by Perez et al. (2013) revealed that “*VMV and CAEV seroprevalence*

*control methods may involve a combination of the following practices: culling of the flock and substitution by uninfected sheep, selective culling of seropositive animals, sheep replacement only with offspring from seronegative ewes, early culling of seropositive animals showing initial clinical symptoms, artificial rearing of lambs separated from the seropositive mother immediately after birth and segregation of the flock into two flocks based on serological status followed by separate management of the resulting flocks to avoid horizontal transmission.”* (Perez et al., 2013).

The present paper aimed to present the preliminary results of MVV and CAEV antibodies seroprevalence in sheep and goats flocks in Romania, in a prospective serological study during the year 2016.

## MATERIALS AND METHODS

There are analyzed 1380 serum samples collected from 1145 sheep and 235 goats. The animals belong to the next counties: Cluj, Sibiu, Ilfov, Constanta, Galati, Giurgiu, Braila, Arges, Bacau, Dambovita, Ialomita, Suceava, Calarasi, Buzau, Vrancea and Vaslui (fig.1).



(Map source: <http://d-maps.com/m/europa/roumanie/roumanic25.gif>)

Figure 1. Assay-sampling Romanian Counties.

The four Romanian regions are marked with colors: Blue – Moldavia, Purple – Dobrogea, Red – Muntenia, and Green – Transylvania

In order to evaluate the presence of SRLVs in flocks, serum samples collected have been pooled on each farm, as follow: 100µl of serum blood obtained from five animals (sheep or goats from the same flock) have been mixed into Eppendorf tube and used in one reaction. The serological examination has been done for

276 pooled sera (229 for sheep and 47 for goats).

The pooled sera have been tested using an ELISA commercial kit (IDEXX CAEV/MVV Total Ab Test, Switzerland) according to the manufacturer's instructions previously described (Gurau et al., 2015).

The serological results were edited and statistically analyzed with Anova: Single Factor data analysis tool. The variation of the serological results obtained in Muntenia, Moldavia, Transylvania and Dobrogea has been statistically validated ( $p < 0.05$ ).

The geospatial analysis was designed in Microsoft Power Map in Excel.

## RESULTS AND DISCUSSIONS

The distribution of the results obtained in 16 counties is presented in table 1. Eleven counties provided positive pooled sera: Braila, Dambovită, Galati, Constanta, Vrancea, Vaslui, Suceava, Giurgiu, Ilfov, Sibiu, and Cluj. Serological prevalence of SRLVs in Romanian regions (Muntenia, Moldavia, Transylvania and Dobrogea) is variable ( $p < 0.05$ ), with significant higher number of positive pooled sera in Moldavia than in the other three (fig. 2-5).

The distribution of the results obtained for each species are presented in tables 2 and 3. The pooled sera of sheep, from ten of the sixteen counties, respectively from Braila, Galati, Constanta, Vrancea, Vaslui, Suceava, Giurgiu, Ilfov, Sibiu, and Cluj, provided positive results. In goats, only the pooled sera from four counties of the nine - Braila, Ilfov, Dambovită and Constanta, provided positive results.

Although the number of goat pooled sera is considerably lower compared to the sheep ones and thereby makes irrelevant a comparative analysis over the infection seroprevalence in the four Romanian regions, the comparative analysis of the seroprevalence in each county where it have been analyzed samples from both species, keep open the issue of the interspecies spread of the SRLVs.

The results provided on sheep and goats samples from Dambovită county, are suggesting the absence of transmission of goat SRLVs to sheep.

To the opposite, the prevalence of positive sera pools, quite uniformly distributed in goats and sheep in Ilfov county, equally suggests that the transmission between sheep and goats, the selection of a variant-specific host SRLVs limited or the presence of multiple variants. Of course, these assumptions will suffer a significant correction when overlapping the results to a rigorous epidemiological surveys, GIS related to the location of the holdings and phylogenetic analysis of samples.

The serological prevalence of SRLVs in sheep pooled samples from Muntenia, Moldavia, Transylvania and Dobrogea regions is variable, with higher prevalence of the positive pooled sera in Transylvania and Moldavia than in Muntenia and Dobrogea ( $p < 0.05$ ) (fig. 6).

The positive results obtained in goat pooled samples have been twice more in Moldavia than in Muntenia and Dobrogea (fig. 7). In this study, we missed goat serum samples from Transylvania.

Table 1. Distribution of the pooled samples according to the flock origin

Results	Braila	Bacau	Buzau	Arges	Dambovită	Galati	Constanta	Calarasi	Vrancea	Vaslui	Ialomita	Suceava	Giurgiu	Ilfov	Sibiu	Cluj	Total
+	11	0	0	0	5	26	2	0	1	1	0	1	3	5	11	68	134
-	54	7	4	3	7	14	7	3	4	3	3	8	3	4	3	10	137
±	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	2	5
<b>Total</b>	<b>65</b>	<b>7</b>	<b>4</b>	<b>5</b>	<b>12</b>	<b>40</b>	<b>9</b>	<b>3</b>	<b>5</b>	<b>4</b>	<b>3</b>	<b>9</b>	<b>6</b>	<b>9</b>	<b>15</b>	<b>80</b>	<b>276</b>

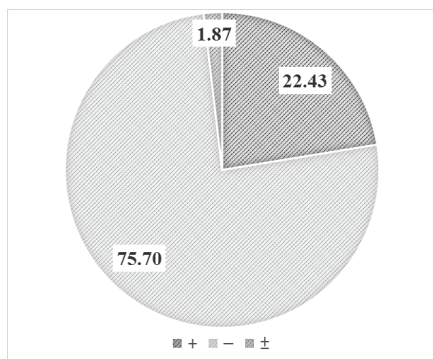


Figure 2. Serological prevalence of SRLVs in Muntenia region

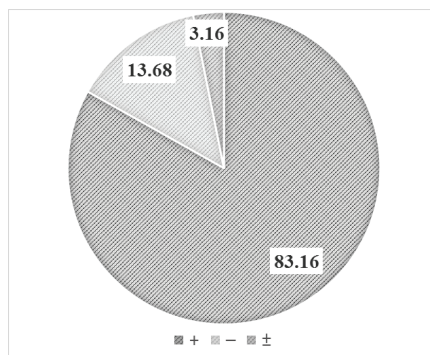


Figure 4. Serological prevalence of SRLVs in Transylvania region

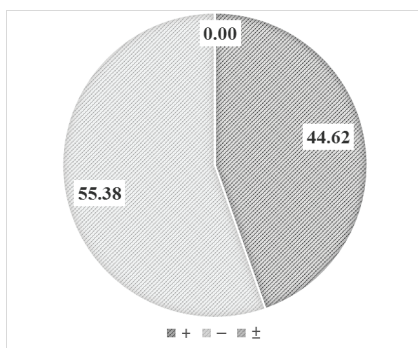


Figure 3. Serological prevalence of SRLVs in Moldavia region

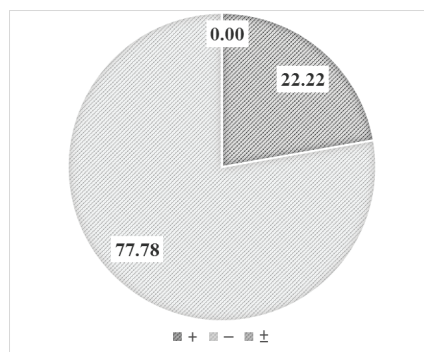


Figure 5. Serological prevalence of SRLVs in Dobrogea region

Table 2. Distribution of the sheep pooled samples according to the flock origin

Result	Muntenia								Moldavia					Transylvania		Dobrogea	Total
	Braila	Buzau	Ialomita	Giurgiu	Arges	Ilfov	Calarasi	Dambovita	Bacau	Galati	Vaslui	Suceava	Vrancea	Cluj	Sibiu	Constanta	
+	8	0	0	2	0	3	0	0	0	26	1	1	1	68	11	1	122
-	38	4	2	2	3	0	3	4	3	14	3	6	4	10	3	3	102
±	0	0	0	1	0	0	0	1	0	0	0	0	0	2	1	0	5
Total	46	4	2	5	3	3	3	5	3	40	4	7	5	80	15	4	229

Table 3. Distribution of the goat pooled samples according to the flock origin

Result	Muntenia								Moldavia					Transylvania		Dobrogea	Total
	Braila	Buzau	Ialomita	Giurgiu	Arges	Ilfov	Calarasi	Dambovita	Bacau	Galati	Vaslui	Suceava	Vrancea	Cluj	Sibiu	Constanta	
+	3	0	0	0	0	2	0	4	0	0	0	0	0	0	0	1	10
-	16	0	1	1	0	4	0	3	4	0	0	2	0	0	0	4	35
±	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Total	19	0	1	1	2	6	0	7	4	0	0	2	0	0	0	5	47

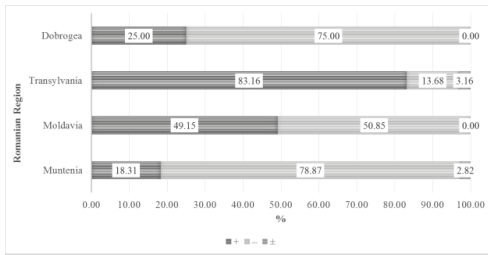


Figure 6. Serological prevalence of SRLVs in Romanian sheep ( $p < 0.05$ )

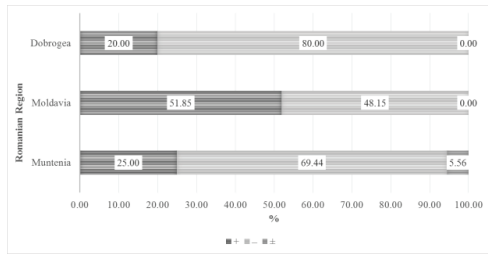


Figure 7. Serological prevalence of SRLVs in Romanian goats ( $p < 0.05$ )

The geospatial distribution of the prevalence of positive pooled sera in Romanian small ruminants is presented in figure 8.

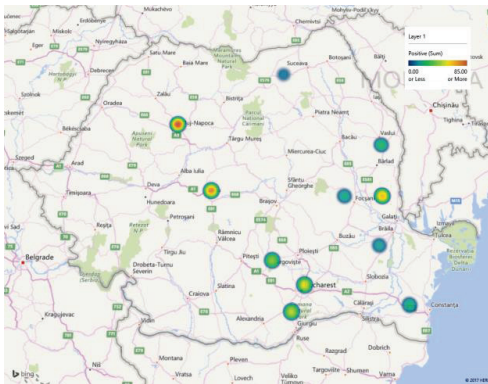


Figure 8. Geospatial distribution of seropositive results of ELISA CAEV/MVV Total Ab (IDEXX, Switzerland).

The geospatial distribution of the positive results between Romanian regions: hotspots in the middle of Transylvania and in the south-east of Moldavia ( $p < 0.05$ )

At national level, the prevalence of goat positive results (40.00%) are quite similar to those reported by Gurau et al. (2015) in only one goat flock located in Braila County (38.46%). However, the prevalence in Moldavian counties is significantly higher. In

both studies, serological positive results have been associated with few clinical cases of CAE (Gurau et al., 2015), but more clinical outbreaks could emerge in the next years, and therefore it is necessary to establish preventive measures. In similar circumstances, Gufler et al. (2007) recommended the introduction of a control or eradication program up to the prevalence of virus on the field, the structure of small ruminant population and the economic aspects (Gufler et al., 2007).

Moreover, if we take in consideration the studies of De Andres et al. (2013), our results could be underestimated; the SRLV strains circulating in different areas can be heterogeneous, and the performance of ELISA tests will vary accordingly. To solve the problem, it was proposed several ELISA-based strategies (De Andres et al., 2013). An alternative to ELISA could be PCR-based strategies and in the near future we are focused in testing with this method.

## CONCLUSION

SRLV infections among Romanian small ruminants should be considered. National authorities could establish a program of surveillance and diagnosis at national level, able to provide a more complete picture of the SRLVs prevalence in each county.

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