SEROLOGICAL RESULTS OF MVV AND CAEV ANTIBODIES IN SAMPLES COLLECTED FROM SHEEP AND GOAT FLOCKS OF ROMANIA

Dan Alexandru Enache¹, Stelian Baraitareanu², Marius Dan³, Maria Rodica Gurau⁴, Camelia Nuşiu⁵, Camelia Sarbu⁶, Doina Danes⁷

Abstract:
INTRODUCTION: Maedi-Visna (MV) and Caprine Arthritis Encephalitis (CAE) are specific diseases of small ruminants caused by lentiretroviruses. These diseases are not a public health concern but they are important due to their economic impact.
OBJECTIVES: The aim of this study is to analyse the results of the serological screening for MVV and CAEV antibodies in samples collected from small ruminants farms located in 14 Romanian counties and the proximity of Bucharest city.
METHODS: The samples were analysed by indirect ELISA, using a commercial diagnosis kit. We investigated 702 serum samples in 160 iELISA tests: 148 pools (2-5 samples/pool) and 12 individual animals.
RESULTS: We noticed 27.50% positive samples that confirm the exposure to MVV and CAEV viruses of small ruminants, but the confirmation is requesting investigation using molecular biology tools or other serological techniques.
CONCLUSION: A reliable picture over the SRLV infections could underlie a national program for monitoring.

UDC Classification: 616.9, DOI: 10.12955/cbup.v7.1444
Keywords: Maedi-Visna; Caprine Arthritis Encephalitis Virus; serology; lentivirus; Retroviridae.

Introduction
Small ruminant disease surveillance and diagnostics are two major activities of the Romanian veterinary services and research. Recent epidemiological studies have targeted zoonotic or emerging infectious diseases whose epidemiological situation is not fully known (Hotea et al., 2016; Danes et al., 2016; Tilibasa & Darabu, 2016; Baraitareanu et al., 2018). Also, Maedi-Visna (MV) and caprine arthritis and encephalitis (CAE) are economically important diseases of sheep and goats whose prevalence must be better known. MV and CAE are specific diseases of small ruminants caused by MV virus (MVV) and CAE virus (CAEV) (de Andres et al., 2005; Leroux et al., 2010; Cruz et al., 2013; Sanjose et al., 2015; Danes et al., 2016). These viruses belong to the Retroviridae family, Lentivirus genera, and they share a similar viral structure (Minguijon et al., 2015) and high genetic variability (Brinkhof, 2008; Herrmann-Hoesing, 2010). Small ruminant lentiviruses (SRLVs) were classified into 5 genotypes and several subtypes (Shah et al., 2004; Deubelbeiss et al., 2014; Danes et al., 2016; Olech et al., 2019).

MV and CAE are not of public health concern but they are quite important because they can decrease productions. Some susceptible animals do not express any clinical sign following the viral exposure, but some could exhibit a respiratory form with interstitial pneumonia (Maedi) or a neurological form with progressive inflammatory disease of the central nervous system (Visna). Also, sheep and goats could be affected by inflammatory processes (located in joints and/or mammary gland) (Grego et al., 2007; Gomez-Lucia et al., 2018). The clinical signs are: coughing, lameness, weight loss and failures to mount (Hamza & Ozkan, 2017). SRLV, having a very long period of incubation (even up to 2-3 years), the clinical signs can appear very late or not at all (Herrmann-Hoesing, 2010).

¹ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; enachedan1990@yahoo.com
² Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; stelianbaraitareanu@fmvb.ro
³ Institute for Control of Biological Products and Veterinary Medicines, Bucharest, Romania; dan.fmarius@yahoo.com
⁴ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; otelea_maria@yahoo.com
⁵ Sanitary Veterinary and Food Safety Directorate of Mureș County, Romania; camelianutiu2001@yahoo.co.uk
⁶ Sanitary Veterinary and Food Safety Directorate of Alba County, Romania; camyyss@yahoo.com
⁷ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; danes.doina@gmail.com
Despite the viral susceptibility of all breeds to SRLVs, the clinical expressions observed is quite variable. While asymptomatic Karakul sheep imported to Iceland never developed any clinical sign (Torsteinsdóttir et al., 1992), some breeds seem to be more susceptible such as: Texel, Border Leicester, Finnish landrace, Assaf (Leginaoikoa et al., 2006; Muz et al., 2013). Other breeds tend to be more resistant such as: Columbia, Rambouillet, and Suffolk (Muz et al., 2013). In goats, Saanen and Toggenburg breeds seem to be more susceptible while Bedouin Black could be resistant to the CAEV infection under natural conditions (Rowe et al., 1992; Perk, 1995).

SRLVs could cross the species barrier and they can affect both sheep and goats (Peterhans et al., 2004). They should be considered specific lentiviruses for small ruminants, considering the other potential imminent source of infection: the wild ruminants (Sanjose et al., 2016). The following ones can be mentioned as susceptible: Ovis aries, Capra aegagrus hircus, Capra ibex, Ovis gmelini, Oreamnos americanus, Ovis orientalis, and Ovis aries musimon (Denner, 2007; Guiguen et al., 2000; Leroux et al., 2010; Ramirez et al., 2013; Sanjose et al., 2016).

SRLVs transmission can be accomplished under the following conditions: direct contact (Leginaoikoa et al., 2006; Pisoni et al., 2007; Pérez et al., 2010), feeding with infected colostrum/milk (Mdurwva et al., 1994; Leginaoikoa et al., 2006; Villoria et al., 2013; Junkiszew et al., 2016; Yang et al., 2017) or feeding on an infected pasture (Gufler, 2004; Gufler et al., 2007). The sexual transmission is not excluded (Al Ahmad et al., 2005; Lamara et al., 2013).

One of the most important problems regarding the diagnosis of SRLVs infection is what type of method to choose, and the most convenient way is to appeal to a serological technique: AGID (agar gel immunodiffusion), ELISA (enzyme-linked immunosorbent assay) or WB (western blotting) (OIE, 2017). Standard PCR should be performed when the serology results are not enough for a proper result, even when it is about seronegative animals or seropositive animals. In addition, sequencing PCR products can be useful to perform phylogenetic analyses (Sanjose et al., 2016; Sanchez et al., 2016; Panneum & Rukkwamsuk, 2017; Kokawa et al., 2017; Yang et al., 2017). The prevalence tends to be higher in the countries which adopted the system of feeding lambs and/or kids with colostrum/milk from a tank. This practice could enhance the transmission (de la Concha-Denier, 1997). All the genotypes, subtypes and recombinant SRLV viruses could contribute to a wider spectrum of cell and host tropism (Sanjose et al., 2016). SRLV seroprevalence is still a challenge that most countries in the world have to confront with. National programs should be implemented to reduce the seroprevalence in every country as, for example, Switzerland did: it began such a programme in 1980s exclusively on goats. The programme became mandatory 8 years later and the results did not delay. The seroprevalence decreased from 60-80% to less than 1% and the clinical cases of arthritis completely disappeared (Blatti-Cardinaux, 2016). Such programs have been successfully enforced in many countries (Rowe & East, 1997). The viruses have been eliminated by culling the seropositive subjects and replacing them with seronegative animals (Silvonen et al., 2000; Peterhans et al., 2004), if possible, from SRLVs free flocks or even areas. The antibody responses could fluctuate during the first 6 months of infection (Larsen et al., 1982) and it is quite necessary to perform regular serological tests and strict control measures as proper choice in order to eradicate the infection in a short period of time (Pérez et al., 2010; Rachid et al., 2013).

The present survey aimed to underline the results of MVV and CAEV seroprevalence in different Romanian sheep and goat flocks from January 2017 to March 2018.

Material and Methods

702 serum samples were analysed (Table 1) from five Romanian county clusters (Figures 1) by using a method described in a previous study (Enache et al., 2017). Briefly, from each subject 3-5 ml of blood was collected, in sterile conditions, from the jugular vein. The samples were stored 2-3 h at the room temperature and 24-48h at 4°C. The initial collection tubes were processed by centrifugation 1200-1500 rpm/15-20 min and serum samples were transferred in sterile Eppendorf tubes. All the serum samples were stored at -20°C until the serological investigation. The serological examination was done for 148 pools (2-5 samples/pool) and 12 individual animals provided by farms located in 14 Romanian counties and the proximity of Bucharest city (Table 2). All samples were tested using an iELISA commercial kit (IDEXX CAEV/MVV Total Ab Test, Switzerland) according to the manufacturer's instructions.
Table 1: Demographic information of the small ruminants included in survey

<table>
<thead>
<tr>
<th>Samples</th>
<th>Adults</th>
<th>Young</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>483</td>
<td>113</td>
<td>607</td>
</tr>
<tr>
<td>Goats</td>
<td>89</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Total</td>
<td>572</td>
<td>118</td>
<td>702</td>
</tr>
</tbody>
</table>

Source: Authors

Figure 1: Spatial distribution of the small ruminants included in survey

Legend: Blue – Oltenia, Red – Ardeal, Orange – Muntenia, Grey – Moldova, Purple - Banat

Source: Author; Map source: http://d-maps.com/m/europa/roumanie/roumanie25.gif

Table 2: County distribution of iELISA tests (pools and individual samples) performed on small ruminants included in survey

<table>
<thead>
<tr>
<th>County</th>
<th>No. pools</th>
<th>No individual samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alba</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Argeş</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Brăila</td>
<td>77</td>
<td>-</td>
</tr>
<tr>
<td>Bucharest and Ilfov region</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Călăraşi</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Dâmboviţa</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Dobţ</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Maramureş</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mureş</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Olt</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Prahova</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Timiş</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Vâlcea</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Vrancea</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Authors

Results and Discussion

Out of 135 sheep iELISA tests, 22.96% (n=31) gave positive results, 4.44% (n=6) were suspect and 72.59% (n=98) negative. While out of 25 goat tests, 52.00% (n=13) gave positive results and 48.00% (n=12) were negative (Table 3, Figure 2).

Over all, the 160 sera samples from small ruminants tested by iELISA assay to detect CAEV/MVV antibodies provided 27.50% (n=44) positive results, 3.75% (n=6) suspect and 68.75% (n=110) negative results (Figure 2).

The spatial distribution of the CAEV/MVV seropositive samples revealed that of 14 counties and the proximity of Bucharest area, we obtained at least one positive pool in 9 of these counties. In addition, these results were mostly located in small ruminant’s populations from south and centre areas of Romania (Figure 3).
Table 3: Results of CAEV/MVV antibodies testing in Romanian sheep and goats herds

<table>
<thead>
<tr>
<th></th>
<th>Sheep pools</th>
<th>Goat pools</th>
<th>Sheep Individual samples</th>
<th>Goat Individual samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females Males Young</td>
<td>Females Males Young</td>
<td>Females Males Young</td>
<td>Females Males Young</td>
</tr>
<tr>
<td>Positive</td>
<td>22 2 1 6</td>
<td>12 0 0 1</td>
<td>22 2 1 6</td>
<td>12 0 0 1</td>
</tr>
<tr>
<td>Suspect</td>
<td>4 2 0 0</td>
<td>0 0 0 0</td>
<td>4 2 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Negative</td>
<td>75 20 0 3</td>
<td>9 1 0 2</td>
<td>75 20 0 3</td>
<td>9 1 0 2</td>
</tr>
</tbody>
</table>

Source: Authors

Figure 2: The prevalence of CAEV/MVV antibodies in sheep (135/160) and goat (25/160) samples

Source: Authors

Figure 2: The prevalence of CAEV/MVV antibodies in small animal samples

Source: Authors

The working methodology approached for this survey does not allow the calculation of epidemiological indicators, but provides valuable information on the spread of the SRLVs across Romania. In fact, the final results could be different due to the following reasons:

- the number of the investigated animals could be variable taking into consideration the animal production category, the counties of origin and, last but not least, the real possibility to collect samples from flocks;
- the individual seropositive animals could be modified because we suppose that a seronegative pool can “hide” one or more seropositive animals due to dilutions;
- the commercial diagnostic kit could have its own limits and we recommend to consider also other diagnostic tools;
- the seronegative young animals should be retested due to the seroconversion.

In this study, we identified six seropositive lambs in a farm located in the proximity of Bucharest city. Unfortunately, it was not possible to collect data regarding their mothers. Considering the infected colostrum/milk as a possible source of contamination, the status of their mothers would be useful to be investigated in order to reveal supplementary data, to support or not the supposition of the sexual transmission. We found, in our previous study seropositive animals from the same flock (Enache et al., 2017).
Based on these results, it becomes obvious the need for a more specific approach – using other different diagnostic tools - AGID or PCR as recommended by the World Organisation for Animal Health (OIE, 2017), to fully characterize the MV/CAE landscape in the Romanian flocks.

**Conclusion**

The obtained results confirm the exposure of the Romanian flocks to MVV or CAEV. The prevalence values found in both species is mirroring the velocity of the spread under natural conditions, without any interference, other than the breeding practices. The high level of the total prevalence must be considered as one of the “hidden” causes of the modest economic performances. A national control program to identify seropositive animals and to eliminate them from the flocks would be a first step in order to prevent the occurrence of outbreaks.

**References**


Lamara, A, Fieni, F., Chatagnon, G., Larrat, M., Dubreil, L., & Chebloune, Y. (2013). Caprine arthritis encephalitis virus (CAEV) replicates productively in cultured epididymal cells from goats. *Comparative Immunology, Microbiology and Infectious Diseases, 36(4)*, 397-404


Muz, D., Oğuzoğlu, T.C., Rosati, S., Reina, R., Bertolotti, L., & Burgu, I. (2013). First molecular characterization of visna/maedi viruses from naturally infected sheep in Turkey. *Archives of Virology, 158*, 559-570


