HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN BLOOD OF LAMBS BORN TO MAEDI-VISNA VIRUS - INFECTED AND UNINFECTED EWES

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Abstract

Selected haematological and biochemical parameters in blood of lambs born to maedi-visna virus (MVV) seropositive and seronegative ewes were investigated. The study included 116 lambs born to ewes with MVV antibodies and 111 lambs born from seronegative mothers. The presence of antibodies was determined by ELISA. Blood samples were taken prior to weaning and post-weaning at 18-23 and 80-88 d of age, respectively. The study revealed that age and serological status had a significant effect on most of investigated variables. Lambs from seropositive ewes had significantly lower average white cell counts ($P \leq 0.05$) than lambs from seronegative ones. A similar relationship was noted for lymphocyte counts; however, granulocyte count was higher in the litter of uninfected ewes. Analysis of red blood cell indices of lambs from seropositive ewes showed significantly higher haematocrit, as well as marked changes in red blood cell volume and corpuscular haemoglobin concentration, compared with those from lambs born to uninfected ewes. There were no significant differences in platelet counts in both groups of lambs, tested on days 18–23; however, significantly lower values were noted in lambs born to seropositive ewes on days 80-88. Lactate dehydrogenase and alkaline phosphatase activities and uric acid concentrations were higher in lambs from infected ewes at both time points.

Key words: ewes, lambs, maedi-visna virus, blood parameters.

Maedi-visna is a chronic disease of sheep caused by the maedi-visna virus (MVV), which belongs to the genus Lenitivirus of the family Retroviridae. The disease is epizootiologically important and results in financial losses caused by reduced lambing, decreased productivity, and restrictions in animal trade (2, 5, 6, 10, 12). The disease can take a neurological or pulmonary form, manifesting clinically as limb pareses, paralyses, or as interstitial pneumonia, dyspnoea, cough, emaciation, and death (10). The infection can be transmitted vertically from mother to offspring through colostrum and milk, as well as horizontally through airborne droplets, or iatrogenically (3, 5). Transplacental infection is also possible, as was demonstrated by the presence of the virus in lambs born to MVV-infected ewes (6). Zoohygienic conditions and stocking density, especially when housed indoors, are also important factors for spreading the infection within a flock. The majority of infected sheep develop specific antibodies against core protein (p25) and transmembrane glycoprotein (gp40) of MVV, which are synthesised immediately after infection and during the late stage of infection, respectively (4). These antibodies can usually be detected 3-10 weeks after infection, whereas clinical symptoms can appear as late as 2-5 years post-infection. Hypochromic anaemia, and a drop in haemoglobin concentration to 70-80 g/L can accompany the clinical symptoms. In addition, leukocytosis, lymphocytosis, and often hypergammaglobulinaemia were seen in diseased animals, as a result of the slow disease process (11). However, little is known about the impact of early MVV infection on the haematological and biochemical parameters of peripheral blood. Examination of these parameters in lambs born to infected ewes could help to determine the impact of the ewes' health status on their progeny.

This study compares haematological and selected biochemical variables in peripheral blood of lambs born to ewes infected with MVV with those of lambs from uninfected ewes.

Material and Methods

The study was performed between 2006 and 2007, in a flock of Suffolk sheep and two synthetic mutton lines called BCP and SCP, monitored for MVV infection by the detection of specific antibodies. MVV antibodies were detected using the ELISA MVV kit (Institute Pourquier, France). The assay was performed...
following the manufacturer’s recommendations. Ewes were considered MVV-positive, if they were seropositive in three consecutive ELISA testing.

Two-hundred and twenty-seven lambs were studied, 116 born to MVV-positive ewes (MVV+ group) and 111 from seronegative ones (MVV- group). The two groups of lambs and ewes were kept isolated from one another during the lambing period, and were maintained under the same nutritional and environmental conditions. Factors such as the approximate date of lambing (± 7 d), similar sex distribution (45%-46% rams), and type of birth (twins 72%-73%) were taken into account when the groups of lambs were categorised.

Blood samples from lambs were taken twice, once during the suckling period, (approximate age 18-23 d), and after separation of the lambs from their mothers at 80-88 d. The following haematological parameters were determined: leukocyte count (WCC), lymphocyte count, monocyte count, granulocyte count, red blood cell (RBC) count, thrombocytes (PLT), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), haemoglobin (HGB), and mean corpuscular haemoglobin concentration (MCHC). All blood counts were carried out using an automated haematological analyser (Abacus Junior VET, Diatron). The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP); and the concentrations of uric acid, glucose, and total protein were determined in serum. These assays were performed using dedicated monotests with an automated analyser (Hitachi 704).

The results were statistically analysed using SAS 9.1.3 software. MVV antibody status, genotype, and lamb age (~20 and ~84 d) were included in a multifactor analysis of variance. Duncan’s test was used to make pair-wise comparisons between mean variable values. Significance was set at P≤0.05.

### Results

Analysis of WBC patterns of lambs at the age of ~20 d (Table 1) showed that offspring born to seropositive mothers were characterised by a statistically lower WCC (P≤0.05) and granulocyte count (P≤0.01), but higher lymphocyte counts (P≤0.05) than lambs born to seronegative ewes. After weaning, the WBC patterns changed in both groups. Total mean WCC increased to 10.66 x 10^9/L and 14.66 x 10^9/L in the MVV+ and MVV- groups, respectively, exceeding the reference values (14). In the post-weaning period, a greater increase in WCC was noted in lambs born to uninfected mothers, compared with the pre-weaning period. In the older lambs (80-88 d old), WCC, lymphocyte, and granulocyte counts were significantly lower in the MVV+ group than in the MVV- group (P≤0.01). When RBC patterns were examined, significant differences were found in terms of age and infection status (Table 2). In the young MVV+ group, RBC, and HCT were higher by 1.25x10^12/L and 5.75%, respectively, compared with lambs from the MVV- group. These differences were statistically significant (P≤0.01). Red blood cells in the MVV+ group had significantly lower MCH and MCHC than MVV- lambs. At 80-88 d, the RBC in both groups increased, reaching 12.85x10^12/L and 14.88x10^12/L in the MVV+ and MVV- lambs, respectively. At this time point, the MVV+ lambs had a higher mean MCV, and lower MCHC than MVV- lambs. The MVV- group had significantly lower HCT, while the MCH did not differ between groups.

The platelet counts in both groups of younger lambs (18-23 d old) were within the reference range (14). In older lambs (80-88 d old), platelet counts were significantly lower, and the MVV+ group was significantly different from the MVV- group (126.1x10^9/L vs. 185.0 x10^9/L). This might have been a result of the MVV infection.

Lamb genotype sporadically influenced the values of the tested parameters, whereas time of examination had a substantial impact, suggesting that there are additional factors at play (Tables 1 and 2).

### Table 1

Leukocyte counts in blood of lambs born to ewes seropositive and seronegative for MVV tested at pre- and post-weaning period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-weaning period</th>
<th>Post-weaning period</th>
<th>Factor effect</th>
<th>Significant interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MVV+ n=51</td>
<td>MVV- n=50</td>
<td>MVV+ n=61</td>
<td>MVV- n=61</td>
</tr>
<tr>
<td>WCC (10^9/L)</td>
<td>7.46±4.32 *</td>
<td>9.04±4.26 *</td>
<td>10.66±4.29 **</td>
<td>14.66±4.98 **</td>
</tr>
<tr>
<td>Lymphocyte count (10^9/L)</td>
<td>4.26±2.42 *</td>
<td>3.45±1.76 *</td>
<td>6.88±3.05 **</td>
<td>9.54±4.35 **</td>
</tr>
<tr>
<td>Monocyte count (10^9/L)</td>
<td>0.33±0.43</td>
<td>0.50±0.79</td>
<td>0.26±0.40</td>
<td>0.23±0.36</td>
</tr>
<tr>
<td>Granulocyte count (10^9/L)</td>
<td>2.88±2.01 **</td>
<td>5.19±3.54 **</td>
<td>3.53±3.21</td>
<td>4.76±3.55 **</td>
</tr>
</tbody>
</table>

WCC - white cell count; * P≤0.05; ** P≤0.01.
### Table 2
Haematological indices, and red blood cell and platelet counts in blood of lambs born to ewes seropositive and seronegative for MVV tested at pre- and post-weaning period

<table>
<thead>
<tr>
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<td>MVV+ n=61</td>
<td>MVV- n=61</td>
</tr>
<tr>
<td>RBC (10^{12}/L)</td>
<td>8.63±2.35**</td>
<td>7.38±2.51**</td>
<td>12.85±1.3</td>
<td>14.88±15.03</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>99.71±11.16</td>
<td>100.8±16.31</td>
<td>112.44±11.11</td>
<td>113.97±10.77</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>26.62±6.35**</td>
<td>20.87±6.04**</td>
<td>34.55±4.41**</td>
<td>31.57±5.42**</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>30.85±5.66</td>
<td>29.18±5.0</td>
<td>27.02±3.26**</td>
<td>24.56±2.52**</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>11.95±4.51**</td>
<td>15.49±6.46**</td>
<td>8.77±0.81</td>
<td>9.64±6.74</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>375.8±106.8**</td>
<td>524.3±18.39**</td>
<td>326.6±15.26**</td>
<td>345.6±61.46**</td>
</tr>
<tr>
<td>PLT (10^9/L)</td>
<td>240.9±148.9</td>
<td>211.4±143.8</td>
<td>126.1±89.43**</td>
<td>185.0±132.9**</td>
</tr>
</tbody>
</table>

RBC - red blood cells; HGB - haemoglobin; HCT - haematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration; PLT – platelets, * P≤0.05; ** P≤0.01.

### Table 3
Biochemical parameters in blood of lambs born to ewes seropositive and seronegative for MVV tested at pre- and post-weaning period

<table>
<thead>
<tr>
<th>Parameter</th>
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<td>MVV+ n=61</td>
<td>MVV- n=61</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>69.91±28.1</td>
<td>65.66±23.4</td>
<td>149.67±80.0</td>
<td>128.46±53.0</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>6.78±5.25</td>
<td>6.68±3.05</td>
<td>24.82±14.38</td>
<td>21.62±13.14</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1367.1±296.7*</td>
<td>1255.4±257.5*</td>
<td>1453.7±369.4*</td>
<td>1295.1±272.7*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>787.7±303.6*</td>
<td>654.1±310.2*</td>
<td>513.5±194.6*</td>
<td>410.8±180.2*</td>
</tr>
<tr>
<td>UA (µmol/L)</td>
<td>362.23±258.14**</td>
<td>229.0±254.57**</td>
<td>50.56±22.6</td>
<td>31.52±16.65</td>
</tr>
<tr>
<td>GLU (mmol/L)</td>
<td>5.16±1.06</td>
<td>5.04±1.05</td>
<td>3.88±0.57</td>
<td>3.8±0.62</td>
</tr>
<tr>
<td>TSP (g/L)</td>
<td>55.5±10.7</td>
<td>58.7±6.8</td>
<td>67.6±5.7</td>
<td>66.6±6.9</td>
</tr>
</tbody>
</table>

AST - aspartate aminotransferase; ALT - alanine aminotransferase; LDH - lactate dehydrogenase; ALP - alkaline phosphatase; UA - uric acid; GLU - glucose; TSP - total serum protein, * P≤0.05; ** P≤0.01.
Enzyme activities and concentrations of uric acid, glucose, and total serum protein are presented in Table 3. Lamb age had an influence on AST and ALT activity. In the older lambs, AST and ALT activities were more than two and three times higher than in younger animals; however, the differences between MVV+ and MVV- offspring were not statistically significant. The activities of LDH and ALP, regardless of age, were higher in lambs born to infected ewes. When analysed according to the age groups, the differences in MVV+ lambs ranged from 111.7 to 158.0 U/L (P<0.05) and from 102.7 to 133.6 U/L (P<0.01) for LDH and ALP, respectively. In all cases, the activity of these enzymes exceeded reference values. The concentration of uric acid was over 7-fold higher in young lambs than older ones. Higher concentrations were noted in the MVV+ group than in MVV- animals, while the differences between the groups were statistically significant (P<0.01) in younger lambs only. Elevated uric acid concentrations in lambs born to infected ewes might be indicative of compromised dietary energy delivery and could result from the lower milk production in MVV-infected ewes. There were significant differences in glucose concentrations and total serum protein between MVV+ and MVV- groups. The range for glucose across both time points was 3.88–5.16 mmol/L for lambs born to seropositive ewes, compared with 3.80–5.04 mmol/L for those from seronegative ewes. Ranges for total serum protein were 55.5–67.6 g/L and 58.7–66.6 g/L, for the MVV+ and MVV- groups, respectively.

Discussion

This study documented different patterns in haematological and biochemical parameters in blood of lambs born to MVV-infected ewes, compared with those born to uninfected ewes. In the first month of life, lambs born to infected ewes showed significantly lower WCC, but higher lymphocyte counts, and lower granulocyte counts than lambs born to uninfected ewes. The different WBC patterns, depended on lamb age and the ewes' MVV status, which indicates growth-related physiological changes, and may also reflect some changes in immunity as a consequence of viral infection. This assumption can be supported by Watt et al. (1992), who showed activation of T cells and an increase in the number of CD8+ γ/δ lymphocytes in the lymph nodes of sheep in the first stage of MVV infection. Maedi-visna virus infection results in typical signs of persistent infection characterised by the continuous presence of viral nucleic acid or viral proteins in the infected cells. Given the fact that monocytes and macrophages, as well as peripheral blood mononuclear cells are the target for MVV, the infection may lead to immune dysfunction, mainly through impairment of cellular immunity (10). The molecular mechanism is related to changes in the production of some cytokines, mainly interferon and tumour necrosis factor, as well as the suppressive activity expressed by viral proteins (rev and vif) on the synthesis of MHC class II antigens, which play a pivotal role in antigen recognition (7). Lambs born to infected ewes had significantly higher HCT and lower MCH and MCHC. The platelet counts in both groups of younger lambs were at the lower end of the reference range, whereas in older animals, a marked decrease was noted, especially in lambs born to infected ewes. The activities of selected enzymes, particularly LDH and ALP, and uric acid concentration were higher in lambs born to infected ewes than those from uninfected animals, irrespective of age. Increased LDH may be indicative of haemolytic anaemia in lambs, while increased ALP activity can be related to rapid skeletal growth (14).

Ewes infected with MVV can transmit the infection to their offspring, mainly through colostrum and milk. This results in lower lambing percentages (8). Arsenault et al. (1) found that MVV infection caused a reduction in birth weight by 0.94 kg, compared with uninfected animals. This was also linked to a higher death rate for lambs between delivery and 30 d post-partum. From a zootechnical perspective, the period between birth and separation from the ewe is the most difficult for lambs. During this period, animals must overcome many stressors to adapt to an independent life, and considerable losses occur. Lipecka et al. (9) showed that lamb mortality may reach 25% during this period. Keen et al. (5) and Pépin et al. (10) suggested that higher lamb mortality in the first month of life might be due to reduced milk production in MVV-infected ewes. This study relied on the infection status of the ewes, without direct determination of infection status of their offspring. Further studies are required to investigate the incidence of MVV seroconversion in lambs at narrower age intervals. Ideally, studies of experimentally inoculated lambs would help elucidate any links between MVV infection and haematological and serum biochemical changes.

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