EFFECT OF EXPERIMENTAL MANNHEIMIA HAEMOLYTICA INFECTION IN SHEEP FED COLOSTRUM AND DAM OR COW MILK AFTER BIRTH ON SERUM IgG

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Abstract

The objective of the study was to determine the effect of experimental Mannheimia haemolytica infection in sheep fed colostrum and dam or cow milk after birth on serum IgG levels. Ten Chios newborn lambs were fed ad libitum colostrum and dam milk at their dam’s side (control group). Other 10 lambs were separated from their dams immediately. These lambs were not allowed to suckle normally but were fed cow milk with feeding bottle (experimental group). When the sheep were one-year-old, the study was continued. The animals were inoculated intratracheally with $1 \times 10^9$ log-phase M. haemolytica organisms. Blood samples were collected from the jugular vein once before bacterial inoculation and on days 1, 4, 7, 10, 13, 16, 19 and 22 after the inoculation. Antibiotic treatment was applied on day 22. Blood samples were also collected on days 1, 4 and 7 after the treatment. IgG levels were significantly higher in the control group than the experimental one on days 0, 1, 4, 7, 10, 13, 16, 19 and on day 1 after treatment. The levels in the control group gradually decreased. In the light of the obtained findings, it may be suggested that the lambs non-suckling their dams might suffer, as one-year-old sheep, from an insufficiency of the immune system, and that the sheep compensated this disorder with own immune systems.

Key words: sheep, colostrum, milk, IgG, Mannheimia haemolytica, immunity.

The immune system is a remarkably adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders (12). Colostrum is the initial secretion in the lactation of the ewe (1, 19). It contains immunoglobulins which provide the lamb, which has little or no immunoglobulins in its serum at birth, with passive immunity to certain infectious diseases. It is also a laxative which helps to remove the meconium from the gut of the lamb and, very important, it is a nutrient source which provides energy for heat production and the prevention of hypothermia (19). It has been reported generally that colostrum has higher fat, protein and immunoglobulin concentrations and a lower lactose concentration than normal milk (26).

In ruminants, transfer of maternal immunity to the foetus is prevented by the placenta. As a result, ruminants are born essentially devoid of Ig and must depend on immunoglobulins absorbed from colostrum for (passive) immunity prior to the development of their own (active) immune systems (8). Immunoglobulins are absorbed intact across the intestinal epithelium and reach the circulation from which they equilibrate throughout the animal tissues. In ruminants, immunoglobulin absorption, reported in different works, is remarkably variable ranging from 16 to 66% (16, 20). Immunoglobulin G (IgG), the most abundant isotype in serum, constitutes about 80% of the total serum immunoglobulins (12). Neonatal morbidity and mortality rates decrease when adequate concentrations of immunoglobulins are received via colostrum (22). Low serum IgG concentrations in newborn calves and lambs are associated with increased disease susceptibility and losses (8).

Pneumonic pasteurellosis is a severe respiratory disease that develops in ruminants (3). It is often fatal. It leads to an acute fibrinonecrotizing pleuropneumonia characterized by an influx of neutrophils into the alveoli; accumulation of fibrinous oedema fluid within the alveoli, pleural surface, and interlobular septa; haemorrhage; vascular thrombosis; and coagulative parenchymal necrosis of the lung (13). Its cause is multifactorial and usually involves various combinations
of stress and accompanying infectious agents. Stressful conditions such as shipment, weaning, and overcrowding are prominent predisposing factors in the development of the disease. Mannheimia (Pasteurella) haemolytica is the most important bacterial species in the development of fatal pneumonic pasteurellosis. M. haemolytica is a Gram-negative bacterium composed of many potentially antigenic proteins, polysaccharides, and lipids (3).

The objective of the study was to determine the effect of experimental M. haemolytica infection in sheep fed colostrum and dam or cow milk after birth on serum IgG levels.

**Material and Methods**

Ten Sakiz newborn lambs were fed ad libitum colostrum and dam milk at their dam’s side (control group). Other 10 lambs were separated from their dams immediately. These lambs were not allowed to suckle normally but were fed commercial cow milk with feeding bottle (experimental group). Each lamb was eartagged with individual identification numbers during the day following birth. When they were one-year-old, the study was continued. Hay, concentrates and water were provided ad libitum. All the sheep were found to be clinically normal on the basis of physical examination at the beginning of the experiment.

*M. haemolytica*, serotype A1 (Cat. No: 103426 T ATCC), was inoculated on brain-heart infusion agar plates and incubated at 37°C for 24 h. Thereafter, the organisms were harvested with phosphate buffered saline (0.9% NaCl) (PBS) and washed with PBS three times. The bacteria were adjusted to 1x10^9 organisms /2 ml using sterile PBS. Inoculum was kept at 4°C.

Sheep (n=20) were inoculated intratracheally with 1x10^9 log-phase of *M. haemolytica* organisms suspended in 2 ml of PBS. Blood samples were collected from the jugular vein once before bacterial inoculation and on days 1, 4, 7, 10, 13, 16, 19 and 22 after the inoculation from all the animals. Antibiotic treatment was applied on day 22 after infection. Blood samples were also collected at days 1, 4 and 7 after the treatment. Serum samples were stored at -70°C until analysis. IgG concentrations were assayed by the ELISA.

In order to measure IgG concentrations separately in the serum of the sheep and lambs, a new sandwich ELISA system was developed. The rabbit anti-sheep IgG antibody (S1265), a peroxidase-conjugated rabbit anti-bovine IgG antibody (A5295), the sheep IgG antibody (S1265), a peroxidase-conjugated rabbit anti-bovine IgG antibody (A5295), and the sheep IgG antibody (S1265) were used. Sheep IgG (0.5 µg/ml, 50 µl/well) served as the standard. Blood serum was diluted (1:50 000, 50 µl/well) and incubated for 1 h at 37°C. A peroxidase-conjugated rabbit anti-bovine IgG antibodies (1:50 000 in PBS-Tween 20, 50 µl/well, 1 h at 37°C) was used as the conjugate. Tetramethylbenzidine (0.2 mg/ml in 0.1 mol acetate-citrate buffer, pH 5 with 0.0005% H_2O_2, 100 µl/well) served as substrate. The enzymatic reaction was stopped after 10 min with 1 M H_2SO_4 (50 µl/well), and the resulting absorbance was measured at 450 nm. Between each step, the ELISA plates were washed 4 times with 0.9% NaCl-Tween (pH=7.2).

Mean serum IgG concentrations for control and experimental groups on each blood sampling day were compared by use of a two-tailed Student’s t-test. Data were analysed by use of factorial and repeated measure analysis of variance between blood sampling days within each group. Results are presented as mean ± standard deviation. All statistical analyses were performed using software package program (SPSS for windows, Standart version 10.0, SPSS Inc., Chicago, Illinois, 1999). A significance level of P<0.05 was employed in the analysis of data from the treatment groups (21).

**Results**

In order to measure sheep IgG antibodies, a sandwich ELISA system was developed. Sheep IgG concentrations could be detected via standard curves ranging from 500 ng/ml to the detection limit of about 10 ng/ml, with intra-assay and inter-assay variabilities in lamb serum between 4.4% and 9.9% (from 7 intervals, mean 6.3) and between 5.8% and 11.0% (from 9 intervals, mean 9.1), respectively (Fig. 1).

Serum IgG levels in both groups are shown in Table 1. The levels were significantly higher in the control group than the experimental group at days 0, 1, 7, 10, 13, 16 and 19, and at days 1 (P<0.05) and 4 (P<0.01) after treatment. The levels in the control group gradually decreased.

**Discussion**

In both animals and humans, the first few months of life are a time of increased susceptibility to infectious diseases. Resistance to pathogenic organisms depends to a large degree on passive antibodies transferred from the mother either transplacentally (humans) or by means of colostrum (ruminants, equines) (10). Antibodies given orally can develop a local effect by neutralization of pathogen stimuli or connection (humans) or by means of colostrum (ruminants, equines) (6). Neonates that fail to receive passive maternal immunity, normally obtained by ingestion of colostrum, are particularly susceptible to infection and sepsis.

Gram-negative septicaemia is an important cause of mortality in sheep, and failure of passive transfer of immunity by colostral ingestion has been positively correlated with sepsis (2).
Fig. 1. Standard curve of the sandwich ELISA for the detection of sheep IgG (inter-assay, n=7, 450 nm).

Table 1
Mean serum IgG levels (mg/ml)

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>19</th>
<th>22 (treatment)</th>
<th>1</th>
<th>4</th>
<th>7</th>
</tr>
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<tr>
<td>Exp</td>
<td>20.57&lt;sup&gt;a&lt;/sup&gt; ±6.31</td>
<td>20.45&lt;sup&gt;a&lt;/sup&gt; ±5.12</td>
<td>19.93&lt;sup&gt;a&lt;/sup&gt; ±4.33</td>
<td>20.53&lt;sup&gt;a&lt;/sup&gt; ±4.30</td>
<td>20.53&lt;sup&gt;a&lt;/sup&gt; ±4.30</td>
<td>21.57&lt;sup&gt;a&lt;/sup&gt; ±6.11</td>
<td>20.70&lt;sup&gt;a&lt;/sup&gt; ±4.60</td>
<td>21.84&lt;sup&gt;a&lt;/sup&gt; ±8.02</td>
<td>19.66&lt;sup&gt;a&lt;/sup&gt; ±5.11</td>
<td>20.53&lt;sup&gt;a&lt;/sup&gt; ±4.30</td>
<td>21.01&lt;sup&gt;a&lt;/sup&gt; ±7.24</td>
<td>22.00&lt;sup&gt;a&lt;/sup&gt; ±6.81</td>
</tr>
<tr>
<td>Ctrl</td>
<td>27.18&lt;sup&gt;ab&lt;/sup&gt; ±7.63</td>
<td>27.23&lt;sup&gt;ab&lt;/sup&gt; ±6.37</td>
<td>27.48&lt;sup&gt;ab&lt;/sup&gt; ±8.57</td>
<td>26.35&lt;sup&gt;ab&lt;/sup&gt; ±8.69</td>
<td>25.26&lt;sup&gt;ab&lt;/sup&gt; ±3.78</td>
<td>26.39&lt;sup&gt;ab&lt;/sup&gt; ±8.19</td>
<td>25.46&lt;sup&gt;ab&lt;/sup&gt; ±5.46</td>
<td>27.00&lt;sup&gt;ab&lt;/sup&gt; ±8.02</td>
<td>26.20&lt;sup&gt;ab&lt;/sup&gt; ±5.65</td>
<td>24.48&lt;sup&gt;ab&lt;/sup&gt; ±6.29</td>
<td>20.46&lt;sup&gt;b&lt;/sup&gt; ±3.83</td>
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± SD, * P<0.05, ** P<0.01 show difference between two groups
<sup>ab</sup> means within the same line with different superscripts differ significantly at P<0.05

Total serum IgG concentrations in foals that receive colostrum would be expected to depend on the concentration of these antibodies in maternal serum and colostrum. Total serum IgG concentration is currently used to evaluate passive transfer of maternal immunity in foals, and foals are said to have had failure of passive transfer if their serum IgG concentration is <2 mg/ml (2). Serum levels of passive antibodies to particular pathogens can be highly variable, however, and also decline with age (10). IgG are involved in antigen opsonisation and enhance phagocytosis by alveolar macrophages. The opsonising effects of serum IgG play major roles preventing infection (27).

Kaltreider (11) and Donachie et al. (5) who showed that inoculation of antigens into the lungs of the animal could result in both local and systemic cell-mediated and humoral immunities. In rabbits experimentally infected intranasally with Pasteurella multocida, serum IgG against the microorganism began to rise 21 to 33 d after the infection and remained elevated until the animals were euthanized 90 d after the infection (14). A high level of antibodies reactive with cilia of the upper respiratory tract was detected in the sera from many of the lambs in flocks affected from Mycoplasma ovipneumoniae (coughing syndrome) (18). Serum IgG anti-Fasciola hepatica titres in sheep increased from 2 weeks after infection and maximum values were reached 8 weeks after infection, tending to decrease slowly thereafter (7). Van Donkersgoed et al. (24) reported significant antibody responses to
recombinant leukotoxin (by 3 months of age) after vaccinating conventional beef calves at 3 and 5 weeks of age. Mice vaccinated with *M. haemolytica* A1 outer membranes had elevated IgG antibody titres to these membranes in serum collected on day 24 (15). Townsends et al. (23) reported production of antibodies to capsular polysaccharide in neonatal colostrum-deprived calves vaccinated with live *M. haemolytica* A1. Vestweber et al. (25), reported severe clinical signs of pneumonia in 1- to 3-week-old calves receiving *M. haemolytica* A1.

In the present study, serum IgG concentrations were found higher in sheep fed as lambs colostrum and dam milk than other group up to day 1 after treatment. Since this elevation in sheep fed colostrum and dam milk was also present before bacterial inoculation, it was suggested that the elevation was due to an infection before the inoculation. Antibiotic treatment lowered this elevation in IgG to the normal level (levels on days 4 and 7 of treatment). So, in the present study, the inoculation of *M. haemolytica* in these doses did not lead to a significant increase in serum IgG concentrations. In the study of Zamri-Saad et al. (27), serum IgG levels in the animals exposed to *M. haemolytica* A2 increased gradually compared to the unexposed control to reach a significantly high level at week 4 post-exposure before declining slightly the following week and increasing again at week 6 post-exposure. Donachie et al. (5) observed the high levels of IgG against *M. haemolytica* within 1 week post-exposure. However, in the present study, *M. haemolytica* did not increase significantly antibody levels in both groups. This finding is similar to the study of Zamri-Saad et al. (27). If the present study had continued for 4 weeks, may be serum IgG concentrations would have significantly risen, but the study lasted briefly and the treatment began on the day 22.

The babies sucking mother had higher antibody levels than the babies fed with bottle after the vaccination or contact with infectious material (17). Hodgins and Shewen (9) reported that antibody titres in vaccinated calves were significantly higher than titres of placebo recipients by 6 week of age. The authors noted significant antibody responses to leucotoxin in colostrum-deprived calves, but peak antibody titres were relatively low compared to responses in older calves (4). Mean serum IgG concentration (mg/ml) in colostrum-fed and colostrum-deprived foals given bacterial lipopolysaccharide was significantly higher (14.3 ± 4.32) in colostrum-fed than that (0.34 ± 0.12) in colostrum-deprived foals (2). In the present study, the serum IgG concentrations in the sheep fed colostrum and dam milk after birth were higher than in the sheep fed cow milk after birth, similarly to the findings of Newman (17). However, this elevation was also present before bacterial inoculation and fell after antibiotic treatment. Therefore, *M. haemolytica* infection at the applied dose did not lead to a remarkable increase in antibody level for 22 d. This showed the presence of an pre-trial infection in the animals of this group. In the light of the obtained findings, it may be suggested that the non-sucking their dam’s lambs might suffer, as the one-year-old sheep, from marked insufficiency of the immune system and that the sheep compensated this disorder with own immune systems.

**References**


