CLINICAL, HAEMATOLOGICAL AND BIOCHEMICAL STUDIES IN GOATS NATURALLY INFECTED WITH MYCOPLASMA AGALACTIAE

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Abstract

The aim of this study was to investigate some clinical, haematological and biochemical parameters in goats with contagious agalactia caused by Mycoplasma agalactiae. Forty seven goats showing clinical signs of contagious agalactia and 20 healthy goats were used in the study. Concentrations of plasma biochemical parameters were analysed by a clinical chemistry analyser, and packed cell volume, haemoglobin concentration, and red blood cell and white blood cell counts were determined using an automatic blood counter. There was positive result in the PCR analysis for Mycolasma agalactiae. The body temperature, pulse and respiration rates, aspartate aminotransferase and lactate dehydrogenase activity in plasma were significantly higher in the infected goats than in the controls, while rumen contractions, plasma total protein, albumin and glucose concentrations were significantly lower. No significant difference relative to white and red blood cell counts, packed cell volume, haemoglobin concentration, alkaline phosphatase and γ-glutamyl transferase activity, and sodium, potassium and chloride concentrations between the groups were found.

Key words: goat, Mycoplasma agalactiae, haematology, biochemistry.

Caprine contagious agalactia (CCA) is one of the most serious disease affecting small ruminants (3, 5, 27). Although the etiologic agent of the classical disease is Mycoplasma agalactiae (3), similar clinical and pathological features can be produced by other mycoplasma species such as Mycoplasma mycoides subsp. mycoides, Mycoplasma capricolum subsp. capricolum and Mycoplasma mycoides subsp. capri (3, 21, 29). Goats seem to be more susceptible to the natural disease than are sheep (17). The disease is rapidly spread by contact between infected and healthy animals. Young animals are most commonly infected with contaminated colostrum or milk (13, 16, 27). The incubation period in the natural disease varies between 7 and 56 d (23). Mycoplasma agalactiae primarily affects the mammary gland, eyes, and joints (7, 22). Anorexia, lethargy, and unwillingness to follow the herd are the first clinical signs. Pregnant animals may abort (17, 30). The economic impact of the disease lies in its high morbidity and resultant loss of milk and meat production rather than in its mortality (6, 17).

Although clinical signs of the disease are characteristic, laboratory confirmation of field diagnosis is essential because of several look alike mycoplasmal and bacterial infections (17). The most suitable samples include milk, mastitic secretions, joints fluids or eye swaps for isolation attempts (16, 17, 22).

The purpose of this study was to determine the variations in clinical, haematological, and biochemical values in goats naturally infected with Mycoplasma agalactiae.

Material and Methods

Animals. The study was performed on 20 healthy non-infected goats (control group) and 47 goats naturally infected with Mycoplasma agalactiae in the province of Elazig, Turkey. None of these animals had been vaccinated against Mycoplasma agalactiae. The clinical signs in the sick animals were mostly mastitis and arthritis, and there was no history of agalactia-related symptoms or signs in the control animals at the time of sampling. Ages of the animals varied between 2 and 3 years and mean body weights 45-55 kg. All the animals were under the same care and feeding conditions. In both groups, the goats were fed straw, barley and concentrated feed. All the goats were clinically examined.

Collection of samples. About 5 ml of milk samples from each female goat were obtained aseptically and collected into sterile tubes for culture and identification by polymerase chain reaction (PCR). Also 10 ml jugular venous blood sample was collected from each of the infected and control goats into vacutainer tubes with ethylene diamine-tetra-acetic acid
(EDTA) for haematological and biochemical analysis. Samples were transported on ice to the Department of Veterinary Microbiology and Internal Disease, University of Firat, Elazig. Plasma was separated by centrifugation (3 000 rpm, for 10 min) and stored at -20°C until being used for analysis.

**Analytical procedures.** Concentrations of plasma biochemical parameters were analysed by a clinical chemistry analyser (Advia 1200, Chemistry System, Bayer-Healthcare, Germany), and blood biochemical indices determined, including total protein, albumin, alkaline phosphatase (AP), aspartate amino transferase (AST), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose, sodium (Na), potassium (K), and chloride (Cl).

Packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell (RBC) and white blood cell (WBC) counts were determined using an automatic blood counter (Sysmex KX-21 N, Japan).

**Culture and PCR test.** Approximately 0.1 ml of milk samples were inoculated into mycoplasma broth base (Oxoid) and mycoplasma agar base (Oxoid), containing mycoplasma supplement G (Oxoid), and incubated in a humid atmosphere of 5% carbon dioxide at 37°C for 7 d. The broths were checked daily for growth and when they became turbid, loopfuls were inoculated into mycoplasma agar base and the plates were incubated under the same conditions.

PCR test for the *Mycoplasma agalactiae* (10) was used for confirmation. The primers, ma-mp 1F and ma-mp 1R derived from ma-mp 81 gene, used for the *Mycoplasma agalactiae* PCR were:
- ma-mp1F 5’-AGCAGCACAAAACTCGAGA-3’,
- ma-mp1R 5’-AACACCTGGATTGTTTGAGT-3’,

PCR products with the molecular size of 176 bp were considered indicative for identification as *M. agalactiae*.

**Statistical analysis.** Statistical analysis was performed using SPSS Ms Windows Release 10.0 programme. Impaired t-test was used for evaluating data between groups. The data were expressed as mean and standart deviation of mean (mean ± SD), and P<0.05, P<0.01 and P< 0.001 were taken as the level of significance.

**Results**

Changes in the clinical, haematological, and biochemical plasma parameters and significant differences of data are presented in Table 1. The clinical, haematological and biochemical plasma parameters obtained for both agalactia and control animals were within the normal reference range (9, 12).

**Clinical signs.** The transient fever, mastitis, arthritis and keratoconjunctivitis observed here were the constant findings in the goats infected with *Mycoplasma agalactiae*. Lameness was especially seen in the male goats. The carpal or tarsal joints were swollen and painful. The udder was filled with connective tissue, and in some cases atrophy developed. The conjunctiva was hyperaemic bilaterally, and vision loss was seen in the some goats due to corneal vascularization. There were statistically significant differences between groups (agalactia and control) relative to body temperature (T), pulse (P) and respiration (R) rates, and rumen contractions (Rc).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Goats infected with <em>M. agalactiae</em></th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>40.1 ± 0.60</td>
<td>39.6 ± 0.37</td>
<td>**</td>
</tr>
<tr>
<td>P (/ min)</td>
<td>92.7 ± 7.14</td>
<td>84.4 ± 7.41</td>
<td>*</td>
</tr>
<tr>
<td>R (/ min)</td>
<td>28.4 ± 4.47</td>
<td>23.2 ± 3.09</td>
<td>**</td>
</tr>
<tr>
<td>Rc (/5 min)</td>
<td>5.7 ± 1.49</td>
<td>8.6 ± 1.4</td>
<td>***</td>
</tr>
<tr>
<td>WBC (x10³/L)</td>
<td>12.06 ± 1.45</td>
<td>11.64 ± 1.07</td>
<td>NS</td>
</tr>
<tr>
<td>RBC (x10⁶/L)</td>
<td>13.27 ± 1.17</td>
<td>12.81 ± 0.94</td>
<td>NS</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>34.04 ± 2.81</td>
<td>32.93 ± 0.96</td>
<td>NS</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.57 ± 0.97</td>
<td>11.16 ± 0.94</td>
<td>NS</td>
</tr>
<tr>
<td>T.protein (g/dl)</td>
<td>6.75 ± 0.27</td>
<td>7.02 ± 0.24</td>
<td>*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.0 ± 0.2</td>
<td>3.29 ± 0.20</td>
<td>**</td>
</tr>
<tr>
<td>AP (U/L)</td>
<td>60.0 ± 9.5</td>
<td>56.6 ± 8.98</td>
<td>NS</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>120.1 ± 13.37</td>
<td>97.8 ± 9.39</td>
<td>***</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>59.9 ± 8.47</td>
<td>56.2 ± 3.67</td>
<td>NS</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>264.9 ± 52.06</td>
<td>213.4 ± 37.42</td>
<td>*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>52.0 ± 5.5</td>
<td>67.1 ± 7.5</td>
<td>***</td>
</tr>
<tr>
<td>Cl (mEq/L)</td>
<td>106.7 ± 6.7</td>
<td>103.0 ± 10.2</td>
<td>NS</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>147.3 ± 4.66</td>
<td>150.3 ± 7.83</td>
<td>NS</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.38 ± 0.27</td>
<td>4.26 ± 0.19</td>
<td>NS</td>
</tr>
</tbody>
</table>

± SD, NS: not significant, *:P<0.05, **:P<0.01, ***:P<0.001
**Haematology and blood biochemistry.**

Significant difference between groups was shown in regard to plasma total protein, albumin, and glucose concentrations, and aspartate aminotransferase and lactate dehydrogenase activity. No statistical difference was observed in white blood cell and red blood cell counts, packed cell volume, haemoglobin concentration, alkaline phosphatase, and γ-glutamyl transferase activity, and sodium, potassium, and chloride concentrations between the groups.

**Isolation and identification of Mycoplasma agalactiae.** Mycoplasma agalactiae was isolated and identified from 17 milk samples collected from the sick goats. The plates were examined under a stereoscopic microscope for ‘fried egg’ colonies. Microscopic observation of agar plate culture revealed the presence of typical ‘fried egg’ shaped colonies of *Mycoplasma*. We could observe the 176 bp band obtained from the DNA amplification of *Mycoplasma agalactiae* using the primers ma-mp1F and ma-mp1R.

**Discussion**

Contagious agalactia in the goats is a very contagious disease, characterized by fever, malaise, mastitis, arthritis, and keratoconjunctivitis. Infection with *Mycoplasma agalactiae* occurs in both male and female goats at all age, and can be unapparent or can cause a mild, acute or chronic disease (8, 23, 25). Although the changes in biochemical and haematological parameters are well-documented in many bacterial and viral diseases with regard to their pathogenesis, these alterations caused by mycoplasma are poorly studied and there are a few reports on clinicopathological changes of the goats experimentally inoculated with *Mycoplasma* species. (4, 11, 19, 26, 28).

This study was undertaken to determine the variations in clinical, haematological, and biochemical values in the goats naturally infected with *Mycoplasma agalactiae*.

Differential diagnosis is necessary to distinguish mastitis and keratoconjunctivitis of mycoplasmal origin from the same conditions caused by other microorganisms (17). Recently, PCR has been accepted as a valuable tool for the diagnosis of mycoplasma infections. The PCR has the advantage of easy use, rapid availability of results, and standardization and is more suitable for processing of a large number of specimens (10). *Mycoplasma agalactiae* was isolated by culture and identified by PCR in this study.

There was a statistically significant increase relative to body temperature and pulse and respiration rates in the goats with contagious agalactia compared to control animals, while a significant decrease in rumen contractions was observed. An onset of a transient febrile syndrome was also reported in the cases when mycoplasmaemia preceded the colonization of the mammary gland, joint lining and conjunctiva mucosa by the infectious agent (1, 2, 15). As body temperature increases, the rate of respiration increases because in animals which cannot sweat over the entire body, heat loss occurs through the respiration. Also, in most febrile diseases the pulse rate increases in direct proportion to the degree of fever. Anorexia and decline of rumen contractions usually occurs secondarily to a primary disease (18).

Although it has been reported that leukopenia, decreased erythrocyte count and haemoglobin concentrations in goats infected with other mycoplasma species, such as *Mycoplasma mycoides* subsp. *mycoides* and *Mycoplasma mycoides* subsp. *capri* (11, 20, 24, 31), there were no observed statistically significant differences relative to WBC, RBC, Hb, and percentage of PCV between the groups in this study.

Blood biochemical examinations revealed that there were significant reductions in serum total protein, albumin and glucose concentrations as compared to control animals. The depletion of serum proteins may be due to the utilization of blood proteins by mycoplasma organisms for their proliferation as well as decreased synthesis of proteins during the disease process. Kumar (14) and Mondal (19) have also reported similar biochemical changes. It was concluded that the declined levels of glucose concentrations in serum of the goats infected with *Mycoplasma agalactiae* might be due to anorexia caused by the primary disease.

Although there was a significant increase in the activity of AST and LDH in the present study, there were no significant changes in alkaline phosphatase, and γ-glutamyl transferase activity, and sodium, potassium, and chloride concentrations in the plasma. The increase in AST and LDH activities might have occurred due to inflammatory changes in the organs, especially joints and mammary glands.

Based on the results obtained in our study we can conclude that the *Mycoplasma agalactiae* infection in goats was not anaemic or septicaemic in nature, although the goats were affected clinically and metabolically. Furthermore, this is the first report on the isolation and identification of *Mycoplasma agalactiae* in goats bred in the province of Elazig, Turkey.

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