Clinico-pathological profile and frequency of *Mycoplasma mycoides* subsp. *capri* infection in goats in northern zone of Khyber-Pakhtunkhwa, Pakistan

*Said Sajjad Ali Shah¹, Umer Sadique¹, Zahoor Ul Hassan¹, Shakoor Ahmad¹, Hamayun Khan¹, Muhammad Kamal Shah¹, Muhammad Israr² and Hanif ur Rahman³*

**Abstract**

Caprine mycoplasmoses are important diseases of goats and occur worldwide. The current study was designed with the objective of investigating the frequency and clinico-pathological profile of caprine mycoplasmosis in two districts in the northern zone of Khyber Pakhtunkhwa, Pakistan. Three hundred nasal swabs were collected, 150 each for Swat and Buner from goats showing clinical signs of respiratory mycoplasmosis. In districts Swat and Buner, 21.3% and 31.3% samples were mycoplasma positive (respectively). All isolates were identified as *Mycoplasma mycoides* subsp. *capri* by biochemical and growth inhibition testing. Blood and serum samples from 30 mycoplasma positive goats were collected for studying hematological and blood biochemistry profile. Hematological results showed that there was a significant decrease (P<0.05) in Total Erythrocyte Count (TEC), Packed Cell Volume (PCV) and hemoglobin concentration while significant increase (P<0.05) in the Mean Corpuscular Hemoglobin and Mean Corpuscular Volume value was recorded. Blood biochemistry revealed that total serum protein and albumin were decreased significantly (P<0.05) while there was a significant increase (P<0.05) in serum glutamic pyruvate transaminase (SGPT) and globulin fraction of serum. It can be concluded that *Mycoplasma mycoides* subsp. *capri* is the most prevalent species of caprine respiratory mycoplasmas in the northern zone of Khyber Pakhtunkhwa.

**Keywords**

Caprine mycoplasmosis, *Mycoplasma mycoides* subsp. *capri*, goat, hematology, biochemical tests

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**Introduction**

Caprine respiratory mycoplasmoses are important diseases of goats throughout the world, particularly in the developing countries of Africa and Asia inflicting heavy economic losses (18, 25,27). These diseases are prevalent in Pakistan causing several outbreaks in the northern and southern regions (4, 22, 24). The pathogenic species of *Mycoplasma mycoides* cluster comprises six different members mainly responsible for disease in small ruminant (11, 12). Among the *M. m.* cluster, *Mycoplasma mycoides* subsp. *Capri (Mmc)* is a very important member responsible for respiratory infections, high morbidity and mortality, which lead to huge economic losses. The primary signs of disease include cough, pneumonia, nasal discharge and lacrimation. Other signs could be pyrexia, diarrhea, mastitis, arthritis, weight loss and occasionally nervous signs and abortion (22, 23). The disease is highly contagious and can easily spread through direct and indirect contact (10).
Due to fastidious nature of mycoplasma and special requirement for growth, limited work is conducted in Pakistan (3, 23). This study was conducted for the isolation and identification of the etiology of caprine mycoplasmosis in the northern Districts of Khyber Pakhtunkhwa, Pakistan through biochemical and growth inhibition test in goats in natural outbreaks. Another objective of the study was to determine the hematological and biochemical profile of goats affected with caprine mycoplasmosis.

Material and Methods

Study area and sample collection
The present study was conducted in districts Swat and Buner of Khyber Pakhtunkhwa. Temperature in both districts ranges from 42°C in summer to 3°C in winter. From both districts, a total of three hundred nasal swabs (150 from each district) were collected from goats having respiratory signs suspected for caprine mycoplasmosis. All these animals were showing typical signs including cough, nasal discharge, lacrimation, conjunctivitis and pyrexia. Blood samples were collected from goats suspected for caprine mycoplasmosis and also from apparently healthy (non-infected) goats. For finding of hematological profile, 3 ml of blood was collected in the vacutainer tubes containing EDTA. For biochemical analysis, 5 ml of blood was collected in the plain tubes, and were allowed to clot.

Isolation and identification of mycoplasmas
Initially, the samples were inoculated in Modified Hayflick’s broth medium providing the required temperature (37°C) and carbon dioxide (concentration 5%). Positive cultures were streaked on Modified Hayflick’s agar medium (24).

After the initial identification through morphological characteristics, different biochemical tests were performed for further confirmation of the isolates: Arginine Hydrolysis, Glucose Fermentation, Casein Hydrolysis, Liquefaction of Coagulated Serum, and Tetrazolium Reduction (aerobically and anaerobically) (19).

Specific antibodies against Mmc were raised in rabbits. On Modified Hayflick’s agar media, 2ml of diluted mycoplasma culture was spread evenly and filter paper disc immersed in hyper-immune sera was placed on Modified Hayflick’s agar. Plates were incubated for 7 days and then observed for a zone of inhibition. Inhibitory zone of 2 mm or more was declared as positive (20).

Hematology
Blood samples were subjected to hematological studies including Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Hemoglobin levels (Hb), Packed Cell Volume (PCV) and Differential Leukocyte Count (DLC) by using Hematology Analyzer (Sysmax KX-21N, Japan). Erythrocyte Indices were also estimated including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Biochemical analysis
Blood samples were collected in the clean plain tubes and centrifuged for 10 min at 3000 rpm. Serum glutamic pyruvate transerase (SGPT), total serum proteins, albumin and globulin were measured by using Biochemistry analyzer (PS-520 Shenzhen Procan Electronics, China).

Statistical Analysis
The collected data were arranged in Microsoft Excel sheet and analyzed through statistical analytical software Statistics version 8.1. Chi square was used to check significant difference among the Districts. Student t-test was applied for hematological and biochemical differences, and means were compared by Duncan’s multiple range at $P \leq 0.05$ level of significance.

Results

Isolation of mycoplasmas
Total of 82 out of 300 samples (27.3%) were positive for mycoplasmas. District-wise prevalence was 31.3% and 21.3% in districts Buner and Swat ($P>0.05$), respectively.

All the isolates give positive reaction for glucose, Tetrazolium Reduction Test, Liquefaction of Coagulated Serum and Casein Hydrolysis Test, while for Arginine Hydrolysis Test none of the isolate was positive. All 82 isolates showed positive reaction for Growth Inhibition Test with antisera specific for Mmc.

Hematology
Table 1 shows that there were significant differences in hematological parameters between infected and non-infected animals. Table 2 shows that there was a significant increase ($P<0.05$) in the level of Total Leucocyte Count (TLC), lymphocytes, neutrophils and monocytes between infected and non-infected goats, while no significant differences ($P>0.05$) were present between infected animals of both districts.

Biochemical analysis
Table 3 shows that there were significant differences ($P<0.05$) in the level of total serum protein, albumin and A/G ratio between infected and non-infected animals. Globulin and SGPT of infected animals were significantly higher ($P<0.05$) than those of non-infected animals.

Discussion and conclusions
Caprine respiratory mycoplasmosis is mostly caused by members of Mycoplasma mycoides cluster. Mycoplasma capricolum subsp. capripneumoniae (Mcnp) affects only the thoracic cavity while in Mmc infections other systems are also involved (21, 22). Clinical signs and symptoms of goats examined in the present study were diarrhea, difficulty in movement, mucous discharge from nose and also noisy cough. Due to intense cold in Buner district, signs were very prominent, which is in accordance with the study carried out by Gelagay et al. (6) and Mekuria et al. (13). Different diagnostic tests are used for the identification and confirmation of species of Mycoplasma. Advanced molecular tests are also used, but even today biochemical tests are useful for the initial identification of mycoplasma.
Table 1. Hematological profile of non-infected and goats infected with Mmc (mean ± standard error)

<table>
<thead>
<tr>
<th>Condition</th>
<th>TEC (×10^6)</th>
<th>Hb (g/dL)</th>
<th>PCV (%)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>5.6±0.1</td>
<td>6.9±0.2</td>
<td>20.7±0.6</td>
<td>36.7±0.9</td>
<td>12.2±0.3</td>
<td>33.5±0.7</td>
</tr>
<tr>
<td>Non infected</td>
<td>10.1±0.1</td>
<td>9.6±0.01</td>
<td>27.2±0.2</td>
<td>26.9±0.5</td>
<td>9.3±0.18</td>
<td>35.2±0.31</td>
</tr>
<tr>
<td>P-value</td>
<td>0.005</td>
<td>0.000</td>
<td>0.021</td>
<td>0.045</td>
<td>0.029</td>
<td>0.956</td>
</tr>
</tbody>
</table>

Table 2. Total Leukocyte Count and Differential Leukocyte Count of non-infected and goats infected with Mmc (mean ± standard error)

<table>
<thead>
<tr>
<th>Condition</th>
<th>TLC (×10^3)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Monocytes+Eosinophils + Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>13.6± 0.13</td>
<td>39.6± 0.68</td>
<td>53.8± 0.62</td>
<td>8.18±0.12</td>
</tr>
<tr>
<td>Non infected</td>
<td>13.05±0.01</td>
<td>35.9±0.06</td>
<td>51.59±0.38</td>
<td>6.8± 0.36</td>
</tr>
<tr>
<td>P-value</td>
<td>0.010</td>
<td>0.004</td>
<td>0.002</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 3. Serum profile of Liver Function Test of non-infected and goats infected with Mmc (mean ± standard error)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total serum protein (g/dL)</th>
<th>Serum Albumin (g/dL)</th>
<th>Serum Globulin (g/dL)</th>
<th>A:G</th>
<th>SGPT (u/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>5.6±0.14</td>
<td>2.5±0.13</td>
<td>3.06±0.06</td>
<td>0.79±0.05</td>
<td>43.5±2.45</td>
</tr>
<tr>
<td>Non infected</td>
<td>6.7±0.05</td>
<td>3.8±0.06</td>
<td>2.8±0.01</td>
<td>1.33±0.02</td>
<td>35.2±0.28</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
<td>0.02</td>
<td>0.001</td>
<td>0.028</td>
<td>0.018</td>
</tr>
</tbody>
</table>

In this study, all isolates of mycoplasmas were identified by biochemical and GIT as Mmc, and this results are similar to those in the previous study of Shehzad et al. (24) from different regions of Pakistan.

Mmc cause septicemic form of disease with marked anemia and fatal consequences (7). In the present study, there was a significant decrease in the level of TEC, Hb and PCV. Infected animals in the study areas were weak and jaundiced on gross appearance. This was suggested true by the decrease in erythrocyte count, hemoglobin level and hematocrit value. Mmc infection produces hyperactive radicals i.e. hydrogen peroxide in the body, which lead to destruction of erythrocytes in the body that ultimately leads to anemia (15). There was a significant increase in Total Leukocyte Count and in most cases, it was due to neutrophilia that reflected acute nature of the disease. Hematological findings were almost similar to the findings of Alafiatayo et al. (1), Metwalli et al. (14) and Nayak and Bhowmik (16). Erythrocytopenia occurs in Mmc infection as stated by Ojo, (17) and Thigpen et al. (26). In the present study, there was a significant increase only in MCV, whereas MCHC and MCH remained normal, so anemia can be named macrocytic normochromic anemia. These findings were in contrast to the findings of Gutierrez et al. (7) who stated that there was no variation in erythrocyte indices in experimentally infected goat kids with Mmc and Mycoplasma mycoides subsp. mycoides LC.

Due to septicemic nature of disease caused by Mmc, different visceral organs are affected with different degree of toxicity. The liver is the most common organ targeted by this species of Mycoplasma that was evident by necrotic foci on its surface on gross examination (15). The hepatotoxicity ultimately increases the enzyme levels in the liver function tests (7, 15,22). Due to the involvement of the liver, there is a significant increase in SGPT. Similar findings were also reported by Gutierrez et al. (7) and Mondal et al. (15). Due to the liver damage , protein synthesis is also affected, which leads to decrease in total serum protein. In the present study, there was a significant reduction in total serum protein and albumin while globulin increased significantly. This reduction may also be due to the fact that Mycoplasma spp. also consumes protein for their proliferation. Increase in globulin might be due to the production of antibodies in response to infection. Normal ratio of albumin and globulin, which is slightly above 1 is also disturbed in the present study. There is a significant decrease in the Albumin/Globulin Ratio. These findings are in accordance with the findings of Kaneko and Cornelius (8) and Kumar et al. (9).
Results of this research indicated that the most prevalent species causing caprine respiratory mycoplasmosis in both districts was Mycoplasma mycoides subsp. capri. Macrocytic normochromic anemia was the most striking feature of the hematological results while biochemical parameters indicated a significant effect on the liver.

Acknowledgement

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References


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Kliničko-patološki profil i frekvenca Mycoplasma mycoides subsp. capri infekcije koza u sjevernim područjima Khyber - Pakhtunkhwa, Pakistan

Sažetak

Uvod i ciljevi

Materijal i metode

Ova studija je načinjena u dva distrikta (Buner i Swat) područja Khyber-Pakhtunkhwa. Ukupno tri stotine nosnih briseva (150 iz svakog distrikta) je prikupljeno od koza koje su pokazivale znakove respiratornih smetnji. Za hematološke i serološke nalaze krv je prikupljena od bolesnih i klinički zdravih koza. Uzorci nosnih briseva su inokulirani u modificiranoj Hayflickovoj podlozi na temperaturi (37°C) i inkubirani u atmosferi sa 5% ugljen dioksida. Nakon morfološke identifikacije, različiti biohemijski testovi (Hidroliza arginina, fermentacija glukoze, hidroliza kazeina, likvefakcija koagulisanog seruma i redukcija tetrazoliuma) su izvedeni za preliminarnu identifikaciju izolata. Za test inhibicije rasta, razrijeđena kultura mikoplazmi je nanesena ravnomjerno na modifikovanu Hayflickovu agar podlogu i postavljeni su filter papir diskovi uronjeni u hiperimuni (Mmc specifičan) serum. Podloge su inkubirane sedam dana te su nakon toga posmatrane zone inhibicije rasta. Inhibitorne zone od 2 mm i veće smatrane su pozitivnom reakcijom. Uzoreci krvi su obrađeni kroz hematološki analizator za hematološke parametre (TEC, Hb, PCV, TLC, DLC) i eritrocitne indikatore (MCH, MCHC i MCV). Na sličan način uzorci seruma su obrađeni kroz biohemijski analizator na SGPT, ukupni serumski protein, serumski albumin i globulin.

Rezultati i interpretacija

Ukupno 82 od 300 uzoraka (27,3%) bili su pozitivni na mikoplazme. Prevalence pojave su bile 31,3% u distriktu Buner i 21,3% u distriktu Swat. Svi izolati davali su pozitivnu reakciju na glukozu, tetrazolium redukcioni test, likvefakciju koagulisanog seruma i test hidrolize kazeina, ni jedan uzorak nije bio pozitivan na hidrolizu arginina, dok je svih 82 izolata dalo pozitivnu reakciju na test inhibicije rasta sa antiserumom specifičnim za Mmc. Zapažene su znatne razlike u hematološkim parametrima i u TLC, DLC između zaraženih i nezaraženih životinja. Znatne razlike (P<0.05) su zapažene i u stepenu ukupnog serumskog proteina, albumina i A/G odnosu između zaraženih i nezaraženih životinja. Globulin i SGPT inficiranih životinja bili su znatno veći (P<0.05) od neinficiranih životinja. Makrocitna normohromna anemija bila je najuočljiviji pokazatelj hematoloških rezultata dok su biohemijski parametri ukazali na znatan utjecaj na jetru.

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