SUMMARY:
Differential count of leucocytes and six lymphocyte subpopulations were investigated by flowcytometry in fourteen cases with leptospirosis. Marked granulocytosis and lymphopenia were found in 100%. The percentage of the monocytes was significantly decreased (mean 6.65%; 0.7 to 14.0%; sd 5.49). Immunophenotypization of lymphocyte subpopulations by flowcytometry was revealed prevalent tendencies for decreasing of the number of total T-lymphocytes, T-helpers, T-suppressors, T-helpers/ T-suppressors index; increasing of the number of the activated T-lymphocytes, total NK-cells, and B-lymphocyte. These data are suggested that cell-mediated immunity plays a role in later stages of the diseases.

Key words: leptospirosis, immunogenesis, flowcytometry, lymphocyte subpopulations, dysregulation.

Leptospirosis is a zoonotic disease which is an important public health threat. There are >250 serologically defined serovars of pathogenic Leptospira currently divided into 15 genomospecies. Infection by Leptospira spp. can lead to widely divergent clinical outcomes: symptomatic infection, common in endemic regions; an undifferentiated febrile illness or an aseptic meningitis syndrome with low morbidity; or fulminant disease with a septic shock-like syndrome, jaundice, renal failure, myocarditis, hemorrhage, meningitis, and death, such as observed during ongoing epidemic of severe leptospirosis in urban Brazil (3). Historically, some leptospiral serovars have been considered to be more virulent than others (hence, serovar designations such as icterohaemorrhagiae). Yet population-based studies indicate that the relationship between infecting serovar and clinical manifestations of the disease remains far from clear; purportedly virulent serovars can cause mild disease. Therefore, it is of substantial importance to delineate mechanisms by which Leptospira activate the immune system so as to point out novel ways to approach the treatment of this possibly fatal illness. Elevated levels of soluble IL-2R, IL-6, and TNF-α have been demonstrated in sera obtained from patients treated for acute leptospirosis. Recently, heat-killed Leptospira was also shown to induce IFN-γ and IL-12 production from human whole blood cultures. Clinical hallmarks of severe leptospirosis can resemble Gram-negative sepsis, with multiorgan failure, refractory hypotension, and death. However, the pathogenic mechanisms from either the host or the pathogen side that results in the clinical manifestations of human leptospirosis remains unclear. Naturally acquired immunity that protects against reinfection by Leptospira does occur and has been shown to be serovar-specific in animal models. It has been assumed that naturally acquired immunity is humorally mediated. It has been proposed that immunity is linked to antibodies directed against oligosaccharides of serovar-specific leptospiral LPS, and that leptospiral LPS stimulation of the innate immune system via a Toll-like receptor 2 (TLR2)-dependent mechanism may be important in leptospirosis. There is also evidence that antibodies specific to Leptospira membrane-associated proteins may play a role in host defense. The recent observation that high grade bacteremia (101–106/mL) in leptospirosis can occur in the presence of moderate or high titer anti-leptospiral agglutinating antibodies makes it plausible that mechanisms other than anti-LPS antibodies play a role in naturally acquired protective immunity (2). The role of cell-mediated immunity in host defense to Leptospira remains poorly understood in both animal models and human disease (2, 4, 5).

The aim of this study is analysis of flowcytometric investigations in cases with leptospirosis.

MATERIALS AND METHODS:
Differential count of leucocytes in fourteen cases with leptospirosis has been investigated by flowcytometry (References: CD3+ – 67-76%; CD3+DR+ – 8-15%; CD4+ – 36-46%; CD8+ – 31-40%; CD4+/CD8+ – 1-1,5; CD19+ – 11-16%; CD56+ – 10-19%) (1). Six lymphocyte subpopulations have been investigated in the same patients.

RESULTS:
Marked granulocytosis was established in all cases (n=14; 100%) (mean 80.31%; 69.81 to 97.00%; sd 10.61). Lymphopenia was found in all (mean 11.71%; 0.50 to 21.50%; sd 8.08). The percentage of the monocytes was significantly decreased (mean 6.65%; 0.7 to 14.0%; sd 5.49). The data were correlated with routine investigations of the blood cells in the same patients (p<0.05). Results from immunophenotypization are presented on the Table 1. T-lymphocytes (CD3+) are decreased in eight, normal in four, and increased in two cases (mean 56.98%; 16.44 to 88.5%; sd 11.95). Activated T-
lymphocytes (CD3+DR+) are increased in thirteen cases, decreased in one (mean 17.63%; 7.3 to 23.6%; sd 2.66). T-helpers (CD4+) are decreased in eleven cases and increased in three (mean 24.18%; 4.19 to 48.6%; sd 6.98). T-suppressors (CD8+) are decreased in twelve and increased in two cases (mean 19.91%; 14.2 to 21.36%; sd 1.78). Total NK-cells (CD56+) are increased in eight patients, normal in four and decreased in two (mean 21.32%; 9.2 to 38.96%; sd 5.92).

DISCUSSION:
The leptospiral invasion and its dissemination turn on intensive immune mechanisms. It is known that the course of the disease is in two phases – acute (septicemic) and immune (followed septicemic) (3). The early immunogenesis in leptospirosis is humoral – IgM antibodies have been produced followed by longer persisting IgG antibodies. Complement IgG antibodies participate in stimulating of bactericidal activity of the macrophages. The host participates with non specific inflammatory mechanisms and with cell-mediated immunity which plays a role in later stages of the diseases (2). This correlates with our observations. At the summarize of the results from flowcytometric immunophenotypization of six lymphocyte subpopulations a prevalent tendency has been found for decreasing of the number of total T-lymphocytes, T-helpers, T-suppressors, T-helpers/ T-suppressors index; increasing of the number of the activated T-lymphocytes, total NK-cells, and B-lymphocyte. These data have been correlated with the observations of Mel’nik GV, et al. (1995) and especially with results of Yamashiro-Kanashiro EH, et al. (1991) which by immunofluorescence observed declining in the CD3+ and CD4+ cell subsets in patients with and without acute renal failure (4, 5). In the literature we had not found data about flowcytometric investigations of lymphocyte subpopulations in leptospirosis.

CONCLUSION:
The cell-mediated immunity plays an important role in pathogenesis of leptospirosis. An investigation by flowcytometry might be facilitating the registration of fine abnormalities in cell subsets. Further researches are needed for elucidation in this respect.

Table 1. Investigations of lymphocyte subpopulations by flowcytometry

<table>
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<tr>
<th>Subpopulations</th>
<th>N (%)</th>
<th>&lt;N</th>
<th>n</th>
<th>%</th>
<th>=N</th>
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<th>&gt;N</th>
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<td>14.29</td>
<td>56.98</td>
<td>11.9</td>
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<tr>
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<td>7.14</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>92.86</td>
<td>17.63</td>
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<td>11</td>
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<td>3</td>
<td>21.43</td>
<td>24.18</td>
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<tr>
<td>CD8+</td>
<td>31-40</td>
<td>12</td>
<td>85.71</td>
<td>-</td>
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<td>14.29</td>
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<tr>
<td>CD4+/CD8+</td>
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<td>7</td>
<td>50.00</td>
<td>5</td>
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<td>2</td>
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</tr>
<tr>
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<td>-</td>
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<td>8</td>
<td>57.14</td>
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REFERENCES:

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