Method of goat immunodiagnosis in arthritis-encephalitis

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Abstract. Currently, serological tests are used in the diagnosis of Caprine Arthritis Encephalitis (CAE), but a more accurate diagnosis of the disease requires the most reliable modern methods of immunodiagnosis. This is important for timely diagnosis and prevention of this disease, especially in commercial production of goat milk. At the present stage, the practice of infectious diseases immunodiagnosis in animals includes the determination of cytokines 6 and 10. The discovered cytokines allow for a different view on the infectious disease pathogenesis, while the previously used diagnostic methods are not excluded from the veterinary practice. At present, during the restoration and renewal of the goat population, new breeds purchased in foreign countries have appeared, and with them have come new diseases not previously prevalent in the Russian Federation. Therefore, practicing veterinarians need to develop the most effective immunodiagnostic tests for these diseases and work out effective control and prevention methods, as well as to be able to assess the risks of new infections or mixed infections. Immunophenotyping is performed in various infectious animal diseases, and immunological monitoring is also possible.

1 Introduction

There are diagnostic methods based on enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) and other serological tests referred to in the OIE Terrestrial Animal Health Code. This methods are aimed at defining the antibody count or the pathogen genotyping itself, but the method description lacks the determination of the Immune System status and the ability to resist the given infection [1-5].

Molecular-biological methods are known to detect CAE virus genome in blood samples and serum of infected animals at early stages of the disease, but they do not reflect the Immune system state, which is to be taken into account for planning of animal treatment and recovery measures. [6-11].

The study objective is to determine the most reliable immunodiagnostic method for goats with arthritis-encephalitis.

The objective is achieved by conducting additional studies to determine different classes of immunoglobulins in comparison with the level of various cytokines reflecting the infectious inflammatory process and their ratio in diseased, infected and healthy animals.

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2 Materials and methods

The scientific and production experiment was conducted at goat farms of the Sverdlovsk region, the Republic of Tajikistan, on the basis of the Department of Infectious and Non-Infectious Pathology of the Ural State Agrarian University, in the UrFANIC of the Ural Branch of the Russian Academy of Sciences, Clinical and Diagnostic Centre of the Ministry of Health of the Russian Federation, and the Institute of Biological Safety Problems and Biotechnology of the TASHN in 2019 and 2022.

The object of the study were 3-month-old goat kids of Alpine, fine-fleece Tajik, Alpine and local goat breeds.

For the study 2 groups of analogues were selected from 40 3-month-old goat kids. The goats were divided into 2 groups: the first group was experimental and included goats with clinical signs of Caprine Arthritis Encephalitis with ELISA (enzyme-linked immunosorbent assay) positive reaction to CAE. The second group included healthy goat kids (Table 1). For the study, blood was collected from goat kids of both groups for immunohaematological examination. CAE was diagnosed by ELISA method in FGBU "VNIIZZh".

The study design is shown in Table 1.

The study of cellular immunity was determined by flow cytometry using monoclonal antibodies (on the analyser Beckman Coulter, USA) on the flow cytometer Cytomics FC 500 (Beckman Coulter, USA).

Table 1. Study design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental ( Diseased according to ELISA diagnosis), n=10</th>
<th>Control (Healthy by ELISA diagnosis), N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Year 2019</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Study Year 2022</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Immunoglobulins were determined by turbidimetric method using test systems and biochemical analyser KONELAB, (Finland).

The cytokine 6 and 10 concentrations in goat serum were determined by solid-phase enzyme-linked immunosorbent assay using test systems (USCN Life Science, China) on Multiskan FC ELISA analyser (Thermo Electronics). Immunophenotyping of B-lymphocytes (CD20+) was performed using monoclonal antibodies by flow laser cytofluorometry on flow cytofluorimeters. The studies were performed according to Medunitsynitsyn N.V. (2004). Digital data was subjected to statistical processing. Microsoft Excel and Microsoft Office 7.0 programmes were used to process the obtained data.

3 Results and discussion

To determine what may have influenced this relatively rapid spread of this infection, assays were made in 2019 and 2022 for serum immunoglobulin levels and measures of immune status in goats. The data is shown in Tables 2 and 3.

Table 2. Immunoglobulin content in goat blood serum

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2019 year</th>
<th>2022 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental, n=10</td>
<td>Control, n=10</td>
</tr>
<tr>
<td>IgG, g/l</td>
<td>15.59±0.43</td>
<td>16.43±0.32</td>
</tr>
<tr>
<td>IgM, g/l</td>
<td>1.63±0.12</td>
<td>2.75±0.13</td>
</tr>
<tr>
<td>IgA, g/l</td>
<td>1.98±0.05</td>
<td>3.15±0.06</td>
</tr>
</tbody>
</table>
According to the data of Table 2, the change in the content of immunoglobulins G, M, A, and E in the control and experimental groups of goats for 2019 and 2022 can be traced. IgG in the experimental group is lower than in the control one and its level decreases faster than in healthy goats by 2022.

In 2022, the IgA in the experimental group is almost half that of the control group. IgA in 2019 in the experimental group was lower than in the control group by about 30%. IgE was detected in small amounts in the serum of the experimental animals, whereas it was basically undetectable in the control group, in 2019 and in 2022.

Table 3 records the quantitative value of lymphocytes, CD4, CD3, CD8, CD4/CD8 and CD20. T-lymphocytes, T-helper, CTCL and B-lymphocytes in the experimental group are an order of magnitude lower than in the control group and continue the downward trend in 2022. However, the percentage of lymphocytes in the experimental group, on the contrary, is higher than in the control one and continues to increase, as does the CD4/CD8 ratio in dynamic monitoring. The results of the conducted studies showed significant differences in both 2019 and 2022 data.

**Table 3. Immune status parameters in goat kids**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2019 year</th>
<th>2022 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental, n=10</td>
<td>Control, n=10</td>
</tr>
<tr>
<td>Lymphocytes,%</td>
<td>65.3±1.97*</td>
<td>61.1±3.52</td>
</tr>
<tr>
<td>T-lymphocytes (CD3), %10^9/l</td>
<td>48.4±3.27*</td>
<td>58.2±2.44</td>
</tr>
<tr>
<td>T-helper cells (CD4), %</td>
<td>31.7±1.74*</td>
<td>59.1±2.53</td>
</tr>
<tr>
<td>CTCL (CD8),%</td>
<td>19.2±3.72*</td>
<td>48.7±1.54</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>1.60</td>
<td>1.22</td>
</tr>
<tr>
<td>B-lymphocytes (CD20), %10^9/l</td>
<td>13.2±0.94</td>
<td>14.8±0.79</td>
</tr>
</tbody>
</table>

*Significance by Student's criterion P<0.05

The analysis of immunological parameters revealed a humoral immunity deficit in cases of CAE, which was compensated by active functioning of T-cell immunity. Among immunocompetent cells, an increase in the number of T-row lymphocytes was observed in cases of CAE, indicating the presence of pathological processes. Quantitative changes were accompanied by an increase in the relative content of T-helper (CD4) and cytotoxic T-lymphocytes (CD8), in connection with which the immunoregulatory complex CD4/CD8 was reduced by 1.5 times compared to the same indicator in healthy goat kids, presented in Table 4.

**Table 4. Serum cytokine concentrations in goat kids in an epizootically unfavourable area (n=40)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2019 year</th>
<th>2022 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental, n=10</td>
<td>Control, n=10</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>91.05</td>
<td>264.71</td>
</tr>
<tr>
<td>Interleukin 10</td>
<td>102.02</td>
<td>346.2</td>
</tr>
</tbody>
</table>

The obtained results are indicative of a unidirectional type of immune system response to the production of IL-6, IL-10, which plays a cardinal role in the pathogenesis of CAE in goat kids as a universal immune response of the macroorganism to the introduction and multiplication of the causative agent of CAE.
To further study the defence properties of the studied animal organism, the blood levels of interleukin 6 and interleukin 10 were tested (Figure 1).

**Fig. 1.** Concentrations of interleukin 6 and interleukin 10 in healthy goat kids and in goat kids with clinical signs of CAE.

Figure 1 shows the content of interleukins in blood in goat kids in dynamics.

The difference in interleukin content may be indicative of reduced immunity in the studied animals, in both 2019 and 2022.

Reduced immunity favours the development of secondary infections. Studies in CAE-unfavourable farms show a worsening of the situation with this infection over time. Studies have revealed ineffectiveness of preventive measures.

The results showed a decrease in the two interleukin fractions in the experimental group by about 3 times both in 2019 and 2022.

In immunological tests for the detection of different immunoglobulin classes, the most reliable results are seen in the content of immunoglobulins M and A. The immune status of diseased and healthy animals can also be traced by the content of immunoglobulin E, but the amount of immunoglobulin E is much lower than that of immunoglobulins M and A.

Immunoglobulin G quantity also varies in the blood of diseased and healthy animals, but the difference is not significant.

According to the blood test results of experimental and control groups in the monitoring study dynamics, the most significant are the determination of the total lymphocyte count, determination of the T-lymphocyte count and their ratio. The B-lymphocyte ratio is insignificant. The comparison of the content of cytokines 6 and 10 in the serum of diseased and healthy animals shows distinct differences.

The conducted immunological tests showed that regardless of the study method, the indicators in diseased animals were significantly different from the healthy group. Studies conducted at 3-year intervals have determined that the morbidity rate has a similar trend and is worsening without comprehensive interventions. Infectious CAE disease reduces the general animal immunity causing the development of immunodeficiency.

The novelty of the study is that the present work shows different Immunological test methods for goat kids on the example of Caprine Arthritis Encephalitis. The results of monitoring studies conducted at three year interval are shown. Comparison of indicators between diseased and healthy animals was carried out, reliability of the revealed differences was determined on the example of one goat breeding farm. The results of various immunological research methods are available and a general pattern of differences in the
immunity of healthy and diseased animals has been determined. The immunity decrease pattern in goat kids of the unfavourable farm for this infection with a tendency to immunodeficiency development and increased morbidity risk of other infectious diseases was revealed. For the first time, an assessment of various immunological diagnostic methods was given on the example of CAE with the risk of further infection with various pathogens.

4 Conclusions

Based on the carried out research analyses, it can be concluded that:
1. The level of immunoglobulins, interleukins 6 and 10, as well as lymphocytes in the blood of the experimental group animals significantly differs from the content of these indicators in healthy animals.
2. Decreasing over time, these parameters reflect a decrease in the resistance of the goat kid's organism to other infections as well, i.e. a secondary immunodeficiency state develops.
3. As a result of clinical, immunological and monitoring studies, it is advised that constant monitoring of immune status and the content of immunoglobulins and interleukins (cytokines) 6 and 10 should be introduced into veterinary practice.

References

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