EVALUATION OF IMMUNE RESPONSE IN SEROPOSITIVE CATTLE FOR *MYCOPLASMA BOVIS*

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Abstract

The aim of the study was to evaluate the changes of selected immunological parameters in seropositive cattle infected with *Mycoplasma bovis*. The study was performed on two age groups of cattle, i.e. young (n=139) and adult (n=21) animals. Within each age group, two subgroups composed of seropositive and seronegative animals for *M. bovis* infection were formed. The serum samples were collected and the following immunological parameters were determined: *Mycoplasma bovis* antibodies, total protein, γ-globulins, and selected acute phase proteins - haptoglobin and serum amyloid A. In *M. bovis* seropositive cattle, the distinct increase in all examined parameters in both age groups was observed as compared with the controls. The results indicated the significant stimulation of immune response in the affected cattle and the performed assays confirmed the real possibility for their use in the screening diagnosis of the bacterial infections.

Key words: cattle, *Mycoplasma bovis*, immune response.

The immune system protects a host organism against different species of both strictly pathogenic and commensal germs. The mechanism of immune response can be nonspecific and antigen-specific. The first called innate is less precise but react faster. It forms first line of the host organism defence. The second called adaptive is more precise against antigen (14). The immune response can be divided into humoral and cellular. In the humoral reaction antibodies produced by B lymphocytes are bound in the form of antibody-antigen complex. The antibodies neutralise bacterial toxins, facilitate phagocytosis of bacteria by white blood cells, and stimulate output of cytotoxic compounds. The cellular reaction helps to eradicate bacteria, which proliferate in the host organism. T lymphocytes stimulate the cellular response by production of amplifier cells (CD4+ cells). These cells release cytokines *i.e.* interferon, tumour necrosis factor, and interleukins. The cytokines activate delayed hypersensitivity reactions that activate macrophages and monocytes, activate the production of enzymes that change inactive compounds into active compounds profitably for macrophages. This transformation increases antibacterial capacity of macrophages, and through the release of cytokines that provoke the proliferation, differentiation, and activation of natural killer and other specific cytotoxic cells such as CD4+ and CD8+ cells (29). *M. bovis* affects the host organism as both an immunostimulant and immunosupressor (27). Our studies have shown the increase in percentage of T lymphocytes and their subsets, *i.e.* CD4+ and CD8+ cells in response to experimentally challenge of calves with *M. bovis* (data not shown). Under these conditions, leukocytosis and the significant rise in percentage of WC4+ (B lymphocytes) were observed too (8). Vanden Bush and Rosenbusch (38) indicated the activation of CD4+, CD8+, and γδ-T cells as a result of incubation with *M. bovis* antigen. On the other hand, an inhibitory effect of *M. bovis* on bovine lymphocytes, such as suppression of their proliferation and induction of lymphocyte apoptosis has been shown in other studies (11, 35, 39).

The acute phase response (APR) is a component of immune response. It is a summation of immunological, behavioural, and metabolic changes in an organism, which responds to inflammation, infection, or trauma (15). Acute phase proteins (APPs) are one of the most important elements of APR (24). They are produced by hepatocytes and their synthesis is mediated by cytokines such as interleukin-1, interleukin-6, and tumour necrosis factor-α (TNF-α). APP level can give an insight into herd health status as well as individual animal condition. It is known that a strong correlations between APP level and the occurrence of disease exists (5). Some data show that several APPs are valuable biomarkers of respiratory infections in calves after viral, bacterial, or combined infections (23). The major APPs in cattle are serum amyloid A (SAA) and haptoglobin (Hp) (32). During APR, Hp concentration in calves can increase significantly (50–100 x) while a rise of SAA is moderate (2–5 x) under these conditions (16).
A concentration of Hp in cattle may play an important role in the diagnosis of different disease, i.e. pneumonia, mastitis, enteritis, peritonitis, endocarditis, or endometritis. High level of SAA in calves is caused by acute inflammation and in less range in the course of chronic inflammatory processes (9).

Infections with *Mycoplasma bovis (M. bovis)* occur in European countries and all over the world (21). Results of a recent study carried out in Poland in 2007-2010 show that the percentage of seropositive calves for *M. bovis* was 76.65% and among them 12.59% of the animals displayed strongly positive results (2). Nowadays, *M. bovis* is one of the most important infectious factors of bovine respiratory disease (BRD), which is responsible for large losses in cattle population (7). *M. bovis* in association with other microorganisms such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* induces mastitis, pneumonia, or arthritis (21, 25).

Gammaglobulins, total protein, SAA, and Hp are the representative parameters of immune response in cattle so the aim of the study was to investigate the changes of these parameters in *M. bovis* positive animals.

**Material and Methods**

**Animals.** The study was performed on two age groups of Black and White Lowland breed cattle: calves at the age from a few weeks to four months (n=139) and adult animals (n=21). Both calves and adult animals were divided into two subgroups: seropositive cattle for *Mycoplasma bovis* and seronegative (control). Animals originated from different farms and regions of Poland and were kept in individual pens for maximum 20 animals. The average herd size in which the laboratory samples were collected had about 100 animals.

**Study protocol and sample collection.** The serum samples were collected from animals and sent to the laboratory for further analysis.

**Test used for the infectious agent detection.** To determine the presence of serum *Mycoplasma bovis* antibodies, the serological tests were carried out using ELISA (Bio-X Diagnostics).

**Serum analysis.** The following serum parameters were determined: total protein, γ-globulins, haptoglobin (Hp), and serum amyloid A (SAA). The concentration of total protein was determined by biuret method (BioSystems S.A., Spain), whereas the analysis of remaining parameters was made using commercial ELISA tests (Bio-X Diagnostics) with regard to γ-globulin concentration or Tridelta Development Limited (Ireland) for Hp and SAA.

**Data analysis.** Results were presented as arithmetic means with standard deviation (means ± SD). Statistical significance of differences between the values recorded in individual subgroups and their controls was compared using Student’s *t*-test at *P*<0.05, *P*<0.01, and *P*<0.001.

**Results**

The presence of *Mycoplasma bovis* antibodies in the sera of animals suspected of infection was confirmed by the serological examination in which the antibody titres were obtained in the range of + to ++++ for calves and + to +++ for adult animals. Animals showing the negative serological results were considered as free of *M. bovis* infection.

The concentration of total protein, Hp, and SAA of *Mycoplasma bovis* seropositive calves was statistically significantly higher (P<0.001) when compared with seronegative calves (Table 1). Similar results were obtained for γ-globulins where statistically significant differences (P<0.01) between both groups were observed (Table 1).

In seropositive adult animals, an increase in the concentration of total protein, γ-globulins, and Hp was observed when compared with the controls (Table 1). However, this rise was not statistically significant. With reference to the content of SAA, a statistically significant (P<0.05) higher values in seropositive cattle was found when compared to seronegative adult animals (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>calves (n = 118)</th>
<th>adult animals (n = 15)</th>
<th>calves (n = 21)</th>
<th>adult animals (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Total protein (g/L)</td>
<td>75.96 ± 11.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.67 ± 11.81</td>
<td>66.75 ± 8.89</td>
<td>82.16 ± 14.38</td>
<td></td>
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<tr>
<td>γ-globulins (µg/mL)</td>
<td>25,959 ± 11,130&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38,299 ± 11,795</td>
<td>18,753 ± 11,060</td>
<td>33,095 ± 15,447</td>
<td></td>
</tr>
<tr>
<td>Hp (mg/mL)</td>
<td>0.326 ± 0.563&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.302 ± 0.336</td>
<td>0.109 ± 0.042</td>
<td>0.254 ± 0.335</td>
<td></td>
</tr>
<tr>
<td>SAA (ng/mL)</td>
<td>44,198 ± 65,841&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48,965 ± 33,663&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15,512 ± 13,533</td>
<td>26,523 ± 12,445</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> *P*<0.05, <sup>b</sup> *P*<0.01, <sup>c</sup> *P*<0.001.
Discussion

*M. bovis* is responsible for large losses in cattle population due to development of clinical diseases, such as pneumonia, arthritis, mastitis, and the others (25). It is also one of the most important causative agents of bovine respiratory syndrome (BRD) among other infectious factors such as bovine herpes virus type 1 (BHV1), bovine respiratory syncytial virus (BRSV) (30), parainfluenza type 3 virus (PI3V), *Mannheimia haemolytica*, *Pasteurella multocida*, or *Histophilus somni* (3).

The high seroprevalence of *M. bovis* infection in cattle in different European countries (1, 20, 26, 34, 37) and outside of Europe, for example in Western Canada (4) was observed. In Switzerland it was up to 50.3% (37), whereas the *M. bovis*-seropositivity rate in cattle averaged 88% in Northern Italy (26), from 10% to 20% in France (20), 11% in Hungary (34), or 22% in Great Britain, respectively (1). For comparison, the percentage of seropositive samples for *M. bovis* in cattle of Polish population investigated in 2007-2010 was more than 76% (2). Due to the fact that the significance of this pathogen in cattle diseases in Poland is so important, the attempt to assess the immune response in seropositive animals for *M. bovis* was undertaken.

An increase in the concentration of total protein in *M. bovis* seropositive cattle probably resulted from the consistent rise in the content of γ-globulins and selected APPs, i.e. Hp and SAA under these conditions. The rise in the examined parameters was particularly well-defined (P<0.01) in seropositive calves. The changes in total protein concentration are connected with a humoral response to infectious microorganisms. The obtained results correspond with the previous findings, where an increase in total protein, γ-globulins, Hp, and SAA concentrations was observed in calves intratracheally inoculated with *M. bovis* (7, 8). Pratić et al. (12) have shown a significant increase in α-globulin content in cases of bronchopneumonia in calves. No significant differences in concentration of β- and γ-globulins between healthy calves and those with clinical signs of bronchopneumonia were observed. Vanden Bush et al. (38) observed an increase in antigen-specific antibody titres in sera of 12-week-old calves experimentally infected with *M. bovis*. That rise was mainly connected with IgG1 production attributed to Th1 response. Under these conditions, a slight rise of IgG2 isotype was shown (38). In adult cattle seronegative for *M. bovis*, the high concentrations of Hp and γ-globulins were observed, when compared with their reference ranges, which normally averaged about 0.1 g/L for Hp (33) and between 16.9 and 22.5 g/L with regard to γ-globulin content in clinically healthy cattle (19). It could be connected with the possibility of concomitant infections, mainly viral, in those cattle. It is very probable because the seroprevalence for PI3V, BRSV, or BHV-1 in affected animals, examined recently in Poland, have remained at 100%, 93.6%, and 16.6%, respectively (31). The increased concentration of γ-globulins in the seronegative adult animals, noted in our study, could also result from the possible noninfectious disturbances such as bovine parasite invasions (28) and even due to environmental factors i.e. stress, preventive manipulations, or feeding changes (31).

*M. bovis* indicated a synergism with other pathogens involved in BRD, such as *Pasteurella multocida*, *Histophilus somni*, and especially with *Mannheimia haemolytica* (3), which has the synergistic effect with reference to colonisation of the lower respiratory tract (17). It is known that infections caused by *Pasteurella multocida* or *Mannheimia haemolytica* are associated with an increase in Hp and SAA concentrations (6, 22) hence a rise in these APPs in seropositive for *M. bovis* animals was the expected.

Humblet et al. (18) showed that individually labelled APPs such as Hp are useful for assessing the health status of animals due to the high specificity, whereas these proteins identified together or with other indicators increase the likelihood of determining the suitability of the use of therapy in calves suffering from bronchopneumonia. Other studies performed on cattle also demonstrate the suitability of the examination of some APPs in monitoring of bovine respiratory diseases (13, 36).

Results of the presented study indicate a stimulatory property of *M. bovis*, which is manifested by the increase in total protein, γ-globulin, Hp, and SAA concentrations. In the early phase of the infection, when antibodies do not appear yet, or only in subclinical cases, a screening monitoring of *M. bovis* infection in cattle using especially Hp and SAA detection supplemented with routine total protein and γ-globulin analysis could be helpful and worth applying in the bacterial infection diagnosis in the future.

References


