Chlamydial infection in clinical cases of enzootic bronchopneumonia of calves (EBC): Serological investigations and phenotypic analysis of peripheral blood leukocyte subpopulations in affected calves based on flow cytometry (FCM)

KRZYSZTOF NIEMCZUK UND DARIUSZ BEDNAREK
National Veterinary Research Institute, Department of Cattle and Sheep Diseases
PL-24 100 Pulawy, Poland

The "enzootic bronchopneumonia of calves", which is also called "bovine respiratory disease complex (BRD)", refers to a complex disease of feedlot young cattle that causes major economic losses to the livestock industry. The disease is characterized by depression, inappetence, fever, cough, nasal discharge and dyspnea. The histopathological lesions observed at necropsy include suppurative (lobular) or fibrinous bronchopneumonia. The aetiology of EBC is multifactorial and generally believed to be an interaction between viruses (BHV-1, PI-3, BVDV, BRSV), mycoplasmas (M. bovis, M. dispar), bacteria (P. haemolytica, P. multocida, Haemophilus somnus, Chlamyphila psittaci, Chlamyphila pecorum) and environmental stress factors (1,2,3). The infectious etiological agents associated with bronchopneumonia are ubiquitous in the cattle population and produce the disease only when the host defenses, especially bacterial clearance by alveolar macrophages, are lowered by stress, nutritional deficiencies, and/or respiratory virus infections.

Chlamydial infectious agents have been known for a long time. Earlier on, Chlamydiaceae as a member of the psittacosis-lymphogranuloma (PL) group were identified as a cause of pneumonia in mice, cats, sheep and goats, and later established as distinct disease entities (4,5). At present, chlamydial agents are thought to produce only mild respiratory infections by themselves, but they may enhance the pathogenicity of concurrent infections. Exposure of calves to a combination of Chlamydia and Mannheimia (Pasteurella) haemolytica results in clinical disease more severe than either agent produces alone (7). However, studies on the role of Chlamydiaceae in the pathogenesis and immunity of bovine bronchopneumonia are too scarce and should be initiated to further our understanding of chlamydial infections in cattle.

In 1999, the genus Chlamydophila was defined in addition to the genus Chlamydia on the basis of phylogenetic analysis of 16S and 23 rRNA genes (4) in the family Chlamydiaceae. The following species were differentiated in the genus Chlamydia: Chlamydia trachomatis, Chlamydia muridarum and Chlamydia suis. In the genus Chlamydophila were included: Chlamydophila psittaci, Chlamydophila abortus, Chlamydophila caviae, Chlamydophila pecorum and Chlamydophila pneumoniae (5,6).

The aim of the study was a comparative analysis of the proportional differences of peripheral blood leukocytes in calves suffering from bronchopneumonia syndrome with high and low antibody titre against Chlamydophila psittaci antigen.

Material and Methods

Animals. Twenty Black-and-White Lowland Breed calves aged 1-3 months and showing typical signs of enzootic bronchopneumonia (fever >40°C, dyspnea, cough, nasal discharge, inappetence, depression) were divided into 2 groups: 10 calves with high antibody titre (over 1:32) against C. psittaci were included into Group I, and 10 calves with low titre (1:4 – 1:16) served as controls (Group II). Calves of each group came from the local veal farms and were kept separately under similar environmental and feeding conditions.

Blood sampling and testing. Blood samples were taken once from calves of both groups from the jugular vein. The blood samples were collected into tubes containing tripotassium salt of ethylenediamine tetraacetic acid (K₃EDTA) as anticoagulant (0.07 mol/ml blood) and additionally some blood was retained without any anticoagulant (serum).

The following parameters were assayed: the total number and differential number of leukocytes (WBC and lymphocytes, mid-size cells, neutrophils), the immunophenotyping of pe-
Peripheral blood leukocytes expressing CD45 (leukocyte common antigen), CD14 (monocyte antigen), CD2 (T-cell antigen), CD4 (T-helper cell antigen), CD8 (T-cytotoxic/suppressor cell antigen) and WC4 (B-cell antigen) surface markers. Moreover, the complement-fixation (CF) test and enzyme immunoassay (EIA) were used to evaluate the antibody response to chlamydial infections in calves affected with enzootic bronchopneumonia based on *Chlamydia psittaci* antigen. The tests are suitable for the detection of *C. psittaci* antibodies in blood serum samples of animals having been in contact with *Chlamydia psittaci*.

The haematological indices were estimated with Celoscope-AutoCounter AC 920 (Swelab Instrument AB, Sweden). The immunophenotype of peripheral blood leukocytes was examined by the use of Coulter Epics 4XL Flow Cytometer (Beckman Coulter Company, USA). In serological examinations, *Chlamydia psittaci* antigen manufactured by the Institute of Virology in Bratislava for CF test and the CHEKIT-Chlamydia enzyme immunoassay kit (Bommeli Diagnostics) for EIA were used.

**Results**

The results of the study (Table 1) have shown that in respiratory diseased calves from Group I the total number of white blood cells was significantly higher (P<0.05) than in the control animals (Group II). The mean values of this parameter were 19.6 x 10^9/l and 14.2 x 10^9/l, respectively. This increase was directly connected with the significant (P<0.01) rise of neutrophil count, and also with distinct increase of a mixed number of monocytes, eosinophiles and basophiles (MID) and an individual number of CD14/CD45-positive cells. The number and percentage of peripheral blood neutrophils were 10.1 x 10^9/l and 51.7% in diseased animals from Group I, and 6.4 x 10^9/l and 45.1%, respectively, in the control calves. Concerning the number of circulating CD14/CD45-positive cells, there were 2.2 x 10^9/l and 11% in Group I of calves and 1.8 x 10^9/l and 12.7% in the control animals. A similar tendency was also noted with regard to changes of the Mid-size cells, with percentage of 5.2 in Group I and 6.3 in Group II, and measured values of 1.0 x 10^9/l and 0.9 x 10^9/l, respectively.

On the other hand, in calves affected with bronchopneumonia from Group I, the number of total peripheral blood lymphocytes was significantly higher (P<0.05) compared with the values obtained in animals from the control group. This increase was caused by the significantly higher number of T-cells (57.2 %, 4.9 x 10^9/l), mainly at the instance of CD8-positive cell subset (22.7%, 1.9 x 10^9/l), and to a lesser degree also regarding the number of Th lymphocytes (2.5 x 10^9/l). In control animals, these mean values were 56 % and 3.9 x 10^9/l for T-lymphocytes, 13.4% and 0.9 x 10^9/l for Ts/c cells, and for Th ones - 32.1% and 1.8 x 10^9/l, respectively. The differences between mean values of CD8-positive cells in both groups of calves were statistically significant at P<0.05 and P<0.01.

Concerning the serological investigations, blood sera collected from the respiratory diseased calves were examined by the micro-method of complement fixation (CF) and the Chekit-Chlamydia enzyme immunoassay test (EIA). Sera of animals with antibody titre of 1:32 (CF) and higher (1:64 – 1:128) were considered as positive, in compliance with valid laboratory veterinary regulations (Manual of Standards 2000). The calves with this antibody titre were included into Group I and the remaining ones into the control group. A species-specific antigen of *Chlamydia psittaci* (previously *Chlamydia psittaci*) was used in the study based on CF. For the additional confirmation of obtained CF results we conducted the Chekit-Chlamydia (Dr Bommeli AG) enzyme immunoassay test.

It was found in the serological studies that over 25 per cent of randomly selected calves affected with bronchopneumonia syndrome, kept in typical field conditions (private beef and veal calf farms), were sero-positive with regard to chlamydial infectious agent.

Based on the obtained results of flow cytometry analysis, it can be concluded that neutrophils (PMNLs) and T lymphocytes, i.e. especially CD8-positive cells, play a significant role in the cellular immune response against *Chlamydia psittaci* co-infection in calves suffering from enzootic bronchopneumonia syndrome, what it has repercussion in their higher numbers likely by the more effective recruitment from reserve marginal pool of these cells in blood vessels and activation of bone marrow proliferation.
Table 1: Phenotypic analysis of peripheral blood leukocyte subpopulations in respiratory diseased calves affected with chlamydial (Group I) and non-chlamydial infectious agents (Group II)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
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<tbody>
<tr>
<td>White blood cells (WBC) (10⁹/l)</td>
<td>19.6 ± 3.2**</td>
<td>14.2 ± 2.4</td>
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<tr>
<td>Total lymphocytes (%)</td>
<td>43.1 ± 6.8</td>
<td>48.6 ± 7.5</td>
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<tr>
<td>CD2-positive cells (%) (T cells) (10⁹/l)</td>
<td>57.2 ± 8.7</td>
<td>56.0 ± 9.1</td>
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<tr>
<td>CD4-positive cells (%) (Th cells) (10⁹/l)</td>
<td>29.9 ± 11.4</td>
<td>32.1 ± 12.5</td>
</tr>
<tr>
<td>CD8-positive cells (%) (Ts/c cells) (10⁹/l)</td>
<td>22.7 ± 5.9**</td>
<td>13.4 ± 4.3</td>
</tr>
<tr>
<td>WC4-positive cells (%) (B cells) (10⁹/l)</td>
<td>14.1 ± 9.1</td>
<td>16.4 ± 7.4</td>
</tr>
<tr>
<td>Mid-size cells (MID) (%)</td>
<td>5.2 ± 1.3</td>
<td>6.3 ± 2.1</td>
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<tr>
<td>CD14/CD45-positive cells (%)</td>
<td>11.0 ± 2.2</td>
<td>12.7 ± 2.7</td>
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<tr>
<td>Neutrophils (PMNL) (%)</td>
<td>51.7 ± 5.2**</td>
<td>45.1 ± 6.1</td>
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Significantly different from control group at *P<0.05, **P<0.01

Groups: I and II (controls)

References