Abstract

Forty Pasteurella multocida strains of serotype B:2,5 were collected from cattle affected with haemorrhagic septicaemia, and 72 strains mainly of serotype A:3, were isolated from calves with changes typical of bronchopneumonia. The outer membrane proteins (OMPs) were prepared by extraction, with 1% sarcosyl from field strains and two reference strains. OMPs were separated electrophoretically at a 10% gradient on polyacrylamide gel in SDS-PAGE. The densitograms of the examined strains were compared. The densitometric analysis showed some qualitative and quantitative differences in the protein electrophoretic profiles of the isolates. The most important differences in molecular weights and optical density of protein bands were noted in the 30 kDa to 38 kDa range. Strains isolated from adult cattle and calves were classified into two and four OMP types, respectively.

Key words: cattle, Pasteurella multocida, outer membrane proteins, electrophoresis.

Pasteurella multocida may cause primary infection, called haemorrhagic septicaemia (septicaemia haemorrhagica bovum), or Bollinger’s disease in older cattle (5). Bollinger’s disease is caused by strains of capsular type B or E according to Carter (4) and somatic antigen 2 recognized by Heddleston scheme (12). P. multocida rods, mostly composed of serotype A:3, are involved in the polyethiological diseases of the respiratory system in calves (10).

Numerous authors have paid increasing attention to the molecular structure of outer membrane proteins (OMPs). These proteins may serve as virulence factors. Moreover, they are involved in adhesion to the epithelial cells of the respiratory system, resistance processes of the bacteriocidal activity of serum, and resistance to phagocytosis (20). Several outer membrane proteins are immunogens and the antibodies against them, demonstrate a strong protective activity. Such antigens may be used as the components of subunit vaccines. The immunogenicity of selected OMPs of P. multocida was demonstrated in calves (6, 8, 19), rabbits (7), and chickens (23). Moreover, the protective action of OMPs of serotype B:2 against haemorrhagic septicaemia was reported (18).

The aim of the study was to determine and to compare electrophoretic profiles of OMPs of P. multocida strains isolated from adult cattle with haemorrhagic septicaemia (HS), calves with the changes typical of bronchopneumonia, and reference strains of the serotypes B:2 and A:3, using SDS-PAGE.

Material and Methods

Bacterial strains. One hundred and twelve field P. multocida strains from two sources were used for the studies. The first group contained 40 strains of serotype B:2,5, isolated from cattle, which died due to haemorrhagic septicaemia in 1958-1962 (21) and 1985-1995 (14). The second group, comprising of 72 strains mostly of serotype A:3, were isolated from swabs from nasal cavity of calves with morbid symptoms in the respiratory system or from internal organs of animals died of bronchopneumonia in 1985-2003. Two reference strains: B850 (serotype B:2) obtained from the IEMVT (Paris) and P1059 (serotype A:3) from the National Disease Laboratory, Ames, USA, were also examined.

Preparation of OMPs from P. multocida strains for SDS-PAGE. The examined strains were cultured on BHI medium (swine organ extract, IDG). The OMPs were obtained according to the modified method of Morton et al. (17) by the extraction with 1% N-Lauroylsarcosine sodium salt solution of bacterial cells previously exposed to ultrasounds. The amount of proteins was evaluated using a Protein Assay Kit (Sigma).

Separation of proteins in SDS-PAGE. Electrophoretical separation of the proteins was
performed in 10% polyacrylamide gels, according to Laemmli (16), as described in a previous study (2).

Gels were photographed using an ImageMaster VDS apparatus (Pharmacia Biotech), and subjected to the analysis of one dimensional separation and Dot Blot programme.

**Results**

The electrophoretic profiles of OMPs of *P. multocida*, serotype B:2,5, usually revealed the presence of proteins with the molecular weight amounting 18, 28, 31 to 32, 36 to 37, 44 to 46, 48 to 50, 56 to 58, 64 to 66, 84 to 86, and 100 to 104 in kDa. All the isolates demonstrated intensely stained bands corresponding to 31 to 32 and 36 to 37 kDa, whereas band weighing 31 to 32 kDa showed the highest OD values. Two protein patterns were distinguished, based on differences in protein fractions (Fig. 1). The isolates of type 1 revealed the presence of the two above-mentioned protein bands; and the isolates of type 2 showed an additional band corresponding to 38 kDa. The two distinguished protein types comprised of 87.5% and 12.5% of the field isolates, serotype B:2,5, respectively. The electrophoregrams obtained from the field isolates of this serotype showed the rate of homology ranging from 57% to 100%.

The examinations of the serotype B:2 reference strain, confirmed the presence of protein bands with various optical density and molecular weights similar to those found in serotype B:2 field isolates (Fig. 1). The electrophoretic profile of the isolates sampled from adult cattle, exhibited a 56% to 100% rate of homology in comparison to the reference strain B:850. The densytometric analysis of the isolates sampled from calves; usually exhibited protein bands weighing 18, 33 to 34, 44 to 46, 48 to 50, 54 to 56, 64 to 66, 80 to 84, and 100 to 104 in kDa. The strains were divided into four types with regard to a protein pattern, and with a molecular weight ranging from 30 kDa to 38 kDa (Fig. 2). The protein type with one band, weighing 33 kDa to 34 kDa and demonstrating the highest optical density, occurred in most isolates. The strains belonging to groups 2 and 3 exhibited an additional band. In the case of group 2, it was a band weighing 36 kDa to 38 kDa, whereas in group it was 3 – 30 kDa. Strain type 4, demonstrated only one intensely stained protein weighing 36 kDa; 88.9% of the strains belonged to the first protein pattern. The remaining distinguished types comprised of 6.9%, 1.4%, and 2.8% isolates, respectively. The electroforegrams of the isolates showed a 46% to 100% rate of homology.

The outer membrane proteins of the reference strain P1059, serotype A:3, exhibited the presence of protein bands similar to those confirmed in field isolates from calves (Fig. 2). The electroforegrams obtained from the field isolates; showed a 41% to 64% rate of homology in comparison to the reference strain.

**Discussion**

Similarly to the results reported by Pati et al. (18) and Tomer et al. (22), the electrophoretic profiles of field isolates of *P. multocida* sampled from adult cattle and calves, and the reference strains, serotypes B:2 and A:3, exhibited 9 to 14 bands with different molecular weights, ranging from 18 kDa to 115 kDa.

The examined field isolates, serotype B:2,5, and reference strain B850 had two intensely stained protein fractions weighing 31 to 32 and 36 to 37 kDa. The presence of these fractions was also confirmed by electroforegrams by other authors (18, 22). On the other hand, Johnson et al. (13) found a high OD value in the 32 kDa band in the profiles of whole-cell type B isolates, whereas the 37 kDa band was the main protein of the strains with A, D, and E capsules.
In the present studies, one protein weighing 33 kDa to 34 kDa and showing the highest optical density; occurred in nearly all the strains sampled from calves and in the reference strain of serotype A:3 (P1059). Few researchers described the proteins of *P. multocida* strains sampled from calves and belonging to serotype A:3 (1, 6, 8). Confer et al. (6) found one main band weighing 35 kDa in the OMPs profiles of strains from calves, whereas Debreuil et al. (9) and Zhao et al. (24) showed the presence of 3 bands weighing about 32, 36, and 39 kDa, in the OMPs profile of reference strain P1059. In our study, like in reports by Abdullahi et al. (1), Dabo et al. (8), and Großmann et al. (11), we stressed the differences in the range of 30 to 38 kDa, which permitted us to distinguish two and four protein patterns in the strains from haemorrhagic septicaemia cases, and in the strains isolated from calves, respectively. On the other hand, Großmann et al. (11) demonstrated 8 various electrophoretic profiles in whole-cell lysates from calf isolates.

These studies were continued by the use of OMPs of the two serotypes of *P. multocida* for immunization of calves (3, 15). The immunization of the animals with the complexes of the OMP antigens; stimulated the production of antibodies specific to the protein fractions detected by ELISA and immunoblotting.

**Fig. 2.** Electrophoretic profiles of OMPs of *P. multocida* strains isolated from calves. M – weight standards

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**References**


