ANALYSIS OF *PASTEURELLA MULTOCIDA* STRAINS ISOLATED FROM CATTLE WITH HAEMORRHAGIC SEPTICAEMIA BY THE USE OF PFGE

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Abstract

Forty *Pasteurella multocida* subsp. *multocida* strains collected from cattle with haemorrhagic septicaemia belonging to serotype B:2, 5 and two reference strains of serotype B:2, were examined. The strains were isolated in the years 1958 to 1962 and 1985 to 1995. Analysis of the polymorphism of restricted DNA fragments by the use of the pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric field electrophoresis were made. The phylogenetic relationship between the examined strains was calculated by the unweighted pair-group method using arithmetic averages After digestion of chromosomal DNA by using ApaI endonuclease, field isolates from HS cases were homogenous. The presence of an additional restrictive fragment in electrophoretic profile was found only in one strain.

Key words: cattle, *Pasteurella multocida*, haemorrhagic septicaemia, PFGE.

Material and Methods

**Bacterial strains.** Forty *P. multocida* subsp. *multocida* strains belonged to serotype B:2, 5 from three focuses were used (2, 4, 5, 11). The first group involved 16 lyophilisates of the strains isolated in 1958-1962 and described by Tereszczuk as type I – mammals corresponding to type I by Roberts (13). The second group comprising of 23 strains isolated in the Olsztyn and Ostroleka Voivodeships from internal organs of the cattle that died of Bollinger’s disease in 1991-1995. Moreover, one strain sampled from a heifer at Ustrzyki Dolne (the Podkarpacie Region) in 1985 was included. Two reference strains of serotype B:2: B850 obtained from the IEMVT in Paris and M1404 from the National Disease Laboratory, Ames, USA, were also examined.

**Preparation of genomic DNA.** The examined *P. multocida* strains were cultured on 5% horse blood agar and incubated for 18 h at 37°C. High molecular weight DNA embedded in agarose was prepared according to the procedure described by Isenberg (10). A restriction endonuclease digestion was performed by incubation of Apal (MBI Fermentas) for 18 h at 30°C.

**Pulsed-field gel electrophoresis.** The electrophoretic separation was carried out in a 1% agarose for PFGE (Bio-Rad). The PFGE technique with contour-clamped homogeneous electric field electrophoresis (CHEF) was carried out on a CHEF DR II (Bio-Rad) apparatus with an initial switch time of 1 s, increasing to 40 s for 23 h. A pulse Marker 50–1000 kb (Sigma) was used as a marker of the molecular weight. Gel was photographed with the use of the GelDoc2000 (Bio-Rad) system for gel registration and analysed by the Quantity One software (Bio-Rad).

**Data analysis.** The philogenetic relatedness of the examined strains was calculated by the unweighted pair-group method using arithmetic averages (UPGMA) with the Dice similarity coefficient and the band position tolerance of 1%.
Results

Except for one strain, all the field isolates of *P. multocida* subsp. *multocida* achieved from bovine haemorrhagic septicaemia cases revealed the same electrophoreogram (Fig. 1) irrespective of the time of isolation. The obtained electrophoretic profiles contained 10 restrictive fragments in the range of 61 kb to 289 kb. In the case of one isolate sampled from Ustrzyki Dolne surrounding in 1985, an additional 319 kb lane was found. The analysis of electrophoretic picture with the use of the unweighted pair-group method using arithmetic averages allowed the determination of the philogenetic relatedness of the restrictive pattern of this isolate in comparison to that in remaining strains at a level of 95% (Fig. 2). All the examined strains belonged to the same clonal group irrespective of the time of isolation.

The analysis of the reference strain B850 isolated in Africa demonstrated the presence of 11 lanes from 61 kb to 335 kb (Fig. 1). The electrophoretic profile was found to comprise 10 restrictive fragments occurring in the Polish isolates of serotype B:2, 5 and an additional lane at a 335 kb level. The restrictive pattern of the second reference strain M1404 from the USA consisted of 10 fragments ranging from 61 kb to 319 kb (Fig. 1). Seven of them occurred in local isolates and B850 strain. The electrophoregram of M1404 strain showed 3 additional lanes at 319 kb (similarly to P1/Rz isolate), 193 kb and 137 kb. On the other hand, 219, 158 and 142 kb restrictive fragments were absent. The relatedness of the electrophoretic reference standard of B850 strain with the Polish isolates was 95% and in the case of P1/Rz – 91%. For M1404 strain, the relatedness was 70% and 76%, respectively. The philogenetic relatedness among restrictive patterns of reference strains was 66%.

Fig. 1. Electrophoretic profiles of the chromosomal DNA field isolates sampled from bovine haemorrhagic septicaemia cases and the reference strains B850 and M1404 (serotype B:2). M – marker

![Fig. 1](image1.jpg)

<table>
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<th>Number of strains</th>
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<th>90%</th>
<th>80%</th>
<th>70%</th>
<th>60%</th>
<th>50%</th>
<th>40%</th>
<th>30%</th>
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Fig. 2. Dendrogram of philogenetic relatedness among the *P. multocida* subsp. *multocida* strains isolated from HS affected cattle.

![Fig. 2](image2.jpg)
Discussion

Townsend et al. (14) analysed with the use of PFGE the polymorphism of the restrictive fragments of the chromosomal DNA of *P. multocida* strains causing haemorrhagic septicaemia. Similarly to the case involving strains from cattle in Poland described above, the Asian isolates of serotype B:2 digested with Smal and NotI endonucleases were markedly homogenous. The North America strains of this serogroup varied remarkably from the Asian isolates.

Similar results were obtained by Aalbæk et al. (1) who studied 37 strains of *P. multocida* serogroup B originating from Danish fallow deer and 9 strains of other animal species. An analysis of the restrictive patterns of strains isolated from fallow deer and achieved with the use of Sall endonuclease, revealed that all the strains demonstrated the same profile. The isolates obtained from other animals were heterogenic.

Strain typing is an essential part of epidemiologic investigation of infections. Genotyping techniques have been used extensively to differentiate epidemiologically significant strains of *P. multocida*. Donnio et al. (6, 7) found 11 restrictive patterns among 13 strains of *P. multocida* subsp. *multocida* with capsular type A or D, producing derrnecrotoxine and isolated it from swine and people. These isolates revealed a significant polymorphism of DNA that, however, did not demonstrate a visible correlation with the host species. Several *P. multocida* strains originating from people and swine revealed the same restrictive pattern. Phenotyping and genotyping failed to describe the host. According to the authors, the data obtained may indicate a possibility of the colonisation of the human organism by microorganisms from the swine reservoir.

Similarly, Blackwood et al. (3) found *P. multocida* strains with the same restrictive profile of chromosomal DNA in people and their domestic animals. The researchers isolated *P. multocida* from a patient, his brother and three cats whereas one cat revealed 2 different strains. The authors identified three profiles by cutting the genome DNA with a restrictive enzyme. They revealed that one electrophoretic pattern was mutual for the brother and all the cats but was not connected with the disease. The second restrictive pattern was present only in one cat. The profile of the strain originating from the patient was also unique.

The studies presented here permitted the improvement of the classification methods of *P. multocida* strains isolated from cattle. These studies will be continued to analyse by PFGE the electrophoretic patterns of *P. multocida* isolated from the cases of enzootic bronchopneumonia in calves. This enables recognizing the epidemiologic state in Poland and improving diagnostics of infections caused by this microorganism.

References

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