

Protein and Antigenic Profile among *Mycoplasma bovis* Field Strains Isolated in Bosnia and Herzegovina

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Abstract

Mycoplasma bovis is a serious, worldwide-spread but often overlooked pathogen causing respiratory disease, mastitis, and arthritis in cattle. In this study we characterize the protein and antigenic profiles of *M. bovis* field strains isolated in Bosnia and Herzegovina by sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting, and analyze possible variations among these strains. Greater differences occurred when comparing field strains with the reference strain PG45. One field strain isolated from lung samples of a heifer was markedly different from strains isolated from nasal swabs taken from cattle raised in another geographic region. A possible correlation may exist between protein and antigen profiles of *M. bovis* field strains, geographic regions and anatomical sites of isolation.

Mycoplasma bovis, cattle, antigen, immunoblotting, protein, SDS-PAGE

Mycoplasma bovis is the most pathogenic bovine mycoplasma in the parts of the world considered to be free of contagious bovine pleuropneumonia. This pathogen primarily causes calf pneumonia, mastitis and arthritis. Other symptoms are seen less frequently. Diseases caused by *M. bovis* are found worldwide, causing significant economic losses. Yet, there are no effective control measures or standard protocols for their routine application available (Nicholas and Ayling 2003).

Although *M. bovis*, like other mycoplasmas, possesses a small genome size, it is still highly invasive and able to colonize organ systems. It is now clear that one of the possible explanations for its ability to avoid bodily defence mechanisms and adapt to different environments in the host is due to phenotypic variations in mycoplasmas (Wise 1993; Razin et al. 1998; Minion 2002). Rosengarten et al. (1994) demonstrated highly variable membrane surface proteins (Vsps) of *M. bovis* which represent a family of antigenically and structurally related lipoproteins. These Vsps represent the predominant antigens recognized by the host immune response during infection, and they can undergo high frequency changes in size and expression. This system is responsible for the survival of these pathogens in the presence of cytolytic antibodies and for their adaptation to specialized environments within their respective hosts (Behrens et al. 1994; Razin et al. 1998). A full understanding of phenotypic variation among *M. bovis* strains should be of great value when developing diagnostic methods and vaccines for the control of infections caused by this organism.

Recently, *M. bovis* has been isolated, for the first time, in Bosnia and Herzegovina (Rifatbegovi et al. 2007). The aim of this study was to characterize the protein and antigenic profile of these *M. bovis* field strains by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and immunoblot and to analyze possible variations among these strains.

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Materials and Methods

M. bovis strains and harvesting

Eight field strains of *M. bovis* (01, 166, 168, 170, 187, 188, 189, 190) recovered in Bosnia and Herzegovina (Rifatbegović et al. 2007) were studied and compared with the reference strain PG 45 and a field strain (2/91) originating from Germany. Field strain 01 was isolated from lung samples of quarantined heifers that died from respiratory disease. The other seven strains were isolated from nasal swabs from fattened cattle with clinical signs of respiratory disease.

For SDS-PAGE and immunoblot, strains were grown in liquid medium (Thiaucourt et al. 1996) until logarithmic phase. After centrifugation at 14,000 g for 20 min at 4 °C, followed by three washes with saline (0.85% of NaCl in distilled water), pellets were resuspended in saline until the turbidity matched that of the McFarland no. 3 standard.

SDS-PAGE

Prior to electrophoresis the protein content of each mycoplasma sample was estimated with a commercial kit (Microprotein-PR/Sigma) following manufacturer's instructions. SDS-PAGE was performed following the procedure described by Laemmli (1970) with 1.5 mm thick 4% stacking and 10% resolving gel. Each sample containing 26 µg of whole cell proteins of mycoplasmas was dissolved in lysis buffer and heated at 96 °C for 5 min. Low molecular weight marker solution (LMW - Amersham Pharmacia Biotech, UK) was prepared according to manufacturer's instructions. All samples and LMW were submitted to electrophoresis at a constant current of 20 mA per gel in a vertical electrophoresis unit (Biorad, Hercules, CA). Coomassie blue stain (Amersham Pharmacia Biotech, UK) was used for visualization of protein bands.

Immunoblotting

Proteins were transferred from gel to a 0.45 µm nitrocellulose membrane (BioTrace NT, Pall Corporation) in a transference unit (Biorad, Hercules, CA) at 70 V for 90 min (Gonçalves et al. 1998). After the transfer, the membrane was blocked by incubating at room temperature for 2 h in a blocking solution (1 mol/l glycine, 1% egg albumin and 5% skimmed milk in PBS). The membrane was incubated overnight at 4 °C with rabbit antisera produced against PG45 strain, diluted at 1 : 200. After washing the membrane three times with PBST (15 min) and once with PBS (10 min), secondary antibodies conjugated with peroxidase were added. The membrane washing was repeated in the same way after 1 h of incubation at room temperature. Once it was completed, the developing solution (4-chloro-*n*-naftol 24 µg, methanol 8 ml, PBS 50 ml, H₂O₂ 30 µl) was added. The reaction was stopped by washing the membrane with distilled water.

Results

SDS-PAGE analysis

The electrophoretic patterns of ten *M. bovis* strains were homogeneous, with some differences in band intensity, expression and sites (Plate XI, Fig. 1). Coomassie blue stain showed 38 to 45 protein bands between 205 and 14.4 kDa, with the main proteins corresponding to molecular masses of about 86, 72, 63, 53, 48, 45, 43, 40, 38, 32 and 27 kDa.

SDS-PAGE results revealed that the reference strain PG45 had a different electrophoretic pattern when compared with the field strains. It was characterized by unique bands at 160, 147 and 30.5 kDa, higher intensity bands in the 80, 67 and 58 kDa position and lack of proteins at 120 and 55 kDa. The 160 kDa band was observed also at the field strain 168, but with very low intensity.

The main difference between the German field strain 2/91 and the other tested strains was the lack of a 58 kDa protein and the expression of a 29 kDa protein. Low intensity of a 86 kDa band was characteristic of this and field strain 01.

Field strain 01 was specific with intense bands at 31 and particularly 32 and 36 kDa. The 31 and 36 kDa bands were only observed at this strain and strain 2/91. Also, strain 01 was distinguished from others by expressing a protein at 69 kDa, and the lack of a 34 kDa band which was expressed by other strains with different intensity.

Immunoblotting analysis

Immunoblotting analysis of the *M. bovis* strains revealed up to 36 bands, with major proteins at 72, 63, 48, 45, 43, 40, 38, 32 and 29 kDa. Some differences regarding antigenic expression and intensity were observed, especially in the lower (25–16 kDa) molecular mass regions (Plate XII, Fig. 2). The reference strain PG45 was the only strain without bands at 39 and 27 kDa positions. The lack of expression at 86 and 58 kDa level was characteristic of reference strain PG45 and German strain 2/91.

At 25–16 kDa position, the Bosnian field strains expressed up to seven bands. In contrast, reference strain PG45 expressed no bands at this level, and strain 2/91 expressed only low intensity band at 23 kDa. The analysis of Bosnian field strains revealed that strain 01 was unique, since it expressed prominent bands at 36 and 31 kDa, and lacked 34, 30, 29, 23 and 16 kDa bands. Field strains 166 and 168 expressed no bands or very low intense bands at 20, 19 and 16 kDa positions.

Discussion

We have detected protein and antigen variability among *M. bovis* strains. Seven out of eight strains isolated in Bosnia and Herzegovina had similar protein profiles obtained by SDS-PAGE, but one field strain (01) showed marked differences. The main differences among field strains in comparison to protein patterns were in the intensity and expression of some bands.

Results revealed that the protein profile of the German field strain 2/91 was similar to those isolated in Bosnia and Herzegovina, particularly with strain 01. Reference strain PG45 was clearly distinguishable from all others by the presence of three specific protein bands and the lack of two.

The protein heterogeneity among field strains does not correlate with results of some authors (Sachse et al. 1992; Poumarat et al. 1994; Filioussis et al. 1999), but antigen heterogeneity has been confirmed in earlier studies (Poumarat et al. 1994; Rosengarten et al. 1994). The same is true for protein characteristics, as differences were noted between field strains and were evident for strain 01. Also, antigen heterogeneity between the two control strains and the field strains were more marked than those obtained by SDS-PAGE. The greatest differences were obtained when comparing the reference strain PG45 with the other ones. Similar results with antigen expression patterns of PG45 strain were reported by Rosengarten et al. (1994). Specific patterns of the PG45 strain were also demonstrated by Thomas et al. (2003) who found a weak adherence rate of reference strain PG45 in contrast to other *M. bovis* strains. These differences could be a consequence of the high number of passages of the reference strain and the different storage conditions during many years of storage.

Strain 01, which was isolated from a heifer lung sample, was markedly different from the other seven field strains, which were all recovered from nasal swabs of cattle of the same farm. The heifer was from another geographic region and had only recently been imported to Bosnia and Herzegovina.

Similar results, correlating between geographic origin and antigen profile of mycoplasmas, were reported by Solsona et al. (1996), who found that the antigen profile of *M. agalactiae* strains could be separated into groups according to geographic origin. Rosengarten et al. (1994) compared the protein profiles of 11 strains of *M. bovis* and found that one strain which had been isolated in Cuba was clearly distinguishable from the other isolates originated from Germany. However, these authors suggested that the geographical origin and site of isolation do not have an important influence on antigen heterogeneity.

This study has demonstrated phenotype heterogeneity among *M. bovis* Bosnia and Herzegovina strains. It has also suggested a possible link between protein and antigen profiles of *M. bovis* field strains and the geographic origin and site of isolation. Since the number of strains used in this study was not large enough to support this type of conclusion, further study is needed to confirm this correlation.

Proteinový a antigenní profil terénních kmenů *Mycoplasma bovis* izolovaných v Bosně a Hercegovině

Mycoplasma bovis je nebezpečný, celosvětově rozšířený, avšak často přehlížený patogen způsobující dýchací onemocnění, mastitidu a artritidu v chovech skotu. V této studii jsou

charakterizovány bílkovinné a antigenní profily terénních typů p vodce *M. bovis* a jeho detekovaných p echodných variant izolovaných v Bosn a Hercegovin pomocí gelové elektroforézy a imunoblotování. V tší rozdílly byly prokázány p i porovnání terénních typů s referenčním typem PG45. Jeden z terénních typů izolovaný z plicních vzorků jalovice byl značně odlišný od typů izolovaných z nosních výtoků skotu chovaného v jiném zeměpisném regionu. Byla zjištěna možná korelace mezi bílkovinnou a antigenní strukturou terénních typů *M. bovis*, zeměpisnými regiony a anatomickou lokalizací odberových míst pro izolaci tohoto patogena.

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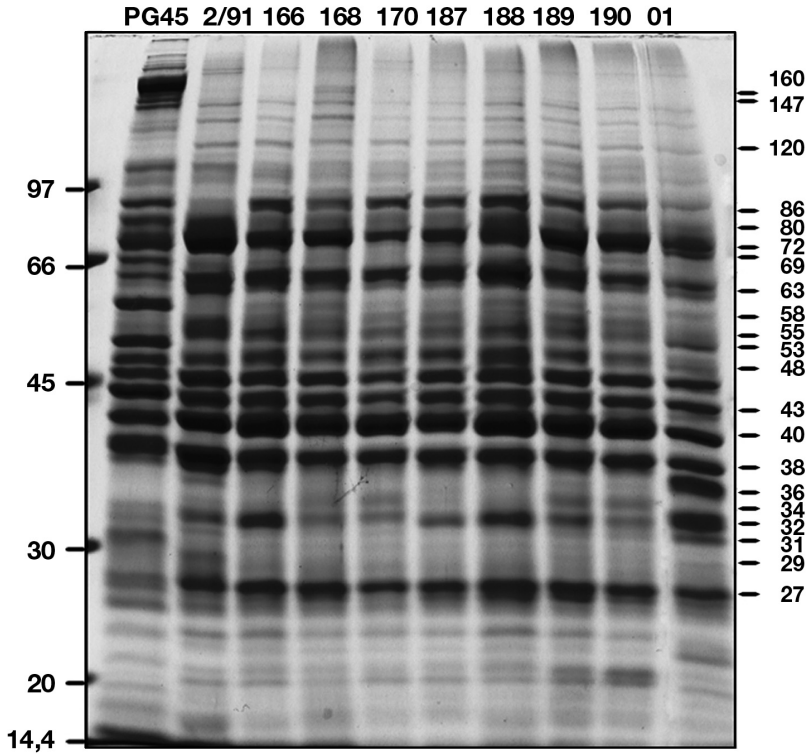


Fig 1. Coomassie blue-stained SDS-PAGE gel of whole-cell proteins from ten *M. bovis* strains (26 µg proteins loaded per track). On the top: names of the analysed strains. Positions of molecular mass standards are indicated (kDa) on the left. Positions of major or bands with some differences in expression or intensity are indicated (kDa) on the right.

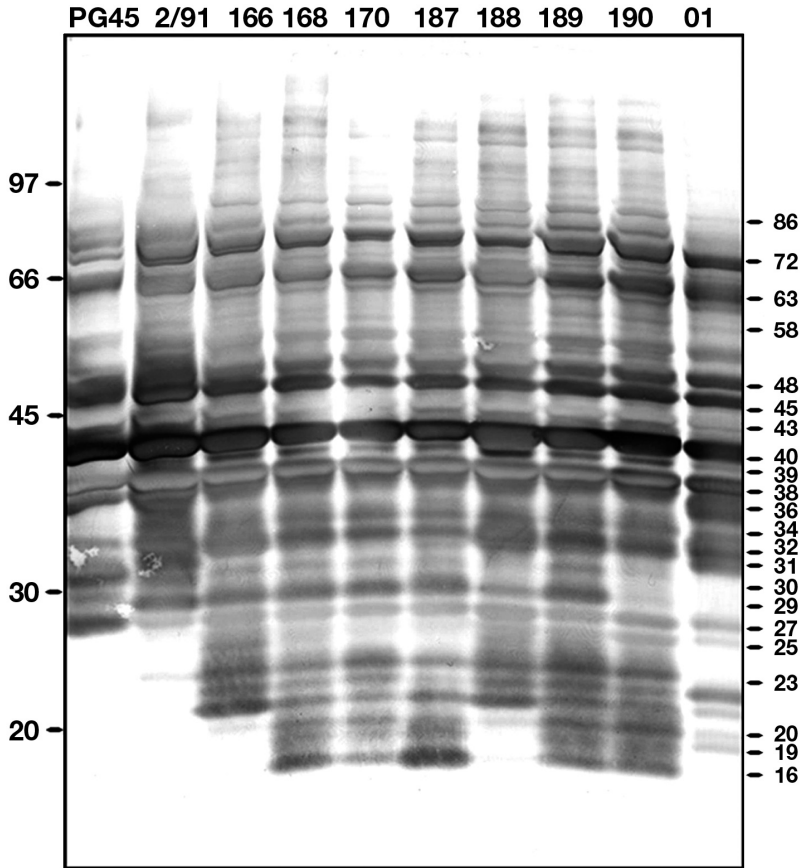


Fig 2. Immunoblot profile of *M. bovis* strains (26 μ g proteins loaded per track). On the top: names of the analyzed strains. Positions of molecular mass standards are indicated (kDa) on the left. Positions of some bands are indicated (kDa) on the right.