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*Short communication*

# A serological and molecular study on the occurrence of mycoplasmas in European bison (*Bison bonasus*) from two areas of Eastern Poland

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## Abstract

European bison (*Bison bonasus*) from two different areas of Eastern Poland showing gross pathology possibly associated with mycoplasma infections were tested for ruminant *Mycoplasma* species using serological and molecular methods. Fifty-five samples, blood or tissue were collected from 28 animals during 2013-2014. Six sera were positive for *Mycoplasma bovis.* The ELISA and complement fixation test for *Mycoplasma mycoides* subsp. *mycoides* gave a few weak reactions, but were negative by immunoblotting and molecular methods.

**Key words**: European bison, ruminant mycoplasma, serology, molecular biology

## Introduction

Approximately 1432 European bison (*Bison bonasus*) are present in Poland. Nearly 60% of them populate two areas of Eastern Poland: the Polish region of the Białowieża Forest (BF) with 559 heads; and the Bieszczady Mountains (BM) with 301 heads (Raczyński 2015). *M. bovis* has previously been linked with disease in bison. In Poland serological evidence in one bison (Krzysiak et al. 2014); pneumonia, laryngitis, arthritis, synovitis and tenosynovitis in feedlot bison (Dyer et al. 2008); arthritis, pleuritis and abscesses in the lung with mortality above 27% (Janardhan et al. 2010); fatal reproductive disorders and abortion in American bison in which bronchopneumonia, pleuritis and coagulation necrosis in the lung were also observed (Register et al. 2013b). Other ruminant *Mycoplasma* species that may cause respiratory disorders and arthritis in domestic and wild ruminants include: *Mycoplasma mycoides* subsp.

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*mycoides (Mmm)*, the causative agent of contagious bovine pleuropneumonia (CBPP), *Mycoplasma agalactiae*, and *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) (Nicholas et al. 2008). Specific antibodies to these mycoplasmas in bison have not been observed (Krzysiak et al. 2014). As gross pathology in European bison has raised the suspicion of *M. bovis* infection, we assessed the current situation and extended the testing to include molecular analyses that would identify the most important ruminant mycoplasma species.

## Materials and Methods

Twenty eight European bison originating from BF and BM were examined in this study. Routine necropsy as decided by the Polish Authority was carried out on three animals which had died, three which were immobilized, and 21 which were sacrificed. 55 samples were examined which comprised of 27 sera, 20 lungs, 3 livers, 1 kidney and 4 nasal swabs. The sera were tested for antibodies to *M. bovis*, *M. agalactiae, Mmm* and *Mccp* using the *M. bovis* ELISA kit (Bio-X Diagnostics, Belgium), *M. agalactiae* Antibody Test Kit (IDEXX), latex agglutination tests for *Mccp* (CapriLAT, APHA, UK) and for *Mmm* (BoviLAT, APHA, UK). A *Mmm* Antibody Test Kit (IDEXX); complement fixation test (CFT) for CBPP (Cirad, France); and immunoblotting method as described by the OIE (2012) were also used for *Mmm*. Mycoplasmal DNA was extracted from organ and nasal swab samples using the QIAmp DNA Mini Kit (Qiagen, Germany). The polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/DGGE) method of McAuliffe et al. (2005) with modifications was used and gels analysed in comparison with the following mycoplasma controls: *M. bovis*, *Mmm*, *M. bovigenitalium, M. canis*, *M. dispar*, *M. arginini*, *M. canadense*, *M. alkalescens*, *M. agalactiae*, *Mccp*, and *M. mycoides* subsp. *capri.* For *M. bovis* DNA and antigen species-specific PCR (Subramaniam et al. 1998) and Pulmotest *Mycoplasma bovis* ELISA kit (Bio-X Diagnostics, Belgium) were used respectively.

## Results and Discussion

Distinct respiratory lesions including pneumonia, emphysema, pleural adhesions, bronchus oedema with fibrinous exudation and caseous foci were observed in 21 European bison. An ovary cyst and nodular focus in the joint was also observed in one bison. None of the respiratory lesions were typical for CBPP, but indicative of *M. bovis* infection. From 27 sera, six were positive for *M. bovis*. The *Mmm* ELISA gave four positive and two doubtful results, one was also doubtful in the CFT. However, none were confirmed by immunoblotting or BoviLAT; and no *Mmm* was detected using molecular tests. These false serological reactions are likely to be due to *M. bovis* cross-reacting antibodies (Goncalves et al. 2008), therefore these bison do not have CBPP. No antibodies to *Mccp* and *M. agalactiae* were detected, which corresponds with our previous work (Krzysiak et al. 2014). Only six sera were positive for *M. bovis*. All seropositive bison originated from BF and showed pneumonia, emphysema and pleural adhesions. Unfortunately, these results were not confirmed by other methods. The Bio-X *M. bovis* ELISA adopted in this study is intended for use in cattle; however, Register et al. (2013a) demonstrated its application for use in bison when they compared it to their in-house ELISA designed for testing bison with agreement at more than 96%. That difference related to two samples which came from bison with an unknown history of exposure to *M. bovis*. In this study, a lack of detection of *M. bovis* antigen, despite gross pathology in seropositive bison, may indicate previous exposure to the infection, possibly from contact with infected cattle, rather than current infection. In Podlaskie province of Poland where BF is situated, *M. bovis* seroprevalence in cattle was assessed at 77% (Bednarek et al. 2012, Dudek and Bednarek 2012). Although the usefulness of the Bio-X *M. bovis* ELISA in bison testing was demonstrated earlier (Register et al. 2013a) the possibility of false positive results in this study cannot be excluded. Nevertheless it has been previously demonstrated that *M. bovis* does infect bison and can cause serious disease problems (Dyer et al. 2008, Janardhan et al. 2010, Register et al. 2013b). Further studies are required to show the true mycoplasma status of bison and to determine the cause of the lesions observed.

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