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## ANTIBODIES AGAINST *MYCOPLASMA BOVIGENITALIUM* IN FREE-LIVING EUROPEAN BISON (*BISON BONASUS*) WITH BALANOPOSTHITIS

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**ABSTRACT:** Since 1980 severe chronic balanoposthitis has been observed in free-living European bison (*Bison bonasus*) in the Białowieża Primeval Forest (Poland). Sera of 50 bison with balanoposthitis and 48 clinically healthy male and 49 female bison were investigated for antibodies against *Mycoplasma bovis* and *M. bovis genitalium* by western blot analysis. Prevalence of antibodies against *M. bovis genitalium* was significantly higher in bison with balanoposthitis than in unaffected male bison. *Mycoplasma bovis genitalium* may play a role in the pathogenesis of balanoposthitis in European bison.

**Key words:** Balanoposthitis, *Bison bonasus*, European bison, *Mycoplasma bovis genitalium*, Poland, serologic survey.

### INTRODUCTION

The largest free-living population of the endangered European bison (*Bison bonasus*) is found in the Białowieża Primeval Forest that stretches across eastern Poland and western Belarus. This population of about 500 animals was founded in 1929/1930 by two cows and one bull (Krasieńska et al., 1998). Since 1980 a chronic disease of the external genital organs has been observed (Piusiński et al., 1997). The predominant pathologic findings are necrotic and ulcerative lesions of the prepuce and penis of bulls from 6 mo to >10 yr old. Histologic findings were described previously (Jakob et al., 2000). Since the start of intensive investigations on this disease in the late 1980s, no female bison with a comparable genital disorder has been found.

The etiology of this disease is unknown; no primary infectious agent has yet been found. Virologic examinations were negative for alphaherpesviruses and various strains of bovine viral diarrhea virus (Borchers et al., 2002). Different bacterial species were isolated, particularly *Corynebacterium* spp. with intensive urealytic activity. Some of these strains were identified as *C. pilosum* and *C. renale*. These

are facultative pathogenic bacteria which are normal flora of the bovine genital system mucosa. In addition, several strains of *Fusobacterium necrophorum* and other *Fusobacterium* spp. were isolated (Jakob et al., 2000). These facultative pathogenic bacteria are normal flora of the intestinal system, especially in the rumen. All these bacteria are believed to play a secondary role in balanoposthitis in bison.

*Mycoplasma* spp. cause various diseases including genital disorders. *Mycoplasma bovis* is one of the most pathogenic bovine mycoplasmas (Pfützner and Sachse, 1996). In cattle, it is associated with diverse clinical manifestations, such as mastitis, arthritis, and pneumonia, as well as genital disorders. *Mycoplasma bovis genitalium* is common in semen, prepuce, vestibule, and vagina of cattle (Parsonson et al., 1974). Pathogenicity was first reported by Blom and Ernø (1967), who isolated *M. bovis genitalium* from a case of bovine seminal vesiculitis. Several cases of spontaneous and experimental infections, especially of the upper genital tract of bulls, have been described (Afshar et al., 1966; Al-Aubaidi et al., 1972; Parsonson et al., 1974; Panangala et al., 1982).

Our objective in this study was to de-

termine whether mycoplasmas play a role in balanoposthitis of European bison. Mycoplasmas are difficult to isolate from tissues highly contaminated with other bacteria. Therefore, we first conducted a serosurvey.

#### MATERIALS AND METHODS

Sera from 147 bison, taken from immobilized or culled animals in the years 1989–2000, were examined for antibodies against *M. bovis* and *M. bovis genitalium* by western blot analysis. A whole cell antigen mixture of three clonal variants expressing VspA, VspB, and VspC derived from *M. bovis* PG45 (Brank et al., 1999) and a whole cell lysate of the *M. bovis genitalium* reference strain PG11 were used in western blot analysis. The procedures for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting of mycoplasma proteins have been described previously (Rosengarten et al., 1994; Brank et al., 1999). Positive control sera from naturally infected bovine bulls with *M. bovis genitalium*-induced balanoposthitis as well as sera from cattle experimentally infected with *M. bovis* were included in this study. Additionally, negative control sera derived from mycoplasma free cattle herds were used.

The *M. bovis genitalium* test results (positive/negative) and the occurrence of balanoposthitis (yes/no) were coded as binary variables. Four stages of balanoposthitis were distinguished: balanoposthitis stage 1) slight to moderate changes around the preputial orifice characterized by crushed hairs and focal zones of hyperkeratosis; stage 2) coagulation necrosis and ulcers in the zone of transition from haired to hairless skin; stage 3) necrosis and ulcers inside the preputial cavity; and stage 4) accumulation of thick exudate and necrotic tissue within the preputial cavity, paraphimosis, constriction of the distal penis, and in some cases necrosis and auto-amputation of the glans penis). Ages of the animals were classified as subadult ( $\leq 2$  yr), reproductive ( $>2$  yr to  $\leq 10$  yr), or old ( $>10$  yr). Potential relationships between the serologic state (dependent variable) and sex, age, and presence of balanoposthitis (independent variables) were evaluated using logistic regression analysis (Hosmer and Lemeshow, 1989) and chi-square tests. Chi-square tests were also used for comparison of the disease stage groups concerning their *M. bovis genitalium* test response rate. Significance level was generally set to  $\alpha=0.05$ . All statistical calculations were performed with the SPSS 9.0 software (SPSS Inc., Chicago, Illinois, USA).

Sera from 50 male bison with balanoposthitis

TABLE 1. Disease stages of male European bison with balanoposthitis and seropositive to *Mycoplasma bovis genitalium*.

	Number of animals examined	Number of animals seropositive to <i>M. bovis genitalium</i>	%
Stage 1 (mild)	23	17	74
Stage 2 (moderate)	8	6	75
Stage 3 (severe)	9	6	67
Stage 4 (extensive)	10	4	40
Total	50	33	66

(group I) in stage 1–4, 48 sera from male bison without balanoposthitis (group II), and 49 sera from female animals (group III) were examined. The animals were between 3 mo and 24 yr old.

#### RESULTS

Sera of three diseased and one healthy bull had antibodies against *M. bovis*, and three sera from diseased bulls were inconclusive. Sera from females were negative for *M. bovis*-antibodies.

In group I, 30 (60%) of 50 sera were positive and 3 (6%) sera were inconclusive for antibodies against *M. bovis genitalium*. These sera were from bulls with disease stages 1–4 (Table 1). Ten (21%) of 48 sera in group II and 21 (43%) of 48 sera from bison in group III had antibodies against *M. bovis genitalium* (Table 2).

The logistic regression model for the occurrence of antibodies as dependent variable and age, sex, and balanoposthitis as independents showed a significant fit to the data (log likelihood ratio test,  $P<0.001$ ). Age (log likelihood ratio test,  $P=0.255$ ) was not significant, as opposed to sex ( $P=0.024$ ) and the presence or absence of balanoposthitis ( $P<0.001$ ). Consequently, age was excluded from further analysis.

According to the logistic regression, the prevalence of a seropositive test results was significantly higher in diseased (17 of 50, 66%) than in non-diseased males (10 of 48, 21%). Seropositive rate of non-diseased males was significantly different (chi-square test,  $P=0.020$ ,  $df=1$ ,  $n=97$ ) from the females (21 of 49, 43%). The

TABLE 2. Antibodies against *Mycoplasma bovis* in different disease stages, ages, and sexes of European bison.

	Males with balanoposthitis	Males without balanoposthitis	Females	Percent
≤2 yr	78% (18/23)	17% (5/30)	43% (10/23)	43%
>2–10 yr	54% (13/24)	25% (2/8)	30% (3/10)	43%
>10 yr	67% (2/3)	20% (3/10)	50% (8/16)	43%
Totals	66% (33/50)	21% (10/48)	43% (21/49)	

prevalence of seropositivity did not differ between the four disease stages (chi-square test,  $P=0.267$ ,  $df=3$ ,  $n=50$ ).

DISCUSSION

Our serologic results suggest that *M. bovis* may play a role in the pathogenesis of balanoposthitis in European bison, whereas *M. bovis* seems to be unimportant. Preliminary data obtained by conventional culture and molecular detection by polymerase chain reaction supports the primary involvement of *M. bovis* in the chronic necrotizing inflammation process of the penis and prepuce (Spergser, unpubl. data). *Mycoplasma bovis* has been associated with infertility, abortion, endometritis, seminal vesiculitis, and impaired spermatozoa motility in cattle (Ruhnke, 1994). However, in cattle herds only few individuals show clinical manifestation of *M. bovis* infection of the genital tract. In spite of clinical evidence that *M. bovis* can cause bovine genital disorders, frequent isolations of this bacterium from the genital tract without visible lesions suggest the influence of strain differences, host factors, or other unrecognized determinants on virulence. Although mycoplasmas usually exhibit a rather strict host specificity, *M. bovis* has been isolated from the genital tract of apparently healthy non-bovine animal species such as dogs, pigs, and horses (Whitford et al., 1994). To our knowledge, isolation of *M. bovis* from bison has not yet been described.

In this study, a significant association between balanoposthitis and exposure to *M. bovis* was found. However, not

all sera from diseased animals had positive reactions in western blot analysis. This is in agreement with previous reports describing a rather inconsistent systemic antibody response in experimentally intra-preputially infected bulls (Ernø, 1972; Ernø and Blom, 1972; Kreusel et al., 1989). Differences in humoral immune responses of infected hosts may reflect complex interactions between mycoplasmas and the host immune system involving mycoplasma-induced specific and non-specific immune reactions. A pathogenic property of mycoplasmas is ability to modulate host immune responsiveness enabling them to suppress or evade host defense mechanisms and establish chronic, persistent infection (Razin et al., 1998). Interestingly, the rate of seropositivity to *M. bovis* decreased in European bison with stage 4 disease, even though this difference was not significant.

In conclusion, *M. bovis* may play a role in the pathogenesis of balanoposthitis in European bison. However, many questions remain about the etiology of this disease.

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