8. Summary

Bacteriological Aspects of Balanoposthitis in European bison (*Bison bonasus*) - description of two novel species *Arcanobacterium bialowiezense* and *Arcanobacterium bonasi*

The Białowieża National Park is situated on both sides of the border between Poland and Belarussia. After the second world war it was separated into the Polish and the Belarussian part.

Today the Polish part comprises 594 km^2 and the Belarussian part 874 km^2. The largest of the few remaining free-ranging populations of European bison exists in the Białowieża Primeval Forest. Since 1980 a chronic necrotising disease of the prepuce and penis (Balanoposthitis) has been observed in this population.

The European (E.) bison belongs to the family *Bovidae* (Gray, 1872), the subfamily *Bovinae* (Gray, 1821) and the genus bison. There are two species classified within the genus bison: the European bison (*Bison bonasus*) and the North American bison (*Bison bison*). The European bison is a threatened species (Appendix III (protected fauna species) of the Bern Convention on the conservation of European wildlife and natural habitats, endangered species on the 2003 IUCN Red List of Threatened Species) and the Balanoposthitis is a serious problem for the present Białowieża population (Anonymous, 2002).

A chronic necrotizing inflammation of the external genital organs of male E. bison was first described in 1980 (Kita et al., 1994). Advanced disease is characterized by crushing of the hairs around the preputial orifice, edema of the surrounding skin, and accumulation of thick exudate and necrotic tissue within the preputial cavity. In addition, paraphimosis, constriction of the distal penis, and in some cases necrosis and auto-amputation of the glans penis, have been observed. Histopathologically, the necrotic tissue is demarcated from the underlying connective tissue by inflammatory cells and granulation tissue. Numerous lymphoid follicles in the surrounding connective tissue are also present (Jakob et al., 2000). Due to the inflammatory and necrotic changes of the prepuce and penis in late stages of Balanoposthitis affected bulls are not able to take part in reproduction. This regular loss of bulls (between 5 and 10% per year) obviously leads to a further reduction of the genetic potential of this threatened species. For this reason the objective of this study was to investigate whether certain bacteria are primarily or secondarily involved in the disease process of the Balanoposthitis.

Additionally, a survey of the physiological bacteriological flora of the male and female E. bison genital tract was conducted. For this purpose genital samples of healthy and diseased bulls as well as female E. bison were examined bacteriologically. From genital swabs of bulls showing Balanoposthitis two new bacteria were isolated which were morphologically similar to *Arcanobacterium* (*A.*) *pyogenes.* However strain W3/01 and strain W106/04 showed no serolysis on Löffler serum Agar and they also differed in enzymatic reactions from *A. pyogenes*. 16S rDNA sequence analysis showed that the two isolates W3/01 and W106/04 shared 97.2% sequence similarity. Highest sequence similarities (95.5-96.1%) to *A. pyogenes* DSM 20630 and *A. benardiae* DSM 9152 were found. The isolates with strain numbers W3/01 and W106/04 were deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig) under the species name of *Arcanobacterium bialowiezense* sp. nov. and *Arcanobacterium bonasi* sp. nov., respectively. *A. bialowiezense* or *A. bonasi* were isolated from 87% of the diseased bulls, but not from healthy bulls or female E. bison. The phylogenetic relationship to *A. pyogenes* underlines a possible role in the etiology and pathogenesis of the Balanoposthitis. It is possible that both isolates have virulence factors similar to *A. pyogenes,* and for this reason they might be involved in the disease process. At this stage it is only possible to hypothesize about the process of the disease and use this as a basis for further studies: Predisposing factors might scarify the tissue resulting in a hyperkeratosis. Possibly a genetic defect due to the low genetic variability leads to a failure of the local immune response (Lünser et al., 2005). This would make it easier for pathogenic microorganisms to invade the skin and proliferate.

In preliminary studies involvement of Fusobacteria was demonstrated (Jakob et al., 2000).

A synergism between *Fusobacterium* (*F.*) spp. and *A. bonasi* and *A. bialowiezense* could further enhance the infection as it has been described for *A. pyogenes und F. necrophorum* (Kaczmarowski, 2003).

The phylogenetic relationship of these two *Arcanobacterium* spp. to *A. pyogenes* leads to the assumption that they might have similar virulence factors: Neuraminidase promotes the adherence of *Arcanobacterium* spp. to skin cells, which enables them to colonize the skin (Esmay et al., 2003). PLO, a hemolytic Exotoxin (Ding, 1996; Billington, 1997), has a cytolytic effect through binding to the membranes of animal cells and building large oligomere pores (Billington, 2000). PLO also activates the complement cascade (Paton, 1984). By overreacting this can lead to inflammation and necrosis (www.mucos.cz).

As DNA of one of the new *Arcanobacterium* spp. was isolated by PCR in a bull at Stage I of the disease it is not possible to exclude a primary etiological role in the disease process.