NECROBACILLOSIS IN FREE-LIVING MALE EUROPEAN BISON IN POLAND

Authors: Jakob, Willi, Schröder, Hans-Dieter, Rudolph, Michael, Krasiński, Zbigniew A., Krasińska, Matgorzata, et al.

Source: Journal of Wildlife Diseases, 36(2) : 248-256

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-36.2.248

Journal of Wildlife Diseases, 36(2), 2000, pp. 248–256

NECROBACILLOSIS IN FREE-LIVING MALE EUROPEAN BISON IN POLAND

Willi Jakob,[1,6] Hans-Dieter Schröder,[1] Michael Rudolph,[1] Zbigniew A. Krasiński,[2] Matgorzata Krasińska,[3] Olaf Wolf,[1] André Lange,[4] John E. Cooper,[5] and Kai Frölich[1]

[1] Institute for Zoo Biology and Wildlife Research Berlin, Alfred-Kowalke-Strasse 17, P.O. Box 601103, 10252 Berlin, Germany
[2] Białowieża National Park, 17-230 Białowieża, Poland
[3] Mammal Research Institute PAS, 17-230 Białowieża, Poland
[4] Tierklinik für Fortpflanzung der Freien Universität Berlin, Königsweg 63, 14163 Berlin, Germany
[5] Wildlife Health Services, P.O. Box 153, Wellingborough, Northants NN8 2ZA, UK
[6] Corresponding author (e-mail: Jakob@izw-berlin.de)

ABSTRACT: In the Białowieża Primeval Forest (Poland) a chronic disease of the external genital organs has been observed in free-living male European bison (*Bison bonasus*) since 1980. Investigations on this disease started in the late 1980s. The most striking findings are necrotic and ulcerative lesions of the prepuce and penis of bison aged from 6 mo to >10 yr. Histologic examination of tissue samples from the prepuce of six bison (9-mo- to 8-yr-old), and from the penis of two bison (3- and 8-yr-old), were characteristic of necrobacillosis. Masses of slender, Gram-negative, rod-like or filamentous bacteria occurred in necrotic tissue. At the periphery of necrotic tissue filamentous bacteria were often arranged in large clusters and strands that advanced towards healthy tissue. Immunolabeling and electron microscopy also suggest that these organisms are *Fusobacterium* sp.

Key words: *Bison bonasus*, European bison, *Fusobacterium* sp., histopathology, necrobacillosis, necrotic posthitis and balanitis.

INTRODUCTION

The entire Białowieża Primeval Forest has an area of 1,468 km^2. After its division following World War II, 594 km^2 belonged to Poland and 874 km^2 to Belarus. Two populations of lowland European bison (*Bison bonasus bonasus*), separated by the state boundary, roam the Białowieża Forest. In 1980 a fence was built along the state border. At the end of 1997, the bison population on the Polish side was 290 and the Belarussian population was 232 animals (Krasińska et al., 1998). In order to prevent overpopulation and severe damage to the vegetation, about 10% of the bison have been culled yearly on the Polish side since the early 1970s (Krasiński et al., 1994).

A chronic disease of the external genital organs has been observed in male bison on the Polish side of the Białowieża Forest since 1980 (Kita et al., 1994; Piusiński et al., 1997). Between 1980 and 1997, 133 male bison 6-mo- to 11-yr-old were found to be affected with this disease. Of these 133 animals, 114 were culled and 19 died (Z. Krasiński, unpubl. data). The same disease is also known to occur in the eastern part of the Białowieża Forest in Belarus (Krasochko et al., 1996; Schildger et al., 1996).

The clinical signs and lesions were described by Polish investigators (Kita et al., 1990, 1994; Piusiński et al., 1996, 1997). 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 (Fig. 1). Dependent on the stage of the disease process, areas of necrosis and ulceration occur at and around the preputial orifice, on the adjoining lamina interna of the prepuce and frequently on the surface of the penis. 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 also are pres-

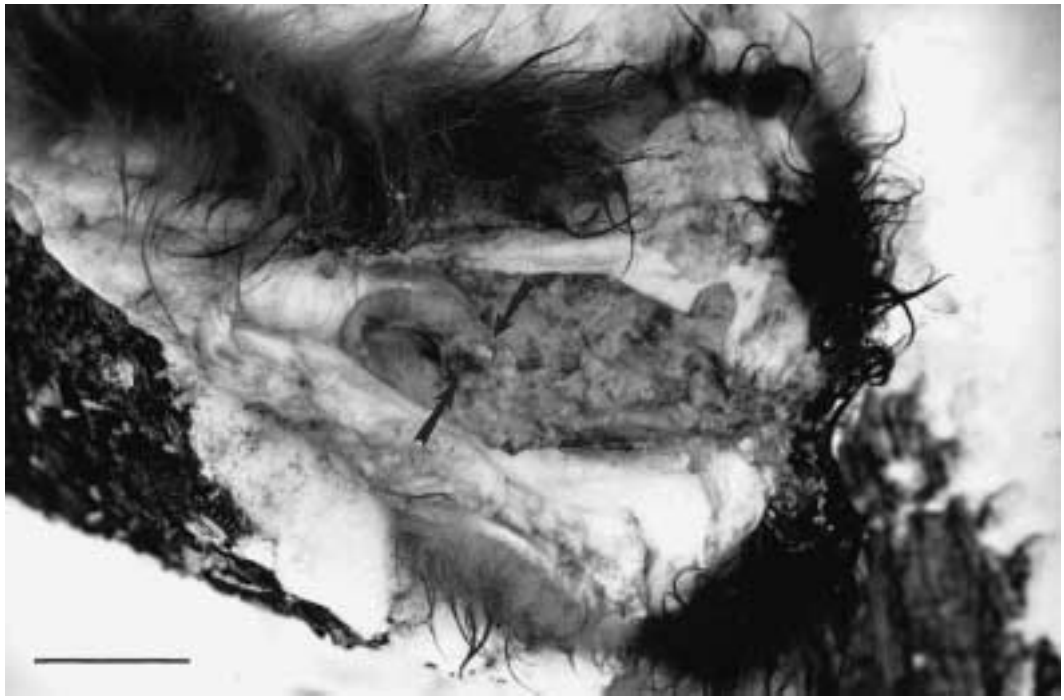

FIGURE 1. Prepuce of a bison with advanced necrotic posthitis. The lamina interna is covered with fissured (yellowish) necrotic tissue. The distal penis is amputated, the penis stump (arrows) is also necrotic. Bar = 5 cm.

ent in advanced disease cases (Piusiński et al., 1997).

The etiology of this disease is still unknown. Several types of bacteria, such as *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Pseudomonas aeruginosa*, and *Escherichia coli* have been cultured. In the 1988–89 season *Fusobacterium necrophorum* also was isolated (Kita et al., 1990). All these bacteria are believed to play a secondary role in the inflammatory process. Hitherto unknown agents or influences capable of inducing an initial inflammation of the preputial skin are assumed to act as a primary cause (Kita et al., 1994; Piusiński et al., 1997).

Our objective in this study was to further examine the character of the histologic tissue changes and the bacteria present in the necrotic tissue in order to obtain more information as to the etiology of this disease.

MATERIALS AND METHODS

The material was collected from male European bison during culling actions on the Polish side of the Białowieża Forest (52°45′N, 23°50′E) in the winter season between 1997 and 1998. Tissue samples from the prepuce and penis of two bulls (3 and 8 yr of age) with advanced lesions were taken for histopathologic examination and fixed in 10% neutral formalin within 2 hr post mortem. In addition, frozen prepuces from a 4-yr-old bull and three young males (9 mo − 1.5 yr) were obtained for examination. The prepuces from the three young males had areas of slight to moderate necrosis at the preputial orifice only. The 4-yr-old bull, however, had extensive necrotic lesions around the preputial orifice and several necrotic areas on the adjoining lamina interna. The largest was about 2–3 cm wide and 4–5 cm long. The penis was not affected in this case.

After thawing, selected tissue samples were taken and fixed in 10% neutral formalin. All samples were embedded in paraffin and 4–5 μm sections were stained with hematoxylin and eosin (H&E) stain. The following stains also were used: Ziehl-Neelsen stain (Romeis, 1989), Gram stain (Brown and Hopps, 1973), Giemsa

stain (Roulet, 1948), and the periodic acid Schiff (PAS) reaction according to McManus, 1948 (Romeis, 1989).

Selected sections from all six cases also were stained by an immunoperoxidase technique (Rabbit IgG VECTASTAIN® *Elite* ABC Kit, Vector Laboratories, Burlingame, California, USA) by using an antibody made in the laboratory against a mixture of *Fusobacterium necrophorum* strains in a rabbit. The working dilution of the antibody was 1:8,000 in pH 7.2 phosphate-buffered saline solution. A preceding treatment of the tissue sections with a 0.1% trypsin (Sigma-Chemie GmbH, Deisenhoven, Germany) solution (15 min at 37 C) was required for staining the fusobacteria. Staining was conducted according to the manufacturer's instructions. Diaminobenzidine (DAB) was used as the chromogen.

For transmission electron microscopy, selected formalin-fixed tissue samples from two bison were transferred to 3% glutaraldehyde, post-fixed with osmium tetroxide, and embedded in glycidether. Semi-thin sections were cut and stained with toluidine blue (Lynn, 1965). Ultra-thin sections stained with lead citrate and uranyl acetate (Plattner and Zingsheim, 1987) were examined with a transmission electron microscope 902 A (Carl Zeiss, Oberkochen, Germany).

For scanning electron microscopy, tissue samples from two bison were fixed in Karnovski solution, post-fixed in 2% osmium tetroxide, dehydrated with ethanol, and critical point dried with carbon dioxide in a critical point dryer (Emitech K 850, Röntgenanalytik Messtechnik GmbH, Taunusstein-Neuhof, Germany). The specimens were then sputter coated in a sputter coater (Emitech K 550, Röntgenanalytik Messtechnik GmbH, Taunusstein-Neuhof, Germany) and examined with a Zeiss 940 scanning electron microscope (Carl Zeiss, Oberkochen, Germany).

As reference material for histology, electron microscopy and immunohistochemistry paraffinated tissue samples from the oral cavity of a fallow deer (D*ama dama dama*) with necrobacillosis were used and treated as described.

Samples also were taken for bacteriology from necrotic areas of three frozen prepuces. Specimens were inoculated onto agar, blood agar, Gassner agar, and phenol-red agar (all from Institut für Immunpräparate und Nährmedien GmbH, Berlin, Germany) and incubated overnight under aerobic conditions. In addition, blood agar plates were incubated under anaerobic conditions for 4 days.

RESULTS

On histologic examination, all samples from the prepuce and penis showed similar lesions which only varied in their extent and severity. Since they did not basically differ from the descriptions given by Polish investigators (Kita et al., 1994; Piusiński et al., 1997), the primary changes will be summarized here.

The slight to moderate changes at the preputial orifice found in three young males were characterized by coagulation necrosis in the zone of transition from the haired to the hairless skin. Between the necrotic areas and the underlying healthy tissue an inflammatory cell layer of varying density was present which mainly consisted of neutrophilic leukocytes. The edge of the epithelium around the necrosis was usually edematous and showed hyperplasia to varying extent. In the necrotic haired skin, the contours of the hair follicles and sebaceous glands were still identifiable. The surface of the necrotic skin was fissured and coated with numerous, mostly round, bacteria which also occurred in the deep gaps and in the hair follicles. In addition, contours of epithelial nests were sometimes identified within the necrotic tissue.

The prepuce of the bulls with advanced lesions had extensive necrotic areas in the lamina interna of the prepuce. The surrounding tissue was more edematous, the edge of the epithelial layer at the periphery of the necrotic areas was hyperplastic and sometimes hyperkeratotic, and epithelial cells in the deep epidermis were often vacuolated. A leukocytic layer that changed to granulation tissue separated necrotic from healthy tissue. Increased fibrosis also was observed in the transition zone to healthy connective tissue in these cases. In addition, multiple lymphoid nodules of varying size occurred in the deep layers of the preputial connective tissue and beneath the more distant epithelium of the preputial skin.

Formalin-fixed material from the penis with areas of necrosis was available from two bulls. Histologically, the necrotic areas were similar to those of the prepuce. One bull additionally showed shallow erosions

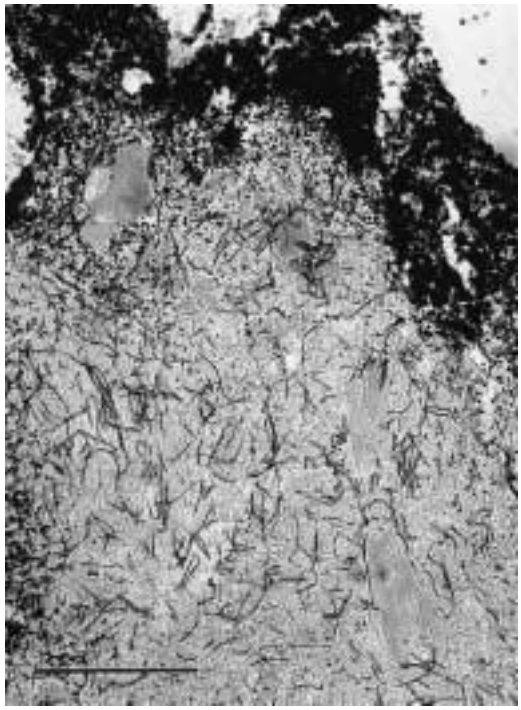

FIGURE 2. Photomicrograph of superficial necrotic preputial tissue showing numerous filamentous bacteria. The surface is coated with masses of round coccoid bacteria of unknown identity. Gram stain. Bar = 50 μm.

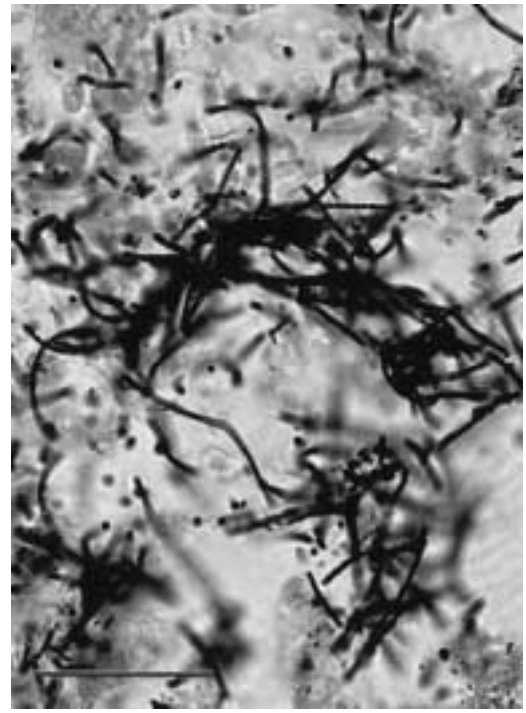

FIGURE 3. Section of superficial necrotic preputial tissue at higher magnification. Irregularly arranged bacteria resembling fusobacteria are clearly visible. Gram stain. Bar = 20 μm.

where the necrotic tissue was largely dislodged. The eroded surface of the penis was infiltrated with inflammatory cells, mainly leucocytes, and contaminated with plant particles. An increased number of lymphoid nodules was also present in the superficial and deep structures of the penis.

Special stains for bacteria showed that the necrotic tissue was infiltrated by numerous bacteria. The fissured surface of the necrotic tissue masses was usually coated with a dense layer of round coccoid bacteria which also were found in deep gaps of the necrotic tissue. In tissue sections stained with Giemsa and Gram stain, numerous slender rod-like bacteria, often in long filaments, were seen within the necrotic tissue (Fig. 2). They were present as multiple, straight or curved thin threads in an irregular distribution (Fig. 3), but were also often arranged in dense parallel strands and large clusters. The latter were usually found deep in the necrotic tissue masses. They often formed a wide front near the leukocytic layer, separated from it by a narrow necrotic zone. These single and clustered bacteria were Gram-negative while the majority of the bacteria on the fissured surfaces were Gram-positive. Among these superficial round organisms, filamentous bacteria were also often detected. These filamentous bacteria were demonstrated by the bacterial stains in the necrotic tissue masses of all six bison that were examined. Fungi were not detected with PAS stains. The Ziehl-Neelsen stain for acid-fast bacteria was also negative in all cases.

Because the morphology of the Gram-negative filamentous bacteria was suggestive of *Fusobacterium* sp., we also tried to stain these bacteria by an immunolabeling method. Suspended cultured *F. necropho-*

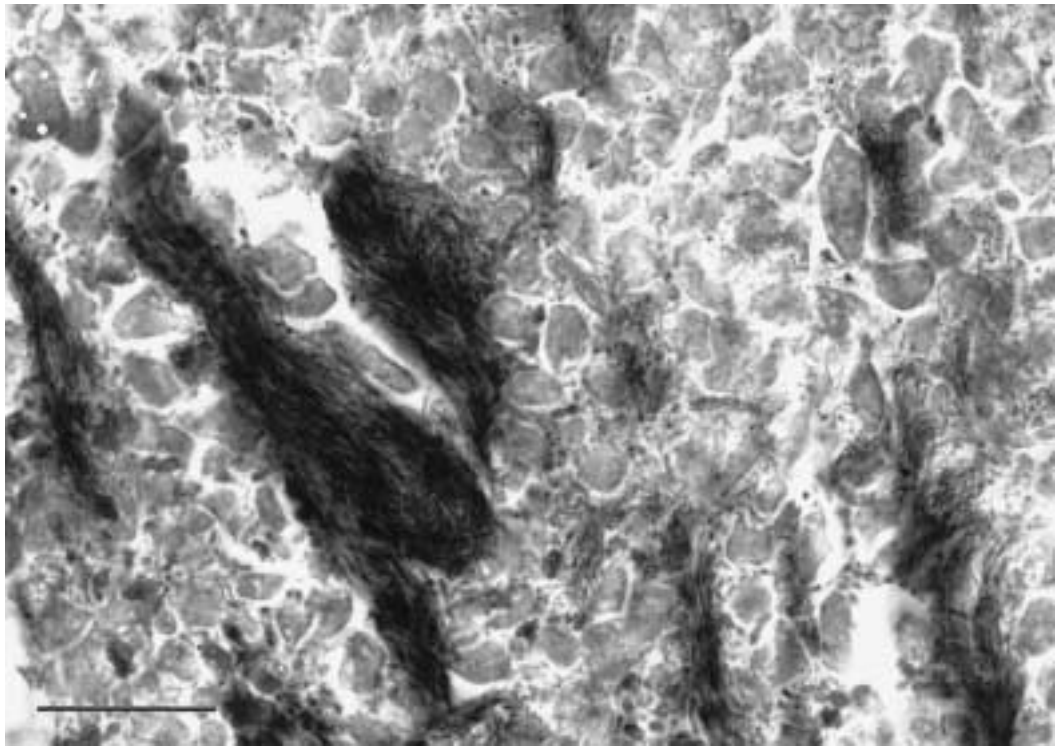

FIGURE 4. Photomicrograph showing clustered fusobacteria stained by an immunolabeling technique. Counterstain with hematoxylin. Bar = 20 μm.

rum killed with formalin, dried onto object slides and post-fixed with formalin, stained well with the prepared antibody. However, when paraffinated tissue was used, the binding of the same antibody to the filamentous bacteria was poor. Only the bacteria arranged in strands or clusters were moderately stained when a preceding trypsinization method was used (Fig. 4). At high dilutions of the primary antibody (1: 8,000 and higher) all background staining was quenched and only the clustered filamentous bacteria were stained. Weak staining of clustered filamentous organisms also was obtained in control sections from the fallow deer after preceding trypsinization.

Large numbers of filamentous bacteria were detectable by scanning electron microscopy. The long filaments displayed constrictions at varying distances which marked the individual cells (Figs. 5, 6). Due to the convoluted surface pattern, it was sometimes difficult to distinguish clearly the single cells. Flagella and pili were not detected on the bacterial surfaces, but the occurrence of blebs was a constant finding. The size of the blebs varied from 30 to 100 nm. Transmission electron microscopy revealed many rod-like bacterial cells with rounded ends in the tissue spaces. The cells had a well-defined cell wall structure with indistinct extra-cellular vesicles that occasionally surrounded the cells. The cytoplasm contained numerous electron-dense ribosomes. The connections between the cell ends were also clearly visible (Fig. 7).

On bacterial cultures, α- and β-hemolytic *Streptococcus* spp., *Corynebacterium* sp. and *Staphylococcus* sp. were isolated from three prepuces.

DISCUSSION

The most striking lesions of the prepuce and penis are more or less extended areas

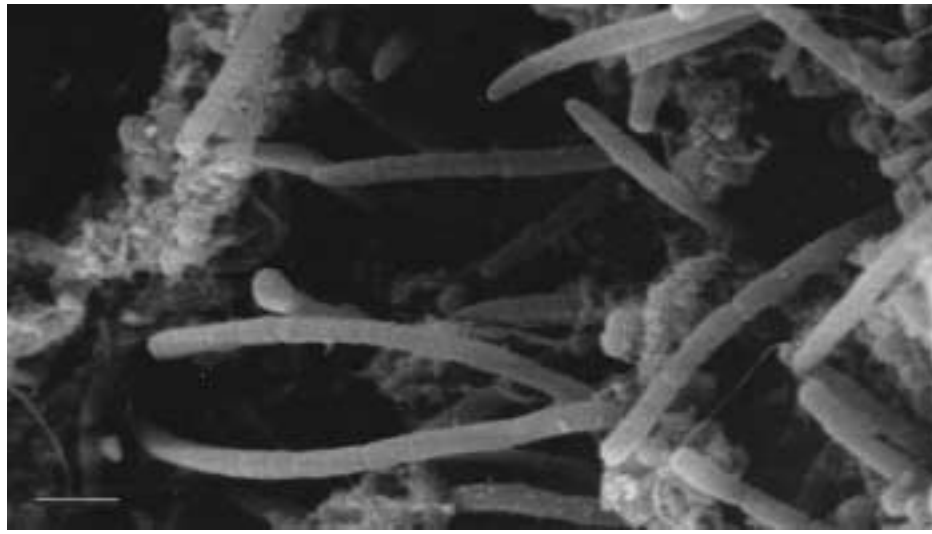

FIGURE 5. Scanning electron micrograph showing filamentous fusobacteria of varying length. Bar = 2 μm.

of tissue necrosis. The morphologic appearance of the lesions is similar to necrobacillosis which occurs in many other animal species and locations. The disease seems to be similar, if not identical, to the *F. necrophorum*-associated necrotic posthitis which was described in feedlot steers and bulls in the United States (Jensen and Mackey, 1971). There are morphological similarities also to the ulcerative ovine posthitis, commonly called sheath rot or pizzle rot. Involvement of *F. necrophorum* in this disease has been suggested, but not yet clarified. Lesions (without histologic description) were reproduced by inoculation into the ovine prepuce of a diphtheroid organism alone or in combination with *F. necrophorum*, but not of *F. necrophorum*

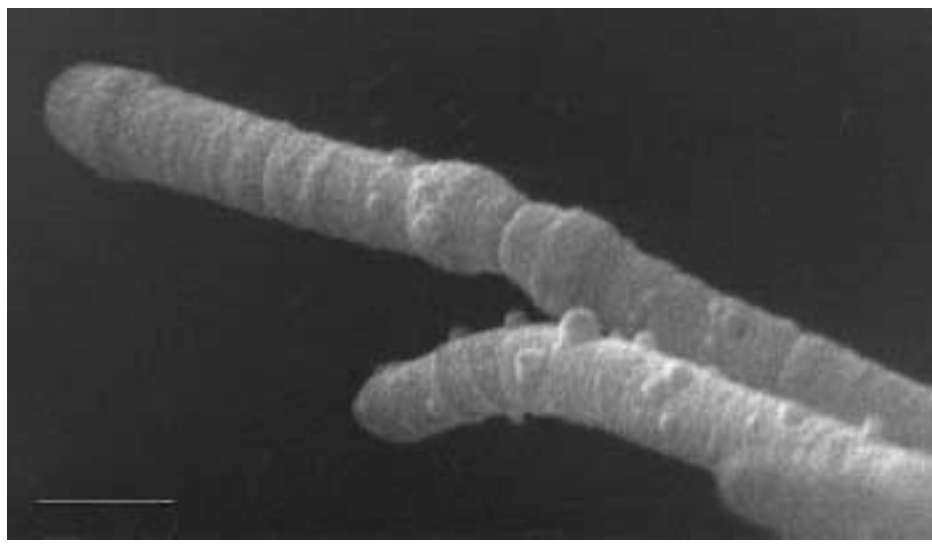

FIGURE 6. High resolution scanning electron micrograph of fusobacteria. The surface is rough and convoluted; bleb formation on the cell surface and a connection area between two cell ends are visible. Bar = 1 μm.

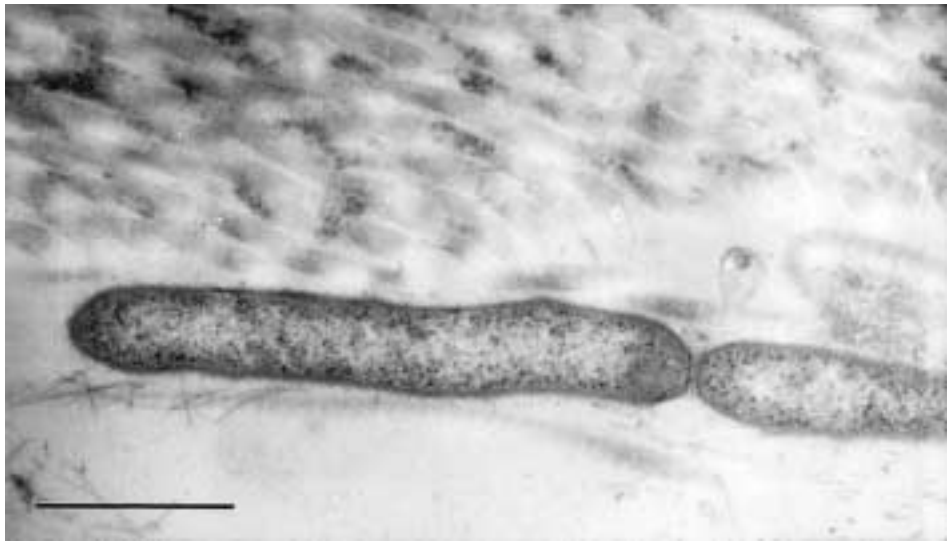

FIGURE 7. Transmission electron micrograph of two longitudinally cut fusobacteria. Numerous ribosomes are seen within the cells. The contact area between the two cells is also clearly visible. Bar = 1 μm.

alone (McMillan and Southcott, 1973). The diphtheroid agent frequently found to be associated with ovine posthitis was identified as *Corynebacterium renale* (Barajas Rojas and Biberstein, 1974).

Rod-like or filamentous Gram-negative bacteria suggestive of *Fusobacterium* sp. were demonstrated by special bacterial stains in necrotic tissue samples from all six bison examined. Staining of these bacteria by immunolabeling was difficult because this technique did not work well in paraffin sections. Weak staining only occurred after pretreatment with trypsin. The reason for it is not known, possibly bacterial capsule formation which also occurs in *F. necrophorum* (Tan et al., 1996) prevented sufficient antibody binding. The rough and convoluted surface structure including bleb formation of the bacteria as shown by high resolution scanning electron microscopy is consistent with the known ultrastructural features of *F. necrophorum* (Garcia et al., 1992). Because the material available for this initial study only included formalin-fixed tissue and frozen prepuces, *Fusobacterium* spp. could not be cultured. However, four strains of *F. necrophorum* were isolated from fresh prepuces of diseased bison obtained in February 1999 (H. Nattermann, unpubl. data).

F. necrophorum is an opportunistic pathogen. It is present in the alimentary tract of animals and humans as a normal inhabitant. The bovine rumen is considered to be an important reservoir for *Fusobacterium* spp. (Smith and Thornton, 1993a; Tan et al., 1996). The organisms can be excreted in the feces (Smith and Thornton, 1993a). Disturbance of the gastrointestinal micro-flora may lead to intestinal multiplication and increased fecal excretion (Smith and Thornton, 1993b). In this way the organisms are scattered in the environment, where under certain favorable conditions, especially in moist pastures or swamps, they can stay alive over a long period of time (Simon and Harris, 1981). Large amounts of excrement accumulates at and around sites where the bison are fed during the winter months. Enrichment of the soil with fecal bacteria, including *Fusobacterium* spp., may occur at these places.

Whether *Fusobacterium* spp. also are members of the normal flora of the preputial cavity of cattle and bison is not yet

known. Infection, however, may take place by exposure of the preputial orifice to feces, manure or moist soil contaminated with these organisms. Insects may also play a role in the transmission of fusobacteria from feces to bison and possibly also from diseased to healthy bison, a mechanism that has been suspected to play a part in the transmission of summer mastitis of cattle in Denmark (Madsen et al., 1991). It has been known for a long time that in most, if not all, cases of necrobacillosis, fusobacteria are found in association with other, usually aerobic bacterial species. A synergism between these bacteria has been suspected and shown experimentally (Smith et al., 1989, 1990, 1991; Tan et al., 1996).

Many questions remain about the pathogenesis of *Fusobacterium* infections in male bison. This includes the type and characteristics of fusobacteria present in the lesions, the kind of aerobic bacteria that may be of importance for supporting growth of fusobacteria, and the role of pre-existent lesions at the preputial orifice for initiating the infection. Another interesting question is why this infection appears only to be found in male bison. Because the bison lay down on the feedgrounds, the prepuce and penis may especially be exposed to soil and feces that may increase the chance of an infection. Without knowledge of the complex etiology and pathogenesis it will be difficult to gain control of this disease problem.

ACKNOWLEDGMENTS

The authors thank H. Nattermann for providing us with cultures of *F. necrophorum* used for the antigen preparation. Special thanks are also due to D. Rehahn, D. Viertel, I. Bockhardt, W. Schütt, M. Biering and B. Paschmionka for technical assistance. The authors also acknowledge the assistance of A. Arasim, M. Szuma, L. Szuma, and H. Zalewska from the Mammal Research Institute of the Polish Academy of Science at Białowieża.

LITERATURE CITED

BARAJAS ROJAS, J. A., AND E. L. BIBERSTEIN. 1974. The diphtheroid agent of ovine posthitis: Its relationship to *Corynebacterium renale*. Journal of Comparative Pathology 84: 301–307.

BROWN, R. C., AND H. C. HOPPS. 1973. Staining of bacteria in tissue sections: A reliable Gram stain method. American Journal of Clinical Pathology 60:234–240.

GARCIA, M. M., S. A. BECKER, B. W. BROOKS, J. N. BERG, AND S. M. FINEGOLD. 1992. Ultrastructure and molecular characterization of *Fusobacterium necrophorum* biovars. Canadian Journal of Veterinary Research 56: 318–325.

JENSEN, R., AND D. R. MACKEY. 1971. Diseases of feedlot cattle, 2nd Edition. Lea and Febiger, Philadelphia, Pennsylvania, 377 pp.

KITA, J., K. DZIĄBA, W. PIUSIŃSKI, K. ANUSZ, Z. LENARTOWICZ, B. KOWALSKI, Z. A. KRASIŃSKI, J. KRUPA, AND S. LEŚNIEWSKI. 1990. A disease of genital organs of free-roaming male European bison in the Białowieża Primeval Forest. Medycyna Weterynaryjna 46: 474–476 [In Polish with English summary.]

———, ———, ———, B. KOWALSKI, Z. LENARTOWICZ, N. KINGSTON, J. DRÓŻDŻ, M. RUTKOWSKA, Z. A. KRASIŃSKI, J. KRUPA, AND S. LEŚNIEWSKI. 1994. Preliminary studies on the diagnosis of a disease of the genital organs of male European bison in the Białowieża Forest of Poland. IUCN Species Survival Commission, Veterinary Specialist Group, Newsletter Number 8: 8–9.

KRASIŃSKA, M., Z. A. KRASIŃSKI, AND A. N. BUNEVICH. 1998. Spatial distribution of the European bison populations in the Polish and Belarussian parts of Białowieża Primeval Forest. *In* Euro-American Mammal Congress, S. Reig (ed.). Universidade de Santiago de Compostela, Santiago de Compostela, Spain, p. 331.

KRASIŃSKI, Z. A., A. N. BUNEVICH, AND M. KRASIŃSKA. 1994. Characteristics of the European bison populations in the Polish and Belarussian parts of the Białowieża Forest. Parki Narodowe i Rezerwaty Przyrody 13: 25–67 [In Polish with English summary.]

KRASOCHKO, P. A., I. A. KRASOCHKO, A. S. SHASHENKO, V. I. GAEVSKII, AND A. N. BUNEVICH. 1996. Clinical findings and immuno-biochemical changes in necrotic balanoposthitis in bison. Veterinarnaya nauka proizvodstvu, 35–45 [In Russian.]

LYNN, J. A. 1965. Rapid toluidine blue staining of Epon embedded and mounted "adjacent" sections. American Journal of Clinical Pathology 44: 57–58.

MADSEN, M., G. HOI SORENSEN, AND S. A. NIELSEN. 1991. Studies on the possible role of cattle nuisance flies, especially *Hydrotaea irritans*, in the transmission of summer mastitis in Denmark. Medical and Veterinary Entomology 5: 421–429.

MCMILLAN K. R., AND W. H. SOUTHCOTT. 1973. Ae-

tiological factors in ovine posthitis. Australian Veterinary Journal 49: 405–408.

PIUSIŃSKI, W., E. MALICKA, W. BIELECKI, B. OSIŃSKA, AND Z. LENARTOWICZ-KUBRAT. 1996. Pathomorphological lesions in bison in the Białowieża Forest. Medycyna Weterynaryjna 52: 386–388 [In Polish with English summary.]

———, W. BIELECKI, E. MALICKA, J. KITA, K. DZIĄBA, B. OSIŃSKA, K. ANUSZ, B. KOWALSKI, AND Z. LENARTOWICZ-KUBRAT. 1997. Pathomorphology and pathogenesis of diseased genital organs (prepuce and penis) of bison in the Białowieża Forest. Medycyna Weterynaryjna 53: 596–600 [In Polish with English summary.]

PLATTNER, H., AND H. P. ZINGSHEIM. 1987. Elektronenmikroskopische Methodik in der Zell- und Molekularbiologie. Gustav Fischer Verlag, Stuttgart, Germany, 335 pp.

ROULET, F. 1948. Methoden der pathologischen Histologie. Springer-Verlag, Wien, Austria, 567 pp.

ROMEIS, B. 1989. Mikroskopische Technik. 17. Auflage, Urban und Schwarzenberg, München, Wien, 697 pp.

SCHILDGER, B.-J., R. WEISS, H. FRANK, W. HERBST, AND N. KREISEL. 1996. Investigations on a reproductive disease in male European bison (*Bison bonasus*) in Belowejskaia-Puscza National Park/CIS. Proceedings of European Association of Zoo- and Wildlife Veterinarians (EAZWV) 1: 247–252.

SIMON, P. C., AND M. HARRIS. 1981. *Fusobacterium necrophorum* (*Sphaerophorus necrophorus*). *In* Handbuch der bakteriellen Infektionen bei Tieren. Band III, H. Blobel and T. Schliesser (eds.). Gustav Fischer Verlag, Jena, Germany, pp. 639–676.

SMITH, G. R., AND E. A. THORNTON. 1993a. The prevalence of *Fusobacterium necrophorum* biovar A in animal faeces. Epidemiology and Infection 110: 327–331.

———, AND ———. 1993b. Effect of disturbance of the gastrointestinal microflora on the faecal excretion of *Fusobacterium necrophorum* biovar A. Epidemiology and Infection 110: 333–337.

———, S. A. BARTON, AND L. M. WALLACE. 1991. Further observations on enhancement of the infectivity of *Fusobacterium necrophorum* by other bacteria. Epidemiology and Infection 106: 305–310.

———, D. TILL, L. M. WALLACE, AND D. E. NOAKES. 1989. Enhancement of the infectivity of *Fusobacterium necrophorum* by other bacteria. Epidemiology and Infection 102: 447–458.

———, L. M. WALLACE, AND D. E. NOAKES. 1990. Experimental observations on the pathogenesis of necrobacillosis. Epidemiology and Infection 104: 73–78.

TAN, Z. L., T. G. NAGARAJA, AND M. M. CHENGAPPA. 1996. *Fusobacterium necrophorum* infections: Virulence factors, pathogenic mechanisms and control measures. Veterinary Research Communications 20: 113–140.

Received for publication 4 January 1999.