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# SEROLOGIC SURVEY FOR BOVINE PATHOGENS IN FREE-RANGING EUROPEAN BISON FROM POLAND

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ABSTRACT: From 1980 to 1983, blood was taken from 60 selected European bison (*Bison bonasus*) in Poland. Serum samples were tested for the presence of antibodies against *Brucella abortus*, 14 serovars of *Leptospira interrogans*, *Chlamydia* spp., *Coxiella burnetii*; foot and mouth disease virus, bovine leukemia virus and bovine herpes virus-1. In addition, an attempt was made to isolate bovine herpes virus-1 from the prepuce of selected bulls. Serological tests suggested chlamydial infection in 28 bison, subclinical Q-fever of a 2-yr-old heifer, subclinical bovine leukemia virus infection in a 12-yr-old bull and bovine herpes virus-1 infection in five bulls and three cows. Attempts to isolate bovine herpes virus-1 were not successful. These results suggest the possibility of cross transmission of several of these bovine pathogens between free-ranging bison and domestic cattle in Poland.

*Key words:* Serology, *Brucella abortus*, *Leptospira interrogans*, *Chlamydia* spp., *Coxiella burnetii*; foot and mouth disease, bovine leukemia virus, bovine herpes virus-1, European bison, *Bison bonasus*, serosurvey.

## INTRODUCTION

The European bison (*Bison bonasus*) once ranged over most of Poland, but by the 11th and 12th centuries they were found only in the larger forests (Krysiak, 1967). At the beginning of the 20th century, European bison were present in only two regions—the Białowieża Primeval Forest and the Caucasua mountains. The last free-living bison in Poland was killed by poachers in April 1919.

In 1929 three bison were purchased from a zoo in Sweden and introduced to an enclosed area in Białowieża. Successful captive breeding allowed bison to be released into the forest as a free-living herd in 1952. Presently, there are over 600 European bison in Poland, of which 230 are in the Białowieża Primeval Forest and the remainder are in the Bieszczady Mountains (Krasiński, 1978; Krasińska et al., 1987). The number of bison and their distribution in Poland has allowed some hunting, but the bison are still carefully monitored in their natural environment.

Since about 1971, approximately 10% of the bison have been eliminated yearly by culling. These animals were used to assess the occurrence of certain infectious diseases which may be common to cattle. Serologic tests have been used elsewhere to study the exposure of wild animals to various pathogens (Zarnke, 1983). The purposes of our study were to test bison for antibodies against bovine viral and bacterial pathogens and evaluate their association with the occurrence of diseases in domestic cattle. During summer and autumn single bison may wander into pastures and farmed land adjacent to the forest. This creates a situation in which the bison may come into contact with organisms pathogenic for domestic cattle, or other animals, by means of naturally fertilized pastures and farmed land. Winter supplementary feeding places, where bison herds receive hay, beets and carrots from farms adjacent to the forest are another possibility of contact with these microorganisms.

## MATERIALS AND METHODS

During culling of herds from 1980 to 1983 blood was collected from 60 bison before they were killed; 19 heifers (females that had calved and were 6 mo to 3 yr of age), 14 cows (2 to 17 yr of age) and 27 bulls (6 mo to 11 yr). These animals came from two free-living herds at Bia-



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łowieża (52°45′N, 23°50′E) and Borki (53°50′N, 21°00′E) and two closed-herds at Komancza (49°20′N, 22°05′E) and Smardzewice (51°30′N, 20°00′E). Selection for culling was made during winter when animals gathered for supplementary feeding. Reasons for removal included poor condition; weak calves, especially those born after October; chronic reproductive problems, especially visible necrotic and/or purulent lesions of the prepuce and penis; advanced lameness in cows and bulls >14-yr-old; tendency to remain near human settlements and attack humans or cause property damage; and presence of abnormal anatomical features.

Serum was collected and frozen at −20 C until examination for antibodies against *Brucella abortus*, 14 serovars of *Leptospira interrogans*, *Chlamydia* spp., *Coxiella burnetii*, foot and mouth diseases virus (FMD), bovine leukemia virus (BLV), bovine herpes virus 1 (BHV-1).

The serum agglutination test (SAT), salt-2-mercaptoethanol tube agglutination test (SMTA) and the complement fixation test (CFT) described by Anczykowski (1960), Hoff and Hamblin (1974) and Alton et al. (1975), respectively, were used to detect antibodies against *B. abortus*. In SAT and SMTA the *B. abortus* agglutination suspension stained by TTC was prepared from strain S-19. In CFT unstained antigen prepared from strain S-19 of *B. abortus* was used (Królak and Błaszczyk, 1987). In both tests antigens were standardized using the second International Standard anti-*Brucella abortus* serum (ISAbS-II). The CFT is considered to be more sensitive than SAT (Mathias and Pinto, 1983).

The microscopic agglutination test (MAT) described previously by Cole et al. (1979) was used to detect antibodies against 14 serovars of *L. interrogans*. The standard serovars were obtained from the WHO Reference Laboratory of the Royal Tropical Institute, Amsterdam, The Netherlands. It has been suggested that serologic reactions in the range of 1:100 in nonvaccinated individuals represent animals with either clinical disease or residual titers to a previous infection (Shotts et al., 1970; Fournier et al., 1986).

The CFT using Antigen pour le diagnostic de l'ornithose psittacose (Nicolass Favre, Institute Pasteur, Paris, France) was used to detect antibodies against *Chlamydia* spp. Titers 1:16 or higher were considered positive (Sadowski and Truszczyński, 1974; Truszczyński and Sadowski, 1978).

The CFT and microagglutination test (MA) described by Anusz et al. (1986) and Fiset et al. (1969), respectively, using phase-II Henzerling antigen (Wytwórnia Surowic i Szczepionek, Kraków, Poland) were used for detection of antibodies against *C. burnetii*. According to the previous investigations (Behymer et al., 1986; Ciecierski et al., 1989) titres of ≥1:8 were considered positive.

CFT and seroneutralization test (SN) as described by Chemyaev and Sobko (1975) and by Smertin and Sviridov (1975), respectively, were applied to detect antibodies against foot and mouth disease.

A commercial enzyme-linked immunosorbent assay (ELISA) (Behringwerke Enzygnost BLV, Marburg, Federal Republic of Germany) was applied to detect antibodies against bovine leukemia virus (BLV). Serum samples were additionally quantitatively analyzed as positive or negative by the agar gel immunodiffusion test (AGIDT) (Kita et al., 1988). The Bovine Leucosis Antigen (Behringwerke AG, Marburg, Federal Republic of Germany) was used.

A commercial ELISA (Behringwerke Enzygnost IBR/IPV, Marburg, Germany) was applied to detect antibodies against bovine herpes virus 1 (BHV-1). Serum samples were diluted 1:20. Additionally the SN test described previously by Saxegard (1970) was used.

An attempt was made to isolate BHV-1 from the prepuce of bulls (Kita, 1978). Specimens were collected both from prepuces with visible lesions and without lesions, using sterile cotton swabs soaked in Hanks' solution with added antibiotics. The material was delivered to the laboratory without cooling and stored at −20 C until examination.

Fetal bovine kidney cell culture was used for isolation of the virus. The cell culture was carried out in Hanks' solution with 10 to 15% calf serum added. The nutritional media was changed to a maintenance media without serum prior to the infection of the cells. This culture was observed daily for cytopathologic effects.

## RESULTS AND DISCUSSION

Antibodies against *B. abortus* were present in 31 sera examined in SAT. Titres of 1:10 (20 IU/ml of serum) were found in 18 samples; 1:20 (40 IU/ml of serum) in 12 samples and 1:40 (80 IU/ml of serum) in one sample. The CFT, considered to be more sensitive than SAT (Mathias and Pinto, 1983), was negative in all animals tested. Additionally, there were no titers in SMTA. It was concluded that the SAT indicated nonspecific agglutination reactions. Such serologic reactions have been observed previously in cattle and swine (Królak and Stryszak, 1978).

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The MAT confirmed the presence of antibodies against *L. interrogans* in 35 sera. The highest titre was 1:40 in six samples that reacted against *L. interrogans* serovars *icterohaemorhagiae*, *hebdomadis*, *bataviae* and *valbuzzi*. Based on these titers we do not believe that any of the bison were infected by serovars of *L. interrogans*. Alternatively, these results of serologic tests for serovars of *L. interrogans* and *B. abortus* may reflect contact with these microorganisms or ones which produce cross-reactions (Olitzki and Godinger, 1963; Diaz et al., 1968; Fournier et al., 1986).

Anti-chlamydia antibodies were detected by CFT in 28 samples with titers of 1:16 in 25 sera and 1:32 in three sera. According to the criteria described earlier 28 bison were considered serologically positive. These may represent new reactivations of old infections, or residual titers connected with previous infections. Complement fixing antibodies are generally detectable only for a short time. Neuvonen and Pyorala, 1981, showed that maximum detectable titers are reached within 4 wk of infection and then decline over a period of a few months.

In seven sera, antibodies against *C. burnetii* were detected by CFT or MA. Of those, one 2-yr-old heifer which had an antibody titer 1:16 in the CFT showed a 1:8 titer in the MA. Based on the earlier described criteria our results indicate an infection of the 2-yr-old heifer. The MA agglutinating antibodies that react with phase II *C. burnetii* antigen are indicative of recent infection.

ELISA confirmed the presence of anti-BLV antibodies in the serum a 12-yr-old bull. This verified earlier AGIDT results. According to criteria used by Kita et al. (1988) in evaluation of BLV infections, the 12-yr-old bull positive in ELISA and AGIDT was considered subclinically infected. This case is interesting, especially since the contact of bison with areas of intensive domestic animal breeding in pastures neighboring forests occupied by bison has been observed. This positive serum sample was collected prior to the eradication of BLV infection from this area in 1985 (W. Kolacz, pers. comm.). Some authors have discussed the potential problem of transfer of BLV by biting and sucking insects (Ferrer, 1979).

The sera of five bulls (aged 9 mo and 2, 4, 8 and 9 yr) and three cows (13-, 17-, 18-yr-old) tested positive in the ELISA for BHV-1. However the SN test did not confirm the results of any of the tested samples. All bulls that were serologically positive to the ELISA, which is the method currently preferred in evaluation of BHV-1 infection (Forschner et al., 1986), had evidently advanced necrotic and purulent lesions of the prepuce and penis. However, seropositive cows did not have any visible lesions of the vulva. Attempts to isolate BHV-1 from the prepuce of the bulls were not successful.

The possibility of bison contracting BHV-1 from local cattle may be suggested by the presence of antibodies against the virus in 38% of the local cattle population in 1987, and 43% in 1988 (W. Kolacz, pers. comm.). Though we do not have information about the prevalence of antibodies against BHV-1 during the 1980 to 1983 period when the samples were collected, the percent of infected animals probably was similar. The lesions of the prepuce may be due to BHV-1 infection, but the possible contribution of other viral and bacterial agents cannot be dismissed.

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