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Bovine tuberculosis in bison (*Bison bonasus caucasicus*) located in Poland

Gruźlica bydlęca u żubrów w Polsce

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S u m m a r y

Bovine tuberculosis (BTB) is a chronic infectious disease. The etiological factor is the bovine bacillus (*Mycobacterium bovis*), which is characterized by the major pathogenicity among mycobacteria belonging to the *Mycobacterium tuberculosis* complex. It occurs mainly in cattle, but also causes tuberculosis in other livestock such as bison (*Bison bonasus caucasicus*), red deer (*Cervus elaphus*), boar (*Sus scrofa*), lynx (*Lynx lynx*), badger (*Males males*), ferret (*Mustela putorius*). Every year tuberculosis it is also recorded in animals kept in zoos and in farmed herds. The cross – contamination may occur at the common meadows and pastures. In adition, *Mycobacterium bovis* has a very high survival in the environment. This article presents three cases of bovine tuberculosis (TB) in bison (*Bison bonasus caucasicus*) located in the Bieszczady National Park (BNP) in Poland. Earlier the studies on the occurrence of tuberculosis in wild animals in this region were conducted by Żórawski and Lipiec and Welz. Among tested animals they found bovine TB in bison and in one badger. The tests used in our laboratory enabled us isolation from bison's organs and characterization of *Mycobacterium bovis* strains.

Key words: bovine tuberculosis, bison, wild animals, *Mycobacterium bovis*

S t r e s z c z e n i e

Gruźlica bydlęca jest przewlekłą chorobą zakaźną, której czynnikiem etiologicznym jest prątek bydlęcy (*Mycobacterium bovis*). Patogen ten charakteryzuje się największą patogennością wśród prątków zaliczanych do kompleksu *Mycobacterium tuberculosis*. Gruźlica bydlęca występuje głownie u bydła, ale powoduje również gruźlicę u zwierząt wolno żyjących, takich jak, żubry, jelenie, dziki, rysie, borsuki, fretki. Co roku gruźlica jest również notowana u zwierząt utrzymywanych w ogrodach zoologicznych oraz na fermach hodowlanych. Zakażeniu sprzyjają wzajemne kontakty, które zdarzają się na pastwiskach i łąkach. Ponadto *Mycobacterium* bovis cechuje się bardzo wysoką przeżywalnością w środowisku. Ten artykuł prezentuje opis trzech przypadków gruźlicy bydlęcej u żubrów w Bieszczadzkim Parku Narodowym. Wcześniej badania nad występowaniem gruźlicy u zwierząt dzikich w tym regionie były prowadzone przez Żórawskiego i Lipca oraz Weltza. Stwierdzili oni występowanie gruźlicy bydlęcej u żubrów i jednego borsuka. Testy stosowane w naszym laboratorium pozwoliły wyizolować i scharakteryzować szczepy *Mycobacterium bovis* wyodrębnione z tkanek żubrów.

Słowa kluczowe: gruźlica bydlęca, żubr, zwierzęta dzikie, *Mycobacterium bovis*

INTRODUCTION

Wild animals are the subject of interest to many research centers (1, 2, 3, 4). **The most important bacterial diseases of bison and other wild species are bovine tuberculosis, Johne's disease (paratuberculosis), yersiniosis, leptospirosis, brucellosis, pasterellosis, anthrax, salmonellosis and colibacillosis** (5). The relative importance of these disease will vary throughout the world depending on time and circumstances, nevertheless wild animals are considered one of the main reservoirs of these microorganisms (6) which may be transmitted directly and indirectly to livestock and humans. Sometimes it is difficult or even impossible to quantify a prevalence of the disease and efficiently fight with it. The poor environmental conditions, excessive density of animals, as well as more frequent occurring of cattle near nature reserves promote the cross contamination (6, 7, 8, 9). Bovine tuberculosis presents the most significant problem with respect to diagnosis, control, trade of live animals and the establishment of wildlife reservoirs of infection (5, 10). Eradication and control of the disease in livestock has been impeded in several countries by the presence of tuberculosis in wild species (11).

The similar situation exists in Poland. The Bieszczady National Park (BNP) is situated around the border

with Ukraine, which still has a problem with the eradication of bovine tuberculosis and many outbreaks of the disease. EFSA (European Food Safety Authority) publishes reports each year, which also place data on the epidemiology of BTB in Europe. Ukraine doesn't provide any information about it and it is not officially bovine TB – free (non-OTF) country in accordance with legislation (12). On the contrary according to Commission Decision 2009/342/EC Poland is regarded as officially free of bovine TB (OTF) from 2009 (12, 13).

Tuberculosis caused by *M. bovis* has a special position among the numerous infectious diseases of wildlife. Many European countries still poses a serious economic and the epidemiological problem with bovine TB (14). This disease is characterized mainly by the symptoms of general weakness and progressive emaciation. The histopathological picture is dominated by granulomatous inflammation extending from the formation of tubercles at the entry point and the surrounding lymph nodes (primary complex) (15, 16, 17, 18, 19). Lesions usually appear as firm nodules, white to yellowish in color, and of a different size (14,16). It progresses to chronic and generalized infection affecting organs and lymph nodes (14, 16, 20, 21). It may lead to formation of large tumors in parenchymal organs and caseous necrosis of lymph nodes. In some cases, the entire structure inside the lymph node capsule is covered by necrosis (20, 21).

MATERIAL AND METHODS

The first case report. In march 2009, the dead bison was found in the BNP (female, ca 7 years old). External examination showed that the animal died one month earlier. Tissues were in good condition because of low temperatures. All tissue samples from this animal (lymph nodes, lungs, pleura, liver, spleen and visceral peritoneum), obtained from a Podkarpackie Provincial Veterinary Officer, were macroscopically examined and collected for further bacteriological examination (22).

The second case report. In March 2010, the same Veterinary Officer sent organs from two bison for further testing. One carcass (female, ca 9 years old) was found in the BNP and was almost entirely eaten by wild carnivorous animals. The remains were only the head with retropharyngeal and neck lymph nodes, bones and the skin. On the place of the death only scant biological material in form of lymph nodes was macroscopically examined and collected for bacteriological examination. The second bison (male, 15 years old) was shot by sanitary services in Park and set of samples in a very good shape was delivered to the laboratory.

Bacteriology examination. Tissue samples with lesions were homogenized and decontaminated in 5% oxalic acid and then flushed twice with a 0,85% NaCl (saline), according to the Instruction of the Central Veterinary Officer (22). The sediments were used for direct microscopical examination, culture and for bioassay.

Direct microscopy. The smears were prepared from tissues as well as sediments and were stained with Ziehl – Neelsen (ZN) method and microscopically examined for acid – fast bacilli (22, 23).

Culture. The sediments were inoculated onto three Stonenbrink (S), two Petragnani (P) and one Lowenstein-Jensen (L.J) slants. All slants were incubated at 37°C for 4-6 weeks, with reading every week. Identification of mycobacteria isolates was based on growth on S, L.J slants and morphology of colony.

Bioassay. Two guinea pigs were used for each trial. The decontaminated sediment was the material for inoculation. The sediment was suspended in physiological saline, and injected intramuscularly on the medial side of the thigh in volume of 1 ml. Post-mortem examination, particularly with emphasis on the presence of characteristic tbc lesions in liver and spleen was done.

RESULTS

In all cases, both tested now and mentioned earlier, the tuberculous changes were observed in all organs sent for examination (20, 21). The pathological studies showed large tumors in liver, spleen and smaller in lungs, pleura and visceral peritoneum. In two cases, the lymph nodes were enlarged 2-3 fold in diameter and caseous yellowish content was present in the cut surface. The entire structure inside the lymph node capsule was covered by necrosis. In direct microscopy examination, acid – fast bacilli weren't seen in any preparations (imprint and smear preparations). After 5 weeks, the abundant growth of *Mycobacterium bovis* was visible only on all ninth Stonenbrinck's slants. Authopsies of laboratory animals showed lesions in the liver and spleen, typical of *Mycobacterium bovis* infection.

DISCUSSION

An occurrence of tuberculosis in big artiodactyla animals was reported in numerous papers and concerned free living and captive American bisons (*Bison bison*) in North America and African buffaloes (*Syncerus caffer*) in Africa (2, 11, 24, 25, 26, 27, 28, 29, 30). It was concluded, that most often changes were situated in lymph nodes of chest and in lungs. Relatively often changes were generalized and they concerned all internal organs (27). The mass occurrence of tuberculosis of free living buffaloes was found already in the first years of 20th century. From among 12.000 slaughtered animals tuberculous changes were found in 6450 animals, therefore exceeded 50% (31). Despite of many attempts of eradication of tuberculosis it has not been managed. In 90's the existence of disease was found in 14 from among 72 hunted animals and tuberculosis affected majority of buffalo herds in Canada as well as USA (South Carolina, New Mexico, Virginia) (32). Presently similar hard situation occurs in many herds of buffaloes, particularly in South Africa (25).

In Poland tuberculosis outbreaks in wildly living ruminants were found in 1996. The case of generalized tuberculosis in young (3 year old) bison (*Bison bonasus*) female was described. Dead female was found in round of the village Dwernik. It originated from the herd

of 30 free living animals. Anatomopathological changes, as tuberculous lesions, were found in lungs, pleura, spleen, liver and mediastinal, bronchial and mesenteric lymph nodes. That kind of generalized lesions was never observed in cattle, even in years 1959-75 during big action of cattle tuberculosis eradication in Poland (19). This case resembled described in literature cases of generalized tuberculosis at bison in North America. The source of infection of the herd was not found, but the hypothesis about capability of transmission of infection from tuberculosis ill cattle using the same pasture was proposed. It resulted from documentation showing that in years 1992-1996 in this region a few tuberculosis outbreaks were found and took place in direct neighborhood of the bison herd region. In the next years a few permissions were issued for shooting of bison in which signs of tuberculosis were observed. The aim was to restrict spreading of disease in this herd. Among 4 animals hunted in 1997, in one case (male, 8 years old) the massive tuberculous lesions in retropharyngeal lymph nodes were found. From these lesions *Mycobacterium bovis* strain was isolated (20). The cases from years 1996-1997 and presented now indicate the ability of strains for induction of quick, generalized tbc process. Taking into account all available information, it is not possible to exclude other species of wild sick tbc animals from these area, particularly badgers, as the source of infection. However there is a lack of data on occurrence of tuberculosis in this species in Poland. It should be emphasized that both in previous and present cases, the carcasses of death animals were the feeding ground of carnivorous animals. Thus it is possible that these species could be next at risk of bovine tuberculosis (21). The obtained results of the research show that attempts of eradication of the disease with the aid of planned shooting do not give satisfactory results (21). Tuberculin tests on this species are practically impossible because of the lack of standarization of tests and necessity of double anesthetizing of examined animals. In field conditions it is impossible.

In the world the examination on the limitation of *Mycobacterium bovis* infections of big ruminants or the species found as a source of infection by using vaccines are conducted (33). It is now from ethical point of view the only way of the counteraction of bovine tuberculosis spreading in wild animals. However the papers from the last years indicate the lack of vaccination efficiency using BCG vaccines (34).

There is no doubt that preventive operations should rely on rigorous control of contacts of bison and cattle freely pastured on the same area. The basic should be tuberculinization of cattle from these area more frequent than dictate obligatory regulations concerning diagnosis and eradication of bovine tuberculosis.

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