

Tuberculosis in Antelopes in a Zoo in Poland – Problem of Public Health

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Abstract

Bovine tuberculosis is an infectious disease that occurs in many species of both domestic and wild animals, as well as those held in captivity. The etiological factor is the acid resistant bacillus (*Mycobacterium bovis* or *Mycobacterium caprae*), which is characterized by the major pathogenicity among mycobacteria belonging to the *Mycobacterium tuberculosis* complex. The material from 8 antelopes from the zoo, suspected for tuberculosis were examined, and *M. bovis* strains were isolated from 6 of them. The spoligotyping method showing spoligo pattern 67676377777600. In Poland, this spoligotype has not been observed so far.

Key words: *Mycobacterium bovis*, antelopes, MTBC, public health, zoo

Mycobacterium bovis is the main cause of tuberculosis in cattle (BTB) and other bovids. It is one of the species belonging to the *Mycobacterium tuberculosis* complex (MTBC) (Rodriguez-Campos *et al.*, 2014). The first complete genome sequence of strain *M. bovis* was described in 2003 (Garnier *et al.*, 2003). *M. bovis* is characterized by natural resistance to pyrazinamide (PZA), one of the most important antibiotics used in the treatment of tuberculosis. PZA resistance phenotype may vary depending on the environment, therefore the test cannot be the basis for species identification (Krajewska *et al.*, 2013). BTB is a quite common zoonotic disease occurring in wild animals kept in zoos and wild animal parks. Tuberculosis caused by members of MTBC has been confirmed, *inter alia*, in Asian elephants (*Elephas maximus*), baboons (*Papio hamadryas*), leopards (*Panthera uncia* and *Panthera pardus*), buffalos (*Bison bonasus*), bison (*Bison bison*), red deers (*Cervus elaphus*) and a sea-lions (*Otaria byrona*) (Thorel *et al.*, 1998; Lewerin *et al.*, 2005; Schmidbauer *et al.*, 2007). This disease is characterized mainly by the symptoms of general weakness, progressive emaciation and respiratory dysfunction. The aim of the study was

to describe the case of tuberculosis in antelopes family (*Addax nasomaculatus*).

The BTB outbreak was detected in the zoo in Chorzów, in 2010. The described herd consisted of 3 males and 5 females, held in the same pavilion. The age of the animals ranged from several months to 10 years (Table I). Incidences of BTB had been reported in that zoo prior to the above mentioned outbreak. Problems with tuberculosis returned in 2010, since two male giraffes were transferred to another zoo, to avoid infection. In October 2010, field veterinarians detected clinical signs of the disease (respiratory track infection and distress) and sudden deaths of the two youngest addaxes (Table I). BTB lesions were observed in the necropsy of both of these antelopes. Lesions were changed as a visible foci of conglomerate lobar pneumonia. The tubercles (from 2 to 8 mm) were observed in the mediastinal lymph nodes with yellowish content in the cut surface. After that incident, the rest of the antelopes were tested using the official bovine PPD, according to the Polish official instruction. All 6 animals were BTB positive, showing a skin fold thickness increase of 4 or more mm and they were euthanized (Table I). The material

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Tabela I

The antelopes family (*Addax nasomaculatus*) and their kinship. Konrad, Mimi and Nora, three antelopes had died earlier – not examined.

Animal name	Data of birth/Sex	Kinship	Place of birth	Clinical symptoms	Tuberculin skin test results
1. TUSIA	2010-03-02 female	Father: Czesio Mother: Matyllda	ZOO Chorzów	respiratory problems	sudden death not tested
2. ARIEL	2010-03-13 male	F: Czesio M: Młoda	ZOO Chorzów	respiratory problems, convulsions	sudden death not tested
3. IWANKA	2000-08-11 female	unknown	ZOO Rotterdam	Zero symptoms	Positive result (4 mm)
4. CZESIO	2004-11-22 male	unknown	ZOO Prague	Zero symptoms	Positive result (4 mm)
5. KOKOLINO	2010-03-15 male	F: Czesio M: Ivanka	ZOO Chorzów	Zero symptoms	Positive result (4 mm)
6. SONIA	2000-12-30 female	F: unknown M: Ivanka (came pregnant)	ZOO Chorzów	Zero symptoms	Positive result (6 mm)
7. MATYLDA	2000-06-08 female	F: Konrad M: Mimi	ZOO Chorzów	Zero symptoms	Positive result (6 mm)
8. MŁODA	2004-05-26 female	F: Konrad M: Mimi	ZOO Chorzów	Zero symptoms	Positive result (8 mm)

for the microbiological examination was collected *post mortem* and appropriate set of lymph nodes and parenchymal organs were derived from a total number of 8. The samples were subjected to laboratory testing, using the standard procedures (Augustynowicz-Kopeć *et al.*, 2011). The GenoType Mycobacterium CM assay (Hain Lifescience, Germany) was performed for the identification of the isolated strains, as recommended by the manufacturer.

Among all the examined antelopes, tuberculous lesions were observed in the thoracic lymph nodes in 5 animals. After 6 weeks a visible growth of acid-fast bacilli in the slants with tissue material from 6 animals was found. The final identification and genotyping of the strains was based on spoligotyping analysis (Kamerbeek *et al.*, 1997). The spoligotyping method indicated that all the isolated strains belong to *M. bovis*, showing a spoligo pattern 67676377777600, which is described in an international spoligo database SpolDB4 as BOV 820. Tuberculosis is a highly contagious disease, transmitted mainly by air passages. The results confirmed the spreading of tuberculosis in a herd of antelopes.

The first case of tuberculosis in a Polish zoological garden was described in 1994 by Kalicki and Rutkowiak. Over the past 20 years, BTB infection was diagnosed in 39 animals belonging to 9 species, including: Alpaca (*Vicugna pacos*), Kob Deffasa (*Kobus ellipsiprymnus deffasa*), Elands (*Taurotragus oryx*), European bison (*Bison bonasus*), Gnu (*Connochaetes taurinus*), Reticulated giraffe (*Giraffa camelopardalis reticulata*), Sitatunga (*Tragelaphus spekei*), Tapir Anta (*Tapirus terrestris*) and Vietnamese pot-bellied pig (*Sus bucculentus*) (Pavlik *et al.*, 2002; Kalicki and Krajewska, 2011; Augustynowicz-Kopeć *et al.*, 2011). The international database SpolDB4 contains 47 strains of the spoligotype

identical with those isolated in antelopes. Among the European strains, 29 originated from Denmark, 6 from Germany, 3 from France and only one from Norway. The SpolDB4 provides information only about the number of the registered strains, reported from different countries. The database stores no information about the clinical material or whether it came from animals or humans.

In the SITVIT WEB, only the strains of 67676377777600 spoligotype, isolated in Germany, are additionally described as originated from cattle.

Only in the case of the 3 strains isolated in France their animal origin is given, however there is no information on what specific animal species they refer to (Haddad *et al.*, 2001), and even the original data presented in the paper does not make it possible to identify the animal species. For the other strains described in the published literature, there is no information indicating their source of isolation. In Poland, this spoligotype has not been observed so far. It seems that with exchange, the problem of tuberculosis returned to the Polish zoos. As regards the case of the confirmation of the infection with *M. bovis* at the zoo, the thorough disinfection of the cages were kept. All the staff should be examined as a person "of contact" by a pulmonologist. The workers of the zoo should have a tuberculin skin test, a chest X-ray and they should be interviewed carefully as regards the occurrence of subfebrile temperature, weaknesses, cough, *etc.* Also, it should be checked if any of the staff suffered from tuberculosis in the past. Perhaps, the sick human is the source of infection. In Poland, the number of tuberculosis patients is slightly lower than in the previous year. The incidence of tuberculosis in Poland is still higher than the average in the European Union countries, Norway and Iceland

(Korzeniewska-Koseła, 2014). The occurrence of the new multi-drug resistant strains complicates the already long-lasting treatment, which sometimes even leads to the death of the patient (Kruczak and Niżankowska-Mogilnicka, 2009; Kozińska *et al.*, 2011). Bovine tuberculosis in zoos, should be reported to the sanitary and epidemiological stations for the sake of public health protection. Early diagnosis in both humans and animals constitutes a very important element of success in the treatment of such dangerous a disease.

Human tuberculosis caused by *M. tuberculosis* and bovine bacillus (*M. bovis* and *M. caprae*) provide a very similar clinical picture, therefore, a well-organized and equipped microbiology laboratory is responsible for the identification of microorganisms (Augustynowicz-Kopeć and Zwolska, 2010). The main transmission route of the human infection with *M. bovis* is usually raw milk from infected cows or a direct contact with an infected animal, with people visiting zoological gardens being a good example of the latter (van der Heever, 1984; Okolo, 1992; Stone *et al.*, 2011).

The data concerning human tuberculosis caused by bovine bacillus (*M. bovis* and *M. caprae*) comes from 25 EU countries, except Greece and France (Osek and Wieczorek, 2013). In 132 confirmed cases of human tuberculosis in the European Union, the greatest numbers were observed in Germany (38 people), the UK (31 cases) and Spain (22). Thirteen EU countries did not report any bovine tuberculosis in humans. As regards the countries outside the EU, 13 cases were noted in Switzerland and 2 cases in Norway. In Poland, bovine tuberculosis in humans has not been diagnosed. The World Health Organization (WHO) estimates that in the countries where human tuberculosis is poorly supervised, 1% of the disease in humans is caused by bovine bacillus.

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