Opis przypadku *Case report*

Problems with the diagnosis of haemotropic mycoplasma – such as infection in the European wisent (*Bison bonasus*)

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Summary

A veterinarian treating wildlife must sometimes provide assistance to a sick animal based on incomplete data. Animals of special biological value are not the same as livestock. Some procedures routinely performed on domestic animals require pharmacological restraint in the veterinary service of large and dangerous wild animals. Therefore, such procedures are performed only in situations of absolute necessity. Such a situation took place in the Gdańsk zoo when a young European wisent (*Bison bonasus*) showed symptoms of haemotropic mycoplasma infection. Hemotropic mycoplasmas cause intravascular infection by attaching to red blood cells and triggering an immune response leading to elimination of the affected cells. This process can result in the development of hemolytic anemia. The case of suspected hemotropic mycoplasmosis in a European wisent described in this paper concerns an almost 2-year-old female, who lost her mother at the age of 11 months and from that time was the lowest in the hierarchy in the group of 10 wisents exposed at the zoo. The disease was manifested by emaciation, marked weakness, protrusion of the third eyelid and severe pallor of the mucous membranes. The sick animal was immobilized. Collected blood showed significant anemia (RBC 1.51 T/l, Ht 0.08 l/l), and microscopic imaging showed characteristic cells adjacent to erythrocytes suggestive of hemotropic mycoplasma infection. The animal was treated with a long-acting oxytetracycline administered three times a week, for 6 weeks. At the end of the 6-week treatment the wisent was immobilized and it was found that the nutritional status of the animal improved, the third eyelid returned to its physiological state and the pallor of the mucous membranes disappeared completely. Examination proved that the red blood cells and hematocrit reached a state that could be interpreted as physiological (RBC 4.89 T/l, Ht 0.26 l/l0, no microscopic changes of RBCs. Unfortunately, the available laboratory methods did not confirm the presence of mycoplasma genetic material. However, the efficacy of the used antibiotic therapy could be a confirmation that the disease was caused by hemotropic mycoplasmas.

Keywords: European wisent, hemotropic mycoplasma

Increasingly improved diagnostic methods facilitate the diagnosis of animal and human diseases. The use of tests based on the level of molecular biology allows for differentiation of pathogens previously completely impossible to diagnose. However, it is not always possible to complete the diagnostic process. Sometimes, for various reasons, it is not possible to identify the species of the pathogen. Particular problems can occur when diagnosing lesser-known pathogens that cause disease in wildlife. A veterinarian treating wildlife must sometimes provide assistance to a sick animal based on incomplete data. Such actions may be based only on observation of disease symptoms and results of easy to do diagnostic tests. From the point of view of highly specialized current science, such situations are often treated as not worth describing. However, they may contribute to possible future in-depth studies to expand diagnostic capabilities, especially for valuable animals belonging to endangered species. Animals of special biological value are not the same as livestock. Some procedures routinely performed on domestic animals require pharmacological restraint in the veterinary service of large and dangerous wild animals. For example, drawing blood from a cow is a relatively simple procedure commonly performed on livestock, but taking blood samples from a wisent

is usually not possible without sedatives or narcotics. Unfortunately, any anesthesia carries a potential risk of complications, including death. Therefore, such procedures are performed only in situations of absolute necessity. This considerably limits the possibility to collect the material for examination. Such a situation took place in the Gdańsk zoo when a young European wisent (*Bison bonasus*) showed symptoms of haemotropic mycoplasma infection. At that time, the material was collected only from the sick animal, other animals from the group were not examined. The measures taken could not confirm the presence of the pathogen genetic material in the blood of the treated animal.

The extent of the occurrence of haemotropic mycoplasma in wild animals in Europe is unknown. There is no available literature on the prevalence of these pathogens in wisent, but available data on cattle in Europe (4) indicate a potential likelihood that these bacteria can be also present in wisent.

Hemotropic mycoplasmas are a group of obligate epieritrocytic bacteria that cause infections in various vertebrate species (including humans). Their characteristic feature is the absence of a cell wall. Hemotropic mycoplasmas cause intravascular infection by attaching to red blood cells and triggering an immune response leading to elimination of the affected cells. This process can result in the development of hemolytic anemia (1). Infection is usually asymptomatic. Symptoms of the disease occur in immunocompromised animals and in humans (3, 2). Genetic material of hemotropic mycoplasma was found in blood-sucking arthropods, so they are suspected to be the vectors of infection (5). Because hemotropic mycoplasmosis is a zoonotic disease, people exposed to environment with infected animals, included arthropods, are most at risk (6).

Material and methods

The case of suspected hemotropic mycoplasmosis in a European wisent described in this paper concerns an almost two-year-old female, who lost her mother at the age of 11 months and from that time was the lowest in the hierarchy in the group of 10 wisents exposed at the Gdańsk zoo. Due to the position in the group, the animal had difficult access to food and was under constant social stress resulting from lack of maternal support. The disease was manifested by emaciation (Fig. 1), marked weakness, protrusion of the third eyelid (Fig. 2) and severe pallor of the mucous membranes. The sick animal, whose body weight was estimated at approximately 120 kg, was immobilized by a mixture of 400 mg xylazine and 400 mg ketamine administrated intramuscularly via a dart. The immobilized animal underwent clinical examination and blood was drawn for laboratory tests. The animal was left in isolation from the group for the duration of the treatment.

Results and discussion

Blood collected from the female showed significant anemia (RBC 1.51 T/l, Ht 0.08 l/l), and microscopic imaging showed characteristic cells adjacent to erythrocytes suggestive of hemotropic mycoplasma infection.

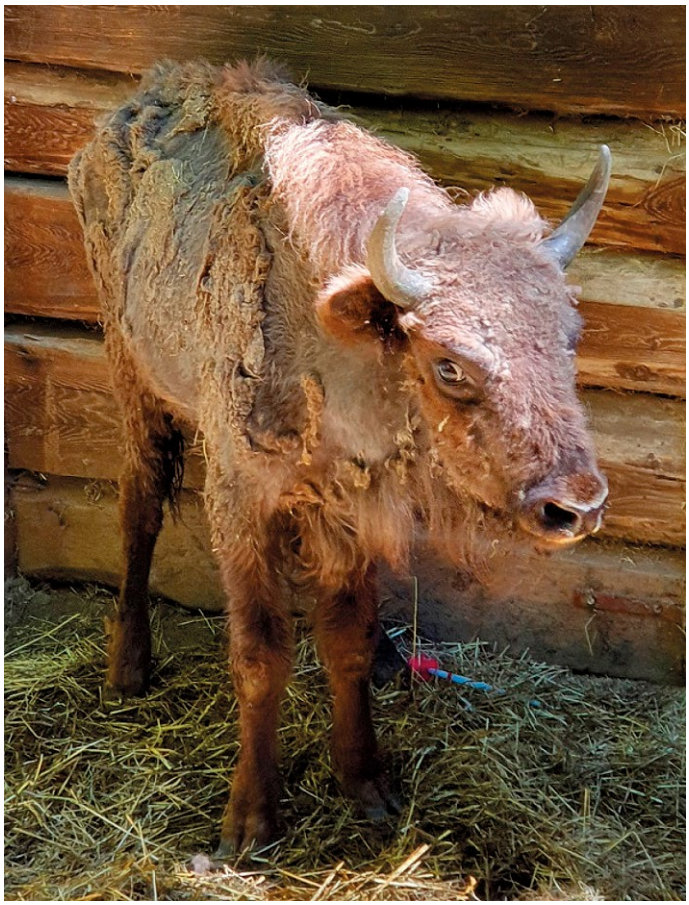

Fig. 1. Clearly visible emaciation of the two-year-old animal

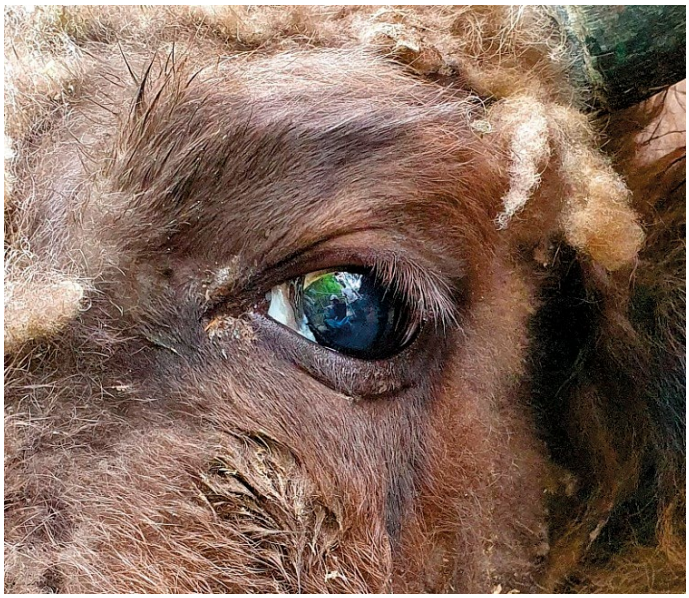

Fig. 2. Pale protruded third eyelid

Due to suspected hemotropic mycoplasma infection, the animal was treated with a long-acting oxytetracycline dihydrate (Oxytet XLA 300 mg/ml). Three times a week (every Monday, Wednesday, and Friday) for 6 weeks, 3000 mg of this drug was administered intramuscularly via a dart.

After the first week of treatment the animal was immobilized again and blood was drawn for examination. Clinical examination revealed markedly reduced protrusion of the third eyelids; however, pallor of the mucous membranes persisted. In the collected blood sample the level of hematological indices improved slightly (RBC 1.92 T/l, Ht 0.11 l/l), but the microscopic examination of red blood cells still showed a picture characteristic of hemotropic mycoplasma infection.

Tab. 1. Changes in morphotic markers of the European wisent with suspected haemotropic mycoplasmosis before, during and after Oxytetracycline treatment

	Before treatment	After the first week of treatment	After six weeks of treatment
WBC G/l	11.20	13.50	5.60
RBC T/l	1.51	1.92	4.89
Hemoglobin mmol/l	1.60	2.10	5.00
Hematocrit l/l	0.08	0.11	0.26
MCV fl	52	59	54
MCHC mmol/l	20.1	18.6	19.1
Platelts G/l	680	984	671
Presence of Mycoplasma-like structures	yes	yes	no

After completion of the six weeks of treatment with long-acting oxytetracycline, the wisent was immobilized for the last time and it was found that the nutritional status of the animal improved, the third eyelids had returned to their physiological state and the pallor of the mucous membranes disappeared completely. Examination proved that the red blood cells and hematocrit reached a state that could be interpreted as physiological (RBC 4.89 T/l, Ht 0.26 l/l), no microscopic changes of RBCs (Tab. 1).

Because hemotropic mycoplasma was suspected, the blood samples containing altered red blood cells collected the first and second time were sent to one of the German universities (where hemotropic mycoplasmas in cattle had been previously tested) for molecular diagnosis; unfortunately, the available laboratory methods did not confirm the presence of mycoplasma genetic material.

European wisents have been exhibited in Gdańsk zoo for over 60 years. The wisent enclosure is directly adjacent to a forest area covered with various species of trees and shrubs, where arthropods characteristic of the Eastern European region are found. A disease similar to the infection caused by haemotropic mycoplasmosis had never been observed before. The disease resembling infection due to hemotropic mycoplasmosis occurred in a young (21-month-old), debilitated individual who had been under social stress for a year due to the death of the calf mother.

The onset of disease symptoms in a debilitated individual is characteristic of infections caused by hemotropic mycoplasmas. The changes in the red blood cell pattern exactly matched that of cells attacked by mycoplasmas. The efficacy of the antibiotic therapy (long-acting oxytetracycline) could be a confirmation that the disease was caused by hemotropic mycoplasmas. Isolation of the affected animal from the group for several weeks was of great importance in the therapeutic process. First, it eliminated stress factors to which the individual, remaining at the lowest level of the group hierarchy, had been exposed. Second, it enabled a definite improvement in the nutritional status.

Bovine diseases caused by haemotropic mycoplasmas have been reported in Europe. Thus, it is highly probable that these pathogens can occur in wisent.

Unfortunately, the genetic material of these bacteria could not be confirmed. Due to the inability to examine the mother of the sick animal, the possibility of transplacentar transmission of the infection could not be checked. The involvement of the vector in the transmission of the pathogen could not be excluded either, as the group of European wisent in the Gdańsk zoo live in an enclosure directly adjacent to the forest. In this forest there are different species of ticks characteristic for the geographical zone of Central Europe.

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