CASE REPORT

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Mycobacterium caprae tuberculosis in a captive lion in Ukraine – Case Report

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Abstract

Introduction and Objective. Because of the armed conflict in Ukraine, companion, farm and captive wild animals have been moved in a simplified procedure across the Polish-Ukrainian border. For that reason, in 2022, Poznań Zoo provided support for almost 200 wild animals before movement to facilities in other countries. The aim of the study is to identify the under-recognized risk of moving animals that may be infected with zoonotic pathogens, such as *Mycobacterium caprae*, between countries.

Materials and Method. Sections of the heart, lungs, and mesentery of 4-year-old lioness from Ukraine were taken for histopathological and bacteriological examination.

Results. Microbiological examinations confirmed the presence of *Mycobacterium caprae*, SB0418 spoligotype.

Conclusion. TB is a zoonotic disease present globally. Movement of captive wild animals from regions with MTBC cases, or lack of MTBC surveillance, such as UA may pose a potential threat to public health.

Key words

Ukraine, zoo, TB, *Mycobacterium caprae*, SB0418, animal tuberculosis, lion, MTBC

INTRODUCTION AND OBJECTIVE

The armed conflict in Ukraine began on 24 February 2022 by the invasion of Russian Federation forces [1], which is an escalation of the war that has been ongoing since 2014 and resulted in 10.3 million inhabitants leaving Ukraine [2]. In addition, animals have also being resettled and many refugees choose to migrate with their companion animals. Due to these extraordinary circumstances, the Chief Veterinary Inspectorate in Poland introduced a simplified procedure for the non-commercial transport of animals across the Polish-Ukrainian border. Non-commercial transport, i.e. one in which refugees transport their own animals, allows up to five dogs, cats or ferrets per person (due to potential transmission of rabies). Small animals, such as hamsters, parrots, rabbits, turtles, and guinea pigs, can be transported without limits. If the animal did not have a microchip or current vaccination for rabies, it was sufficient to provide a written declaration at the border that the dog or cat would be chipped and vaccinated after reaching the final destination [3]. Later, this procedure was simplified: animals were microchipped and vaccinated at the expense of the Polish state. However, homeless dogs or cats were not allowed to cross the border under this procedure.

The war also impacted animal shelters and zoos. Hundreds of wild animals were abandoned in Ukraine. In 2022, Poznań Zoo in Poland provided support for almost 200 animals from Ukrainian zoos. Lions, tigers, bears (two brown and one Himalayan), wolves, caracals, arctic foxes, a leopard, a deer and a monkey were accepted in Poland. Most of these animals have since been moved to facilities in other countries. This was made possible through the support of many institutions and individuals. Poznań Zoo has been cooperating closely with, among others, Natalia Popova, who runs an asylum near Kyiv, as well as with the IFAW (International Fund for Animal Welfare) foundation and the Wildcat Sanctuary in the USA. During the evacuation, wild felids also arrived at

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Poznań Zoo. Four lion cubs, removed at birth, were rescued, placed in quarantine in Poznań, the capital of Greater Poland, and then moved to a sanctuary in the USA.

The aim of this study was to identify the under-recognized risk of moving animals that may be infected with zoonotic pathogens, such as *Mycobacterium caprae,* between countries.

CASE REPORT

A 4-year-old lioness (*Panthera leo*) arrived at Poznań Zoo in Poland, together with other animals rescued from a facility near Kyiv in Ukraine. At the time of the arrival of the lioness, it did not exhibit any clinical signs of illness, apart from a slim body condition. Three months after arrival, the lioness died. Before its death, the animal showed no specific symptoms other than an extremely lethargic condition. A necropsy was performed and sections of the heart, lungs, and mesentery were taken for histopathological and bacteriological examination.

Histopathology. The histopathological examination of the tissue samples from the lioness was performed at the LABOKLIN Diagnostic Laboratory in Warsaw, Poland. Tissue sections were stained using haematoxylin and eosin (HE), periodic acid-schiff (PAS) and Ziehl–Neelsen methods [4, 5].

Mycobacterial culture. A portion of the mesenteric lymph node was submitted for bacteriological examination. After decontamination with 5% oxalic acid for 30 min., the sample was plated in triplicate onto Stonebrink (S) and Petragnani (P) solid media (homemade) and incubated at 37 °C. The ingredients used to make the S and P media: eggs, sodium pyruvate (Fisher BioReagents, Geel, Belgium); monopotassium phosphate, disodium phosphate, malachite gree, potato starch (Chempur, Piekary Śląskie, Poland); crystal violet, glycerine (POCH, Gliwice, Poland) and Pepton Casitone (BD Le Pont de Claix, France). The culture growth was visually assessed weekly. The media and reagents were prepared by the Culture Media Unit of the National Veterinary Research Institute in Poland (NVRI), as previously was described by Krajewska [6].

MGIT TBcIdentification Test®. The BD MGIT TBc Identification Test (Becton Dickinson, Franklin Lakes, NJ, USA), a rapid immunochromatographic assay, was performed to determine qualitative detection antigen of mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC) [7].

Mycobacterial genotyping methods. Genomic DNA the bacterial culture was isolated based on a previously described protocol [8]. Strain genotyping was performed by spoligotyping (Ocimum Biosolutions, Hyderabad, India) using commercially available membranes according to the standard protocol (https://gentaur.es/wp-content/uploads/2015/03/Spoligotyping-Manual.pdf). The *M. tuberculosis* H37Rv and *M. bovis* BCG reference strains were used as quality controls in each run. Spoligotype shared type (ST) and phylogenetic lineage were assigned according to the SITVIT2 and Mbovis.org database [9].

Phenotypic drug resistance. Conventional drug susceptibility testing (DST) was performed using the standard 1% proportion method on a liquid medium using a Bactec MGIT 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA), following the WHO recommendations. The *M. tuberculosis* H37Rv reference strain was used as a quality control. The range and concentrations used for the specific drugs were as follows: isoniazid (INH), 0.10 μg/mL; rifampicin (RIF), 1.00 μg/mL; (ethambutol) EMB, 5.00 μg/mL; streptomycin (STR), 1.00 μg/mL; and pyrazinamide (PZA), 100 μg/mL [10].

RESULTS

General examination revealed severe emaciation with no subcutaneous fat tissue present. The lioness's lungs were pale and had scattered spots of atelectasis (Fig. 1). A large amount of straw-yellow fluid was observed in the peritoneal cavity. Extensive cauliflower-like infiltrates with creamy-yellow purulent content were visible on the mesentery of the small intestine. The mesenteric vessels were enlarged and engorged with blood. No other lesions were found.

Since the primary histopathological changes were found in the lungs, further research was focused on tuberculosis (TB). Multi-focal, extensive atelectasis and clusters of epithelioid macrophages and small lymphocytes were observed. Confluent nodular clusters of epithelioid macrophages with small mononuclear leukocytes, fewer neutrophils and sporadic fibrocytes, were also present in mesenteric adipose tissue. PAS staining was negative although Ziehl-Neelsen staining revealed acid-fast bacilli in the cytoplasm of numerous epithelioid macrophages.

Bacterial colonies were present on both Stonebrink and Petragnani media, and were described as rough, whitish colonies (Fig. 2). A sample from the colonies was used for the MGIT TBc Identification Test®, which was positive for MTBC. Further characterization by spoligotyping identified the strain as *Mycobacterium caprae* spoligotype 200003777377600, lineage BOV_4-CAPRAE 647 and corresponding with M.bovis.org database as SB0418 spoligotype. Phenotypic drug sensitivity testing showed that the isolate was susceptible to the antimicrobials tested.

DISCUSSION

During recent years, significant progress has been made in the fight against TB on a global and regional scale. Unfortunately, optimal control of the disease in the former USSR countries, especially in Ukraine and Russia, is still lacking**.** Despite some decline in human TB morbidity and mortality rates in these regions, the eastern areas of the WHO European Region still face a high burden of TB, and represent the highest burden of multidrug-resistant (MDR) TB in the world [11]. Ukraine has one of the highest global MDR TB incidence rates and incidences of latent TB infection (behind India, China and Russia) [12]. This has been attributed to overcrowded prisons, inadequate outpatient care for patients with TB, ongoing conflicts, and the growing number of HIV infections [13].

A search of the available literature revealed a lack of information on animal tuberculosis in Ukraine. The latest articles on the occurrence of animal tuberculosis in Ukraine

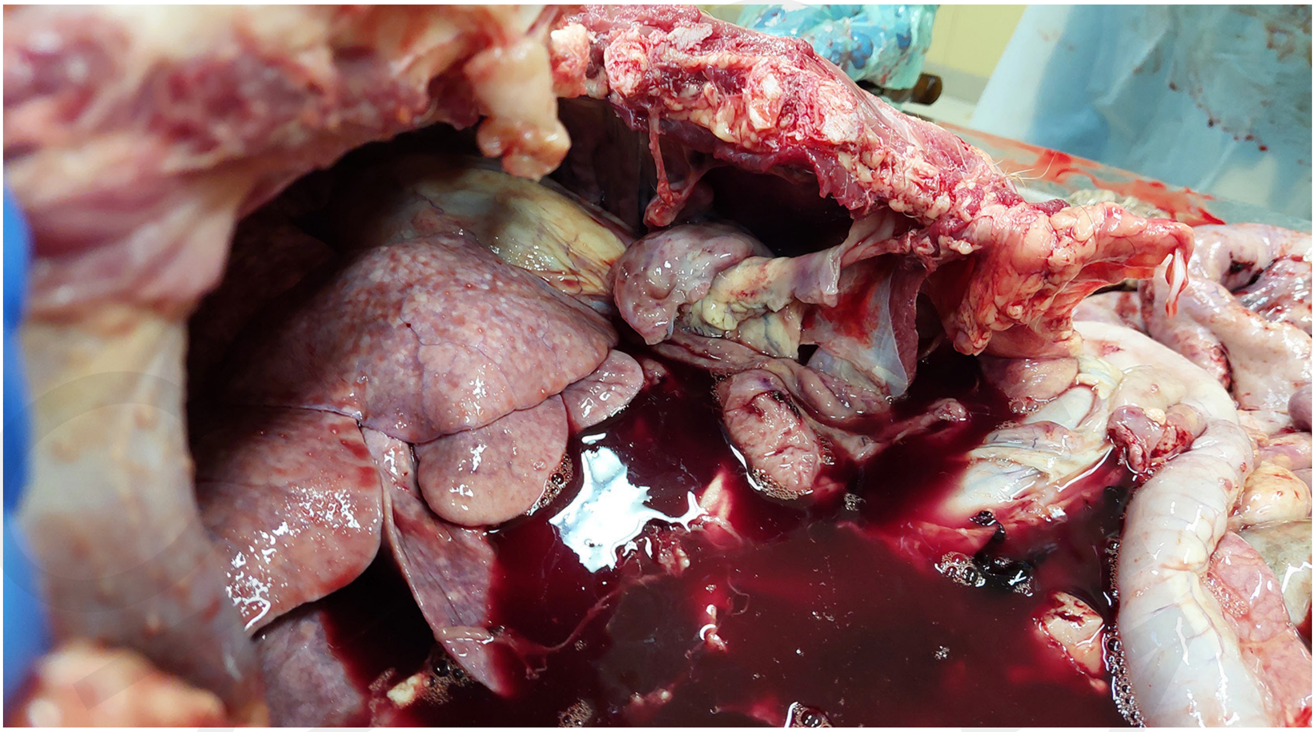

Figure 1. Pale lungs with scattered spots of atelectasis

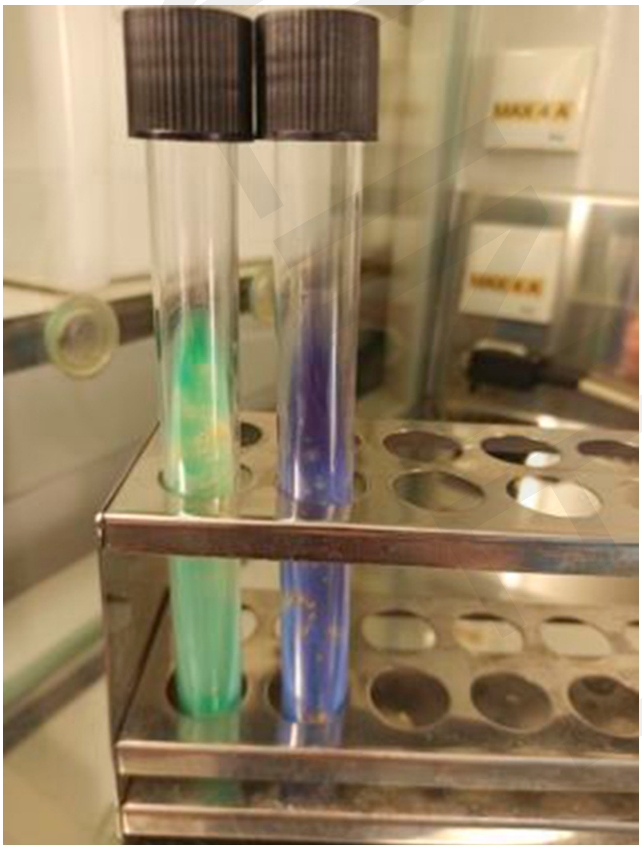

Figure 2. Mycobacterium caprae growth on Stonebrink and Petragnani media

were published in the 1990s [14]. However, in 2019, two cases of *Mycobacterium bovis* infection in cats were reported in Germany [15] and Italy [16]. These animals either came from Ukraine or had contact with animals from Ukraine, suggesting that animal tuberculosis is present in the country.

Mycobacterium caprae (one of bovine bacilli), a member of the *Mycobacterium tuberculosis* complex (MTBC), has been mainly isolated from goats (*Capra aegagrus hircus*) and domestic cattle (*Bos primigenius taurus*) [17]. There are also known cases in other animal species, such as camel (*Camelus dromedarius*) or Borneo elephant (*Elephas maximus borneensis*) [18, 19]. *M. caprae* was described for the first time in the Polish animal population in 2011 and is still reported as the causative agent of animal tuberculosis [20]. Further research showed that the reservoir of this species in Poland are free-living animals [21]. Despite the a very low incidence of human TB disease due to *Mycobacterium caprae*, its zoonotic potential poses a serious threat to public health [22, 23]. In 2022, 130 cases (0.034 cases/100,000 population) confirmed human cases of TB due to *Mycobacterium bovis* and *Mycobacterium caprae* were reported in the European Union (UE) [24]. The summary of zoonotic tuberculosis statistics relating to humans in EU between 2018–2022 is presented in Table 1.

Table 1. Summary of TB due to *Mycobacterium bovis* and *Mycobacterium caprae* statistics relating to humans, EU, 2018–2022

Humans	**2022**	**2021**	**2020**	**2019**	**2018**
No. of confirmed *M. bovis* cases	125	106	95	141	168
No. of confirmed *M. caprae* cases	5	9	4	11	13
Total*	**130**	**115**	**99**	**152**	**181**

*according with The European Union One Health 2022 Zoonoses Report [24]

So far, one case of pulmonary TB disease due to *M. caprae* in human has been described in Poland. The patient came from the southern region of the country and the source of infection in this case could not be determined [25]. Papaventsis et al. described this same kind of TB in a 65-year-old goat breeder from northern Greece [26]. Here, the *M. caprae* transmission between goats and breeder has not been confirmed. The veterinary office investigation identified no tuberculin skin test (TST) reactors among >300 goats of the epidemiologically-linked herd [26].

To the best of our knowledge, this is the first case of wild animal tuberculosis caused by *Mycobacterium caprae* from Ukraine. The SB0418 spoligotype was identified based on the presence of spacers 30–33 [27]. These was first introduced into the Mbovis.org database in 2003 by Belgians researchers, with this strain reported as being present in Central Europe [28, 29]. This strain has also been found outside Europe in the Maghgrebian countries, isolated from cattle with tuberculosis in Tunisia [27].

Based on the presence of *M. caprae* in the mesenteric lymph nodes, it suggested that the lion was infected orally. It should be emphasized, however, that mycobacteria can also enter the body through droplets. Tuberculosis resulting from ingestion of infected meat has been recognized in wild and domestic felids [15, 30, 31]. Transmission of *Mycobacterium bovis* from infected prey, such as African buffalo, to lions has been supported by genotyping [32]. Development of tuberculosis in captive wild and domestic felids has been reported to be associated with unintentional feeding *M. bovis* contaminated commercial raw diets or livestock carcasses [33, 34]. Since *M. caprae* has been isolated from cattle in Ukraine, it is hypothesized that this lion may have been infected by feeding meat from infected cattle [35]. Hence, to protect animal welfare and public health, animals kept in zoos or in a private animal collection should be tested regularly to minimize the risk of infection for humans [36, 37]. This case highlights the importance of screening domestic and wild carnivores for tuberculosis.

CONCLUSIONS

Tuberculosis is a zoonotic disease present globally. Movement of companion, farm and captive wild animals from regions with *M. tuberculosis* complex cases, or lack of MTBC surveillance, such as Ukraine, may pose a potential threat to public health. Recommendations for mandatory tuberculosis testing for all animals from Ukraine, especially those from zoos, should be implemented.

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