Artykuł przeglądowy *Review*

Bacillus anthracis infection in humans and animals

ZUZANNA CZEKAJ[1], MAŁGORZATA D. KLIMOWICZ-BODYS[2], RAFAŁ KANIAK[1], MAGDALENA FLOREK[3], KRZYSZTOF RYPUŁA[2]

[1]Student Science Club "AnthraX", Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wrocław, Poland

[2]Division of Infectious Diseases of Animals and Veterinary Administration, Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wrocław, Poland

[3]Division of Microbiology, Department of Pathology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, ul. Norwida 31, 50-375 Wrocław, Poland

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Czekaj Z., Klimowicz-Bodys M. D., Kaniak R., Florek M., Rypuła K.

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Summary

***Bacillus anthracis* is the zoonotic agent that causes the disease known as anthrax. This pathogen has always been high on the list of potential agents with respect to biological warfare and bioweapons. *B. anthracis* causes severe respiratory, and cutaneous as well as gastrointestinal infections in humans. Among animals, the group most susceptible to infection are ruminants, in which the most common route of infection is local damage to the mucous membrane of the oral cavity and/or gastrointestinal tract, less often the development of a widespread disease as a result of inhalation of spores. This review describes various features associated with pathogenicity-related factors of *B. anthracis*, pathogenesis of anthrax, human and animal forms of the disease, and the possibility of preventing and treating the disease. The article also mentions the potential use of the bacteria for bioterrorism as a biological weapon.**

Keywords: *Bacillus anthracis*, pathogenesis, anthrax, treatment and prophylaxis

Bacillus anthracis is an obligate pathogen and a dreadful, fatal zoonotic causative agent of disease that was known in antiquity by the name of the pustular disease. Currently this disease has many names, including charbon, woolsorters' disease, ragpickers' disease, malignant carbuncle, malignant pustule and Siberian ulceror, and the most recognisable anthrax. *B. anthracis* belongs to the phylum *Firmicutes*, the family *Bacillaceae*, the genus *Bacillus*, and the *Bacillus cereus* group (45, 51). It is a Gram-positive rod-shaped, immobile, aerobic, or facultatively anaerobic bacterium. The bacilli are large (1-1.5 µm wide by 3-8 µm in length) and occur singly or in short chains that resemble bamboo sticks (21).

An essential biological feature of *B. anthracis* is the ability to occur in two morphologically and physiologically distinct forms, as a dormant spore and as an actively proliferating vegetative cell. Spores are infectious agents for both animals and humans. The ability to form the spores allows the bacteria to exist for many years in a resisting state in unfavourable environmental conditions (21). Due to the long-dormant spore periods, this pathogen evolves very slowly and is exceptionally genetically and phenotypically homogeneous. This bacterium replicates during short periods of infection, which are terminated by host death or by clearance of the bacteria by the immune system or proper and effective therapy (51).

B. anthracis has been identified by The Centers for Disease Control and Prevention (CDC) as a Category A agent, a high-priority organism that poses a risk to national security (https://emergency.cdc.gov/agent/agentlist-category.asp). Naturally occurring anthrax infections occur mainly in herbivores after ingestion of spore-contaminated feed or soil. The disease can be transmitted from animal to animal or from animal to human. There is no scientific evidence that anthrax is transmitted from human to human, but it's possible that anthrax skin lesions may play the role in spreading the infection by direct or indirect contact with lesions (32, https://apps.who.int/iris/handle/10665/97503). Notably, *B. anthracis* is also a bioterrorism agent, as has been proven by several episodes of intentional or accidental spore release (31, 45).

Historically, at the turn of the 19th and 20th centuries, anthrax was categorised as an infectious disease with

very high mortality rates in wildlife and livestock. The most significant outbreak is thought to have occurred in South Africa in 1923, claiming between 30,000 and 60,000 animals (21). Subsequently, the significance of the disease declined as a result of the development of a vaccine, effective treatment protocols and the introduction of anthrax control and prevention programmes. Despite considerable efforts to prevent *B. anthracis* infections, this bacterium still poses a significant epidemic threat. Today, anthrax cases are reported worldwide; however, in highly industrialised countries, such as the United States of America or Great Britain, low numbers of cases in livestock with sporadic human cases are observed. Anthrax may be an acute and often fatal endemic disease in agricultural regions of Central and South America, sub-Saharan Africa, central and Southwestern Asia, southern and eastern Europe and the Caribbean (4, https://www.cdc.gov/anthrax/basics/index.html). In certain countries, this disease is referred to as re-emerging disease caused by an unfavourable change in living conditions and standards, a breakdown in veterinary services, or political unrest.

It has been reported that the disease occurs most frequently in areas with neutral or alkaline, calcareous soils (36). Countries with a clear division between the dry and rainy seasons show a higher incidence of anthrax during the dry season, especially towards the end of the season, when there is excessive congregation of animals at a single watering hole or grazing closer to the soil when grasses are short and thinned (https://www.ncbi.nlm.nih.gov/books/NBK507773/). It is worth noting that the incidence of anthrax, particularly in developing countries, is probably underestimated, as the symptoms of anthrax were sometimes confused with those accompanying malaria, typhoid fever or diarrhoea in humans or tick-borne diseases in animals. In these situations, steps were not taken to limit the spread of the infection and the disease remained out of control (36). According to the World Health Organisation (WHO), there are between 2,000 and 20,000 cases of the disease worldwide each year (https://www.who.int/publications/i/item/9789241547536). The most recent outbreak of anthrax in humans and animals occurred in Zambia, in nine out of ten country provinces. The first human cases were reported in early May 2023. During the same period, domestic animals (cattle and goats) and wild animals (hippos) died of unknown causes in the area. Further cases in humans and farm animals were reported in June. A total of 26 people were hospitalized at that time. The probable cause of the disease was the consumption of meat from hippos infected with *B. anthracis*. As of November, there have been 684 suspected human cases, including four deaths (https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON497). An anthrax outbreak was also confirmed in November 2023 in Uganda. The disease most likely appeared in humans in June, after consuming the meat of a dead cow. By December, 25 suspected cases, 7 probable cases and 16 confirmed cases were reported, including 13 fatalities, and 3 recoveries. The number of dead animals was 50, although there is no information on the number of sick or suspicious animals (https://reliefweb.int/report/uganda/uganda-anthrax-outbreak-dref-operation-mdrug049). In Poland, anthrax last occurred in 2009.

Table 1 shows the anthrax cases in animals worldwide that have been reported in the WAHIS (World Animal Health Information System), from 01.01. 2023 to 04.03.2024 (https://wahis.woah.org/#/event-management).

This article aims to provide an overview of current information on the morphology, virulence, and pathogenicity of *B. anthracis* and the possibility of prophylaxis and antimicrobial therapy for infection caused by this bacterium.

Pathogenicity-related characteristics of *B. anthracis*

S-layer and capsule. The cell envelope of *B. anthracis* has a complex architecture and dynamic composition. The surface of these bacilli is unique due to the presence of two structures that have not been found in many other bacteria, such as an S-layer and a capsule (12, 22).

Outside the cell wall is a surface layer (S-layer), a proteinaceous paracrystalline sheath composed of surface array protein (Sap) and extractable antigen 1 (EA-1) with *B. anthracis* S-layer associated proteins (BSLs). Sap is the main component of the S-layer associated with the exponential phase of growth of the bacillus. This protein is gradually displaced by EA-1, which is the main S-layer protein (SLP), forming the stationary phase S-layer. Sap and EA-1 are both major surface antigens. The presence of an S-layer is not required for normal capsulation of bacilli. This suggests that the capsule is anchored to the cell wall's murein independently of the S-layer proteins. However, the S-layer may modify the fine structure of the capsule (12, 22). The S-layer plays multiple functions, such as protective and selective coat and an adhesive surface (24, 60).

The outermost component of a cell envelope of a fully virulent *B. anthracis* is a plasmid-encoded capsule consisting of an anionic polymer of γ-linked D-glutamic acid residues (PDGA) covering the S-layer. The size of the polyglutamine chains differs depending on the growth conditions of the bacilli. The capsule is covalently linked to the peptidoglycan of the cell wall (47) and its synthesis depends on environmental factors that mimic the mammalian host (11, 12, 22, 63). The capsule expression is activated and modulated by CO_2 presence, bicarbonate fluctuations and, which is obvious, transcription factors (12). The capsule is very weakly immunogenic. The fully encapsulated wild-type Ames strain reduces the release of cytokines such as IL-10, IL-23 and TNF-α, which is a very

Tab. 1. Anthrax cases in animals worldwide reported in the WAHIS system from 01.01.2023 to 04.03.2024

Country	Location	Date of last occurence	Date of present event	Source of event or origin of infection	Species	Numer of animals					
						susceptible	case	death	killed and dissposed of	slaughtered/killed for commercial use	vaccinated
Kyrgyzstan[1]	Kara-Suu	–	03.01.23	Unknown or inconclusive	Cattle	1	1	1	0	0	150
Kazakhstan[1]	Tamdy	–	01.06.23	Unknown or inconclusive	Cattle	8554	1	1	–	–	8554
Spain[2]	Cangas de Onis	17.12.22	14.06.23	Contaminated pastures	Cattle	61	8	8	–	–	53
Nigeria[2]*	Gajiri, Suleja LGA	31.12.04	27.06.23	Introduction of new live animals	Cattle	100	20	13	0	7	0
					Sheep	73	15	10	0	0	0
	Marina		16.07.23		Cattle	10	0	0	0	0	0
					Sheep	14	9	9	0	0	0
					Goats	4	0	0	0	0	0
					Camelidae	1	1	0	0	0	0
	Oko Oba		18.07.23		Cattle	999	3	0	3	0	0
Spain[2]	Cangas de Onis	14.06.23	26.06.23	Contaminated pastures	Cattle	65	7	7	–	–	59
Zambia[1]*	Kanchindu and Siameja Veterinary Camp	–	30.06.23	Contact with wild species	Cattle	30 000	10	10	0	0	0
					Goats	35 000	3	3	0	0	0
					Hippopotamus (wild)	3	3	3	0	0	0
	Nalolo District		31.07.23		Cattle	150	17	15	2	0	0
	Limulunga		04.08.23		Cattle	200	2	2	0	0	0
	Kazungula District		16.09.23		Cattle	33 000	110	110	0	0	0
Kyrgyzstan[2]*	Kaarman	05.01.23	31.07.23	Unknown or inconclusive	Cattle	80	1	1	0	0	130
Romania[2]	Tiganasi	20.12.22	17.07.23	Unknown or inconclusive	Cattle	26	0	0	0	0	26
					Goats	83	1	0	0	1	83
					Sheep	585	0	0	0	0	585
	Ungheni		21.08.23		Cattle	4	1	0	0	1	3
					Goats	1	0	0	0	0	1
Kazakhstan[2]	Ushkarasu	02.09.19	31.08.23	Unknown or inconclusive	Cattle	1075	23	23	–	–	1075
					Sheep/Goats	778	–	–	–	–	778
					Equidae	233	–	–	–	–	233
					Swine	16	–	–	–	–	16
Azerbaijan[2]	Aligulular	07.08.22	04.09.23	Unknown or inconclusive	Sheep	471	4	4	0	0	467
Italy[2]	Recigliano	23.11.20	12.10.23	Unknown or inconclusive	Sheep	120	5	1	–	–	–
Kazakhstan[2]	Topar	19.09.16	24.10.23	Unknown or inconclusive	Cattle	546	1	1	–	–	546
					Sheep/Goats	1751	–	–	–	–	1751
					Equidae	132	–	–	–	–	132
					Swine	61	–	–	–	–	61
Mozambique[1]*	Vanduzi-Sede	–	25.02.24	Contaminated pastures	Cattle	233	18	6	0	0	–
Total						114 430	264	228	5	9	14 703

Explanations: [1] – first occurrence in a zone or a compartment; [2] – recurrence of an eradicated disease; * – during eradication (on-going)

effective way to inhibit the adaptive immune response (30). Recent studies have shown that encapsulation protects *B. anthracis* from phagocytosis and inhibits dendric cell maturation by shielding pro-inflammatory components on the bacillus surface (30). Therefore, the capsule is essential for virulence and is a primary mechanism of reducing immune cell invasion and mediates the invasive stage of the infection (53, 56,

65). It is found in animals infected with the a1 virulent strain, and represents one of the major virulence factors (18, 42).

Toxins. The vegetative form of *B. anthracis* produces and secretes three main virulence factors encoded by two plasmids: pXO1 (containing genes encoding three anthrax exotoxins which are: the oedema factor, EF; the lethal factor, LF; and the protective antigen, PA) and pXO2 (with genes encoding the biosynthesis and formation of the poly-γ-d-glutamic acid capsule) (21, 53). PA, LF, and EF individually are non-toxic. PA is the cellular binding moiety, while LF and EF are the catalytic moieties of the toxins: lethal toxin (LT) and oedema toxin (ET), respectively (39, 42). LF is a zinc-dependent metalloprotease that cleaves and inactivates mitogen-activated protein kinases (MAPKK or MKK/MEK). At the same time, EF is a highly efficient calmodulin (CaM)-dependent adenylyl cyclase that converts ATP to cAMP in the cytoplasm of host cells (23, 39, 50).

PA binds to its cellular receptors, mainly anthrax toxin receptor 1 (ANTXR1), tumour endothelial marker-8 (TEM-8), anthrax toxin receptor 2 (ANTXR2) or capillary morphogenesis protein-2 (CMG-2), and to a lesser extent LDL receptor protein-6 (LRP-6) and integrin β1 (66). After binding, PA is proteolytically processed by furin proteases, forming a competent oligomeric structure (heptamer or octamer). Each heptamer or octamer binds 3 or 4 molecules EF and/or LF, respectively (70). The formed complexes PA/LF and/or PA/EF are internalised through a receptor-mediated endocytic pathway. In host cell endosomes, low pH induces the conversion of the PA oligomer prepore to a protein-conducting channel through which LF and EF are translocated into the cytoplasm to exert their toxic effect (39, 66). In endosomes, toxin complexes can be translocated to intraluminal vesicles (ILVs), where LF and EF are sequestered (14, 70). In this case, two significant advantages are observed; first, in the ILV lumen, exotoxins are protected from lysosomal enzymes, and second, by this route, exotoxins can escape outside the cell as an exosome (with the help of Rab11 and Rab35 GTPases) to then enter the next cell without using a receptor, which does not stimulate the immune system. Host death is mainly the result of bacterial sepsis and toxaemia, caused by a high concentration of toxins in the blood, affecting multiple organs (23, 27).

Small amounts of ET and/or LT are produced, making them difficult to detect, but their effects on the body are biochemically amplified, making even small amounts dangerous (66). Liu et al. (39) emphasise that LT and ET cause cell and tissue damage at late stages of infection, and also play a significant role in the impairment of the innate immune response in the early stage of infection. After germination, the toxins paralyse the immune response, targeting macrophages, neutrophils, and other myeloid cells, thus paving the way for bacterial replication. The lethal toxin (LT) disrupts crucial signalling pathways for regulating the host cell cycle, proliferation, and cellular stress defence (25). In rats, LT caused progressive hypotension, and haemoconcentration (15), while in canines, this toxin caused progressive shock associated with reduced central venous pressure that persisted in non-survivors and was associated with lactic acidosis (61). *In vitro*, lethal toxin disrupts endothelial cell function by stimulating apoptosis of these cells and alterations in actin fibres, and by activating mast cells. It is believed that the lethal toxin may affect the function of the heart muscle by reducing the function of cardiomyocytes and may alter peripheral vasculature function (62). The oedema toxin (ET) leads to the loss of chloride ions and water from the cell and causes intense oedema in surrounding tissue associated with anthrax disease (25). In canines, ET causes vasodilation leading to a reduction of central venous arterial and blood pressure, increased urine output, and hyponatremia (62).

Some authors consider AtxA (anthrax toxin activator) to be one of the main virulence factors of *B. anthracis* alongside toxins and capsular polypeptides (8). AtxA, a pXO1-encoded protein, activates the transcription of toxin and capsule genes in particular conditions such as the temperature of 37°C, and the presence of CO_2 and bicarbonate (44, 49). AtxA regulates the expression of three toxins genes: *pagA* (protective antigen), *cya* (oedema factor), and *lef* (lethal factor) as well as the capsule biosynthetic operon – *capBCADE*. Moreover, this regulatory protein also affects a large number of different genes on the chromosome and plasmids. For this reason, AtxA is named a global or a master regulator (6). An additional function of AtxA is to modulate the sporulation of *B. anthracis* by controlling *skiA* (*pXO2-0075*) gene transcript levels (69). An increased level of AtxA protein has been observed to lead to increased *skiA* transcription, which results in delayed and decreased sporulation. However, *skiA* is able to impact atxA expression. Dale et al. (17) suggested that elevated expression of a known AtxA-regulated sporulation inhibitor, *skiA*, is a mechanism developed by *B. anthracis* to dampen premature sporulation during host infection.

B. anthracis also secretes a toxin of the cholesterol-dependent cytolysin (CDC) family, anthrolysin O (ALO). This haemolysin is lethal to human primary polymorpho-nuclear leukocytes (PMNs), monocytes, monocyte-derived macrophages (MDMs), lymphocytes, and many others. Moreover, it exhibits lytic activity against phagocytes, reduces the barrier function of human polarised epithelial cells, and may be one of the tools used by *B. anthracis* to destabilise and break the membrane of phagosomes (5, 47, 65). Other enzymes induced under strictly anaerobic conditions and expressed in the early stages of infection within macrophages are three putative phospholipases C which are phosphatidylcholine-specific (PC-PLC),

a sphingomyelinase (SMase), and phosphatidylinositol-specific PLC (PI-PLC) (33).

Pathogenesis. In the case of aspiration of *B. anthracis* spores into the lungs, they disseminate from the alveoli to the regional lymph nodes and then into the bloodstream, contributing to the development of septicaemia in the later stage of the disease. Four hypothetical models of spore escape from the alveoli have been proposed. The first two are named the Trojan horse model, mainly concerning human respiratory infections. Spores are phagocytosed by alveolar macrophages or dendritic cells (Trojan horse), followed by spore germination in the phagolysosome (50). Phagocytes transport the spores into the tracheobronchial and mediastinal lymph nodes, where germination occurs, followed by the expression of the genes encoding virulence factors such as AtxA, LF, EF, and PA as well as the formation of a capsule (5, 47). In order to cause systemic infection, bacilli escape from phagocytes and replicate extracellularly. Banks et al. (2) demonstrated that the expression of the anthrax toxin receptor (ANTXR) in macrophages contributes significantly to the ability of germinated *B. anthracis* spores to kill macrophages from within efficiently. The ANTXR is a critical component of macrophage killing. However, other virulence factors also likely influence macrophage killing by phagocytosed anthrax spores. Although the Trojan horse model is the best-defined pattern of dissemination of *B. anthracis*, this model is still being redefined and re-evaluated (https://www.ncbi.nlm.nih.gov/books/NBK507773). The third hypothetical model assumes that spores do not need carrier cells, but rather they are transported from the apex to the basolateral side of the alveolar epithelium. This transport is transcellular. Germination begins after the spores have reached the lymph nodes via lymphatic vessels (50). The fourth hypothetical model suggests that some spores germinate within the alveoli and, as vegetative forms, begin to produce virulence factors that help to subdue the innate immune response in the alveoli and break the epithelial barrier, allowing both spores and vegetative forms to escape from the alveoli and access the lymphatics. This mechanism has been called a 'jail-break' model (50).

The 'jail-break' model was also introduced to describe pathogenesis in cutaneous and gastrointestinal anthrax infections in humans. In the cutaneous form, spores enter into the skin at the site, and its continuity is broken (also due to the attack by insects such as mosquitoes or *Stomoxys calcitrans* flies that have ingested anthrax-infected blood) (21). Gastrointestinal (and oropharyngeal) anthrax results from the consumption of spore-contaminated feed, water, or food (48). The vegetative bacilli are able to grow at the spore entry site itself (Peyer's patches or dermis in the gastrointestinal or dermal form of the disease, respectively). The jail-break model suggests that spores may germinate at the initial entry site, such as nasopharyngeal-associated lymphoid tissue (NALT) or/and gut-associated lymphoid tissues (GALT), and start producing virulence factors. Continuous growth increases the concentration of exotoxins and proteases, leading to action to dampen the immune response and damage to the epithelial/endothelial lining, effectively allowing vegetative bacilli to drain into the regional lymph node. Once such a lymph node becomes overwhelmed with exotoxins and bacteria emanating from the initial site of infection, vegetative bacteria gain access to the bloodstream without the help of phagocytic transport. The bacteria disseminate via a haematological route to various target tissues and organs (mainly lungs, kidneys, and spleen, and sometimes to the brain causing meningitis), which are typically highly vascularized as the bacteria are also present in the lymph nodes, continuing to replicate and constantly releasing virulence factors, causing bacteraemia, and eventually leading to host death. When bacilli enter the blood, they multiply, causing vascular damage resulting in petechia, haemorrhages, and plasma infiltration into tissues (https://www.ncbi.nlm.nih.gov/books/NBK507773).

Anthrax in humans

The most severe type of anthrax in humans is inhalation anthrax, also called the pulmonary form of anthrax. Inhalation disease has the highest mortality rate (45% with aggressive antibiotic treatment and approximately 100% without); however, it is a very rare form of anthrax (< 5%). This type of disease occurs when a person has breathed in the spores of the bacteria. Typically, the infection develops within a week (1 to 6 days) after exposure. However, sometimes the incubation period is up to 60 days with a non-specific prodromal phase, including symptoms such as fever, sweats, nausea, vomiting, malaise, chest pain, or non-productive cough. In the next stage of disease development, haemorrhagic lymphadenitis and mediastinitis occur, and progression to bacteraemia results from bacterial replication in the mediastinal lymph nodes. During this period, fever, dyspnoea, and stridor are observed due to increased lymphadenopathy in the respiratory tract, and eventually, respiratory failure and hemodynamic collapse occur. In almost 50% of cases, meningitis develops, manifesting as headache, disorientation, and coma. The time from the onset of the disease to death is 1 to 10 days (48, https://www.cdc.gov/anthrax/basics/index.html).

The second form is gastrointestinal (intestinal and oropharyngeal) anthrax, which has a lower mortality rate than the inhalational form of infection (40% with proper treatment and 50% of patients with oropharyngeal disease) and is relatively rare (< 5% of anthrax cases). The incubation period is usually several days (1 to 5 days). In oropharyngeal anthrax, spores settle in the throat area and cause ulcers. The disease is accompanied by symptoms such as fever and swelling of the neck. In the intestinal form, spores deposit

and cause ulcerative lesions in the small and/or large intestines. Patients often report non-specific gastrointestinal symptoms (lack of appetite, nausea, vomiting, abdominal pain, or diarrhoea), while more severe cases develop fever, ascites, increased abdominal circumference, and shock. Furthermore, this form can lead to ascites or intestinal perforation, resulting in death within 2-3 days (14, 47).

Cutaneous anthrax is the least severe (mortality < 1% with antibiotic treatment and 20% without) and the most common form of the disease in humans (> 95%). The incubation period is 1 to 12 days. In many cases, the disease affects the skin of the head, neck, or limbs and can take two forms. The first focal form is the so-called black pustule (*pustula maligna*), i.e. a vesicle filled with dark, transparent fluid that, with time, bursts leaving a black scab around which swelling and numerous purple vesicles can be observed. The second diffuse form is the so-called malignant oedema (*oedema malignum*), forming a non-painful but hard and deep inflammatory infiltrate. Both forms are accompanied by enlargement of the surrounding lymph nodes. In rare cases, the eye-lids are involved, resulting in necrosis and ectropion (retraction), which is an inducator for reconstructive surgery. In addition, headache, malaise, and a slight fever are observed. In a minimal number of cases, when the disease becomes generalised, death may also occur (39, 47, 67).

The fourth form is anthrax associated with intravenous drug use described relatively recently. This type of disease manifests itself as an atypical soft tissue infection accompanied by disproportionate tissue oedema, slight pain, and occasionally fever. Sometimes, addicts have a rapid course of the disease (resembling systemic anthrax and toxaemia) with a fatal outcome. More than 30% of patients infected with anthrax through use of an intravenous drug die (as a result of septic shock and meningitis despite treatment). This form is more challenging to diagnose and more dangerous than cutaneous anthrax (27, 47).

Anthrax in animals

The pathogenesis and pathology of anthrax in animals are excellently reviewed in many publications (2, 4, 9, 21, 22, 34, 46, 64, 67). Ruminants are the most susceptible group in *Mammalia*, and small ruminants (primarily sheep) are less resistant than cattle. Most clinical cases of anthrax in ruminants occur during the grazing season. Cattle can swallow spores directly from the soil while grazing or from plants growing on contaminated soil. Local injuries to the oral mucosa and/or the gastrointestinal tract contribute to the development of local inflammation that may eventually lead to bacteraemia. Spore inhalation disease development is less common. Animal by-products such as hides, slaughterhouse material, and bone meal from endemic areas may contain *B. anthracis* spores, pos-

Tab. 2. Anthrax in cattle: clinical symptoms and gross pathology findings

Disease form	Clinical signs	Post-mortem lesions
Per-acute	– sudden staggering and recumbency without preceding visible signs; – convulsions or spasms followed by death within minutes; – sometimes fever; wheezing; congestion of the mucous membranes; – dying animals might bleed from the nose, mouth, vulva and anus	– dark, unclotted blood, also in the nose and rectum; – a delayed or incomplete *rigor mortis*; – rapid putrefactive decomposition; – swollen spleen with extensive petechiae (*tumorlienis anthracocicus*); – haemorrhage at mucosae, serosae, and into subcutaneous stroma; – bloody fluid in body cavities; – a friable and limp myocardium; – serous oedemas in the mesentery, mediastinum, kidneys; – subcutaneous oedemas and ulcerative and haemorrhagic changes in the rectum and pharynx, lymphangitis; – dark-red stained, swollen lymph nodes
Acute	– septicaemia, – high fever (> 40°C); – excitation or somnolence; – depression; – lack of appetite; – dyspnoea; – cyanotic mucosae or congestion of the mucous membranes; – stasis in the rumen; – colic; – ulcers on the tongue or elsewhere in the oral cavity; – abortions, decrease in milk yield; – oedematous swellings in the neck region or the chest, abdomen or flanks; – death due to shock occurs within 48-72 hours	

Tab. 3. Anthrax in domestic small ruminants (sheep and goats): clinical symptoms and gross pathology findings

Disease form	Clinical signs	Post-mortem lesions
Per-acute	– death occurs within minutes with convulsion or spasm	– obliterated structure is visible; – degeneration of parenchymal organs (especially the heart); – liver and kidneys also congested with congestion; – congestion and petechiae in the lungs, brain and meninges, in which there are sometimes haemorrhages leading to sudden death (*apoplexia intermeningealis*); – occasionally, haemorrhagic necrotizing enteritis in the form of diffuse or focal carbuncles (*pustula maligna*); – the serous membrane at the site of the carbuncle is oedematous, with petechiae and covered with fibrin
Acute	– high fever; – lack of appetite; – dyspnoea; – muscle trembling and grinding of teeth; – sometimes reddish foam from the mouth shortly before death; – rarely anthrax carbuncles (*pustula maligna*) develop as limited haemorrhagic necrotizing inflammation of the skin and subcutaneous tissue	

Tab. 4. Anthrax in horses: clinical symptoms and gross pathology findings

Disease form	Clinical signs	Post-mortem lesions
Per-acute	– sudden death	– incomplete or absent *rigor mortis*; – gross pathology resembles the pathology of ruminants; – perforation of the intestinal wall; – swollen draining lymph nodes of the gastrointestinal tract; – bloody infiltrates and oedema in the submucosa at the ventral chest and abdomen; – swelling of the throat, neck, chest, shoulder, and mammary gland; – systemic haemorrhagic oedema, – haemorrhagic fluid in the body cavities; – enlargement of lymphoid tissue – including splenomegaly
Acute	– predominant clinical sign of anthrax in live horses: subcutaneous oedema (particularly in the inguinal, preputial, and ventral thoracic and abdominal areas); – high fever (40-41°C); – tachypnoea; – dyspnoea; – tachycardia; – severe depression; – cyanosis; – colic; – bloody diarrhoea; – local oedemas with necrotic centres; – and bloody exudates from body orifices; – muscle tremors; – sepsis leading to death of the animal within 2-3 days	

Tab. 5. Anthrax in swine and companion animals – clinical symptoms and gross pathology findings

Disease form	Clinical signs	Post-mortem lesions
Pharyngeal	– pharyngitis; – hot painful oedemas in the head and chest regions; – lethargy; – lack of appetite; – vomiting; – fever (41.7°C); – animals may recover spontaneously or die due to suffocation	– a gelatinous consistency of the cervical tissues, separated by straw-coloured, pink, or red haemorrhagic fluid; – fibrinous exudate on the necrotic tonsils; – inflammation and swelling of the pharyngeal mucosa; – enlarged, necrotic, haemorrhagic, or yellow in the cross-section of the mandibular and pharyngeal lymph nodes; – haemorrhagic lymphangitis; – dry, encapsulated, grey-yellowish foci within the regional lymph nodes
Intestinal	– lethargy; – lack of appetite; – vomiting; – ataxia; – fever (41.9°C); – dysphagia; – cyanosis; – black necrotizing papule of the skin and mucosae; – icterus; – vomiting; – diarrhoea (sometimes bloody); – death follows in severe cases	– segmental necrotizing enterocolitis (ileum and colon); – haemorrhagic enteritis with ulceration; – fibrinous-necrotic exudate on the intestinal mucosa; – fibrin deposits on the intestinal serosa; – a large amount of pink fluid that clots in the air in the peritoneal cavity; – enlarged mesentery lymph nodes – mesenteric oedemas with haemorrhaging and serous infiltrated, gelatinous, with numerous petechiae; – black ulcers in the liver, spleen, and kidneys; – parenchymal degeneration and congestion in the liver and kidneys; – necrosis in the liver; – petechiae in the kidneys
Septicemic	– sudden death or – as lethargy, tremor, and fever followed shortly by death	– variable amounts of serous-blooded peritoneal fluid; – serous and renal ecchymoses; – enlargement of the spleen; – lymphadenopathy

ing an infectious risk to animals that come into contact with these materials. Insects that feed on blood can also transmit the infection. The incubation period in ruminants ranges from 3 to 5 days, but it can also be from 1 to 14 days (4, 64). Typical clinical symptoms and gross pathological findings in cattle are presented in Table 2, whereas clinical signs and post-mortem lesions in sheep and goats are presented in Table 3.

Horses appear to be less susceptible to anthrax than ruminants. Anthrax outbreaks have been noted after long periods of unusually warm and dry spring weather followed by heavy rainfall. The disease is almost always fatal. In most cases, death occurs within 2-4 days of infection. Horses either die suddenly or develop acute disease. The disease develops due to aspiration of spores into the respiratory system or ingestion into the digestive tract. Infections by blood-sucking insects are also possible. The incubation period is probably a few days (34). Clinical symptoms and gross pathology findings in horses are presented in Table 4.

In confined swine, anthrax infections are sporadic, and non-seasonal, and they are usually the result of feeding with feed contaminated with spores. Direct transmission from pig to pig is rare. Insects can spread the disease. Pigs are relatively resistant to infection with *B. anthracis*, and often subclinical disease may occur, and local changes are possible. The incubation period in swine ranges from 1 to 8 days (9).

Isolated infections in dogs and cats are generally seen during severe anthrax outbreaks in livestock. Dogs and cats are considered relatively resistant to inhalation anthrax, and the disease is most often the result of being fed raw meat from contaminated carcasses (46). Clinical symptoms and gross pathology findings in swine and companion animals are presented in Table 5.

Treatment and prevention of anthrax in humans

The European Medicines Agency and its Scientific Committee, the Committee for Human Medicinal Products (EMA/CHMP), produced a guidance document on using medicinal products for the treatment and prophylaxis of biological agents that might be used as weapons of bioterrorism. The first version of the guidance was published in 2002. The last update was made on 18th November 2014 (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/biological-chemical-threats). This guidance document is concerned with selecting medicinal products that are potentially useful for post-exposure prophylaxis and/or treatment of infectious diseases (including anthrax disease) in the context of biological warfare. Centers for Disease Control and Prevention Expert Panel Meetings on Prevention and Treatment of Anthrax in Adults emphasised that such phenomena as toxin production, the potential for antimicrobial resistance, the high prevalence of meningitis, and the presence of latent spores when selecting postexposure prophylaxis or anthrax therapy with combination antimicrobials, have to be taken into account (28).

EMA/CHMP has recommended the use of ciprofloxacin as first-line treatment or post-exposure prophylaxis for children and adults. At the same time, other quinolones such as ofloxacin and levofloxacin can only be given to adults. Doxycycline and penicillin G are suggested as alternative therapies if proven to be susceptible, although penicillin is not bactericidal against *B. anthracis*. Oral amoxicillin is also a late-stage treatment option if the patient's condition improves and the bacterial susceptibility to the antibiotic has been confirmed. *B. anthracis* strains are resistant to sulfamethoxazole, trimethoprim, cefuroxime, sodium cefotaxime, aztreonam, and ceftazidime, so these drugs should not be used to treat or prevent anthrax infection. Due to the very high mortality associated with inhalation anthrax, it is recommended to use at least two antimicrobials that may be effective. *In vitro*, synergy has been demonstrated between ciprofloxacin or doxycycline and protein synthesis inhibitors (rifampicin, chloramphenicol, clindamycin, clarithromycin, erythromycin, gentamicin, and streptomycin) and vancomycin. However, there is insufficient data to confirm these agents' utility in treating inhalational anthrax (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/biological-chemical-threats).

Meningitis and haemorrhagic brain parenchymal infection have been observed in nearly half of the patients with systemic anthrax. Potential meningitis should always be considered in these cases, and antimicrobials with activity against *B. anthracis* and good penetration into the central nervous system should be used (55). Anthrax meningitis or meningoencephalitis can be treated with at least three antimicrobial drugs (one of them should be clindamycin) (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/biological-chemical-threats). When meningitis is suspected, or when meningitis cannot be ruled out, the participants of the 'Centers for Disease Control and Prevention Expert Panel Meetings on Prevention and Treatment of Anthrax in Adults' recommend the use of more than three antimicrobial agents with activity against *B. anthracis*, including that, more than one should have bactericidal activity and more than one should be a protein synthesis inhibitor (28). Recommendations for using antimicrobial agents in the treatment and post-exposure prophylaxis of anthrax in adults are presented in Table 6.

Treatment or post-exposure prophylaxis should continue for 60 days for inhalation anthrax, and 7 to 10 days for uncomplicated cutaneous anthrax. However, if a risk of inhalation anthrax cannot be ruled out, prophylaxis should continue for 60 days. In more severe cases of cutaneous anthrax with signs of systemic disease, combination treatment should be considered (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/biological-chemical-threats). For patients with systemic anthrax, but without signs of meningitis, intravenous combination treatment can be provided for longer than two weeks or until the patient is clinically stable. When anthrax meningitis is suspected, or when meningitis cannot be ruled out, intravenous combination treatment should be given for longer than two or even for three weeks (28).

Tab. 6. Recommendations for the use of antimicrobial agents in the treatment and post-exposure prophylaxis of anthrax in adults. Based on 'EMA/CHMP Guidance document on use of medicinal products for the treatment and prophylaxis of biological agents that might be used as weapons of bioterrorism'

Drug	Treatment dosage	Post-exposure prophylaxis dosage	Interval (hours)	Comments
Ciprofloxacin	400 mg IV followed by 500 mg PO	500 mg PO	12	First-line treatment and as alternative for oral follow-up or prophylaxis
Ofloxacin	400 mg IV followed by 400 mg PO	400 mg PO	12	Alternative to ciprofloxacin
Levofloxacin	500 mg IV followed by 500 mg PO	500 mg PO	24	
Doxycycline	100 mg IV followed by 100 mg PO	100 mg PO	12	Alternative first-line treatment (and follow-up) or prophylaxis; susceptibility must be confirmed
Amoxicilin	1 g IV followed by 500 mg PO	500 mg PO	8	
Panicillin G	2.4-3 g IV		4	Alternative first-line treatment; susceptibility must be confirmed

Explanations: IV – intravenous; PO – orally

Due to the very rapid course of the disease, even intensive antimicrobial therapy and supportive therapy may be ineffective in reducing the high mortality rates for inhalational anthrax. This is likely due to constant exposure to anthrax toxins. Therefore, anti-toxin therapies are necessary to neutralise toxins produced by the bacilli and reduce mortality. Polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) in combination with antimicrobials are used for this purpose. In the first case, human IgG is isolated from the plasma of volunteers vaccinated against anthrax. Human hyperimmune immunoglobulin, for treating toxaemia associated with inhalational anthrax patients in combination with antimicrobial therapy was approved by The United States Food and Drug Administration (FDA) in 2015. This polyclonal antiserum has been registered as Anthrax Immune Globulin Intravenous (AIGIV) (https://www.fda.gov/vaccines-blood-biologics/approved-blood-products/anthrasil).

In the second case two mAbs are used. In December 2012, the FDA approved the first monoclonal antibody against the protective agent, raxibacumab, to treat inhalational anthrax as a preventive measure (https://wayback.archive-it.org/7993/20170111193902/; https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm332341.htm). On 15th October 2014, the EMA granted this mAb as an 'orphan medicine' for treating inhalation anthrax disease (EU/3/14/1352) (https://doi.org/10.3201/eid2002.130687). Raxibacumab is an anti-PA human recombinant IgG1λ mAb, and has a molecular weight of 146,000 Da. The pharmacological activity of raxibacumab is based on binding to PA domain IV and blocking toxin binding to its receptors (35, 40). Combining raxibacumab with anthrax vaccine adsorbed (AVA) should also be considered, which may provide additional benefits in post-exposure prophylaxis against inhalation anthrax (58).

In March 2016, the FDA approved the second mAb (obiltoxaximab) to treat and prevent inhalational anthrax (https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-inhalation-anthrax). In Europe, this product was authorised under 'exceptional circumstances' by the EMA and designated as 'orphan medicine' on 24th August 2018 (EU/3/18/2065). The product received a marketing authorization valid throughout the European Union on 18th November 2020 (https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3182065). Obiltoxaximab is a chimeric IgG1(κ) monoclonal antibody, a large protein molecule with a molecular weight of 148,000 Da. Its pharmacological activity is based on the neutralisation of circulating PA by preventing binding to the host cell receptors and preventing LF and EF from entering cells, and thus preventing the progression of the disease (40).

Both products are indicated in adult and paediatric patients for treatment and post-exposure prophylaxis when alternative therapies are unavailable or inappropriate. Premedication with antihistamine is recommended before administration of monoclonal antibodies. Raymond et al., comparing the efficacy of three antitoxins (AIGIV, raxibacumab, and obiltoxaximab) in a New Zealand White rabbit model of inhalation anthrax, noted that in the post-licensure study, mAbs were better than AIGIV. However, neither mAb was better than the other. Thus, monoclonal antibodies are more effective than polyclonal antibodies. However, they should be used in combination with antibiotics (59). The recommendations for using approved monoclonal antibodies in the treatment and post-exposure prophylaxis of anthrax in adult and paediatric patients are presented in Table 7.

Tab. 7. Recommendations for the use of approved monoclonal antibodies (mAb) in the treatment and post-exposure prophylaxis of anthrax in adult and paediatric patients

mAb	Dosage (inone-time single IV infusion)	Time (h)	Comments
Raxibacumab	< 15 kg: 80 mg/kg bw 15 to 50 kg: 60 mg/kg bw > 50 kg: 40 mg/kg bw	2.25	Does not cross the blood-brain barrier and does not prevent or treat anthrax meningitis
Obiltoxaximab	< 15 kg: 32 mg/kg bw 15 to 40 kg: 24 mg/kg bw > 40 kg: 16 mg/kg bw	> 1.5	

Explanations: IV – intravenous; bw – body weight

There are two licensed human anthrax vaccines in the western world: anthrax vaccine adsorbed (AVA) and anthrax vaccine precipitated (AVP). Both vaccines are cell-free and contain either live or dead *B. anthracis*. AVA was initially approved by the FDA in 1970 to prevent anthrax disease in people at high risk of exposure. However, in 2015 the FDA approved this product for use after known or suspected *B. anthracis* exposure. AVA is manufactured from sterile culture filtrates of the toxigenic, non-encapsulated *B. anthracis* strain V770-NP1-R that is adsorbed onto aluminium hydroxide, and contains an unquantified amount of PA and only trace amounts of LF and EF (7, 38).

Anthrax vaccine precipitated (AVP) is a cell-free filtrate of the toxigenic, non-capsulated *B. anthracis*

Tab. 8. Pre-exposure and post-exposure administration of human anthrax vaccine

Vaccine		Vaccination schedule
BioThrax® (AVA)	PrEP	Primary immunization: IM injections at 0, 1, 6, 12 and 18 months Booster immunization: IM once every 12 months
	PEP	SC injections at 0, 2 and 4 weeks
AVP		Primary immunization: IM injections at 0, 3, 6 and 32 weeks Booster immunization: IM once every 12 months

Explanations: AVA – anthrax vaccine adsorbed; AVP – Anthrax vaccine precipitated; PrEP – Pre-exposure prophylaxis; PEP – Post-exposure prophylaxis; IM – intramuscularly; SC – subcutaneously

Sterne 34F2 strain, precipitated with aluminium potassium sulphate (Alum). This vaccine contains the three major toxin components of anthrax, with approximately 7.9 μg/mL of PA, 1.9 μg/mL of LF, and low but detectable amounts of EF. Adverse reactions are rare, and even if they occur after the first dose, this does not mean that they will occur after subsequent doses (10, 19). This regimen is mainly used as post-exposure prophylaxis together with antibiotic therapy. The most common side effects include tenderness, pain, erythema, swelling, muscle aches, fatigue, and headaches. Furthermore, recent studies recommend that in people at low risk of exposure, booster doses should be given every 3 years (13). Pre-exposure and post-exposure administration of both types of anthrax vaccines is presented in Table 8.

The vaccines listed above are capable of generating a protective immune response, but work continues to improve anthrax vaccines, including the possible development of a vaccine that incorporates multiple antigens presented by *B. anthracis* and targets different aspects of the organism's pathogenicity (16).

Prevention and treatment of anthrax in animals

Anthrax is listed on Old Classification of Diseases Notifiable to the OIE – list B (transmissible diseases that are considered to be of socio-economic and/or public health importance within countries and that are significant in the international trade of animals and animal products). In many countries, the treatment of this disease is prohibited, regardless of the species of animal in which it is diagnosed. The primary countermeasures regarding human health are the control of anthrax in animals and the testing of meat and other zoonotic materials.

An essential aspect in the prevention of anthrax infections in animals is proper management and vaccinations of the herd and vaccination of susceptible animals in the case of an anthrax outbreak. The first vaccine was developed by Max Sterne in 1937 and is still the most widely used vaccine worldwide (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access). The vaccine is a live, non-encapsulated, spore former, held in suspension. A natural loss of the pXO2 plasmid characterises the original Sterne strain (34F2). This variant is incapable of forming a capsule, which makes it less virulent compared to other strains; however, it is able to stimulate a protective immune response. In Central and Eastern Europe, an equivalent pXO2– derivative (strain 55) is the active ingredient of the present vaccine for farm animals. For prophylactic vaccination, the recommended dose for cattle and horses is a minimum of 2-10 × 10^6 and for sheep, goats, and pigs, it is 1-5 × 10^6 culturable spores. In horses, revaccination may be required 1 month after the first dose. Immunity should be good for at least one year, and an annual booster is recommended (https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access). The vaccines contain attenuated spores; therefore, antimicrobials should not be administered before and after vaccination as they may reduce the effectiveness of vaccination. Vaccination should be repeated if antibiotics are used before or after vaccination (56).

In the case of zoonoses such as anthrax, vaccinations and treatment with antimicrobials are not enough. An essential aspect of preventing anthrax outbreaks is the use of appropriate control and biosecurity procedures: owners consent to providing information about the occurrence of the disease in the herd to the relevant control authorities, remembering to observe quarantine when trading the herd (after vaccination, before leaving the farm, and in the case of slaughter). Any contaminated material such as (faeces, corpses, or bedding) should be removed immediately (burning is most effective). It is obvious the disinfection of used equipment, drinkers, feeders, rooms, pens, and compartments in which animals and equipment are kept, and the isolation of sick or suspicious animals, have a huge impact (https://apps.who.int/iris/handle/10665/97503).

Anthrax is a very rapid disease, so very rarely, only if the disease is recognised early, can antibiotics with constant veterinary care help. The penicillin group is considered the first line of defence against anthrax due to the susceptibility of most naturally occurring strains (https://apps.who.int/iris/handle/10665/97503). Penicillin and oxytetracycline are the antibiotics of choice in most cases of *B. anthracis* infection in farm animals (1, 9). Other combinations of antibiotics used include penicillin and tetracycline in cattle (64), or penicillin and streptomycin in horses (34). For companion animals such as dogs, tetracycline, penicillin, and ciprofloxacin/enrofloxacin are recommended (46); however, their effectiveness needs to be better documented, and it is challenging to select the best treatment (37).

Various studies have been carried out to test the effectiveness of antibiotics in treating anthrax in animals. Doxycycline and ciprofloxacin, which are commonly referred to as the antimicrobial agents of choice in human infections, have been studied, among others (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/biological-chemical-threats). Other authors administered doxycycline, tetracycline, ciprofloxacin, ofloxacin, imipenem, gentamicin, and erythromycin to guinea pigs to test drug effectiveness in preventing the development of respiratory anthrax (68). These studies have shown that administering antibiotics can prevent the development of fatal anthrax-induced respiratory disease, and doxycycline and ciprofloxacin have been shown to be the most effective. Unfortunately, not all antibiotics have proved to be sufficiently compelling, so only vaccination in combination with antibiotic therapy ensures long-term protective immunity and prevents disease recurrence.

Anthrax as a biological weapon

Currently, anthrax is classified by the CDC as a Category A pathogen, and all cases of the inhalable form should be treated as a bioterrorism event to be reported to local authorities and the CDC (51, https://www.ncbi.nlm.nih.gov/books/NBK507773; https://www.cdc.gov/anthrax/bioterrorism/index.html). *B. anthracis*, as a biological weapon, is not an invention of recent years. The attractiveness of anthrax as a biological weapon was noticed by scientists very early, even during World War I, by the Germans. The documented use of *B. anthracis* as a biological weapon occurred in 1937 when Japan used it against Chinese civilians in Manchuria. After World War II, the United States, Great Britain, and the Soviet Union continued their offensive biological warfare programs for various periods (54). The Soviet Union was the last to end its program in the 1990s officially (41). A technically advanced and successful attack took place in September 2001 in the US, known as 'anthrax letters' following the 9 September 2001 World Trade Center assault in New York. An unknown number of envelopes containing anthrax spores were sent through the US postal service to news agencies in New York or Congress offices in Washington (54). Twelve postal carriers were among the twenty-two persons who contracted anthrax; five of these 22 people passed away afterwards (https://www.cdc.gov/anthrax/bioterrorism/threat.html). The specific strain in this case was traced to a facility run by the US army at Fort Detrick, but the attackers are still anonymous (3). Methods created before and throughout the inquiry were applied in the microbiological forensic study of the *B. anthracis* spores found in the "anthrax letters". The inquiry hastened the creation of several novel tests and techniques, many of which are now well-verified and easily accessible (29). It is worth noting that the "anthrax letter" resulted in a small number of infections compared to other pathogens we face daily. Still, this situation had a substantial political and psychological impact on society. Before the anthrax attack in 2001, there was no developed strategy to combat such biological weapons' attacks. Only after that situation did US hospitals develop many action plans for fighting such situations in the future (26).

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Corresponding authors: Zuzanna Czekaj, Student Science Club of Animal Infectious Diseases "AnthraX", Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wroclaw, Poland; e-mail: 115804@student.upwr.edu.pl

Małgorzata D. Klimowicz-Bodys, Division of Infectious Diseases of Animals and Veterinary Administration, Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences,pl. Grunwaldzki 45, 50-366 Wroclaw, Poland; e-mail: malgorzata.klimowicz-bodys@upwr.edu.pl