ANTHRAX IN ANIMALS - MANIFESTATION, DIAGNOSTICS AND PREVENTION

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Citation: Yordanov, S. & Dimitrova, A. (2023). Anthrax in animals - manifestation, diagnostics and prevention. *Bulgarian Journal of Animal Husbandry*, *60*(3), 37-47 (Bg).

Abstract

Anthrax is a deadly disease for animals and humans, which occurs mainly acutely - in a septicemic form, with inflammatory-necrotic processes in different organs. It is caused by *Bacillus anthracis*. The disease is widespread in Asia, Africa, Central and South America and southern Europe. In our country, until the end of the 20th century, anthrax in animals was a serious problem, but with the implementation of systematic, annual vaccinations of susceptible animals in stationary outbreaks and strict diagnostic control in the National Reference Laboratory (NRL), the incidence dropped sharply and now only single cases.

The article presents the most important characteristics of the causative agent, epizootological features of the infection, pathogenesis and clinical manifestations of the disease. The diagnostic methods used and the possibilities for immunoprophylaxis and control of anthrax in animals were reviewed, in accordance with the requirements of Ordinance No. 6/October 5, 2020.

Modern molecular methods for detection and identification of *B. anthracis* are indicated. The application of *B. anthracis* produced lethal factor (LF) and protective antigen (PA), as an alternative in cancer therapy is described. Anthrax is discussed in the context of the One Health concept.

Key words: anthrax; *Bacillus anthracis*; animals; epizootology; manifestations; prevention; control

Anthrax is a sporadically or enzootically manifested deadly disease of animals and humans, which occurs mainly acutely - in a septicemic form and less often subacutely - with local inflammatory-necrotic processes in different organs, with a tendency to generalization. In pigs, the disease occurs as a local asymptomatic infection and, in very rare cases, as acute septicemia, or neck edema. It is caused by *Bacillus anthracis* /WHO, 2008; Ordinance 6/ 5 Oct. 2020/.

History and distribution. Anthrax is a disease known since ancient times. It is believed to be mentioned in ancient Indian texts and the works of Homer and Virgil. In „Natural history", Pliny the Elder calls it a carbuncle. In 1607, an epidemic broke out on the Old Continent, during which about 60,000 humans died. Andreevsky described an epidemic in the Urals (1786-1788), from where the disease was called Siberian ulcer. The name anthrax (coal) was imposed, because of the dark, almost black color, that the blood acquires after the death of animals and people. For the first time, anthrax bacilli were found in the blood of dead animals in Germany in 1849 by Polender, in France by Davin and Rojer in 1850, and in Russia in 1855 Brauel proved its etiological role. The bacills were isolated in pure culture by R. Koch in 1876. Russian scientists Chernogorov and Vorontsov in 1894 proved for the first time the susceptibility of pigs to anthrax and succeeded in reproducing the disease. Pasteur (1881) made the first successful attempts to obtain a vaccine with weakened cultures of the causative agent. In the 30s of the 20th century, the Max-Sterne´s vaccine was successfully applied in the USA, and analogues of it in the Sovi-

et Union (Vachev, 1977; Andreev and Andreev, 1984; Arsov, 1984; Koehler, 2002; Whiteman, 2004; WHO, 2008; Popova, 2009).

The disease is widespread in Asia, Africa, Central and South America and southern Europe. It is less common in the USA and other northern countries. Anthrax in pigs was first reported in 1952-53, with the infection being introduced with contaminated feed (Whiteman, 2004). Later, swine anthrax was found in almost all countries of the world. In the last quarter of the 20th century, the disease was found in the USA, Morocco, India, Congo and other countries. In our country, in the first half of the 20th century, animal anthrax was a serious problem, but with the implementation of systematic annual vaccinations of susceptible animals in stationary outbreaks and strict diagnostic control of the disease, the incidence dropped sharply (Dimitrov and Kebedjiev, 1972; Arsov, 1984; Koeler et al., 2002; Straw et al., 2006; Bonovska et al., 2011; Ivanova et al., 2011; Bonovska, 2012). Anthrax should be considered as a disease of great health and economic importance (WHO, 2008).

Etiology. *Bacillus anthracis* is a Gram-positive, nonmotile rod, aerobe, and facultative anaerobe of the *Bacillus genus* of the *Bacillaceae* family. Morphologically, it is one of the largest pathogenic microorganisms in this group - it has a length of 4 to 8 μm, and a width of 1-1.5 μm. When viewed under a microscope, it appears in the form of chains similar to bamboo sticks. On the blood agar does not cause hemolysis. In a stitch on an agar column, it grows in the shape of a Christmas tree, turned upside down. *Bacillus anthracis* has high biochemical activity with pronounced carbolytic and proteolytic properties. Breaks down sugars into acids without forming gas. Forms ammonia and hydrogen sulfide and does not reduce nitrates. In the body of animals and in nutritious media, in a CO2-rich atmosphere, it forms large capsules, and in the presence of oxygen and a temperature of 12 to 42°C - highly resistant spores. Spores are not formed in unopened carcasses. *B. anthracis* possesses capsular, somatic and protective antigen. The capsular antigen has antiphagocytic activity. Somatic antigen is a thermostable, species-specific hapten. The protective antigen is a thermostable protein, responsible for the formation of specific antibodies (Arsov, 1984; Kaloyanov et al., 1992; WHO, 2008; Popova, 2009; Ivanova et al., 2011; Bonovska, 2012).

In the process of its vital activity in the body of infected animals, the anthrax bacillus produces an exotoxin, consisting of three components, having antigenic properties. The produced exotoxin includes a protective antigen, edema- and lethal factor - proteins causing a dermonecrotic reaction, pulmonary edema and severe hypoxia on the background of pronounced hypoglycemia. Complex exotoxin induces the production of incomplete antibodies (Durmishev et al., 1992; Straw et al., 2006; Popova, 2009; Komitova, 2012).

The virulence of *B. anthracis* is determined by two factors of pathogenicity – a phagocytosis-inhibiting capsule and anthrax toxin. For the manifestation of the full virulence of the organism, the presence of both factors is necessary (Luna et al., 2006).

The vegetative forms of *B. anthracis* are poorly resistant and are particularly sensitive to heat (in warm weather they die in 2-4 days), to antibiotics, phytoncides and disinfectants, such as 2-4% NaOH, 3-5% chlorine lime, 3- 5% formalin, etc. Gastric juice kills them in 20 min. Spores are very persistent and persist for decades. The preservation of food products and technical raw materials by salting, freezing and drying helps to preserve the spores. For disinfection, agents with a pronounced sporicidal effect are used 10-20% chlorine lime, 4-7% formalin, 5% sulfur-cresol mixture, etc. For skin disinfection, Schattenfroh's solution (1-2% HCl + 10% NaCl) is most widely used. Biothermal treatment of manure and direct sunlight kill spores in several days, and boiling water in 30-40 min (Vachev, 1977; Durmishev et al., 1992; Popova, 2009; Whiteman, 2004; Straw et al., 2006).

Epizootological features. Under natural conditions, all herbivores and omnivores, and sometimes carnivores are susceptible and suffer from anthrax. Goats, sheep and deer are most susceptible, followed by cattle, buffalo, camels and horses. Pigs are relatively more resistant - they get

sick from the local (throat) form of anthrax and sometimes get sick again. According to Vachev (1977), anthrax in pigs should be considered in connection with the occurrence of the disease in other types of animals, and according to Taylor (1995), also when taking feed contaminated with spores. Dogs can become ill after ingesting large amounts of infected carcass material. Birds have innate immunity, but under certain conditions and large quantities of a highly virulent pathogen, it can be overcome. Of the test animals, white mice, guinea pigs and rabbits are the most susceptible. The person is also highly receptive (Andreev and Andreev, 1984; Durmishev et al., 1992; Taylor, 1995; Whiteman, 2004).

The sources of infection are the sick and the carcasses of animals that died from anthrax. The bacills are released with the bloody discharges from the natural openings of the sick in the dying stage of the disease. In carcasses, anthrax bacills multiply a lot until they rot. An important role in the epizootology of the disease is played by infected areas of pastures, watering holes, areas around pits for carcases and other places called "damned fields". They usually occur, when carcasses are opened. Animals, including pigs, are most often infected alimentarily by consuming infected feed, or water and by blood-sucking insects, which are mechanical carriers of the infection. Very rarely, infection can occur by an aerogenic route. The disease is spread through predators, birds of prey and contaminated feed. From one country to another, the infection is transferred by importing skins, bones, wool, etc. Anthrax has a pronounced enzootic character and occurs as a stationary infection, showing an emphasized summer seasonality (Vachev, 1977; Arsov, 1984; Taylor, 1995; Bonovska, 2012).

Pathogenesis. The causative agent penetrates through microlesions of the mucous membrane, injured skin, or respiratory tract. At the site of penetration, in dendritic cells, or macrophages, anthrax spores germinate in vegetative forms, multiply intensively and reach regional lymph nodes. Here, they produce toxins and damage the endothelium and lymphatic vessels. A lethal factor in exotoxin is responsible for death in animals. Edema and tissue necrosis occur. Local, serous-hemorrhagic inflammation occurs. When inhaled, the spores reach the alveoli and through the macrophages to the tracheobronchial lymph nodes, where they change into vegetative forms and multiply. Through the lymphatic system, bacteria enter the blood flow and cause toxemia and sepsis. The exotoxin produced by *B. anthracis* causes tissue edema and necrosis, but most commonly- pulmonary edema with severe hypoxia and marked hypoglycemia. A lethal factor in exotoxin is responsible for death in pigs. The skin form is manifested by the so-called anthrax carbuncle. Due to necrosis of the surface epithelium, a black, coal-like crust (anthrax/charcoal) forms. In more resistant animals, the infectious process is localized on the gateway. A papule appears, which turns into a blister, which turns into a pustule with a necrotic center. A red inflammatory zone forms around the bubble (Taylor, 1995; Whiteman, 2004; Straw et al., 2006; WHO, 2008).

The infective form of *B. anthracis* is the spore. Spores germinate in a host organism (human or animal) to produce vegetative forms, that multiply rapidly and express the anthrax toxins and the poly-D-glutamic acid capsule - the major pathogenicity factors, encoded by genes, located in the virulence plasmids, respectively pXO1 and pXO2. Anthrax toxins consist of three synergistically acting proteins: protective antigen (PA), edema factor (EF) and lethal factor (LF). PA in combination with EF forms an edematoxin, and PA in combination with LF forms a lethal toxin. Toxins are responsible for the characteristic signs and clinical symptoms of the disease (Zasada, 2020).

In pigs, infection occurs most often via the alimentary route and the causative agent multiplies locally, usually affecting the pharyngeal and regional lymph nodes. Septicemic and intestinal form of the disease occurs very rarely. In more resistant animals, the process remains local, and in more susceptible bacills spread through the lymph and blood throughout the body and cause serous-hemorrhagic edema, containing anthrax capsules and exotoxins, which cause metabolic and anatomical disorders characteristic of secondary shock - paralysis of the respiratory cen-

ter, hypoxia, etc. A lethal factor in exotoxin is responsible for death in pigs (Vachev, 1977; Arsov, 1984; Taylor, 1995; Komitova, 2012; Bonovska, 2012).

Clinical manifestations. The clinical manifestation of the disease is caused by the R form of *B. anthracis*. The incubation period varies from 1 day to 2 weeks, with an average of 3 to 5 days. Anthrax comes in three forms. The peracute form occurs with manifestations of cerebral apoplexy. Healthy looking, animals fall to the ground, kick and emit bloody foamy discharge from the nose and anus. Tremor, difficulty breathing, weak pulse and cyanosis of the mucous membranes may be observed. Death occurs in 15-20 min. The acute form proceeds as severe septicemia with high fever, rapid breathing, fatigue, reddened conjunctivae, sometimes with hemorrhages on the mucous membranes, constipation, and then diarrhea. Secretions and excreta are often mixed with blood. Inflammatory edema appear, hot and painful, most often in the area of the chest and abdomen. Abortions may occur. Death comes quickly. The subacute form manifests itself like the acute one, but takes longer (5-8 days). It usually ends fatally, but there are also cases of recovery. A private case of the manifestation of the subacute form is the skin form (carbuncular), usually on the "gateway", with the appearance of edema in the form of a carbuncle, warm and painful, bluish-red with a cone-shaped tip, which later necrotizes and becomes in an ulcer. It can also be in the area of the mouth, in the form of vesicles (Pustula maligna), more often in cattle and horses. In pigs, the infection usually occurs locally, affecting the submandibular lymph nodes and tonsils. The temperature is 41-42°C, there is edema in the throat area, which goes to the neck and reaches the chest (anthrax angina). This edema makes swallowing and breathing difficult. Visible mucous membranes in the head area are cyanotic. Animals die of suffocation after 1 to 5 days. In free-range pigs, the disease can manifest itself in a septicemic form, without edema in the throat, as well as in a subclinical form (Andreev and Andreev, 1984; Arsov, 1984; Taylor, 1995; Whiteman, 2004; Straw et al., 2006; Bonovska, 2012).

Pathological changes. The carcass of an anthrax-died animal is severely swollen, with bloody, foamy oozing from the body openings. There is no cadaveric stiffness, there is a strong cyanosis of the visible mucous membranes, sometimes with hemorrhages and edema in the head, neck and abdomen. Anthrax and suspected anthrax corpses are not autopsied, because opening them poses the risk of human infection, contamination of the environment and the emergence of permanent outbreaks of the disease. In cases of autopsy, (which is allowed in exceptional cases), the following changes are found as follows: 1. Subcutaneous and subserous connective tissue in the area of the pharynx and pharyngeal, and peripharyngeal tissue are saturated with fibrous infiltrates of a yellowish color, with superficial hemorrhages; 2. Regional lymph nodes, primarily retropharyngeal and cervical, are enlarged, with hemorrhagic and dry gray-brown necrotic outbreaks; 3. The spleen is greatly enlarged with rounded edges, sometimes with a ruptured capsule and softened pulp, dark in color; 4. The blood is thick, dark red, uncoagulated, and tar-like in dead corpses; 5. Local inflammations are found in some areas of the skin, and carbuncles in the small intestine; 6. The parenchymal organs are hyperemic and friable, and a dull red exudate is found in the abdominal and thoracic cavity. The changes are more pronounced in the subacute form of the disease, while in the peracute form they may be absent, or not characteristic. In pigs, the pharyngeal form is mainly manifested, in which serous-hemorrhagic infiltration of the tonsils, pharyngeal mucosa and submucosal connective tissue is observed. Regional, retropharyngeal, and cervical lymph nodes are edematous and hemorrhagic. In cases of the intestinal form of anthrax, there is always peritonitis with increased bloody peritoneal fluid. The mesentery is edematous, confluent, and may have reddened areas around the blood vessels. There are focal areas of severe diphtheroid enteritis. The mesenteric lymph nodes are enlarged and reddened. (Arsov, 1984; Taylor, 1995; Whiteman, 2004; Straw et al., 2006; WHO, 2008).

Laboratory diagnosis. Anthrax must be laboratory confirmed. A piece of the ear of the dead

animal, obtained after applying a double ligature to prevent the outflow of blood, is sent as material for microbiological examination. A swab, soaked in strong solutions of disinfectants, with a sporicidal effect is placed at the site of the cut. If the suspicion of anthrax arose during the autopsy, pieces of the spleen, liver, kidneys, heart, carbuncles and lymph nodes are sent to the laboratory. In hot weather, it is recommended to send dried smears on glass slides, lumps of sugar or pieces of chalk soaked in blood. If anthrax outbreaks are suspected, samples of soil, or silt are sent. The label "Caution Infectious Material" is placed on the package, and the cover letter is not placed inside. The samples are sent to the nearest accredited anthrax laboratory, and in the case of a positive result - for confirmation at the National Reference Laboratory (NRL).

Microscopic examination includes microscopy of preparations (smears), stained by Gram and Klett, or by Romanowski-Giemsa. Blue, or blue-violet colored streptobacills, or short chains are observed. In more recent studies, in an anthrax-endemic area, Aminu et al., (2020) demonstrated that microscopy of azure B-stained blood smears, instead of polychrome methylene blue (PMB), was more sensitive and specific for confirming *B. anthracis* and this method, being more accurate than others, can be used in field conditions. In cultural research, the samples are cultured simultaneously in ordinary nutrient broth and on nutrient agar and blood agar, or Tomov's selective medium. Blood is sown directly. When examining feces, or intestinal contents, they are previously diluted in physiological solution, heated for 30 min at 75ºC, to kill the side microflora, and then inoculated. Samples of skin, bristles, or wool are cut into pieces in broth, heated for 30 min at 80ºC and cultures are made from the sediment. In specialized (reference) laboratories with pure cultures, biological testing can be carried out on experimental animals, most often white mice and guinea pigs. Serological methods include: reaction precipitation, according to Ascoli and Valenti (warm and cold Ascoli), or immunodiffusion in agar-gel. When examining blood and materials from internal organs, warm Ascoli is applied. For ear, skin, hair and soil samples, cold Ascoli is more suitable. An immunofluorescence assay is performed to detect anthrax spores in the external environment. In recent years, agar-gel immunodiffusion, indirect hemagglutination, ELISA - to detect antibodies against the lethal and swelling factor, and molecular biological methods (PCR - identification) have also been applied. In terms of differential diagnosis, the following should be taken into account: anaerobic toxic infections, pasteurellosis, erysipelas, Classical swine fever, African swine fever and some poisonings (Arsov, 1984, Durmishev et al., 1992; Taylor, 1995; Whiteman, 2004; WHO, 2008; Bonovska et al., 2011; Bonovska, 2012; Aminu et al., 2020). Demonstration of encapsulated *B. anthracis,* in stained smears of blood from fresh anthrax-infected animals is diagnostic for anthrax. All the same, if the animal has been dead for >24 hours, the capsule may not be readily detected in a blood smear. Culture of *B. anthracis*, or detection of virulence factors and chromosomal genes of *B. anthracis*, are also definitive for the diagnosis of anthrax. Detection of virulence factors and chromosomal DNA is a more reliable option than culture, which may be subject to overgrowth with contaminants, especially when the animal has been dead for some time (Hornitzky, M. and J. Muller, 2010).

Microscopy using azure B instead of polychrome methylene blue (PMB), is highly sensitive and specific for detecting *B.anthracis,* in blood smears from animal carcasses and is easier to use due to the availability of Azure B. Tissue samples from carcasses were more readily available than blood smears and *B.anthracis* was detected by them, with high sensitivity and specificity using qPCR. In the event of a suspected case of anthrax in an animal, smear specimens (when available) for use in microscopy and PCR and skin tissues for PCR can provide accurate diagnostic results (Aminu et al., 2020).

The invention of the DNA amplification method using the polymerase chain reaction (PCR) by Kary B. Mullis, in 1983, provided new possibilities for the identification of microorganisms. This was also reflected in the identification of *B. anthracis*. DNA amplification-based methods have some advantages, such as a lack of the necessity

to culture the microorganisms and the possibility to test inactivated samples, which make these methods safer than conventional methods (Zasada, 2020). The most widely used genetic markers for *B. anthracis* identification are located on anthrax virulence plasmids pXO1 and pXO2. There are usually genes coding components of anthrax toxin (protective antigen, edema factor, lethal factor), located on pXO1 and genes coding the capsule, located on pXO2.

The use of DNA amplification-based PCR (Polymerase Chain Reaction) and Real-time PCR tests can be used for the definitive and rapid diagnosis of *B. anthracis* in clinical and environmental specimens. Diagnostic targets include especific DNA regions in the following genes: pagA (pXO1), capB (pXO2), capC (pXO2) and Ba813 (chromosomal) (Zasada et al., 2018; Bentahir et al., 2018; Tamborrini et al., 2010; Zasada, 2020). It has been reported that newer isothermal DNA amplification techniques, such as RPA (recombinase polymerase amplification), HDA (helicase-dependent amplification) and LAMP (loop-mediated isothermal amplification) can be used. Rapid DNA-based methods are particularly useful for the confirmation of the cause of infection in patients, who have been treated with antibiotics, which would prevent the bacteria from growing on culture (Cohen et al., 2016; Zasada et al., 2015; Kim et al., 2015).

Ortatatli et al. (2019), applied two PCR methods [classical (wet) and lyophilized (dry)], with the same mixtures to determine the efficiency and detection limits of *B. anthracis* DNA. Primers specific for three target genes (cap, pag, sap) of *B. anthracis* were used. When comparing the efficiency of the two PCR methods, the PCR results of the sap gene region with 2 different DNA concentrations showed statistical significance in favor of the conventional wet PCR method. For sufficient DNA extraction, researchers recommend that DNA extraction for biomolecular diagnostic tests, such as PCR be performed using bacteria that are cultured, or enriched in liquid media.

The difficulties in identification of *B. anthracis* are related to the high phenotypic and genetic similarity of this species to Bacillus cereus and other closely related species. Other genetically, closely related species of *Bacillus* genus, that are widely distributed in the environment include Bacillus thuringiensis, Bacillus mycoides, Bacillus pseudomycoides, and Bacillus weihenstephanensis. The genome similarity between *B. anthracis* and *B. cereus, B. thuringiensis, B. mycoides, B. pseudomycoides,* and *B. weihenstephanensis* is so significant, that all these species have been included in one bacterial group called *B.cereus* Group *sensu lato* (Zasada, 2020). The BA5345 marker with TaqMan probes in a real-time PCR assay can be used successfully, as a chromosomal marker in the routine identification of B. anthracis; furthermore, the detection of plasmid markers indicates virulence of the tested strains. In order to achieve a complete identification of *B. anthracis*, it seems that first the chromosomal sequence should be found and, in the next step, the pag and cap genes located on the plasmids should be identified to determine the virulence of the tested isolates (Cieślik et al, 2015).

Real-time PCR has some advantages compared to PCR, such as the possibility of quantification of the synthesized product and the lack of post-PCR manipulations for detection of results. Newertheless, both techniques require precise temperature changes, and therefore dedicated apparatuses are necessary. This disadvantage might be overcome by isothermal DNA amplification methods (Zasada, 2020).

Braun et al. (2021) recommend a variant of RT-PCR. The assay can be performed as a real-time PCR, with only DNA as template, or as a real-time RT-version using both cellular pools of nucleic acids (DNA and RNA) as template. The assay is highly species specific, does not give false positives and is sensitive with LoD of about 0.6 copies/µL (DNA only), and about 1.3 copies/µL (DNA+RNA). With the high abundance of 16S rRNA moieties in cells, this assay could be expected to facilitate the detection of B. anthracis by PCR. While standard PCR assays are well established for the identification of B. anthracis from pure culture, the exceptional sensitivity of the new 16S rRNA-based assay can be applied, when detection of small remnants of the pathogen is required.

B. anthracis can be divided into separate genotypes. Whole genome sequencing (WGS) can be used to identify different genotypes and has become an integral part of surveillance and outbreak investigations (ECDC, 2022). Two publications have provided updated frameworks for B. anthracis genotyping, based on two different, but congruent, high-resolution methods: core genome multilocus sequence typing (cgMLST) and single nucleotide polymorphisms (SNP) analysis (Bruce et al., 2020; Abdel-Glil et al., 2021). The authors' work can guide epidemiologists in future anthrax outbreaks and facilitate international collaboration in case of multi-country events.

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has been applied for identification of microorganisms and, currently, is broadly used in medical and veterinary diagnostic laboratories (Zasada, 2020). Wei et al. (2021) defined (MALDI-TOF MS as a rapid, highly accurate and highly sensitive and low-cost, high-throughput method that is a reliable tool for screening *Bacillus anthracis* strains and Bacillus cereus. Application of DNA sequencing enables detection-specific single nucleotide polymorphisms (SNPs), specific for *B.anthracis* in selected chromosomal markers. Newertheless, the use of DNA sequencing is complicated in the field. Therefore, the use of various molecular probes, HRM, as well as PCR-RFLP, for detection of specific point mutations has been proposed; for example, PCR-RFLP of SG-850 marker and RSI-PCR of plcR marker (Zasada, 2020). In recent years there has been a focus on the development of biosensors capable of the rapid and specific detection of B. anthracis. There are currently four types of biosensor platforms: Genosensors (Nucleic Acid Probes), Immunosensors (Antibody Probes), Aptasensors (Aptamers) and Peptide-Nucleic Acid Chimera Probes (PNAs). They employ a range of signal generation approaches, which include electrochemical (amperometric, potentiometric and conductometric), optical and piezoelectric. Genosensors work on the principle that the binding of pathogen-specific DNA to a probe generates a signal. To date, genosensors have been developed, which target pagA, lef and BA813 (Zasada, 2020; Raveendran et al., 2016; Ziółkowski et al., 2018; Zhang et al., 2011).

Measures for the prevention of anthrax disease

1. General preventive and sanitary-hygienic measures

Anthrax in animals is subject to mandatory registration and announcement in accordance with the terms and conditions of Ordinance No. 23 of 2005 (SG, No. 6 of 2006).

Considering the zoonotic nature of the disease, the Bulgarian Food Safety Agency (BFSA) coordinates and communicates the measures taken, with the state health control authorities of the Ministry of Health, according to the Law on veterinary medical activity (LVMA, 2006). In stationary anthrax outbreaks and threatened territories, general preventive and sanitary-hygienic measures are carried out, depending on the epizootic situation (Ordinance, 2020, Chapter Two, Section I, Art. 6, 7 and 8).

2. Specific veterinary preventive measures

Specific veterinary preventive measures include vaccination of susceptible animals and carrying out diagnostic and curative-prophylactic treatments with veterinary medicinal products, according to the World Health Organization (WHO) Guidelines for the Surveillance and Control of Anthrax in Humans and Animals and the Program for the Prevention, Surveillance, Control and eradication of animal diseases and zoonoses, under Art. 118 of the LVMA. BFSA annually organizes prophylactic vaccination of cattle, sheep and horses against anthrax. Such vaccination of buffaloes, goats and pigs may be carried out, only at the discretion of the BFSA. For active immunization of susceptible animals, a highly immunogenic, avirulent anthrax vaccine containing a non-encapsulated variant of *B. anthracis* is used in our country. There is also a method of passive immunization (seroprophylaxis), by injecting hyperimmune serum at 0.1 - 0.2 ml/kg body weight. Immunity lasts about two weeks. It is applied as a first measure to protect healthy animals in herds, where the disease has broken out. Milk from farms with sick and suspected anthrax animals, as well as from those

with complications after vaccination against anthrax, is not consumed by humans and animals. In case of a positive ascolization result, the official veterinarian (OV) takes the measures provided for such a case in Chapter Two from Ordinance 6/5 October, 2020

Measures in case of suspicion and confirmation of anthrax in livestock facilities

In the case of suspicion of the presence of a disease manifested by a decrease in productivity for unclear reasons, or in the event of a sudden death of animals, measures provided for in Chapter Three of the Ordinance of October 6/5, 2020 are taken. The same include measures for: notification, isolation of sick animals, termination of slaughter, autopsies and blood manipulations; as well as treating the sick animals, disposing of the corpses of the dead and disposing of the milk; the regrouping and movement of animals is prohibited, the access of outsiders and means of transport is restricted, and preliminary and final disinfection is carried out in all endangered places.

Therapy includes administration of a specific serum (250-300 ml for horses and cattle, 100-150 ml for sheep and goats; 30-60 ml for pig, i.m. and repeated after 6-7 hours); antibiotics (penicillins x 20-40,000 UI/kg body weight per 24 hours, etc.) and other symptomatic agents. In the case of the acute and subacute form, the treatment gives results if it is applied in time (Vachev, 1977; Arsov, 1984; Taylor, 1995), but such treatment is no longer applied in practice. Livestock facilities, for which there is a suspicion, or a confirmed outbreak of anthrax are placed under foreclosure and a plan is drawn up to contain and eradicate the outbreak, in accordance with Ordinance on the order and manner of announcing and registering infectious animal diseases. The foreclosure is lifted after 20 days have passed since the last case of a sick, or dead animal and vaccination of all unvaccinated animals.

Anthrax in the context of the One Health concept. The epidemiology of anthrax includes an environmental component, as well as livestock, wildlife, and human components. This makes anthrax an ideal example to discuss in the context of One Health. Many outbreaks of anthrax in wildlife go undetected or underreported due to inadequate surveillance and hardship. Human disease is usually acquired accidentally, during anthrax outbreaks among domestic animals and wild animals. An exception is the intentional targeting of people with anthrax in the course of biological warfare or bioterrorism. In the context of the One Health concept, a contaminated environment is periodically a source of infection for a variety of hosts, humans, domestic animals, or wildlife. Accidental hosts are most commonly infected by handling, or eating carcasses of infected ungulates, but may also rarely become infected by contact with contaminated animal products (eg, hides and wool), or contaminated biting insects. In areas, where anthrax is endemic in wildlife, or where there is an active outbreak of anthrax in wildlife, the organisms may be transmitted from this source to neighboring livestock populations through water runoff and dispersal by birds and other scavengers, and through flies (Bengis & Frean, 2014). The main source of environmental contamination from anthrax spores is the carcass of an animal that died from anthrax. To minimize spore formation, carcasses should not be opened and preferably disposed of intact. Keeping carcasses intact until significant decay has occurred will theoretically kill most vegetative anthrax bacilli and reduce their ability to form spores.

More than 95% of anthrax cases in people with the cutaneous form of anthrax result from handling infected cadavers, or the skin, hair, flesh, or bones of such cadavers. B. anthracis is not invasive and requires a lesion for infection. The risk of gastrointestinal anthrax can occur if individuals eat meat from animals infected with anthrax. Inhalation of infectious doses becomes significant in occupations, involving the processing of animal by-products for the production of goods (industrial anthrax). These include leather tanning, wool production, animal hair, carpet production, bone processing and other similar industries, where the potential for aerosol of significant numbers of spores increases the risk of exposure to infectious doses (OIE, 2018).

Controlling anthrax in humans depends primarily on effective control of the disease in animals. The prevention of human anthrax is based on the control of animal infection, education of animal owners and occupational risk groups. For example, ill, or dead animals ought not be slaughtered, skinned, or butchered for consumption of their meats, or have their products used. The control of infections in animals consists of close surveillance, vaccination of animals against anthrax and good veterinary practices, which include the burying, or cremation of infected animal carcasses and the use of effective decontamination and disinfection procedures (Doganay et al., 2023).

Application of *B. anthracis*-produced LF and PA in oncology. Bachran et al. (2016) describe anthrax toxin as a versatile and powerful tool in biomedicine, and specifically in antitumor therapies. The idea of targeting anthrax toxin to tumor cells has led to its successful use in tumor therapy in animal models. Protein toxins, delivered to the cancer cell interfere with protein synthesis, cause DNA damage, and induce apoptosis. The receptor-binding component of the toxin must be cleaved by furin-like proteases, to be activated and deliver the enzymatic moieties of lethal factor and edema factor to the cytosol of cells. Alteration of the protease cleavage site allows activation of the toxin selectively in response to the presence of tumor-associated proteases.

B. anthracis produces three secretory proteins; lethal factor (LF), protective antigen (PA) and edema factor (EF). LF has the ability to check the proliferation of mammary tumors, mainly depending on the mitogen-activated protein kinase kinase (MAPK) signaling pathway. In evaluating the therapeutic potential of recombinant LF (rLF), recombinant PA (rPA) and lethal toxin (rLF + rPA = LeTx) on primary ductal mammary carcinoma cells, Khandia et al. (2017) found a significant reduction in tumor cell proliferation and revealed that rLF, or LeTx could be used as a therapy option against primary ductal mammary carcinoma cells. The protective antigen (PA) protein, produced by *B. anthracis* has been shown to be a potential option for targeted delivery of drug molecules, or toxins to tumors. PA is a component of anthrax toxin, facilitating the translocation of toxin enzymes into the cell cytosol through highly specific and selective interactions with two highly conserved anthrax toxin receptors, known as tumor endothelial marker 8 (TEM8) and capillary morphogenesis gene 2 (CMG2).

Crawford et al. (2019) identified PA as a highly specific targeting agent for tumors overexpressing anthrax toxin receptors, such as cutaneous squamous cell carcinoma (SCC). The MEK /mitogen-activated protein kinase kinase/ family of protein kinases plays a key role in regulating cellular responses to mitogens, as well as environmental stress. Inappropriate activation of these kinases contributes to tumorigenesis. In contrast, anthrax lethal factor, the major virulence factor of anthrax toxin, has been shown to selectively inactivate MEKs.

Bodart et al. (2002) highlighted the potential role of MEK signaling, in using anthrax lethal toxin to treat cancer and provided evidence to support the argument that, as a potent inhibitor of multiple MEK signaling pathways, anthrax lethal toxin represents a novel and potentially useful chemotherapeutic agent.

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