ANTHRAX IN THE HORSE

T. S. Mair* and G. R. Pearson[†]

Bell Equine Veterinary Clinic, Mereworth, Maidstone, Kent ME18 5GS; and [†]Department of Clinical Veterinary Science, School of Veterinary Science, Bristol University, Langford House, Langford, Bristol BS40 5DU, UK.

Keywords: horse; anthrax; *Bacillus anthracis*

Summary

Anthrax is a highly contagious disease of domestic animals, wild animals and man caused by *Bacillus anthracis*. In most animals it results in a rapidly fatal septicaemia and 'sudden death'. The organism exists in vegetative and spore states. Sporulation occurs during exposure to air, and the spores can survive in the environment for many years. The source of *B. anthracis* spores in infections is usually the soil, but spores have also been found in hay and feed. Infection usually occurs by ingestion, although inhalation and cutaneous penetration can also occur. Although peracute infection and 'sudden death' may occur, many horses develop an acute infection resulting in signs of colic, fever, dyspnoea and subcutaneous oedema. The disease typically progresses rapidly, with death within 24–48 h. Treatment with i.v. penicillin or oxytetracycline can be effective so long as it is initiated early.

Introduction

Anthrax (German: *milzbrand*; French: *charbon*) is an important peracute, acute or sub-acute highly contagious disease of domestic animals, wild animals and man caused by *Bacillus anthracis* (de Vas and Turnbull 2004; Long 2007). In most animals it results in a rapidly fatal septicaemia and 'sudden death'. Anthrax most commonly develops in domestic and wild herbivores, such as cattle, sheep, goats, antelope and deer (Shadomy and Smith 2008). Anthrax is of particular topical importance because of its bioterrorist potential (Crupi *et al.* 2003).

Aetiology and epidemiology

Bacillus anthracis is a large, spore-producing, Gram-positive rod (approximately 1 μm in diameter and 3–6 μm long) that can be cultured under both aerobic and nonaerobic conditions on most laboratory media. Optimum growth occurs at 37ºC. On agar plates, the anthrax organisms form surface colonies with a ground glass appearance, and the margins of the colonies are irregular under low magnification ('Medusa-head colonies').

The bacillus was first isolated from animals that died of anthrax in 1877 by Robert Koch (Koch 1877); he was able to infect mice with the spores, thus demonstrating Koch's postulates for the first time (Shadomy and Smith 2008). The bacillus is nonhaemolytic, has a capsule, and is nonmotile. In infected tissues, the large bacilli occur individually or grow in chains (2–6 organisms) and the characteristic capsule is visible when stained with polychrome methylene blue (McFadyean reaction) (Parry *et al.* 1983; Quinn and Turnbull 1998). The capsule stains pink, in contrast to the body of the organism, which stains blue. *B. anthracis* can be differentiated from other bacilli on the basis of the presence of a capsule, susceptibility to penicillin, lack of motility, absence or delayed haemolysis when cultured on blood agar, and susceptibility to γ phage (Shadomy and Smith 2008). The organism exists in vegetative and spore states. The organism sporulates during nutrient deprivation and exposure to air, as occurs during *in vitro* cultivation or when an infected carcase is disrupted by scavengers or during necropsy. Sporulation allows the organism to survive in the environment for many years, and spores are highly resistant to extremes of temperature, UV light and many chemical disinfectants. Spores can survive the tanning and processing of hides, and have been

---

*Author to whom correspondence should be addressed.

shown to remain viable for more than 50 years (Umeno and Nobata 1938). Spores germinate when exposed to 65°C for 15 min, and change into the vegetative bacillary form. In contrast to spores, the vegetative bacilli are susceptible to desiccation, high temperatures and chemical disinfectants. Anthrax has a worldwide distribution, but it is most common in agricultural regions of South and Central America, sub-Saharan Africa, central and southwestern Asia, and southern and eastern Europe (Hugh-Jones 1999).

The source of *B. anthracis* spores in infections is usually the soil, which is considered to be the natural reservoir of *B. anthracis*, but spores have also been found in hay and feed (e.g. from contaminated bone meal and vegetable proteins such as groundnut). Wool and hair wastes, cleanings used in fertilisers, and tannery effluents can also be sources of infection (Timoney *et al.* 1988). Outbreaks typically occur in the summer during periods of hot, dry weather that follow heavy rain and flooding. Additionally, outbreaks may be triggered by disruption of the soil in areas where anthrax-infected carcases have been buried (Shadomy and Smith 2008). Natural outbreaks often occur in cycles, with the primary infection involving one or more animals that have recently been introduced into an area. After they die, the infected carcases contaminate the soil with *B. anthracis*. The next cycle of infection may involve multiple animals that are exposed to the contaminated soil (or carcases). Most natural cases of anthrax in horses are associated with disease in cattle.

Outbreaks of anthrax in animals are often associated with low-lying areas with soil that has high moisture and organic contents, and alkaline pH (Dragon and Rennie 1995). Such areas have been described as 'incubator areas', indicating favourable conditions that permit spore germination and vegetative multiplication (van Ness and Stein 1956). As the area dries out, resporulation occurs. Grazing animals become infected when they ingest *B. anthracis* spores on vegetation. Vegetative bacilli are shed in blood and other discharges from infected animals that are dying or dead; the bacilli then sporulate and contaminate the surrounding soil and water sources (Shadomy and Smith 2008). Within the intact carcases of animals that have died of anthrax, the vegetative bacilli cannot compete with anaerobic bacteria, and they usually die within 2–3 days because of the putrefactive processes in the decomposing carcases (Hugh-Jones and de Vos 2002). Thus, only if the carcase is disrupted, or discharges are released, will the vegetative bacilli be able to reach an aerobic environment and sporulate.

In man, anthrax primarily develops following exposure to infected animals, tissues or products from infected animals (Brachman *et al.* 1996). Anthrax exposure can be divided into either agricultural (nonindustrial) or industrial routes (Shadomy and Smith 2008). Agricultural exposure occurs among people with direct contact with sick or dying *B. anthracis*-infected animals or through handling the carcases or tissues of such animals. Industrial exposure results from cutaneous inoculation or inhalation of particles containing anthrax spores that are generated during the processing of contaminated hides, hair or wool.

Pathogenesis

Infection usually occurs by ingestion, although inhalation and cutaneous penetration can also occur. Infection by biting flies may also be possible (Cousineau and McClenaghan 1965). Following entry into a host by ingestion, inhalation or introduction through the skin, the spores are triggered to germinate.

Susceptibility to the disease varies among different species with herbivores being most susceptible. Pigs are susceptible, but unlike other species they may survive the disease. When spores are ingested, germination and production of vegetative bacilli occurs either in the mucosa of the pharynx or in the intestinal tract. The organisms multiply in an oedematous focus near the site of primary invasion, and then spread via lymphatic channels to lymph nodes. Replication and multiplication continues in the regional lymph nodes, followed by spread to the bloodstream, with resultant bacteraemia, septicaemia and dissemination to multiple organs. The organism is filtered out by the spleen until splenic clearance capacity is overwhelmed. Uncontrolled multiplication continues in the blood until the animal dies. At death, 80% of the organisms are in the blood and 20% are in the spleen, which is often greatly enlarged (Timoney *et al.* 1988).

Proliferation of lethal toxin is the usual cause of death. The bacterium has 3 main virulence factors: the poly-D-glutamic acid capsule and 2 protein

exotoxins (oedema toxin, ET, and lethal toxin, LT). The 3 virulence factors are encoded on 2 plasmids, pXO1 and pXO2. The poly-D-glutamic acid capsule protects it against complement and phagocytosis. The pXO1 plasmid encodes for 3 components that comprise the 2 exotoxins: protective antigen, lethal factor and oedema factor. Protective antigen (PA) allows the toxin to bind to cell surfaces and is responsible for inducing protective immunity against *B. anthracis* infection. PA combines with either oedema factor or lethal factor to form ET or LT respectively. Lethal toxin mediates the lethal effects of the toxin by virtue of hypoxic tissue injury, liver failure and shock. Oedema toxin causes the extravasation of intercellular fluids into subcutaneous, peritoneal and tissue spaces; it also impairs host defences, including inhibition of neutrophil function and phagocytosis. The pOX2 plasmid contains genes for the capsule, which is weakly antigenic and protects vegetative bacilli from phagocytosis by host macrophages.

Clinical features

Nonhuman animals most often develop anthrax following ingestion of spore-contaminated foodstuffs. Herbivores (e.g. cattle, sheep and goats) are considered to be most susceptible. Horses are susceptible, and in some outbreaks, the infection rate has been reported to be higher in horses than in cattle (Fox *et al.* 1977). It has been suggested that horses may either be more susceptible than cattle to anthrax, or they may graze in a manner that results in the greater ingestion of contaminated soil (Fox *et al.* 1977).

The clinical manifestations of the disease depend on the manner of infection. When it occurs through the respiratory or gastrointestinal tract with no visible evidence of localisation, its principal character is its sudden onset and rapidly fatal course (Timoney *et al.* 1988). Three different forms of anthrax are recognised in man and animals (Turnbull 2002; Shadomy and Smith 2008). Cutaneous disease is seen most commonly in people working with animal hides (this form is unusual in domestic animals); it results in localised gelatinous oedema leading to a 'malignant pustule'. The organism can then disseminate from the necrotic ulcer to cause septicaemia and death. Pulmonary anthracic disease occurs in man and animals following the inhalation of spores. This results in a fatal acute haemorrhagic mediastinitis. Gastrointestinal disease follows oral ingestion of the organism; following penetration of the mucosa, local pustule formation and ulceration is followed by systemic dissemination.

The incubation period following exposure to *B. anthracis* spores typically ranges from 3–7 days (range <1 day–14 days). Peracute infection may occur in horses and other herbivores, and results in 'sudden death' (death within 1–2 h). Alternatively, horses can develop an acute infection resulting in signs of depression, colic, fever, dyspnoea, congested mucosae and tachycardia. Subcutaneous oedema of the ventral neck, thorax and abdomen may occur, and there may be haemorrhagic discharges from body orifices. The disease typically progresses rapidly, with death within 24–48 h.

In swine and dogs, anthrax generally assumes a localised form with pharyngeal involvement and gastroenteritis (Timoney *et al.* 1988). There is often severe inflammatory oedema of the tissues of the head and neck, which can cause suffocation as a result of severe oedema of the glottis.

The principle lesions in animals dying from anthrax include widespread oedema, necrosis, haemorrhage and haemorrhagic discharges from the orifices. Rapid *post mortem* decomposition follows with bloating and incomplete development of *rigor mortis*, often with petechiae and ecchymoses (Gleiser 1967). If anthrax is suspected as the cause of death in any animal, the carcase should not be incised so as to prevent sporulation and dissemination of the spores (Shadomy and Smith 2008).

Diagnosis

Necropsy of suspected anthrax cases should not be performed. Diagnosis should be attempted by methods that release a minimum number of anthrax organisms into the environment. Exposure to atmospheric oxygen will allow the formation of spores, which will be extremely difficult to destroy. Blood samples or dried blood smears should be obtained from a peripheral vessel such as an ear vein. Since fulminant anthrax is usually associated with an overwhelming bacteraemia, *B. anthracis* can usually be identified in blood smears. Swabs or smears of local oedematous lesions can also be

examined. Samples should if possible be obtained within a few hours of death. Polychrome methylene blue (McFadyean reaction) or Giemsa stains should be used. Laboratory personnel should be aware that anthrax spores are not necessarily killed by the heat required to fix smears on microscope slides (Timoney *et al.* 1988). A fluorescent antibody test, agar-gel precipitin technique or PCR can also be used. The organism is readily isolated in culture if antimicrobial therapy has not been initiated. However, in view of the public health risk, culture of *B. anthracis* should only be performed by suitably specialised state laboratories.

Treatment

Prompt and aggressive treatment of infected animals is necessary because of the rapidly progressive, and often fatal nature of the disease (Shadomy and Smith 2008). Penicillin has generally been considered the treatment of choice for many years, but variable penicillin resistance and inducible β–lactamase production by *B. anthracis* isolates have been reported (Lightfoot *et al.* 1990). Failure of penicillin treatment in animals has been reported (Bailey 1954). However, high doses of penicillin administered i.v. (44,000 iu/kg bwt q. 6 h) would be expected to be efficacious in most cases. Oxytetracycline administered i.v. (6.6 mg/kg bwt q. 12 h) is also likely be effective. Antimicrobial treatment should be continued for at least 5 days.

In many countries, anthrax is considered to be a notifiable or reportable disease, and the state veterinary services should be informed of all suspect or confirmed cases. Affected premises should be quarantined, and all infected carcases, as well as contaminated bedding, soil etc., should be incinerated. If incineration is not feasible, infected carcases should be buried at least 2 m deep with a covering of quick lime (calcium oxide) or chloride of lime (calcium hydroxide, calcium chloride, and calcium hypochlorite, with 25% active chlorine) and soil. All items that become contaminated with *B. anthracis* should undergo decontamination. The spores resist steaming or boiling at 100ºC for 5 min, but can be destroyed by steam sterilisation under pressure at 121ºC for 30 min (i.e. autoclaving) or by ethylene oxide sterilisation with a contact time of at least 18 h (Shadomy and Smith 2008). The spores are also resistant to disinfectants such as 5% phenol or mercuric chloride. A 2–3% solution of formalin is effective if applied at a temperature of 40ºC, and a 0.25% solution is effective when applied for 6 h at 60ºC (Timoney *et al.* 1988).

Vaccination

The Sterne vaccine (Sterne 1937) is produced by growing virulent anthrax strains on 50% serum agar in an atmosphere of 10–30% CO_2. The vaccine is used to prevent anthrax in cattle but is not recommended for use in horses. It is not available in many countries. Injection site reactions and severe oedema have been reported following the use of this vaccine in horses (Mongoh *et al.* 2008).

References

Bailey, W.W. (1954) Antibiotic therapy in anthrax. *J. Am. vet. med. Ass.* **124**, 296-300.

Brachman, P.S., Kaufman, A.F. and Dalldorf, F.G. (1996) Industrial inhalation of anthrax. *Bacteriol. Rev.* **30**, 646-659.

Cousineau, S.G. and McClenaghan, R.J. (1965) Anthrax in bison in the northwest territories. *Can. vet. J.* **6**, 22-24

Crupi, R.S., Asnis, D.S., Lee, C.C., Santucci, T., Marino, M.J. and Flanz, B.J. (2003) Meeting the challenge of bioterrorism: lessons learned from West Nile virus and anthrax. *Am. J. emerg. Med.* **21**, 77-79.

De Vas, V. and Turnbull, P.C. (2004) Anthrax. In: *Infectious Diseases of Livestock*, 2nd edn., Eds: J.A.W. Coetzer and R.C. Tustin, Oxford University Press, Cape Town. pp 1788-1818.

Dragon, D.C. and Rennie, R.P. (1995) The ecology of anthrax spores: tough but not invincible. *Can. vet. J.* **36**, 295-301.

Hugh-Jones, M. (1999) 1996-1997 global anthrax report. *J. appl. Microbiol.* **87**, 189-191.

Hugh-Jones, M. and de Vos, V. (2002) Anthrax and wildlife. *Rev. Sci. Tech.* **21**, 359-383.

Fox, M.D., Boyce, J.M., Kaufman, A.F., Young, J.B. and Whitford, H.W. (1977) An epizootiologic study of anthrax in Falls County, Texas. *J. Am. vet. med. Ass.* **170**, 327-333.

Gleiser, C.A. (1967) Pathology of anthrax infection in animal hosts. *Fed. Proc.* **26**, 1518-1521

Koch, R. (1877) The aetiology of anthrax based on the ontogeny of the anthrax bacillus. *Med. Classics* (1937) **2**, 787-820. Originally published in German in: *Beitrage zur Biologie der Pflanzen* **2**, 277-282.

Lightfoot, N.F., Scott, R.J.D. and Turnbull, P.C.B. (1990) Antimicrobial susceptibility of *Bacillus anthracis. Salisbury Med. Bull., Suppl.* **68**, 95-98.

Long, M.T. (2007) Anthrax. In: *Equine Infectious Diseases*, Eds: D.C. Sellon and M.T. Long, Saunders Elsevier, Philadelphia. pp 273-275.

Mongoh, M.N., Dyer, N.W., Stoltenow, C.L. and Khaitsa, M.L. (2008) Risk factors associated with anthrax outbreak in animals in North Dakota, 2005: a retrospective case-control study. *Public Health Rep.* **123**, 352-359.

Parry, J.A., Turnbull, P.C.B. and Gibson, J.R. (1983) *A Colour Atlas of* Bacillus *Species,* Wolfe Medical Publications, London.

Quinn, C. and Turnbull, P. (1998) Anthrax. In: *Topley and Wilson's Microbiology and Microbial Infection,* Eds: W.J. Hausler and M. Sussman, Edward Arnold, London. pp 799-818.

Shadomy, S.V. and Smith, T.L. (2008) Anthrax. *J. Am. vet. med. Ass.* **233**, 63-72.

Timoney, J.F., Gillespie, J.H., Scott, F.W. and Barlough, J.E. (1988) *Bacillus anthracis*. In: *Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals,* 8th edn., Comstock Publishing Associates, Ithaca. pp 206-211.

Turnbull, P.C. (2002) Introduction: anthrax history, disease and ecology. *Curr. Top. Microbiol. Immunol.* **271**, 1-19.

Umeno, S. and Nobata, R. (1938) On viability of anthrax spores. *J. Jpn. Soc. vet. Sci.* **17**, 221-223.

Van Ness, G. and Stein, C.D. (1956) Soils of the United States favourable for anthrax. *J. Am. vet. med. Ass.* **128**, 7-9.