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TETANUS IN ANIMALS — SUMMARY OF KNOWLEDGE

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ABSTRACT

Tetanus is a neurologic non-transmissible disease (often fatal) of humans and other animals with a worldwide occurrence. *Clostridium tetani* is the spore producing bacillus which causes the bacterial disease. In deep penetrating wounds the spores germinate and produce a toxin called tetanospasmin. The main characteristic sign of tetanus is a spastic paralysis. A diagnosis is usually based on the clinical signs because the detection in the wound and the cultivation of *C. tetani* is very difficult. Between animal species there is considerable variability in the susceptibility to the bacillus. The most sensitive animal species to the neurotoxin are horses. Sheep and cattle are less sensitive and tetanus in these animal species are less common. Tetanus in cats and dogs are rare and dogs are less sensitive than cats. Clinically two forms of tetanus have been recognized, i. e. localized and generalized. The available treatment is not specific because the toxin in neuronal cells cannot be accessed by antitoxin antibodies. The aim of the therapy is to: neutralise the unbound neurotoxin, inhibit *C. tetani* growth in the wound, and provide supportive care to mitigate the effects of the neurotoxin. The treatment is difficult with an unclear prognosis.

Key words: animal; *Clostridium tetani*; toxin; spasm; treatment

INTRODUCTION

Of the clostridial neurotoxins, botulinum neurotoxin and tetanus neurotoxin are the most potent toxins [15]. Their extraordinary toxicity is caused by neurospecificity and metalloprotease activity, which results in the paralysis. Tetanus is potentially a fatal disease and in some countries it is still very active. Tetanus is a traumatic clostridiosis, an infection in humans and other animals with worldwide distribution. It is a neurologic, toxic, non-transmissible disease characterised by the spastic paralysis caused by the neurotoxin which has been identified as tetanospasmin. Tetanospasmin is produced by an anaerobic bacteria *Clostridium tetani* in a deep wound under anaerobic conditions. Various animal species are differently susceptible to this infection [22, 30, 38, 35].

Clostridium tetani

Clostridium tetani is an anaerobic, spore-forming bacillus. The vegetative form of *C. tetani* is a thin motile rod 0.3—0.6 μm wide and 3—12 μm long. The cell is typically a peritrichous flagella, however some strains are non-motile and non-flagellated. Young cultures are gram-positive, however older cultures can lose the Gram coloration. *C. tetani* can be cultured on blood agar, media with peptones or tissue extracts mostly under anaerobic conditions and at an optimum temperature of 37 °C. Some strains are non-toxigenic and do not produce the toxin [35, 41]. Under adverse conditions, the spores are produced in the process of sporulation; these are—in contrast with the vegetative forms of *C. tetani*—very durable. The vegetative forms are sensitive to disposal and they are vulnerable to disinfection, heat and changes in the environmental pH. The spores are able to survive exposure to various environmental conditions and resistant to disinfectants, such as ethanol and phenols. They can be destroyed by autoclaving at 120 °C for 15—20 minutes. The spores are round and have a terminal with the characteristic shape of a „tennis racket" (0.7 μm × 0.5 μm) and can survive in the soil for 10 years or more [22, 23, 40].

The formation of the spores is variable and depends on the strain type. *C. tetani* spores occur worldwide and they are most often found in soil rich in organic matter in hot and humid environments, but also in the dust, on plants, in contaminated feed, in animal digestive system and the faeces. These bacteria occur in the intestine of animals but they are not the part of the normal digestive flora and do not result in infections. *C. tetani* is not an invasive bacterium and it is not able to enter healthy cells but a deep wound with damaged and necrotizing tissue is a favourable environment for *C. tetani* growth [10, 22, 42].

Toxins

When the spores reach an appropriate anaerobic conditions in the organism, they happen to become pathogenic; then they germinate and multiply in the contaminated wound. Deep wounds without air access and with the presence of necrotic tissue favour spore germination. Deep penetrating injuries (injury to paw or limb, dog bite, biting of contaminated objects in replacement of deciduous tooth), castration, puncture and surgical wounds, and umbilical wounds in neonates are among the most common cases of tetanus. The contaminated wound can be very small and difficult to find. Locally, exotoxins are produced at the site of the wound. *C. tetani* produces two main toxins, neurotoxin (tetanospasmin) and hemolysin (tetanolysin). The others are tetanolepsin and nonspasmogenic toxins. The role of tetanolepsin in the pathogenesis of tetanus is not clinically significant. The influence of the nonspasmogenic toxin is not completely known; it may be involved in blocking transmission in peripheral neuromuscular junctions and it may be the cause of peripheral nervous system paralysis [1, 4, 22, 35]. Tetanolysin causes changes in the permeability of liposomes and biological membranes and can affect a variety of cells including: erythrocytes, neutrophils, macrophages, fibroblasts and others. The effect of this oxygen-sensitive hemolysin results in local tissue necrosis [4, 35].

Tetanospasmin (Tetanus neurotoxin—TeNT) is synthesized as a single-chain polypeptide with a molecular weight of 150 000 Da, encoding with the gene localized on the plasmid. In the form of a single-chain it is weakly active. The toxin must be activated by clostridial or exogenous host proteases to a form consisting of the light (~50 kDa) and heavy chains (~100 kDa), which are bound by a disulphide bridge [20, 33, 38]. One serotype of tetanospasmin is known, but small variations in the amino acid sequence of TeNT proteins without a significant effect on their antigenicity has been reported [12, 27].

Tetanospasmin ascends the axons of motor nerves, which are located adjoining to the contaminated wound and after reaching the cell bodies within the spinal cord, it climbs to the brain. Short nerves are affected before longer nerves and that is the reason why cranial nerves are typically involved before limb nerves [4, 36]. Tetanospasmin permeate also by the lymph to the blood stream. There is a possibility that the circulating toxin might be able to cross the blood-brain barrier and it could bind to axon terminals at distant sites. Tetanospasmin inhibits the release of the inhibitory neurotransmitters glycine and γ-aminobutyric acid (GABA) at interneurons in the spinal cord and brain. This process results in the classical sign of tetanus—continual excitation of the nerves. The high toxicity of TeNT is due to very fast binding to neurons and the activity of enzymes responsible for the cleavage of the necessary proteins to block neurotransmitter release with ensuing neuroparalysis [13, 22, 35, 37].

Tetanus in animals

Different animal species differ in their sensitivity to neurotoxins. The most susceptible species are the horse,

guinea pig, monkey, then sheep, mouse, goat, and human; whereas carnivores such as cats and dogs are less sensitive and birds and poultry are the most resistant species [41]. The interspecies difference in sensitivity and resistance is related to the relative difficulty for the toxins to penetrate and bind to the nerves in different species [1]. The tetanus wound can be very small and sometimes it is difficult to find it. Tetanus may develop even after the wound has completely healed. The parts of the body with a deep wound in contact with soil have the greatest risk for tetanus formation. Accidental wounds and punctures with iron, wooden object or prickly plants at the end of the limbs, the lower side of the trunk and the abdomen are most dangerous. The umbilical infections in new-borns, mainly in lambs and foals are a common predisposing factor for the formation of tetanus. Puerperal tetanus arises after the contamination of the vaginal and uterus mucosa following a complicated delivery. Another risk factor for tetanus is the biting of various objects during replacement of deciduous teeth in dogs. The contamination with *C. tetani* can also occur during castration, injection, ear tagging, shearing and many other traumatic interventions in the body [6, 9, 11, 16, 31].

Tetanus can be observed in non-immunized horses quite often and various forms have been recorded [46]. Cattle are less susceptible than horses and sheep, but outbreaks of tetanus have been reported. General risk factors are accidental wounds, surgical castration, dehorning, and drug injections [11, 16]. In swine, tetanus is observed more often in piglets and is fatal. In cats and dogs, tetanus is rare. Cats are approximately 10 times more resistant than dogs and dogs are 600 times more resistant to tetanus than horses [22].

Signs of tetanus

Tetanus signs are characterized by spastic paralysis and by hyperactivity of voluntary muscles. With tetanus, there are two situations affecting the muscles reported, i. e. rigidity and tetanic spasms. Muscle rigidity are the tonic, involuntary, prolonged contractions. Tetanic spasms are shorter lasting muscle contractions, which can be triggered and amplified by sensory stimulations (light, touch, noise and other) [8, 45].

The incubation period of tetanus lasts 3 to 18 days and depends on the infective dose, local anaerobic conditions and the distance from the tetanic lesion to the central nervous system [28, 36]. Neuro-toxins are absorbed at the neuromuscular endplate and disseminate via retrograde axonal transport. Short nerves are involved before longer nerves. This is why the cranial nerves are preferentially affected over the nerves responsible for the extremities. Clinically, two forms of tetanus have been observed, i. e. localized and generalized [8, 36].

Localized tetanus is rare and may precede generalized tetanus. It causes painful spasm of muscles adjacent to the wound site. Next to the site of infection, one muscle or limb becomes stiff. Subsequently, the stiffness spreads to the opposite limb. If the localized tetanus is not treated, it can affect the entire central nervous system. It may occur in relatively resistant animals, most often in feline patients [1, 46].

Generalized tetanus has a difficult course and involves a large number of symptoms (Table 1). Untreated tetanus ends in the death due to paralysis of the diaphragm and respiratory muscles [8, 24].

Table 1. Clinical signs of tetanus [1, 3, 9, 34, 35, 44, 45]

CLINICAL SIGNS OF TETANUS			
Muscle rigidity	Trismus	Dyspnoe	Contracted facial musculature
Hyperextension of the limbs	Dysphagia	Hyperaesthezia	Erect ears
Stiff gait	Anorexia	Hypersensitivity to auditory or tactile stimulation	Miosis, enophthalmos, prolaps 3rd eyelid
Inability to rise or stand	Regurgitation	Urethral and anal sphincter hypertonicity	Wrinkled forehead
Tetraplegia	Vomiting	Opisthotonos	Risus sardonicus
Extension of the tail	Ptyalism	Torticollis	Swelling of the face



Fig. 1. Characteristic ear position—typical sign of a tetanic dog

Dogs with generalized tetanus are characterised by rigidity of the face muscles that quickly progresses to severe muscle rigidity, a stiff gait and the inability to rise or stand, hyperextension of the limbs and the tail. Typical representative signs of tetanus in dogs include contracted facial musculature, unambiguous ear attitude (Fig. 1) and wrinkled forehead. Dogs commonly have reflex muscle spasms associated with auditory or tactile stimulation and may vocalize during spasms because of pain [1, 3, 9, 22, 34, 35, 44]. They may appear to have some complications associated with: respiratory compromise (laryngospasm, aspiration pneumonia, and central respiratory arrest), autonomic dysfunction (dysuria, urine retention, constipation, and hyperthermia) and protracted paralysis (coxofemoral luxation, and decubitus ulcers). Failure in these different systems may result in some infections; for example, infection of the urinary or respiratory tract and others. Common complications are megaoesophagus and hiatal hernia [1, 2, 6, 9, 14, 21, 28, 32, 47].

Diagnosis

The diagnosis of tetanus in animals is usually based on: the clinical signs, case history, presence of the wound, and progression of the disorder. The overall picture of tetanus disease involves marked muscle rigidity and spastic paralysis. In some cases, a wound may not be detected due to a long incubation period. The detection of *C. tetani* in contaminated wounds is very problematic because of specific culture conditions and low bacterial counts. *C. tetani* must be cultured on specific media in compliance with strict anaerobic culture conditions [13, 22]. The detection of tetanospasmin can be used for diagnostics. It can be detected in the culture supernatant by a mouse bioassay and the typical spastic paralysis observed in mice. Tetanospasmin can be detected by ELISA with specific polyclonal or monoclonal antibodies [29]. In the serum it is possible to identify the circulating antibodies against the tetanus toxin. The minimal level of antibodies in humans considered as protective is equal to > 0.01 $IU.l^{-1}$, but tetanus cases have been reported also at this concentration. The determination of antibody titres in dog serum may be unreliable [17, 22]. Detection of *C. tetani* is possible by the PCR methods; the limit was estimated to be 10 *C. tetani* in the sample [26]. Localized tetanus can be diagnosed by electromyography. Changes in the values of haematological and biochemical parameters are nonspecific for the diagnosis. [13, 18].

One must rule out toxicities in a differential diagnosis (included poisoning with strychnine, organophosphates, metaldehyde and drugs), immune-mediated polymyositis, spinal trauma, meningoencephalitis, subarachnoid haemorrhage or cyst, acute cerebellar disease, and disorders of calcium homeostasis. The determination of diagnosis is the most difficult at the onset of the disease and in the apparent absence of the wound [22, 35, 44].

Management of tetanus treatment

Treatment of animals with tetanus consists of three main steps (Table 2): neutralising the circulating tetanospasmin, inhibiting the growth of *Clostridium tetani* and alleviating the symptoms of the neurotoxin effects. All three phases of therapy happen simultaneously. The treatment of tetanus is complicated and protracted; it must be complex and the prognosis is unclear. For neutralising the tetanus, antitoxin is administered which is used only to bind the circulating toxin. But tetanospasmin is traveling within axons and it is not known what role the circulating toxin plays in the progression of tetanus. It is possible, that antibiotics have a greater effect than antitoxins and the possibility of a cure is assumed even without the administration of antitoxins. One hundred units of antitoxin per 1 kg of body weight are recommended (maximum dose of 20 000 IU) given by a slow, over ten minutes intravenous administration. Repeated dose of antitoxin is not recommended because of

Table 2. Therapy of tetanus [1, 28, 35, 39, 43, 45]

Management of antitetanic therapy	Group of drugs	Drugs
Neutralising the circulating tetanospasmin	Antitoxin	Tetanus antitoxin
Inhibiting the growth of *Clostridium tetani*	Antimicrobial drugs	Penicillin G Amoxicillin-Clavulanate Clindamycin Tetracycline Metronidazole
Alleviating the symptoms of the neurotoxin effect	Sedatives and muscle relaxants	Diazepam Methocarbamol Phenothiazines Magnesium sulphate

the risk of anaphylactic and anaphylactoid-type reactions. An intradermal testing dose of antitoxin (0.1—0.2 ml) is needed to predict hypersensitivity, however, it does not guarantee complete certainty. In case of anaphylaxis, doses of adrenalin, corticosteroids and antihistamines should be given immediately [6, 9, 22, 35].

Antibiotics arrest the growth and reproduction of *C. tetani* and thus also multiplication of the neurotoxin [30]. At the same time, antibiotics suppress secondary infections. The first administration of antimicrobials should be injected intravenously to ensure the necessary level of antibiotics in the blood as soon as possible. At the same time, the wound should be cleaned of any foreign material such as devitalised and necrotic tissue. This action may also help reduced the production of toxins. At surgical wound debridement, veterinarians must consider the risk of patient anaesthesia. The antibiotic of first option is procaine-penicillin G at a dose of 20—100,000 IU.kg^{-1} administered intravenously, intramuscularly or subcutaneously every 6—12 hours. Other antibiotics, which may also be effective are: tetracycline at a dose of 22 mg.kg^{-1} orally every eight hours, clindamycin at a dose of 3—10 mg.kg^{-1} every eight to twelve hours intravenously, intramuscularly or orally, and amoxicillin-clavulanate at a dose of 12 mg.kg^{-1} orally or subcutaneously every twelve hours [22, 28, 35, 45]. In human patients, metronidazole is used and that has achieved good results in dogs also. Metronidazole rapidly achieves therapeutic concentrations in all body fluids and tissues, including abscess cavities. It has a strict anaerobic spectrum of activity (bactericidal against anaerobes) and is not affected by enzyme activity or the local pH in the organism. In dog patients, a dose of 10 mg.kg^{-1} every eight to twelve hours orally or intravenously is preferred. The disadvantage of metronidazole is a higher risk of toxicity [3, 5].

In the case of severe generalised tetanus, the patient must be sedated for muscle relaxation. A combination of acepromazine with buprenorphine and imidazolam or diazepam is used to control painful convulsions and muscle spasms. Diazepam is a muscle-relaxant and combines both anticonvulsant and anxiolytic activities. As a supportive treatment in the suppression of muscle spasms, magnesium sulphate ($MgSO_4$) has been proven successful, as a calcium channel blocker providing muscle relaxation [1, 28, 39, 43].

The animal patient is hypersensitive to auditory or tactile stimulus, and therefore it should be kept in a quiet, dark room. In order to reduce the risk of possible associated health problems, the patient must be placed on a soft pad and turned frequently to prevent pressure sores. It is necessary to ensure basic life functions, such as control of urination and defecation, complement fluid therapy and nutritional support [18, 32].

The recovery from tetanus is slow. Untreated tetanus is usually fatal due to respiratory failure. Cured tetanus infection and low concentration of tetanospasmin in blood does not usually induce an immune response. Vaccination is the only way to protect against tetanus. For prevention of disease, the tetanus-toxoid-containing vaccine is used; it contains tetanus toxoid, a non-toxic form of tetanospasmin. The first *C. tetani* vaccine strain was derived from a laboratory strain collected by G. W. McCoy around 1917—1919 at the US Public Health Service, Hygienic Laboratory [7, 17, 19, 25].

CONCLUSIONS

Tetanus is a neurological disease caused by the action of the neurotoxin tetanospasmin which is responsible for spastic paralysis. The bacterial agent *C. tetani* produces the neurotoxin in the wound under anaerobic conditions. Between animal species, there is considerable variability in the susceptibility to tetanospasmin. Out of all animal species, the horses are the most sensitive to the neurotoxin. Tetanus in cats and dogs is rare and they are the least susceptible of mammals.

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