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Tetanus in animals

Michel R. Popoff¹

Bacterial Toxins, Institut Pasteur, Paris, France.

¹Corresponding author: Michel R. Popoff, Bacterial Toxins, Institut Pasteur, 25 rue du Dr Roux,
75724 Paris cedex 15, France. mpopoff@pasteur.fr

Running title: Tetanus in animals

12 **Abstract.** Tetanus is a neurologic disease of humans and animals characterized by spastic
13 paralysis. Tetanus is caused by tetanus toxin (TeNT) produced by *Clostridium tetani*, an
14 environmental soilborne, gram-positive, sporulating bacterium. The disease most often results
15 from wound contamination by soil containing *C. tetani* spores. Horses, sheep, and humans are
16 highly sensitive to TeNT, whereas cattle, dogs, and cats are more resistant. The diagnosis of
17 tetanus is mainly based on the characteristic clinical signs. Identification of *C. tetani* at the
18 wound site is often difficult.

19

20 **Key words:** animals; *Clostridium tetani*; diagnosis; tetanus; tetanus toxin.

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Introduction

Tetanus is a ubiquitous neurologic disease, characterized by spastic paralysis, that has a worldwide distribution in humans and animals. Tetanus is caused by the neurotoxin, tetanus toxin (TeNT). TeNT is produced by *Clostridium tetani*, an environmental soilborne, sporulating, anaerobic bacterium. Tetanus is not a transmissible disease. Infection and resultant clinical disease often result from wound contamination with *C. tetani* spores.^{7,28}

Clostridium tetani

C. tetani is a gram-positive rod (0.3–0.6 µm wide and 3–12 µm long). Gram-positive staining is characteristic in young cultures, but *C. tetani* can lose the Gram coloration in cultures older than 24 h. Most strains of *C. tetani* have peritrichous flagella, which cause swarming growth on agar medium. However, some strains are non-flagellated and nonmotile. When cultured under anaerobic conditions, motile strains swarm over the entire surface of the agar leading to a transparent film. Discrete colonies (2–5 mm) can be obtained with media containing 3–4% agar. On blood agar, colonies are slightly raised, semi-translucent gray with irregular rough margins and surrounded by a narrow zone of hemolysis.⁵² *C. tetani* grows fairly well on the usual media containing peptones or tissue extracts.

C. tetani spores are round and terminal, giving a characteristic shape usually termed “drumstick.” Spore formation is variable according to the strain. At pH 7 or above and at temperatures near 37°C, sporulation starts within 24 h of culture and usually continues for 4–12 d. Sporulation does not occur above 41°C and is slow at pH <6. Sporulation depends on the composition of the culture medium.¹¹ Spores generally survive moderate heating (75–80°C for 10 min) but usually are destroyed within 1 h at 100°C.

44 *C. tetani* spores can germinate under anaerobic or aerobic conditions in complex media
45 such as liver broth. *C. tetani* spore germination occurs in a wide range of redox potential Eh from
46 –100 mV to +580 mV, but subsequent vegetative growth cannot be obtained with Eh above +300
47 mV.²⁶

48 The optimum temperature for growth is 37°C. Moderate growth of *C. tetani* is obtained at
49 30°C. Incubation at 25°C or 45°C induces no or poor growth. Moderate-to-abundant gas is
50 produced in peptone–yeast extract–glucose medium according to the *C. tetani* strain.

51 **Distribution of *C. tetani* in the environment**

52 *C. tetani* is a ubiquitous organism that is commonly found in soil samples from all parts of the
53 world. The frequency of its isolation varies according to the different investigations. Surveys in
54 Japan, Canada, Brazil, and the United States have identified 30–42% positive samples.⁵³ Several
55 factors influence the different frequencies of *C. tetani* isolation from soil, including pH,
56 temperature, moisture, and amount and type of organic materials. Thus, germination and
57 multiplication of *C. tetani* preferentially favor neutral or alkaline soils, with temperatures >20°C
58 and humidity reaching at least 15%.⁵²

59 Geographical distribution of *C. tetani* shows a higher presence in warm humid regions,
60 with the incidence of tetanus highest in West and Central Africa, Southeast Asia, India, the
61 Pacific Islands, and the southern United States. In cooler regions of the world (e.g., Canada,
62 Norway, England, Finland, Sweden, and others), the incidence of *C. tetani* is lower.⁵² This
63 bacterium can be detected in the intestine of animals but does not represent a significant part of
64 the normal digestive flora. For example, the overall contamination rate of soil samples with *C.*
65 *tetani* in the Durban, South Africa region, was estimated to be 28%. The recovery rate of *C.*
66 *tetani* in 118 fecal samples from horses in this region was 5.9%.⁵⁷ An investigation in southern

67 India in 2009 showed that 2.6% of 115 soil samples contained *C. tetani*; this organism was not
68 identified in 59 aquatic samples.⁴⁹ In the Okinawa Prefecture in Japan, 18.6% of 290 soil
69 samples in 1992 were positive for *C. tetani*.³⁴ In Kanagawa Prefecture, another region of Japan,
70 the prevalence of *C. tetani* was 22.9% from 35 soil samples. The contamination was higher in
71 samples from mountains than from fields, private gardens, or public roads.²⁷ A previous
72 investigation showed a high prevalence of *C. tetani* in soil samples in Japan ranging from 20% in
73 roadsides to 30% in school and hospital grounds, 53% in house yards, and 85% in fields, ponds,
74 rivers, and wet shores.²⁰ Excluding Japan, no country-wide systematic investigations of *C. tetani*
75 in soil have been performed, to our knowledge, but because tetanus occurs worldwide, all soils
76 are presumed to contain this pathogen.

77 Different surfaces and objects contaminated with soil particles, dust, or feces may contain
78 *C. tetani*. Toxigenic strains of *C. tetani* have also been isolated in hospitals from catgut, cotton
79 wool, dust and air samples, human skin, and wounds.¹¹

80 ***C. tetani* toxins**

81 *C. tetani* produces a neurotoxin, TeNT, and a hemolysin called tetanolysin. Tetanolysin is a pore-
82 forming toxin that belongs to the cholesterol-dependent cytolysin (CDC) family, the prototype of
83 which is perfringolysin from *C. perfringens*. CDCs form large pores on the membrane surface of
84 target cells, resulting in oligomerization of 40–70 toxin monomers and membrane damage.
85 Tetanolysin may facilitate local tissue colonization and resistance to macrophages.^{32,58}

86 TeNT is synthesized as a single-chain, 150-kDa protein that is poorly active. The toxin is
87 proteolytically activated in the extra-bacterial medium either by clostridial proteases or by
88 exogenous proteases from the host. The proteolytic cleavage occurs at the one-third N-terminal
89 of the molecule. Active TeNT consists of a light chain (L; the N-terminal ~50 kDa) and a heavy

90 chain (H; the C-terminal ~100 kDa). The L and H chains are linked by a disulfide bridge. The
91 TeNT structure is similar to that of botulinum neurotoxin (BoNT) type E. The structures of
92 BoNTs and TeNT have 3 distinct domains: 1) L chain containing α -helices and β -strands, and
93 including the catalytic zinc-binding motif; 2) the N-terminal part of the H chain (H_N) forming 2
94 unusually long and twisted α -helices; and 3) the C-terminal part of the H chain (H_C) consisting of
95 2 distinct subdomains (H_{CN} and H_{CC}). The H_C region is involved in the recognition of the
96 membrane receptor and internalization of the toxin into the cell. Similar to BoNT/E, the L chain
97 and H_C domains are located on the same side of the translocation (H_N) domain.³⁹

98 **Mode of action of the tetanus toxin and pathophysiology of tetanus**

99 Tetanus occurs after the toxin is secreted by *C. tetani* that has contaminated a wound. Bacterially
100 contaminated wounds can sometimes be small and difficult to find. Tetanus may even develop
101 after wound healing. Contamination of wounds in parts of the body that are in contact with the
102 soil are most at risk of producing tetanus. Examples include accidental wounds or punctures by
103 prickly plants at the end of the limbs, the lower side of the trunk, and the abdomen. Umbilical
104 infections are common predisposing factors for tetanus in newborns, notably in lambs and foals.
105 Surgical wounds can be contaminated with *C. tetani* spores and are commonly observed with
106 castration, tail docking, and ear surgery. Contaminated vaccinations or injection sites or
107 contaminated wounds incurred during shearing are also common issues that have caused tetanus.
108 Puerperal tetanus occurs after contamination of the vaginal mucosa and uterus during difficult
109 delivery.^{10,14,19,38}

110 Deep wounds with little exposure to air and the presence of necrotic tissue (ensuring
111 anaerobic conditions) favor spore germination, *C. tetani* growth, and subsequent TeNT
112 production. *C. tetani* is not an invasive bacterium and cannot enter healthy cells. However, the

113 presence of damaged and lysed cells in a wound is a favorable substrate for *C. tetani* growth and
114 toxin production. Tetanus is experimentally produced in laboratory animals by intramuscular
115 injection of *C. tetani* spores in the presence of a necrotizing agent such as calcium chloride.

116 TeNT diffuses locally in the site of infection and interacts with demyelinated nerve
117 endings. TeNT recognizes specific receptors on neuronal cell membranes consisting of a
118 ganglioside part and a membrane protein. TeNT binds with a high affinity to gangliosides
119 (preferentially GT1b and GD1b) by 2 carbohydrate-binding sites on the C-terminal domain of
120 the toxin H chain.^{13,48} TeNT recognizes the extracellular matrix protein nidogen at the
121 neuromuscular junction.⁶ However, the TeNT receptor on neuronal cell membranes is not yet
122 known. TeNT bound to its receptors is internalized by receptor-mediated endocytosis. The toxin
123 is then routed to endocytic vesicles that are not acidified. These vesicles undergo retrograde
124 transport to the neuronal cell bodies in the central nervous system (CNS).^{8,9,15,16,36,37} In the CNS,
125 TeNT is delivered to the extracellular space and enters the final target neurons that are inhibitory
126 interneurons involved in the regulation of motor neuron activity. TeNT enters target inhibitory
127 neurons via vesicles that are acidified, thus permitting the delivery of the L chain into the
128 cytosol, where it inhibits the regulated release of glycine and gamma-aminobutyric acid
129 (GABA). Acidification of the vesicle lumen triggers a conformational change of the neurotoxin
130 and subsequent translocation of the L chain into the cytosol from the endocytic vesicle. The
131 precise mechanism of L chain translocation into the cytosol is still a matter of debate. The
132 disulfide bond between the L and H chains has a crucial role in the translocation process. The
133 disulfide bond is reduced after translocation allowing the delivery of L chain in the cytosol.^{22-24,35}
134 The TeNT L chain is a zinc-dependent metalloprotease that cleaves synaptobrevin or VAMP
135 (vesicle-associated membrane protein), one of the 3 proteins of the SNARE (soluble N-

136 ethylmaleimide-sensitive factor attachment protein receptor) complexes involved in the evoked
137 release of neurotransmitter. Therefore, TeNT blocks the release of glycine and GABA, thus
138 disrupting the negative controls exerted by the inhibitory interneurons onto the motor neuron,
139 turning on excessive firing of the motor neurons, and ensuing muscle contraction (reviewed in
140 previous reports^{40,44,45,47,50}; Fig. 1). Thereby, tetanus is characterized by spastic paralysis and
141 death by paralysis of diaphragm and respiratory muscles.

142 **Clinical signs**

143 Although all animal species are susceptible to tetanus, there is considerable variability in
144 susceptibility between species. The most susceptible species are the horse, guinea pig, monkey,
145 sheep, mouse, goat, and human, whereas carnivores such as cats and dogs are less vulnerable,
146 and birds are resistant (Table 1). Unlike sheep and goats, cattle are quite resistant. Interestingly,
147 poikilothermic animals such as frogs are resistant to tetanus intoxication when maintained at low
148 temperature (<18°C), despite large amounts of TeNT in the circulating body fluids, but
149 susceptible when exposed to higher temperatures ($\geq 27^\circ\text{C}$). The protective effects of cooling have
150 been attributed to a retardation of the binding rate of TeNT on target neurons and inhibition of its
151 action.⁵²

152 Tetanus is characterized by hyperactivity of voluntary muscles leading to rigidity and
153 tetanic spasms. Rigidity consists of tonic, involuntary, and prolonged muscle contractions,
154 whereas spasms are shorter-lasting muscle contractions usually triggered by sensory stimulations
155 (reflex spasms) such as touch, light, or noise. The signs of spastic paralysis are characteristic,
156 and the diagnosis is often based on this clinical observation. The difficulty in diagnosing tetanus
157 is often at the early onset of tetanus when misdiagnosis with other paralytic diseases such as
158 myopathies can occur.^{1,7,54}

159 Tetanus can appear as either a systemic (generalized) or localized disease process. In
160 systemic tetanus, increasing spasticity of the muscles of mastication occurs initially, followed by
161 progressive spastic paralysis of the muscles of the trunk, and upper and lower limbs. Rigidity of
162 the masseter and temporal muscles (trismus) leads to the inability to open the mouth. Later,
163 generalized tetanic spasms develop. Spastic paralysis of the extensors of the neck and back is
164 accompanied by opisthotonus. Neonatal tetanus often occurs as a generalized form and is
165 observed in newborns <1-mo-old.⁷

166 In localized tetanus, the muscles of the infected region become painful and then later
167 spastic. Local tetanus is more likely to be observed in those animal species such as dogs that are
168 relatively resistant to the toxin.¹

169 The autonomic nervous system is also affected by episodes of tachycardia, hypertension,
170 and sweating, alternating with bradycardia and hypotension. Death occurs because of respiratory
171 failure as a result of the spastic paralysis of the diaphragm and laryngeal and other respiratory
172 muscles. Clinical signs may last for weeks.^{7,28}

173 ***Horses***

174 Tetanus is common in non-immunized horses. Different forms of tetanus can be observed in
175 horses. In the acute form, spastic paralysis rapidly spreads from the head (muscles of
176 mastication, ears, third eyelid) to the respiratory muscles and then to the limbs. Generalized
177 convulsions are accompanied by sweating. Death can occur in 1–2 d because of respiratory
178 failure. In the subacute forms, signs develop in 1–3 wk. Some animals may recover.
179 Hyperesthesia and prolapse of the third eyelid are common early signs. Eating and swallowing
180 are difficult because of paralysis of the mastication muscles. The nostrils are often flared, and the
181 ears are held stiffly in a vertical position. The muscles of the neck, back, and tail will be very

182 tense, so that the tail is often raised vertically. The limbs become stiff, and the head is in an
183 opisthotonus position. The body is in extension, with a global feature comparable to that of a
184 wooden horse. The look of the horse is anxious and expresses pain. The face is tense because of
185 trismus. Respiration is fast and respiratory movements painful. The pulse is normal; although,
186 during the crisis of tetanic spasm, the pulse and respiratory rates are faster. Most cases end with
187 the death of the horse. In a retrospective analysis of 176 tetanus cases in horses in Europe
188 between 2000 and 2014, the mortality rate was 68.2%. Prognostic indicators of survival include
189 voluntary eating of soft food and drinking.⁵⁶ In localized tetanus, the muscle contractions are less
190 intense and localized to a group of muscles such as those of a limb. This form can last several
191 weeks, and the animals can recover.⁵⁶

192 *Sheep*

193 Tetanus occurs mainly as sporadic cases, but outbreaks have been described in lambs and sheep.⁴
194 Newborn lambs can be affected by tetanus following soil contamination of the umbilicus. Dog
195 bites, penetration of the oral mucosa by fibrous plant thorns, and surgical interventions such as
196 castration and tail docking are also responsible for tetanus in sheep. A tetanus outbreak has been
197 reported in sheep subsequent to ear tagging. In an outbreak in Brazil, 50 of 2,830 (1.7%) sheep
198 treated subcutaneously with an anthelmintic contaminated with *C. tetani* developed clinical signs
199 of tetanus.¹⁹ The predominant sign in sheep is stiffness of the limbs with a rigid gait. The tail is
200 often stiff and straight. Feeding is difficult or impossible because of contraction of the
201 masticatory muscles. The ears are stiff and in a horizontal position. The third eyelid is often
202 prolapsed. As clinical signs progress, affected animals collapse into lateral recumbency. Other
203 findings include tachycardia, dyspnea with dilated/flared nostrils, mild fever, teeth grinding, mild

204 bloat, and anxiety. The mortality rate is 50%. Administration of penicillin G (20,000 IU/kg/5 d)
205 can significantly reduce mortality.³⁸

206 *Cattle*

207 In cattle, the signs are comparable to those observed in horses, but the muscular hyperactivity in
208 response to stimuli is less pronounced. Affected animals initially develop overall stiffness with
209 deviation and straightening of the tail to one side. Later, the head and neck go into extension.
210 The ears become stiff and point backward; prolapse of the third eyelid is often observed. Bloat is
211 common. Trismus is pronounced. In severe disease, the thoracic and abdominal muscles are
212 spastic, and respiration is painful and loud. Severely affected animals die of respiratory failure in
213 5–9 d.¹⁹

214 Although less susceptible to tetanus than horses and sheep, outbreaks of tetanus in cattle
215 have been reported (reviewed previously¹⁹). Common risk factors are accidental wounds,
216 surgical castration, dehorning, and drug injections.¹⁴ An outbreak of tetanus in 6- to 8-mo-old
217 calves occurred in Brazil in 2001 following subcutaneous administration of an anthelmintic
218 (disophenol) contaminated with *C. tetani*; 297 of 4,504 (6.6%) cattle died 12–18 d after
219 anthelmintic injection. In this case, calves displayed stiffness, rigidity of neck and limbs, bloat,
220 straight pulled-back ears, and prolapse of the third eyelid. As the clinical disease progressed, the
221 animals developed teeth grinding, hypersensitivity to environmental stimuli, tachycardia,
222 hyperthermia, recumbency with extension of the limbs, convulsions, opisthotonus, and death.¹⁹

223 *Swine*

224 Tetanus in pigs is most often observed in piglets, which develop a generalized disease that is
225 often fatal. Affected animals develop a stiff gait and have difficulty walking. Tetanic spasms
226 often develop rapidly. Feeding is difficult or impossible because of trismus. The ears stand stiffly

227 on the head. Respiration quickly becomes difficult with fast shallow breaths. Death often occurs
228 rapidly as a result of respiratory failure.⁵²

229 *Dogs and cats*

230 Tetanus is rare in dogs and cats. These 2 species (particularly cats) are resistant to TeNT. Often,
231 clinical disease (particularly in cats) is localized and mild. The incubation period is usually 5–10
232 d. Spastic paralysis is observed in certain groups of muscles, causing trismus, stiff limbs, and
233 stretching of the body because of large spinal extensor muscle spasm. Muscle spasms are
234 relatively mild. The ears are usually erect and close together, and the skin of the forehead is often
235 wrinkled because of muscle tension. Hyperthermia is common.^{1,54}

236 In a retrospective series of 61 cases of tetanus in dogs, the source of the wound infection
237 was identified in 58 of the 61 cases: 54% of wounds were in the thoracic limbs, 19% in the
238 pelvic limbs (limb wounds were most often digital or nail wounds), 14% in the oral cavity
239 mostly associated with teeth, and 13% in head, thorax, or abdomen including one post-surgical
240 intervention (ovariohysterectomy). The mean duration between identification of wound and
241 onset of signs was 15.2 d ranging from 0 to 30 d.⁵¹ The most common initial clinical signs are
242 ocular and facial abnormalities. Signs progress rapidly to rigidity of localized muscles to
243 generalized spastic paralysis including contracted facial musculature, trismus, retracted lips,
244 wrinkled forehead, rictus grin, miosis, enophthalmos, prolapse of the third eyelid, erect ears,
245 hyperextension of the limbs, stiff gait, then lateral decubitus and respiratory distress. The animals
246 usually are hyperthermic and have low heart rate and low blood pressure.^{1,51,54} Young dogs are
247 more likely to develop severe disease than older animals. Aberrant heart rate or blood pressure
248 often suggests a poor prognosis.¹⁰ In the cohort of 61 cases, the mean survival time was 6.9 d,
249 but ranged from 2 to 11 d.⁵¹ Mortality was 18–50%.^{5,10,51} Approximately half of surviving dogs

250 develop sleep-associated disorders consisting of permanent muscular spasms including rapid eye
251 movement and repeated episodes of vocalization.⁵¹ The differential diagnosis includes
252 polymyositis, strychnine intoxication, spinal trauma, hypocalcemia, and meningoencephalitis.⁵⁴

253 **Diagnosis**

254 The diagnosis of tetanus is essentially achieved by identification of the characteristic spastic
255 paralytic signs. Strychnine intoxication shows a similar clinical picture and is the only condition
256 that mimics tetanus. However, the presence of a suspected wound can support a diagnosis of
257 tetanus. The onset of tetanus signs such as walking with a stiff gait can be confused with a
258 myopathy.^{7,54} A diagnosis of focal tetanus in a dog can also be supported by electromyography.¹⁸

259 Identification of TeNT is often difficult and usually not detectable in biological samples.
260 Very low levels of TeNT are needed for animals to develop signs of tetanus.³ The detection of
261 TeNT in serum samples from humans or animals with tetanus is reported only rarely.¹⁷ Isolation
262 of *C. tetani* from contaminated wounds is low.

263 **Detection of *C. tetani* in contaminated wounds**

264 Isolation of *C. tetani* at the point of entry consists of enrichment culture from tissues or exudate
265 from the suspected wound. Enrichment cultures are performed in rich medium for anaerobic
266 bacteria such as TGY (trypticase, 30 g/L; glucose 5 g/L; yeast extract 20 g/L; cysteine HCl 0.5
267 g/L; pH 7.5) or fortified cooked meat medium (FCMM; 12.5% cooked meat medium [Difco,
268 Detroit, MI], 0.5% calcium carbonate, 1% ammonium sulfate, 1% yeast extract, 0.8% glucose,
269 0.5% soluble starch, and 0.1% cysteine–HCl, pH 7.6).⁵⁵ Optionally, inoculated enrichment media
270 are heated at 60°C for 30 min for spore selection. After 1–5 d incubation at 37°C in anaerobic
271 conditions, *C. tetani* can be detected either by identification of TeNT in the enrichment culture
272 supernatant or by detection of *tent*-containing clostridia. It is noteworthy that detection of *C.*

273 *tetani* in suspected wounds is often problematic. For example, clostridia were cultured in only 4
274 of 2 wound samples from dogs with clinical signs of tetanus.⁵¹

275 **Identification of TeNT in culture supernatant**

276 TeNT can be detected in the culture supernatant by the mouse bioassay. Culture supernatant (0.2
277 mL) is injected intramuscularly in mice, and the animals are observed for typical spastic
278 paralysis for 1–4 d. TeNT can be detected by ELISA with specific polyclonal or monoclonal
279 antibodies.³³ TeNT can be detected by its proteolytic activity towards its specific substrate
280 VAMP using specific antibodies to the cleaved form of VAMP.³¹

281 **Identification of *tent*-containing clostridia**

282 A sensitive method of *C. tetani* detection consists of PCR based on *tent* identification. Several
283 PCR methods specific to *C. tetani* including standard and real-time PCR have been described
284 (Table 2). Bacterial DNA is commonly extracted from enrichment broth cultures or colonies on
285 agar plates with commercial DNA extraction kits.^{2,25,41-43} In conventional PCR, the readout is
286 performed by electrophoresis of DNA on an agar gel stained with ethidium bromide. More
287 accurately, the sequencing of PCR products gives unambiguous results. Real-time PCR offers the
288 advantage of being rapid and highly sensitive, with immediate readout of results.

289 A procedure including DNA extraction from soil samples with a commercial DNA
290 extraction kit (SoilMaster DNA extraction kit; Epicentre Biotechnologies, Madison, WI) and
291 real-time PCR without enrichment culture has been developed. The limit of detection was
292 estimated to be 10 *C. tetani* in the sample.²⁹

293 **Tetanus serology**

294 Antibodies against TeNT are often not detectable in nonvaccinated humans or animals that have
295 recovered from naturally acquired tetanus. The low concentration of TeNT in natural disease

296 does not usually induce an immune response. Surprisingly, tetanus cases have been observed in
297 humans having antibody concentrations >0.01 IU/L, the minimal level considered as protective
298 (reviewed previously²¹).

299 **Prophylaxis, treatment, and control**

300 Medical prevention consists of immunization with formaldehyde-inactivated TeNT. Two
301 injections at a 3- to 4-wk interval are required to induce effective immunity. No specific
302 treatment is available for tetanus. TeNT antibodies prevent free TeNT from entering neurons via
303 the serum, but toxin that has been taken into neuronal cells cannot be accessed by antitoxin
304 antibodies.^{7,12,56}

305 Wound debridement and cleaning, antibiotic use, and injection of TeNT immunoglobulin
306 are recommended when a risk of tetanus is suspected. Penicillin G and metronidazole are the
307 antimicrobials of choice to treat infection but are ineffective against existing disease.
308 Nonspecific treatment includes sedation and muscle relaxation, and supportive nursing care
309 (feeding, maintaining hydration, and preventing soiling).

310 Standard treatment of tetanus in dogs consists of tetanus antitoxin administered
311 intravenously, penicillin G (25 mg/kg/7 d), sedation with diazepam–acepromazine or
312 chlorpromazine–phenobarbital, hospitalization in a dark and quiet room, and intravenous fluid
313 therapy to maintain hydration.^{1,54}

314 In neonates, good hygienic practices, notably disinfection of the umbilicus, are important
315 in the prevention of tetanus. Surgical interventions (tail docking and castration) must be
316 performed with sterilized materials and in appropriate conditions of hygiene.

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- 447

448 **Table 1.** Sensitivity of animal species to tetanus toxin. Relative minimum lethal doses compared
 449 to guinea pig lethal dose for various animal species, equivalent body weight basis (according to
 450 previous studies^{53,58}).

Species	Minimum guinea pig lethal dose
Horse	0.5
Guinea pig	1
Monkey	2–4
Sheep	2
Mouse	2–6
Goat	12
Cattle*	Unmeasurable low value
Rabbit	4–900
Dog	300–600
Cat	960–7,200
Goose	6,000
Pigeon	6,000–24,000
Hen	180,000–360,000
Human ⁴⁶	10 mouse lethal doses/kg
Human ¹²	2.5 ng/kg

451 * Réthy LA. Unpublished.

452 **Table 2.** Primers and PCR methods of *Clostridium tetani* detection in biological or environmental samples. Enrichment culture and
 453 subsequent DNA extraction are commonly used prior to PCR amplification.

Primer	Sequence	PCR	Reference
P476	ATGCCAATAACCATAAATAATTTTAGATATAG	Conventional PCR	43
P477	TTCATCTTGAAATGGTTCTTCTG		
F3	GATAAAGATGCATCTTTAGGATT	LAMP PCR	30,41
B3	TCTTCTTCATTATCAACCCAAC		
FIP	AGTTGCTTGCAATTAATATATCCCTAGTAGGTACCCATAATGGTCA		
BIP	AACATGTGATTGGTACTTTGTACCTTATGTGTCTATGGTGTGTTG		
TET1	CCTAGTTTCAAACTTATTGGCTTATGTAA	Conventional PCR	29
TET2	CATAATTCTCCTCCTAAATCTGTTAATGAT		
Forward	CTGGATTGTTGGGTTGATAATG	Conventional PCR	25,42
Reverse	ATTTGTCCATCCTTCATCTGTAGG		
TQ TET1	CCTAGTTTCAAACTTATTGGCTTATGTAA	Real-time PCR	2
TQ TET2	CATAATTCTCCTCCTAAATCTGTTAATGATG		

454 LAMP = loop-mediated isothermal amplification.

455 **Figure 1.** Structure of tetanus toxin (TeNT) and schematic representation of its mode of action.

456 H_C = receptor binding domain; H_N = translocation domain; L_C = light chain or enzymatic

457 domain containing the enzymatic site. TeNT is transported from contaminated wound to the

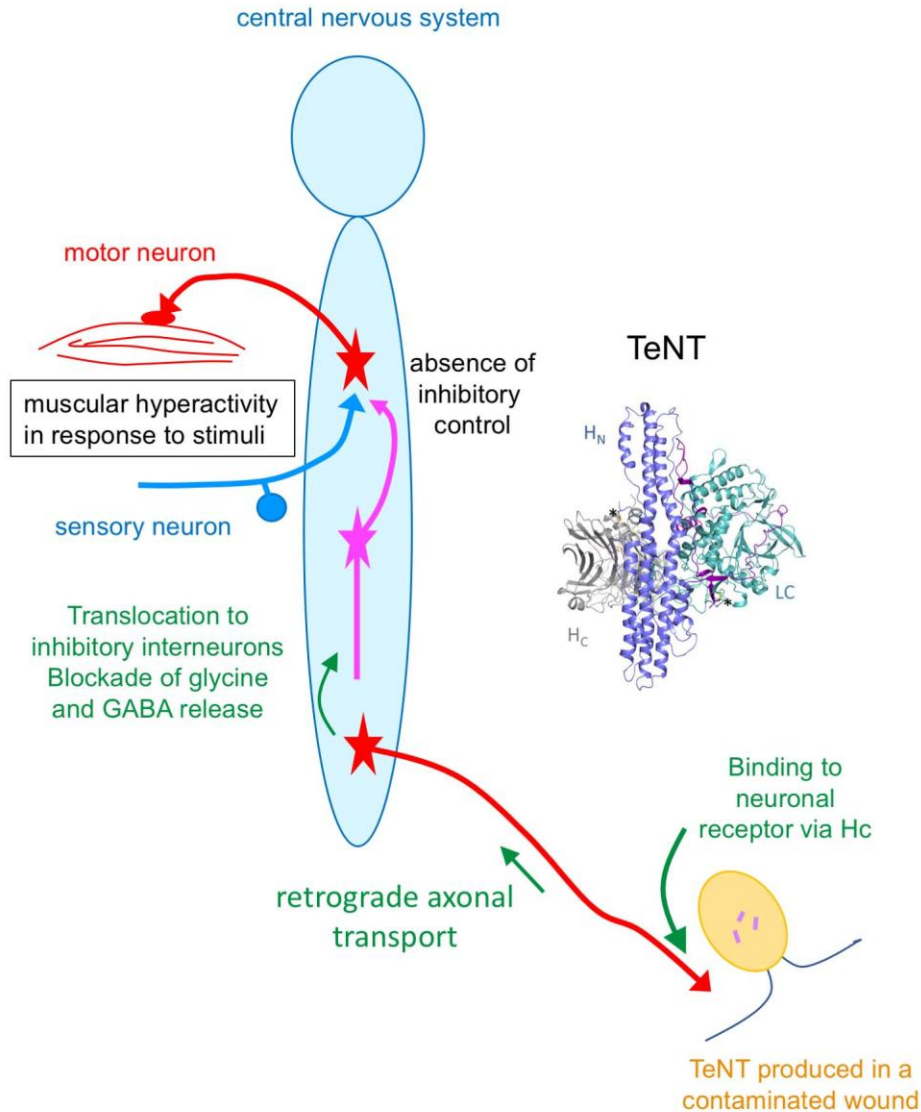
458 central nervous system by retrograde axonal transport through motor neurons and possibly

459 sensitive neurons. In the spinal cord and brain, TeNT targets inhibitory interneurons blocking

460 the release of glycine and GABA. This results in muscular hyperactivity subsequent to stimuli

461 (touch, light, noise, temperature, ...) as a result of the absence of inhibitory control.

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