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Diagnosis and treatment of umbilical cord-derived tetanus in neonatal calves

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Abstract: In this study, umbilical cord-derived neonatal tetanus in calves was identified in Turkey. Four calves with tetanus-specific history and clinical findings were used. Blood samples were taken before and after treatment, and clinical findings were recorded. A tetanus-specific treatment procedure was performed. However, the calves died from not responding to treatment. Pretreatment white blood cell, peripheral blood polymorphonuclear leukocyte, creatine kinase (CK), and lactate dehydrogenase (LDH) levels were determined to be high. Aspartate aminotransferase, alanine aminotransferase, CK, and LDH activities were found to increase after treatment compared to before treatment. Gram-positive terminal spore-forming bacilli were observed in bacterioscopic examination of the necrotic tissue and swab samples from the umbilical cord region. The umbilical cord region was determined to be infected in all calves. In histopathological examination, pyogranuloma formation was observed in the wound area, and in Gram staining agents morphologically concordant with *Clostridium tetani* in necrotic material were observed. Blood serum of the calves was inoculated into mice. All mice died within 2 days after the inoculation, showing tetanus-specific clinical findings. As a result, bacterioscopy and histopathology of the umbilical cord region may be useful for diagnosis in addition to clinical findings. Mice trials may be used in confirming the diagnosis.

Key words: Calves, histopathology, inoculation, tetanus

1. Introduction

Tetanus is a toxemic disease, and the disease is caused by the toxins of the causative agent *Clostridium tetani*, which is an anaerobic, spore-forming, gram-positive bacterium (1). The incubation time varies from a few days to a month or more (2). Tetanus in ruminants is generally observed sporadically. Tetanus in young farm animals may be rarely seen as outbreaks (3,4). The mortality rate is over 80% in young ruminants (4).

Tetanus in young ruminants and/or calves was reported to follow castration, shearing, tail docking, elastrator bands, dehorning, or infected umbilical stalks and injections of pharmaceuticals and vaccines (1,3). Neonatal tetanus is usually observed in calves, and it occurs due to umbilical cord infection as a result of unsanitary birthing conditions (2,4). Clinical signs appear between 2 weeks and 1 month after bacterial inoculation (1). The diagnosis of neonatal tetanus in calves is usually made on the basis of history and clinical findings (5,6). However, the clinical picture might sometimes be confused with meningitis, cerebrocortical necrosis, hypomagnesemic tetany, strychnine poisoning, acute muscle dystrophy, and enterotoxemia (1,4). Therefore, we aimed to clarify two issues in this study. First, in order to confirm the clinical diagnosis of umbilical cord-derived neonatal tetanus in calves, we aimed to determine the diagnostic significance of bacterioscopy and histopathology in infected areas and tissues with in vivo serum antitoxin neutralization assays and in vivo mouse experiments. Second, clinical, hematological, and biochemical findings of the disease in standard treatment were used to evaluate the process. Moreover, umbilical cord-derived tetanus in neonatal calves was defined for the first time in Turkey in this study.

2. Materials and methods

This study included four calves exhibiting clinical signs of tetanus including stiffness in the body (neck, legs), steepening of ears and tails, inappetence or anorexia, and

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lateral recumbency, admitted to Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Internal Diseases. All calves were aged between 10 and 25 days old, two were Swiss brown and two were Simmental, and one was male and three were female.

In anamnesis it was reported that the umbilical cord was ruptured and bled at birth in one of the calves and the umbilical cords were cut for the others after birth. However, it was stated that the umbilical cord was not disinfected in all calves, other processes such as dehorning and castration were not carried out, and no wounds or cuts occurred. Clinical examination of all calves was performed and data were recorded. In clinical examination, there was no evidence of any injury that could cause the entry of an infective agent, but the umbilical cord region was determined to be infected in all calves.

2.1. Treatment

Calves with tetanus were hospitalized in quiet and dark rooms. As treatment, tetanus antitoxin (15,000 IU, IV, twice at 12-h intervals), crystalline penicillin G potassium (50,000 IU/kg body weight, IV, 6-h intervals), and penicillin G benzathine (50,000 IU/kg body weight, IM, single doses) were administered. Following administration of tetanus antitoxin, necrotic parts of wounds in the umbilical region were removed with surgical debridement. Dressings of wounds were then done with 3% oxygenated water. Additionally, tetanus antitoxin (1500 IU) and crystallized penicillin (500,000 IU) were locally injected around the wound. The wounds were left open. As a tranquilizer and an anticonvulsant, xylazine HCl (Rompun, Bayer, Turkey; 0.1 mg/ kg body weight every 12 h intramuscularly) and diazepam (Diazem ampule, Deva, Turkey; 0.2 mg/kg body weight daily 4–6 times intravenously) were administered to the calves. Isotonic sodium chloride solution plus 5% dextrose and lactated Ringer's solution were infused at a constant infusion rate during hospitalization.

2.2. Hematological and biochemical analysis

Blood samples were taken before treatment and at 24 and 48 h after treatment. Hematological analyses were performed using a veterinary hematology device (IDEXX VetAutoread, QBC Diagnostics, Inc., Port Matilda, PA, USA). Biochemical parameters were analyzed using an autoanalyzer (Automatic Analyzer 912, Roche-Hitachi, Mannheim, Germany). Serum cardiac troponin T (cTn-T) values were determined using an immunoassay analyzer (Roche-Hitachi Elecsys 2010).

2.3. Bacterioscopy

Necrotic tissue and swab samples from the umbilical cords of the calves were taken into sterile containers under aseptic conditions. Bacterioscopy of samples was performed with Giemsa and Gram staining methods.

2.4. Mouse experiments

The presence of tetanus antitoxin immunoglobulins in serum samples of animals suspected of tetanus was investigated with a serum neutralization test using an intradermal application technique in mice. Additionally, the developments of specific clinical signs of tetanus in mice were evaluated by injecting serum samples at 1/10 dilutions subcutaneously. Mouse experiments (n=3) were conducted at the Refik Saydam Hygiene Center (Ankara, Turkey).

2.5. Histopathological examinations

Necropsies of the dead calves were conducted. For histopathologic examination, local (wound areas) and systemic tissue samples were taken. Tissue samples were fixed in 10% buffered formaldehyde. Hematoxylin-eosin (H&E) and Brown and Brenn Gram stainings were performed for histopathological examination and detection of microorganisms, respectively.

2.6. Statistical analysis

Pretreatment findings of the calves with tetanus were compared with the reference values for calves or cattle (1). Data were not normally distributed. Therefore, the Kruskal–Wallis test was used to determine the statistical difference between before and after treatment (24 and 48 h) in calves with tetanus, and in the case of the detection of a difference among groups by the Kruskal–Wallis test, the groups were compared in pairs using the Mann–Whitney U test. Statistical significance was determined at $P < 0.05$. All data were given as arithmetic mean ± standard error of the mean (SEM).

3. Results

3.1. Clinical findings

Retrocollis, opisthotonus, tetanic contractions, rigidity of the body, stiffness of the muscles, steepening of ears and tail, hypersalivation, hypersensitivity, and respiratory difficulties were observed in all calves. In addition, partial or total trismus, foaming at the mouth, cyanosis of the conjunctiva mucosa, standing with support or inability to stand, lateral recumbency, rectal prolapse, absence of defecation, and inappetence or anorexia were found in some calves (Figures 1a–1d).

In the period from 24 h after treatment until death, in addition to the increase in the severity of the pretreatment clinical findings in all the calves, it was observed that undetected severe respiratory distress symptoms (foaming at the mouth, increase in salivation, cyanosis in conjunctival mucosa), lateral recumbency, reduction or cessation of defecation and urination, and tenesmus or rectal prolapse that could not be detected in some calves during the pretreatment period had developed or the degree had increased. Prior to death, severe respiratory

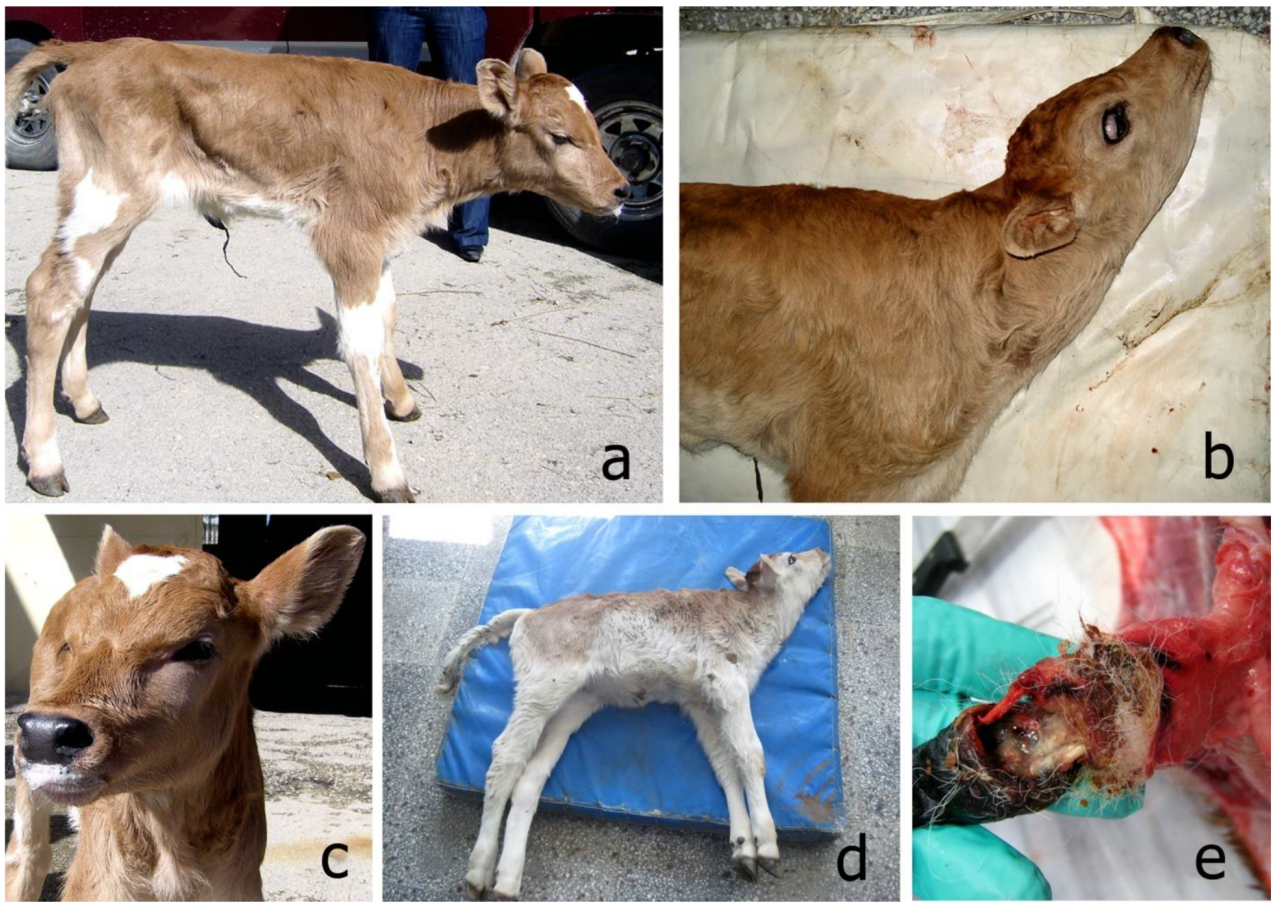

Figure 1. Clinical appearance: rigidity of the body (a); opisthotonus (b); trismus, steepening in the ears, foaming at the mouth (c); inability to stand, steepening in the ears and tail (d); infected and necrotic umbilical cord (e).

distress, tachypnea, hypersalivation, and full trismus developed. Death occurred at 56–65 h after hospitalization.

Pretreatment body temperature, heart rate, and respiratory rate in calves with tetanus were determined to be in the reference ranges reported for calves (1). However, it was determined that the respiratory rate increased significantly ($P = 0.016$) at the posttreatment 24th hour (88.50 ± 11.5 breaths/min) and at the 48th hour (60.75 ± 10.6 breaths/min) compared to the pretreatment value (35.00 ± 8.50 breaths/min), and body temperature and heart rate did not vary.

3.2. Hematological findings

According to the reference values, pretreatment white blood cell (WBC) and peripheral blood polymorphonuclear leukocyte (PBPL) numbers were determined to be high. No statistical difference was determined during pretreatment in the other hematological parameters compared to the reference values, or during posttreatment in all the parameters compared to the pretreatment period.

3.3. Biochemical findings

Serum enzyme activities in the calves with tetanus are presented in the Table. No difference was determined in the other biochemical parameters (total protein, albumin, glucose, blood urea nitrogen, creatinine, total bilirubin, unconjugated bilirubin, conjugated bilirubin, uric acid, gamma-glutamyl transferase, creatine kinase (CK)-MB, Ca, P, Mg, Na, K, Cl, and cTn-T).

3.4. Bacterioscopic findings

Gram-positive terminal spore-forming bacilli were observed in bacterioscopic examination of the necrotic tissue and swab samples performed with the Giemsa and Gram methods.

3.5. Histopathologic findings

Wounds were detected in the umbilical cords in the calves in necropsy (Figure 1e). In H&E staining, the wound area was observed to develop from pyogranuloma with living or necrotic neutrophils located in the middle (Figures 2a and 2b). In Gram staining, agents morphologically concordant

Table. Biochemical results of calves with tetanus (mean ± SEM).

Parameters	RV (7,8)		Calves with tetanus (n = 4)			P-value
	Range	Mean	BT	AT (24 h)	AT (48 h)	
AST (U/L)	78–132 (7)	31.5 ± 6.2 (8) 38.7 ± 16.1 (8)	70.60 ± 24.5[a]	112.6 ± 23.2[ab]	206.3 ± 42.1[b]	0.035
ALT (U/L)	11–40 (7)	27 ± 14 (7)	14.45 ± 4.51[a]	30.37 ± 4.62[ab]	61.40 ± 17.5[b]	0.036
CK (U/L)	44–228 (7)	99.6 ± 69.7 (8) 168.8 ± 211 (8)	579.9 ± 150.0[a]	1103.4 ± 174.4[ab]	1503.6 ± 161.3[b]	0.047
LDH (U/L)	692–1445 (7)	451.3 ± 108.8 (8) 503.1 ± 160.9 (8)	1747.7 ± 229.7[a]	2246.0 ± 167.3[ab]	2783.7 ± 177.0[b]	0.043

Statistical significance between different letters in the same row ($P < 0.05$).
Reference values (RV), before treatment (BT), after treatment (AT).

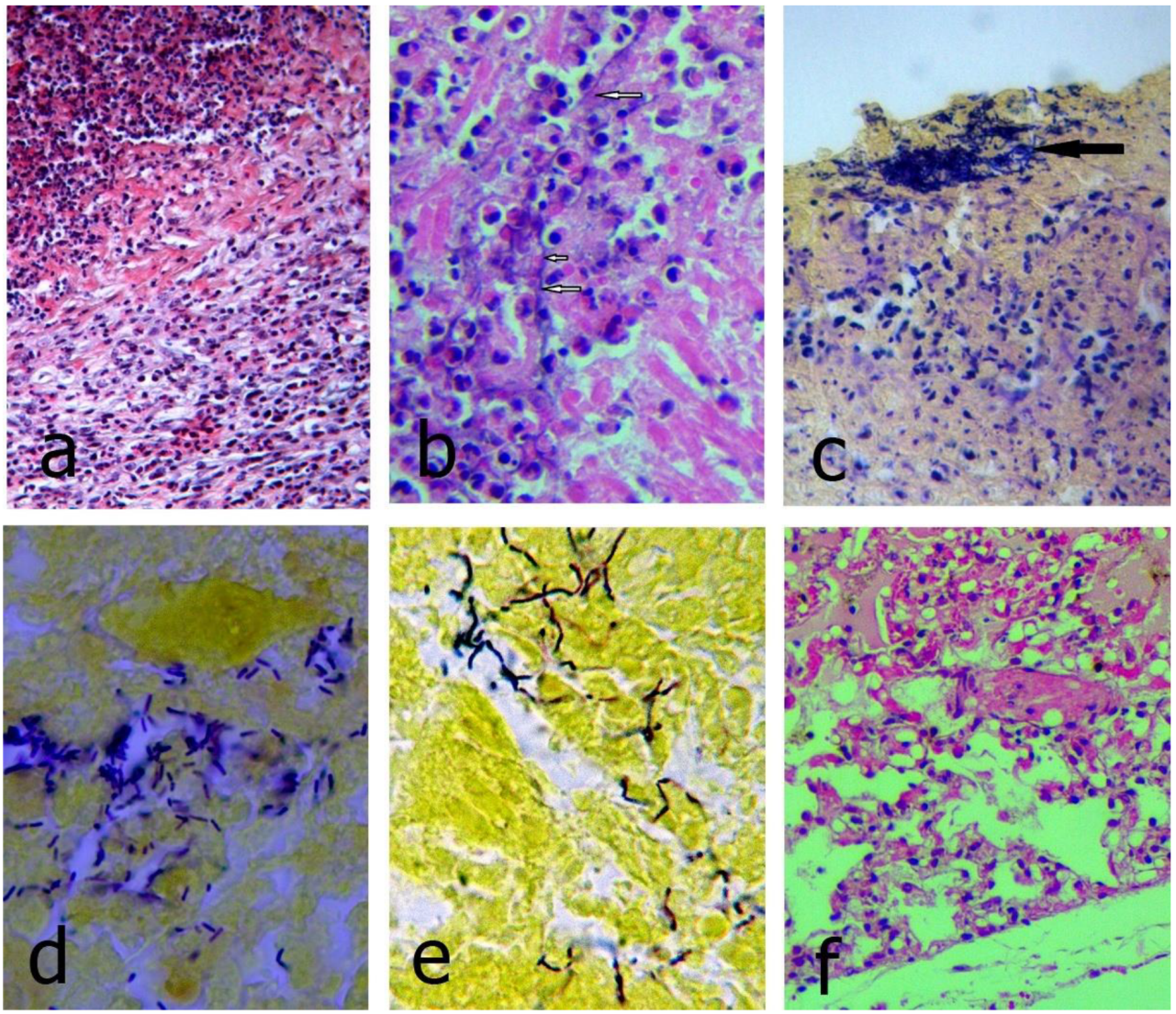

Figure 2. Histopathology: pyogranulomatous-necrotic foci, H&E (a); organism in the necrotic neutrophil cluster, H&E (b); characteristic gram-positive organism morphologically consistent with *Clostridium tetani* in necrotic areas, Brown and Brenn Gram staining (c, d, e); alveolar edema, H&E (f). Scale bars: a = 100 µm, b = 50 µm, c = 50 µm, d = 20 µm, e = 15 µm, f = 100 µm.

with *C. tetani* in necrotic material were observed (Figures 2c–2e). In addition, degeneration of the heart muscle and pulmonary edema were observed (Figure 2f).

3.6. Mouse experiments

In the serum neutralization test in mice, tetanus antitoxin immunoglobulins at ≥250 IU were detected not to exist in calf serum samples. However, after the hypodermic injection of 1/10 dilutions of calf serum samples, the mice died within 2 days at the latest after the development of clinical findings specific to tetanus (ear, waist, and hind leg paralysis).

4. Discussion

Information regarding the insertion site and diagnosis of infection in neonatal tetanus is quite limited. The infection insertion site often cannot be identified specifically. The diagnosis is usually made based on clinical findings. In this study, similar to several others (3,5,6,9), umbilical cord-derived neonatal tetanus was diagnosed tentatively with history and disease-specific clinical findings. However, the importance of other diagnostic parameters and the efficacy of treatment in neonatal tetanus were also examined.

Microscopic or culture examination of *C. tetani* in the suppuration and necrotic tissue at the infection insertion site is recommended for the diagnosis of tetanus. However, it was reported that isolation of *C. tetani* may take a long time (10). In this study, gram-positive terminal spore-forming bacilli concordant with *C. tetani* were detected in the bacterioscopy of the swab samples taken from the infected navel regions. This finding confirms that the umbilical cord is the insertion site of the agent and can be used to diagnose tetanus in a short period of time (30 min). In this study, histopathology and systematic necropsy of the umbilical cord region tissues were also performed for the first time. In histopathological examination, pyogranuloma formation was observed in the wound area (Figure 2a). In Gram staining, agents morphologically concordant with *C. tetani* in necrotic material were observed (Figures 2c–2e). In addition to the bacterioscopy, this finding supports the diagnosis of umbilical cord-derived neonatal tetanus. Lung edema in the present study (Figure 1f) may be explained by the findings of Smith (1), who stated that frothy mucinous salivation due to impaired swallowing function causes respiratory distress.

Diagnosis in mice trials is confirmed by the death of mice after the development of typical tetanus symptoms after inoculation of suspected materials such as subcutaneous tissue lesions (11). In this study, blood sera of calves clinically diagnosed with tetanus were inoculated into the mice for the first time. All mice died within 2 days at the latest after inoculation with clinical findings specific to tetanus. Thus, the diagnosis of tetanus in the calves was confirmed by the mice trials.

It is reported that the determination of protective serum antitoxin antibody levels has limited use in the diagnosis of tetanus, and its levels at the time of the diagnosis decision are not always available (12). However, determination of these levels requires a long time, ranging from days to weeks (10). Similarly, in this study, it was determined that tetanus antitoxin immunoglobulin was not found in calves in examinations made by neutralization test. This may be due to the lack of maternal antibodies, the weakness of the animal in the neonatal period in terms of immune response, the incubation period of the disease not being sufficient in terms of immunoglobulin synthesis, and the immune response not being at a nonspecific level.

It was reported that no specific abnormalities were observed in blood parameters in tetanus (3,4). However, neutrophilic leukocytosis and left shift were reported to be observed due to the wounds in dogs and cats (13). In this study, similar to that of Greene (13), increases in pretreatment WBC and PBPL counts were observed compared to the reference values. This indicates neutrophilic leukocytosis and can be considered as the hematological reflection of umbilical cord infection.

Except for some increases in muscle enzyme activity (Table), no changes with tetanus were reported to be observed for other biochemical parameters (13). Similarly, in this study, levels of muscle-derived enzymes aspartate aminotransferase (AST), CK, and lactate dehydrogenase (LDH) were determined to be higher in pretreatment measurements compared to reference values (Table). As reported in other studies (10,13), this can be explained by constant hypertonicity and prolonged recumbency-related muscle damage. In addition, the continuing increases in enzyme activity after treatment (Table) may be considered as poor prognostic indicators.

Objectives of the treatment of tetanus include neutralization of residual or unbound toxin, elimination of the infective agent, controlling of muscular spasms, maintenance of hydration and nutrition, and applications of supportive therapy (2–4). In this study, treatments reported by several researchers (2–4) were performed. However, the calves did not respond to treatment and all calves died on the third day of the treatment. In this study, the death of all calves despite the tetanus antitoxin application in treatment, as reported by several other researchers (2,4,6), can be explained by the underwhelming tetanus antitoxin application due to limited or very little effect after the emergence of the clinical findings. The tetanus antitoxin can neutralize only circulating or unbound toxins; it cannot neutralize the toxins that bind to nerve cells (9). In this study, antitoxin application of 15,000 IU as recommended by Divers and Peek (9) was administered twice at an interval of 12 h, but all the calves died. This may indicate that the tetanus antitoxin dose

used in this study may have been inadequate considering the top tetanus antitoxin doses reported in other studies. Regarding the antitoxin dose recommended for tetanus, different values such as 1000–5000 IU/kg body weight (1), 1500–300,000 IU/animal (9), or 100 IU/kg body weight (3) have been discussed. However, as in calves treated with 1500 IU (14) and 15,000 IU (15) antitoxin doses, failure to respond to treatment at the doses of 1000 IU (6) and 15,000 IU (15) was also mentioned. This can indicate that there is still not a standard dose of tetanus antitoxin.

In conclusion, in supporting the diagnosis of umbilical-derived neonatal tetanus, in addition to clinical findings, navel region bacterioscopy can be useful in a short time (30 min) and navel region tissue histopathology can be useful in longer periods (several hours). Mouse experiments can be used for the exact confirmation of the diagnosis. However, muscle-derived enzyme activities increasing despite treatment may indicate a poor prognosis. Due to difficulties in the supply of antitoxin, the high cost of treatment, the course of the disease, and high mortality rates despite treatment in neonatal tetanus, tetanus vaccination practices as well as the improvement of hygienic conditions and umbilical cord disinfection for protection in disease-observed regions can be recommended.

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