



Diagnosis and Therapeutic Management of Tetanus in Female Buffalo Calf at Tandojam, Sindh, Pakistan

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ABSTRACT

A female buffalo calf with wound on left leg just below the knee joint suffering from high and persistent fever, anorexia, difficult mastication and urination, stiffness in neck muscle and with some degree of bloat was brought to department of veterinary medicine faculty of animal husbandry and veterinary sciences, Sindh agriculture university, Tandojam, Pakistan, and admitted. The calf was diagnosed to be suffering from tetanus based on clear cut symptoms of high fever, stiff muscles, urine retention and fixed jaws. The Graham's staining of the fresh smear revealed gram+ve rod shape bacteria that appeared like drumsticks. Furthermore, the *Clostridium tetani* was cultured and isolated from the deep necrotic tissue of the wound. The calf was treated with high doses of procaine penicillin, anti-tetanus serum, sedative, meloxicam and intravenous fluid electrolyte therapy (Dextrose 5%). The calf was feed through stomach tube and the urinary catheter was administered to ease out the problem of urine retention. After continues therapeutics management, the calf recovered in two weeks.

Key words: Buffalo calf, *Clostridium tetani*, Diagnosis, Therapeutic management

CASE REPORT
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INTRODUCTION

The tetanus and gas gangrene are rarely encounter in adult population; injury being the single most common cause of these infections in adulthood. Neonatal tetanus (NT) is used as an indicator of overall tetanus burden in study area (2015). Tetanus's disease causing organism is the bacterium (*Clostridium Tetani*) that is the similar family of organisms which causes blackleg (Lewis, 2015). During sporulation, the vegetative form of the organism develops into a spore form, giving a characteristic drumsticks appearance in blood smear. The spores of this organism are very resistant to disinfectants, for example acidified phenol takes about two hours to kill them. Moreover, in spore form they can survive for many years in soil (Radostits et al., 2007).

It is a sporadic and ubiquitous disease that occurs throughout the world (Smith, 2002). Being anaerobic bacteria the tetanus bacilli do not multiply in normal tissues or even in damaged tissues with the same oxidation - reduction potential to that of normal blood. Both necrosis and low oxygen tension in injured tissues provide a sufficient anaerobic environment for multiplication of bacteria producing toxins (Bizzini, 1986). Two types of toxins, tetanospamin and hemolysin are generally produced by *Clostridium tetani* (Montecucco and Schiavo, 1995). Tetanus is an infection categorized by muscle spasms. In the most well-known type, the spasms begin in the jaw and after that progress to the rest part of the body. These spasms remain a few minutes each time and occur repeatedly for three to four weeks (Atkinson et al., 2012). This bacterium is generally present in feces of animals or in soil contaminated with these feces, and enters to the body of the susceptible animals through wound contamination, in calves the wound due to castration is also, one of the major causes for entrance of bacteria to the body (Connor, 1993; Radostitset al., 2007). Tetanus in young calf due to contamination of umbilicus after parturition can also, induced mortality (Suleman, 1982). An injured area with low oxygen tension it multiplies leading to necrosis of the deep tissues providing a much favorable environment for persistence of bacteria, the bacterial cell thus ruptures and neurotoxins are released the from where the toxins enter to the motor or sensory nerve. If the amount of toxins is high they get entry to the blood and lymphatic vessels and reach to Central Nerves System (CNS) where they block the release of neurotransmitters causing all groups

of the muscles to contract simultaneously (Montecucco and Schiavo,1995). The initial clinical signs such as stiffness of neck muscles with extended head mostly appear after 24 hours. Other clinical signs include twitching and tremors of the muscles, firmly fixed jaws (Lock jaw), protrusion of third eyelid, and lameness with alert expressions, hyperesthesia, erected ears and dilated nostrils. Bloat can also, occur because the rumen stops working. Later signs include collapse, lying on side with legs held stiffly out, spasm and death (Smith, 2002; Radostits et al., 2007).

CASE REPORT

A six months old female buffalo calf was brought to the clinic in department of veterinary surgery, faculty of animal husbandry and veterinary sciences, Sindh Agriculture University Tandojam Pakistan. The calf had fallen in farm and sustained an injury on his left leg just below the knee joint. The surgeon cleaned the wound and inserted a simple suture, none of the tetanus anti-toxoid and antibiotics were given. According to the farmer, a horse and donkey in his farm were kept for bringing fodder in the same area where the animals are reared. After two days, the calf was depressed and there was some degree of lameness. No evidence of fracture was noted after performing an X ray. Therefore, the case was referred to department of veterinary medicine tandojam Pakistan, the rectal temperature of the calf was higher (106.8⁰F) and persistent at the same degree after giving antipyretics. On the other hand there was problem with mastication and urination, with some degree of bloat which was not corrected after use of carminative mixture. Keeping in view the poor prognosis after symptomatic treatment the patient was admitted. On next day, the rectal temperature of the calf was persistent at 107⁰F, and the calf was depressed and anorexic. There was stiffness of the neck and jaw muscles, both the ears were erected with alert and anxious expressions. After thirty minutes there was generalized increase in muscle stiffness with lock jaw, prolapse of the third eyelid and stiffness of the hind legs causing staggering in gait. Additional signs exhibited by the animal included dilation of nostrils, hyperesthesia, drooling of saliva from mouth and urine retention.

Diagnosis

On the basis of the above mentioned clinical signs exhibited by the calf, the case was subjected to laboratory diagnosis. The wound area was palpated and there was some pus containing swelling. Therefore, the suture was opened and a fresh smear was prepared from the deep necrotic tissues. The tissue sample from the same site was also, taken for culture and isolation of *Clostridium tetani*.

Smear preparation

In order to prepare a fresh smear, a clean glass slide was taken and touched with the deep tissues of the site. The slide was then immersed in absolute alcohol to fix and then air dried at room temperature (Congera and Lucile, 2009).

Graham's staining technique

First the prepared slide was flooded with basic crystal violet for one minute and washed with water. Then Graham's iodine was applied for one minute and washed with water. After this the slide was decolorized with alcohol for 15 seconds and washed with water. At last the counter stain "safranin" was applied for 30 minutes, washed with water and dried. Using the compound microscope the slide was examined under oil immersion lens (100×) as used by (Ali et al., 2005). After detailed examination of the slide under microscope, a violet colure Graham +verod shaped bacteria was appeared like drumsticks.

Culture and isolation of bacteria

In order to confirm the causative bacterium, it was isolated for which the bacteria were first cultured on blood agar medium. To prepare the blood agar, a fresh defibrinated sheep's blood (5 to 10 ml) was used. The nutrient agar was cooled up to 45⁰C and autoclaved for 24 hours. After that, it was mixed with the required amount of sheep's blood. The prepared medium was poured into sterilized Petri dishes and incubates for overnight. Streak plate method was used to isolate *Clostridium tetani*. A drop of pure culture was placed near one end of the petri dish; the platinum loop was flamed and then cooled by touching on sterile surface of the medium. The petri dish was held in left hand within six inch diameter of the flame. Using the sterile lobe from the surface of the culture on the edge the bacteria were streaked on half of the plate in parallel position. The lobe was again sterilized and cooled, the plate was then rotated at 45⁰angle and the remaining ¼ of the petri plate was streaked from the last line. The plate was rotated and streaked the remaining portion of the plate. The streaked plates were then placed in an anaerobic jar and air tight and then incubated in 37⁰C in an anaerobic incubator as per procedure of Calvin et al. (1969). After detail examination of the plate, single sized, plate, grayish and translucent colonies with irregular margins were observed. Thin zones of beta hemolysis were also noted around the colonies. On the basis of characteristics of colonies it was confirmed that the causative agent was *Clostridium tetani* (Tina, 2010).

Treatment

First of all the affected animal was transferred to an isolated room and cotton plugs were applied in both ears to reduce hyperesthesia. The wound was excised widely; all the contaminated tissues were removed and packed with gauze soaked in hydrogen peroxide. Then multi vitamin and dextrose were administered at 1500 International Unit (IU) intravenously in jugular vein. The sedative (xylase) was administered at the dose of 0.1 mg/kg body weight. After this 30000 IU per kg of body weight of penicillin G procaine was given intramuscularly for 12 days. An I/V injection of ATS (Anti-Tetanus Serum) 1500 IU was also administered (Bhikane et al., 2005). Chlorpromazine 50 mg/kg and meloxicam (Diclostar) 0.6 mg/kg body weight were also used. Intravenous fluid therapy (dextrose 5%) 150 ml/kg body weight was continued for eight days. The feed was given through a stomach tube and urinary catheter was administered to ease out the problem of urine retention.

RESULTS AND DISCUSSION

After continues therapeutic management the calf was capable to stand at 4th day of treatment, while muscle stiffness especially that of neck region was persistent for several days; however the calf was completely recovered at 2nd week. These results corroborate with those reported by Das et al. (2011). The patient in the present case was completely anorexic, therefore the calf was fed through stomach tube, which is fully in line with that of Lombard and Zadnik (2013) while the sedative caused the relaxation of the skeletal and deep body muscles including diaphragm and intercostal muscles that helped in normal respiration. Anti-tetanus serum has the effect to neutralize the toxins and thus prevented the central nerve system. The Meloxicam provided relief from pain and inflammation. These findings are in accordance to that of Radostits et al. (2007) and Bhikane et al. (2005) but the interesting thing in therapeutic management of the present case was use of urinary catheter and feeding of the calf with stomach tube most of the clinicians avoid these things and thus a lot of mortalities may occur due to severe debility and rupture of the urinary bladder. Furthermore, in the current case penicillin was used as antibacterial. According to Smith et al. (1964) penicillin provide a good prophylaxis against tetanus for injured patients who have not immunized against tetanus.

CONCLUSION

From the current case it is concluded that it is necessary in tetanus to take the history about urination and nutrition status of the animals. If the animal has not urinated for a long time, the urinary catheter should be used to ease out the problem of urine retention. Also stomach tube should be used in case of firmly fixed jaws for feeding of the animals to avoid debility.

Competing interests

The authors have no competing interests to declare.

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