Sudden Death Due to Myocardial Necrosis in Imported Calves

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ABSTRACT

This case report documents acute death in 36 out of 233 predominantly Simmental breed calves imported to Israel from Eastern Europe. The cause of death was attributed to myocardial necrosis revealed at post mortem examination. The calves were orally administered with doxcycline at high dosage in their country of origin. Based on previous reported cases and the lack of other pathological evidence, high dose of doxycycline was presumed to be the cause of the myocardial changes. The gross pathology and histopathological findings are described and the possible differential diagnoses are discussed.

Key words: Calves, Doxycycline, Toxicosis, Myocardial necrosis.

INTRODUCTION

Doxycycline is a semi synthetic derivate of tetracycline (1). It is a broad-spectrum antibiotic, widely used in feedlot cattle in the treatment and prophylaxis of infectious respiratory diseases. The tetracyclines are relatively safe drugs. Adverse effects may be attributed to their disturbance of intestinal flora and their toxic effects on liver and kidney cells. Fatal anaphylaxis has occasionally been recorded (1,2).

This case report presents postmortem and histopathology findings of 36 calves, imported to Israel from Eastern Europe which died suddenly or suffered respiratory distress shortly before death. Pathological examination revealed severe myocardial and tongue muscle degeneration and necrosis. Investigation as to previous medication received, traced back to the oral use of doxycycline at 3-5 times the recommended dose and is presumed to be the cause of these changes.

MATERIALS AND METHODS

On May 2011, 233 calves, between the ages of 2-4 months were imported to Israel from Eastern Europe. They immediately entered a quarantine station in the center of Israel, according to Israeli importation regulations.

The calves were of beef-breeds, largely represented by Simmental. Six days after arriving by airplane, 8 calves began to show signs of dyspnea, tachypnea, sialorrea, seizures and sudden death. Over a period of nearly a month, additional 28 calves had died. All the calves were sent for a full post mortem examination at the Kimron Veterinary Institute in Bet Dagan, Israel. Samples from all organs including hind limb muscles and tongue muscle were collected in 10% normal buffered formalin, embedded in paraffin wax, sectioned at 3 micron and stained with Hematoxylin Eosin (H&E). Samples from heart muscle and larynx were taken for a PCR test for foot and mouth disease.

RESULTS

Pathological and Histopathological findings

At necropsy, all calves were in good physical condition. Gross findings were confined to the heart muscle, lungs, skeletal muscles and tongue.

There were marked pale to whitish discolorations of the myocardium that involved large areas of the septum and the left ventricular free wall (Figures1-2). Some lesions were softer and relatively friable while others were gritty on cut surface. Additional findings were mild cranial pneumonia in 5 calves and pulmonary edema in 10 calves.

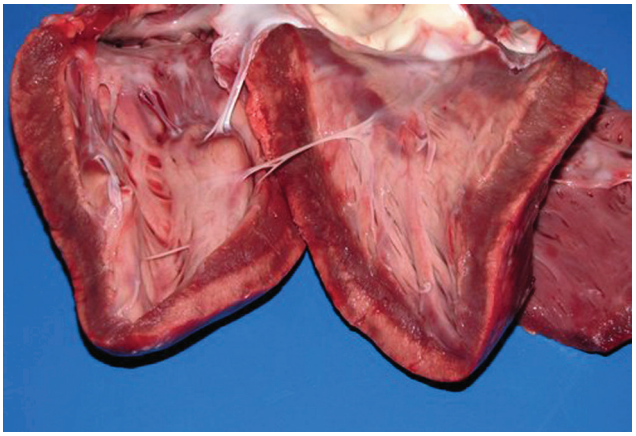

Figure 1: Myocardial necrosis, extensive pale areas in the subepicardium and subendocardium of the left ventricle.

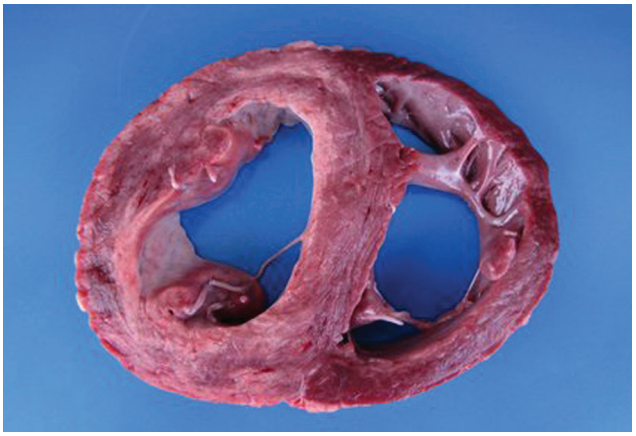

Figure 2: Myocardial necrosis cross section, pale arias of the left ventricular myocardium.

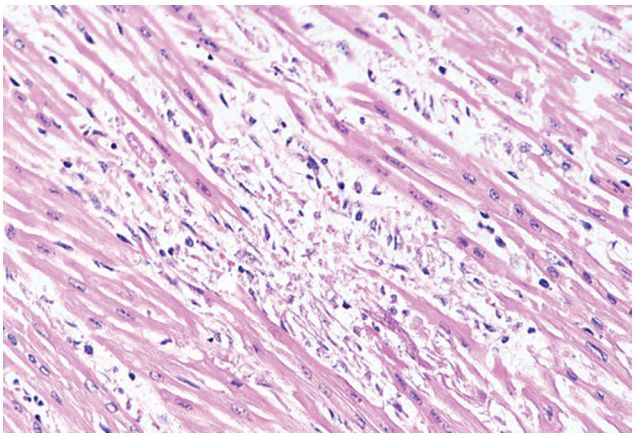

Figure 3: Acute focal myocardial necrosis. H&E stain X 10

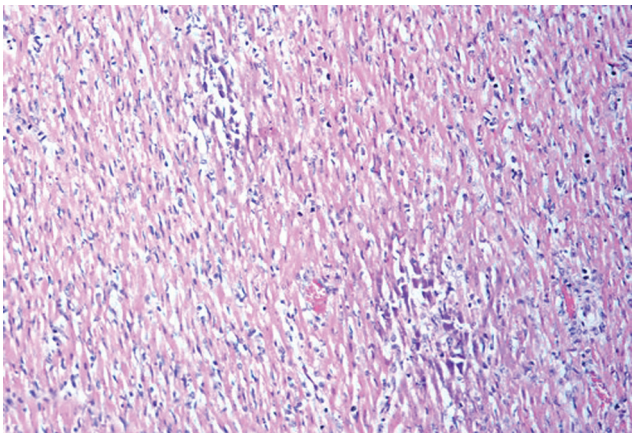

Figure 4: Multifocal acute myocardial necrosis with mineralization. H&E stain X 4

Microscopically, myocytes showed two patterns of necrosis probably in relation with chronological time of injury. In areas of recent necrosis, cells appeared swollen and hypereosinophilic (hyaline degeneration) with pyknotic nuclei. Some necrotic myofibers had scattered basophilic granules, which probably represent calcified mitochondria (Figures 3-7) (3). In the second pattern of necrosis, affected myocytes had a "shredded" appearance; This is due to hypercontraction and the formation of multiple transversal oriented bars of disrupted contractile material (3). Necrotic areas were variably infiltrated by macrophages and few neutrophils. In calves that survived the longest, there was proliferation of fibroblasts and deposition of connective tissue products (collagen and elastic fibers).

Microscopic changes in the tongue and skeletal muscles were similar to the lesions described in the heart muscle, although not to the same extent.

DISCUSSION

Myocardial degeneration and necrosis as well as skeletal muscle necrosis are well documented in veterinary pathology, especially in cattle, pigs, small ruminants and wild animals. Amongst the various causes, the more common are vitamin E/Selenium deficiency, Ionophores toxicity, various calcinogenic plants and gossypol toxicosis (3).

In this case, an inquiry as to the clinical history of the calves, revealed that they were orally administered with doxycycline at the dose of 15-25mg/kg (3-5 times higher than the recommended dosage (recommended dosage 5 mg/kg q12 h) (1,4). The drug was admixed in the milk, in a non-

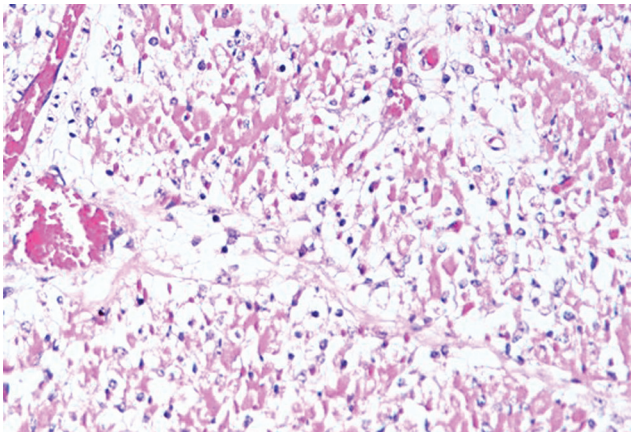

Figure 5: Heart. Severe myocardial degeneration and necrosis with mild infiltration of macrophages and neutrophils. (H&E; X20).

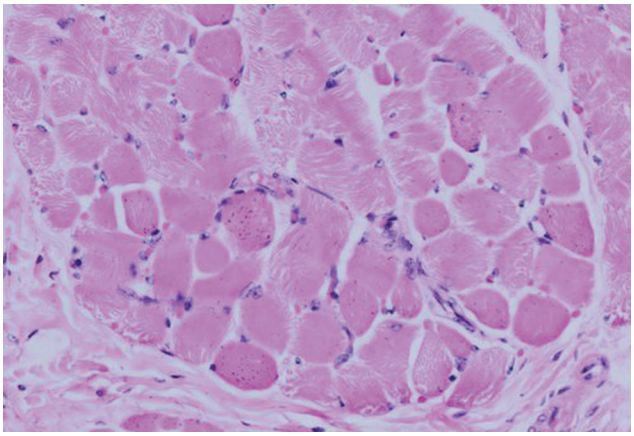

Figure 6: Tongue, hypereosinophilic fibers with fine calcium granules (H&E; X20).

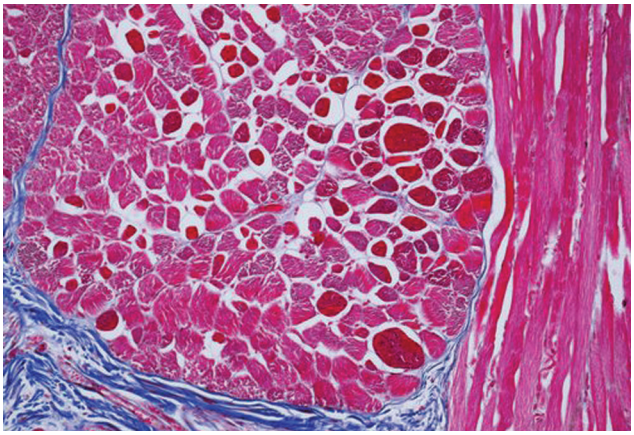

Figure7: Tongue, hypereosinophilic round fibers of various diameter. trichrome stain X10

homogenous manner, which probably resulted in some calves receiving higher dosage than others. This treatment began 4 days prior to their arrival, and was initiated because some calves presented respiratory signs for which an infectious agent was suspected.

Cases of acute doxycycline cardiotoxicity with sudden death have been reported in cattle in Israel, Canada, The Netherlands and Belgium (4-8).

Doxycycline is a semi-synthetic derivate of tetracycline (1,2). Tetracyclines are classic broad spectrum bacteriostatic antibiotics because of their activity against gram (+) and gram negative aerobic and anaerobic bacteria, and are generally useful in the treatment of bovine pneumonias and also in their prophylaxis, especially in feedlots (2). However, adverse effects have been reported: One of the complications following oral therapy is alteration in the normal flora of microorganisms in the digestive tract. (1,2). Furthermore, although not well documented in veterinary medicine, Tetracyclines are also known to induce dose-related functional changes in renal tubules in several species. (1,2,4)

Doxycycline, however, is not eliminated by the same path as other tetracyclines and does not accumulate in the blood if renal failure occurs. It is excreted in feces as an inactive conjugate or chelate, thus has little effect on the microflora of the gastrointestinal tract (1); It is therefore one of the safest tetracyclines in use (1). Tetracyclines have been used for many years in managing infectious diseases in food animals because of their low cost, broad antimicrobial activity and ease of administration (2).

Acute cardiotoxicity from other causes, results in similar pathological lesions:

Ionophores (monensin in particular) are biologically active compounds derived from S*treptomyces cinnamonensis*. Monensin selectively complexes Na+ and pass it through the lipid bilayer of the sarcolemma into the myocyte (9). Toxicity ensues from excessive influxes of Na+ and Ca+, leading to degeneration and necrosis of cardiac and skeletal muscle (3,9,10). Monensin is toxic to many species, in particular to horses (9). In cattle, it is used as feed additives (10,11), promoting food utilization by altering rumen fermentation to reduce energy loses associated with formation of volatile fatty acids. A few cases of known feeding errors and suspected monensin toxicity have been reported in the literature (9,10,11). Cattle deaths have occurred when errors

have resulted in an excessive concentration of monensin in a small quantity of feed (10). However, in an experimental dosing in steers and heifers, no deaths occurred before day 3 after high-level single-dose toxic exposure (22.4 and 39.8 mg/kg), suggesting a delayed cardiac toxicity (9,10,12).

Vitamin E/Selenium deficiency results in degeneration and necrosis of both skeletal and cardiac myocytes (3,13,14). The lesion is caused by lack of the depressant effect of vitamin E on oxidation in mitochondria and the lack of protection by the selenium-containing enzyme gluthatione peroxidase (13). Enlargement and degeneration of mitochondria are accompanied by degeneration of other components of the cell (3,13,14). Grossly, myocardial mineralization is a prominent feature in vitamin E-selenium in sheep and cattle (3) as occurs in vitamin D toxicity and calcinogenic plant toxicosis (3).

Foot and mouth disease in neonatal calves and lambs can cause myocarditis and myocardial necrosis and is highly fatal (3,14). The distinction between myocardial necrosis and myocarditis with necrosis of myocytes is somewhat arbitrary: inflammatory cells will invade necrotic myocardium, but in acute myocarditis, inflammatory cells are intimately associated with necrotic myocardial cells and adjacent myocytes may appear normal (3).

Reports of myocardial necrosis and skeletal muscle degeneration in calves due to doxycycline have been emerging sporadically over the past decade. Despite an extensive therapeutic use of this drug both in veterinary and human medicine, necrotic cardiomyopathy was not reported after oral treatment in any other species (12). Curiously, Brihum *et al*. have unsuccessfully attempted to experimentally induce myocardial lesions in calves using high dosage of doxycycline but failed (12); they postulated that formerly reported outbreaks of doxycycline-poisoning were not due exclusively to doxycycline overdosing, but some other exacerbating factors may have played a role in the development of this process (12). They suggest an association with vitamin E/selenium deficiency as a possible exacerbating factor based on low blood levels measured in some calves (8,12), and similar post mortem findings in both etiologies, although as previously mentioned mineralization is an important feature in nutritional cardiomyopathies. We do, however concur with the notion that other causes may play a role in the pathogenesis of doxycycline cardiotoxicity. Various authors suggest a possible interaction with other drugs (6,12) or stress related situations (12). In our case no other medicinal compounds were used to the best of our knowledge; However, the calves were indeed exposed to stressing factors (long transport, handling, change in feeding habits), which may have triggered an excessive burden on the already malfunctioning heart muscle.

Finally, the fact that not all the animals developed clinical signs may be explained by a possible variation among calves tolerance to doxycycline (12). The mechanism of doxycycline-induced muscle fiber degeneration is not well understood. Tetracyclines are known to bind calcium, but doxycycline has a lower affinity for calcium (1,2,4). In the literature, doxycycline is reported to have cytotoxic and anti- proliferative characteristics in various types of cancer, such as colorectal and breast cancer in humans and osteosarcoma in the dog (15-20) as well as a positive effect on periodontal regeneration (21). It is therefore possible that the cytotoxic effect is at the base of myocytes damage, but this is a theory that no doubt requires extensive experiments, as are the reasons for reported occurrence only in calves.

Because myocardial necrosis is a common outcome in many pathologic situations and diseases, a careful review of the animal's history and clinical data is required. In the present case, foot-and-mouth disease was ruled out using PCR and there was no history of Ionophore additives in the feed. Although blood levels of vitamin E or selenium were not measured, lesions in these diseases appear as highly mineralized both in gross and in histopathology sections and this was not seen in this case. However, it is appropriate to consider that potentially low levels of vitamin E or selenium may have contributed to the necrotic effect exercised by the doxycycline.

We believe that there is strong evidence to suggest that doxycycline was the primary cause of myocardial necrosis and sudden death in these calves. This relatively common outcome in calves merits further experimental and genetic investigation.

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