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Accidental and experimental salinomycin poisoning in rabbits[1]

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ABSTRACT.- Peixoto P.V., Nogueira V.A., Gonzaléz A.P., Tokarnia C.H. & França T.N. 2009. **Accidental and experimental salinomycin poisoning in rabbits.** *Pesquisa Veterinária Brasileira 29(9):695-699.* Projeto Sanidade Animal Embrapa/UFRRJ, Seropédica, RJ 23890-000, Brazil. E-mail: pfpeixoto@terra.com.br

An outbreak of salinomycin poisoning in rabbits is described. At least 27 out of 2,000 rabbits reared on a farm died after the coccidiostatic drug sulfaquinoxaline was substituted by salinomycin in the feed. An average of 26.9ppm salinomycin was detected in the ration given to the rabbits. Clinical signs included anorexia, apathy and bradykinesia, which progressed to incoordination and recumbency. Gross lesions consisted of pale areas in the skeletal muscles. The histopathological findings showed severe necrotic degenerative myopathy in association with infiltration of neutrophils and macrophages. One rabbit exhibited similar alterations in the myocardium. Mineralization was observed in the affected skeletal muscles in some cases. In order to verify if the poisoning was due to salinomycin, 20 rabbits were divided into five groups and a ration containing the drug at doses of 10, 25, 50, 75 and 100ppm was given. The administration of doses higher than 50ppm resulted in manifestation of the clinical signs seen in the outbreak of poisoning. It was concluded, that probably an error related to the mixture of salinomycin in the feed was the cause of deaths in the spontaneous outbreak of poisoning on the rabbit farm.

INDEX TERMS: Salinomycin, poisoning, toxic myopathy, rabbits.

RESUMO.- [**Intoxicações natural e experimental por salinomicina em coelhos.**] Relata-se, pela primeira vez, um surto de intoxicação por salinomicina em coelhos. De 2000 animais, no mínimo 27 morreram após troca do coccidiostático sulfaquinoxalina pela salinomicina. A análise de parte da ração detectou 26,9ppm de salinomicina. Os sinais clínicos observados foram anorexia, apatia e lentidão com evolução para incoordenação dos movimentos e decúbito. As lesões macroscópicas consistiram de áreas pálidas na musculatura esquelética. O exame histopatológico evidenciou miopatia degenerativo-necrótica. Adicionalmente, verificou-se reação inflamatória constituída por neutrófilos e macrófagos. Um coelho apresentou lesões similares no miocárdio. Em alguns casos, mineralização estava presente nos músculos esqueléticos afetados. Vinte coelhos experimentais foram divididos em 5 grupos que receberam 10, 25, 50, 75 e 100ppm de salinomicina por via oral, com a finalidade de reproduzir a intoxicação. Os animais que receberam a partir de 50ppm de salinomicina apresentaram sinais clínicos semelhantes aos observados no surto espontâneo. Nossos resultados indicam que, provavelmente, erro na mistura da substância à ração causou a morte dos coelhos.

TERMOS DE INDEXAÇÃO: Salinomicina, intoxicação, miopatia tóxica, coelhos.

INTRODUCTION

Salinomycin, an antibiotic of the ionophore group, is used to control coccidiosis and also to promote weight gain in poultry, rabbits, cattle and swine (Novilla 1992). Ionophores modify the cell membrane permeability, facilitate the influx

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of ions, but may cause severe functional and morphological disturbances in cells (Novilla 1992, Kawazoe 2000). Poisoning by ionophore antibiotics can occur as a consequence of excessive ingestion due to improper mixing of the drug with the feed (Ganter et al. 1989), mistakes in the calculation of dosages (Rollinson et al. 1987), administration to more susceptible species (Griffiths et al. 1989, Salles et al. 1994), or use in combination with other drugs that potentiate their effects (Ganter et al. 1995). Salinomycin poisoning has been reported to occur in horses, cattle, broiler chickens, cats, turkeys and swine (Frigg et al. 1983, Wanner 1984, Amstel & Guthrie 1986, Potter et al. 1986, Gava et al. 1997, Van der Linde-Sipman et al. 1999). High mortality of rabbits that received the ionophore narasin has been described in Brazil (Salles et al. 1994). We are unaware of any report describing salinomycin poisoning in rabbits. The objective of this paper was to describe the clinical, anatomical and histopathological aspects of unintentional and experimental poisonings by salinomycin in rabbits.

MATERIALS AND METHODS

Accidental poisoning. Twenty-seven rabbits of a total of 2,000 animals from a farm located in the county of Mendes, RJ, Brazil, were received at the Veterinary Pathology Section of Projeto Sanidade Animal Embrapa / Universidade Federal Rural do Rio de Janeiro, Seropédica, in midst 1989. Information concerning the medical history of the rabbits was obtained from the owner, who visited the institution several times. Samples of the feed used in the farm were sent to Pfizer laboratory in the city of São Paulo, where they were qualitatively and quantitatively analyzed by high performance liquid chromatography (HPLC).

Experimental poisoning. The experiments were performed at the above mentioned Veterinary Pathology Section, and at the Veterinary Pathology Section of Universidade Federal de Santa Maria, RS. Twenty New Zealand rabbits in finishing phase weighing 2 to 3 kg were kept in individual cages and received water *ad libitum*, *Brachiaria* and coccidiostatic-free feed. Two animals were used as controls. The rabbits were divided into five groups (I to V) of four animals. Each group received salinomycin at a dose of 10, 25, 50, 75 or 100ppm through the oral route by means of a feeding tube. The dosages were chosen with basis on the daily feed intake of rabbits in finishing phase, i.e., around 150g/day. The rabbits that exhibited alterations were subjected to a detailed clinical examination. The rabbits which had received the doses of 50 and 75ppm were euthanized (diethyl ether) on the 9th day of the experiment.

The animals from the accidental poisoning outbreak and those submitted to experimental poisoning were necropsied. Fragments of the muscles and diverse organs were collected, fixed in 10% formalin, processed for histology, and stained with hematoxylin and eosin. The Masson's technique was used to stain muscle sections.

RESULTS

Accidental poisoning

In November 1989, rabbits of a farm located in the county of Mendes, RJ, Brazil, developed a disease characterized by diarrhea after the commercial feed formulation was changed. The owner had requested the feed manufacturer to modify the ration for a better control of coccidiosis, which caused significant economical losses at that time. Consequently, the coccidiostatic sulfaquinoxaline was substituted by salinomycin. Clinically, the animals showed anorexia, apathy, incapacity to raise the head and bradykinesia that progressed to incoordination. Later, the rabbits became recumbent at/in bizarre positions (Fig.1a,b). Generalized loss of muscle tone was also evident. Some rabbits showed extreme muscular flaccidity, to the point that they were unable to make any movement and remained in the position they were left when placed on the floor. There were dirty, with matted hair in the posterior region, and semi-liquid feces in the perianal region. Finally, the animals were unable to make any movement and died within 48 to 72 hours. The macroscopic examination of the 27 rabbits revealed a lighter color of the muscles throughout the body in several cases. One rabbit exhibited dark urine. In two animals, part of the small intestine was filled with a mucous-gelatinous substance,

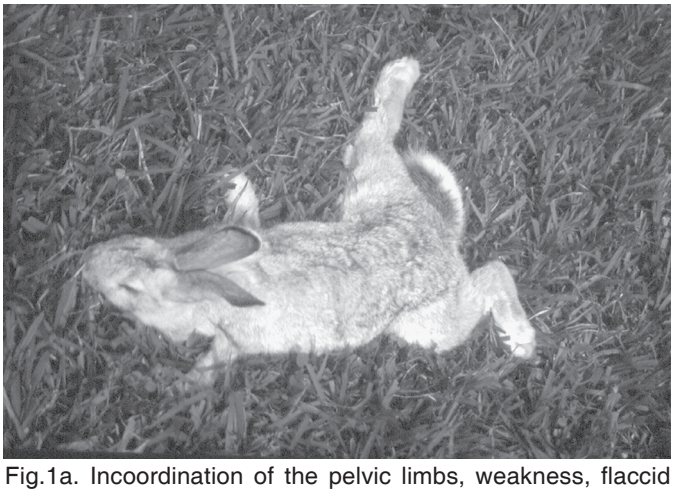

Fig.1a. Incoordination of the pelvic limbs, weakness, flaccid paralysis of the skeletal musculature, inability to make movements, and sternal/abdominal decubitus.

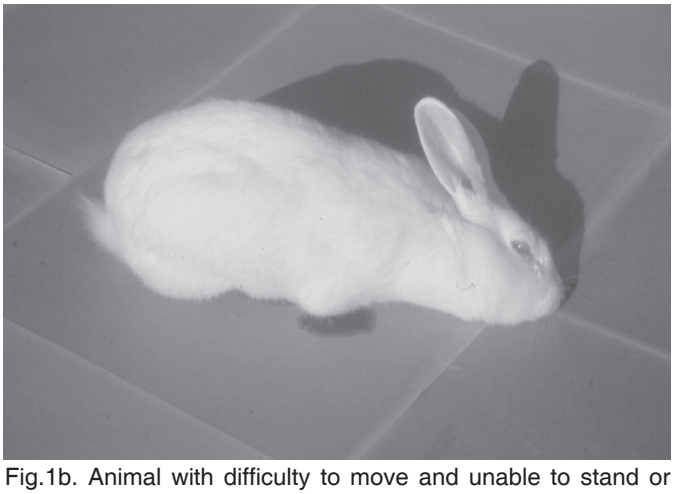

Fig.1b. Animal with difficulty to move and unable to stand or raise the head because of the lesions in the cervical muscle.

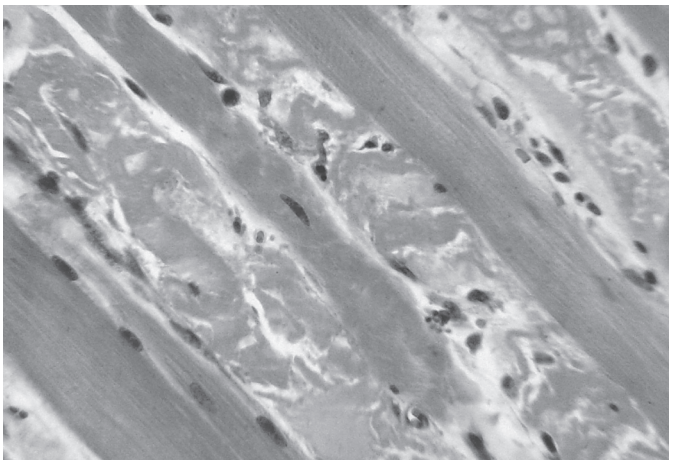

Fig.2. Swelling, increase in eosinophilic staining intensity, coagulative necrosis, mild mixed inflammatory infiltrate in the longissimus dorsi muscle. HE, obj.25x

the contents of the cecum were semi-liquid, and the liver showed a nutmeg appearance.

The histopathological examination revealed areas with necrotic degenerative alterations involving a variable number of muscle fibers. The lesions were almost diffuse in some sites, and were observed in individual or small groups of muscle fibers in other areas. Coagulative necrosis was characterized by presence of fibers with homogeneously eosinophilic cytoplasmic zones with vitreous aspect, loss of striation and adjacent nuclei, or picnotic nuclei. Several animals exhibited floccular (coagulative) necrosis (Fig.2) with formation of granules or eosinophilic cytoplasmic inclusions, sometimes vacuolated, in portions of skeletal muscle fibers. Muscle fiber rupture and segmental hypercontraction (fibers with a "wavy" aspect) were also observed. Sometimes, pale muscle fibers with a swollen appearance apparently undergoing lysis were present. Moderate edema between the myofibers, moderate satellite cell proliferation and mild to moderate inflammatory infiltrates, composed of neutrophils and macrophages between the injured muscle fibers, were sometimes noticeable. There were also regenerating fibers with several central nuclei lined up, mitotic figures in satellite cells, and proliferation of elongated myoblasts. Invasion of hyaline fibers by macrophages and satellite cells was also observed. Additionally, mineralization, characterized by deposition of granular or crystalline basophilic material in areas containing necrotic myofibers, was seen. Similar lesions were found in the heart of one animal, but in this case there was no mineralization.

Experimental poisoning

The animals that received salinomycin at a dose 50ppm exhibited mild anorexia and apathy; those that ingested feed containing 75ppm of the drug showed moderate apathy and marked weight loss. Both groups were euthanized (diethyl ether) on the 9th day of the experiment. Three animals of the group given 100ppm salinomycin died spontaneously on the 4th day of the experiment, and another rabbit died on the 5th day. At the beginning of the 3rd day of the experiment, all of these rabbits were already taking up abnormal positions. Initially they placed their hind limbs before the forelimbs and showed signs of apathy, with marked anorexia and mild tachypnea. The picture progressed rapidly to a state of apparent motor incoordination, marked tachypnea and, later, generalized flaccid paralysis and death. The musculature of the animals of group V (100ppm) was generally lighter than normal, with a whitish color similar to that of fish flesh. Noteworthy macroscopic alterations were not found in the other groups. The microscopic examination of the rabbits that received 100ppm salinomycin revealed the presence of alterations similar to those found in the animals that died due to accidental poisoning. The lesions were conspicuous in the thigh, cervical, longissimus dorsi, and abdominal muscles; they were characterized by degeneration, hyaline and flocular necrosis, presence of inflammatory infiltrates composed mainly of macrophages and neutrophils, and satellite cell proliferation. The cardiac muscle and the smooth musculature of the esophagus, bladder and digestive tract did not exhibit noteworthy alterations. Similar lesions were observed in the animals of group IV (75ppm); however, their intensity was moderate. Few necrotic muscle fibers were found in the rabbits that ingested feed containing 50ppm salinomycin. The histopathological evaluation of the animals belonging to group I (10ppm) and II (25ppm) did not reveal any alterations. Some animals exhibited pulmonary edema, congestion of the kidneys, spleen and lungs, and liver with nutmeg appearance.

DISCUSSION

The diagnosis of poisoning by salinomycin as the cause of the mortality observed in the rabbit farm in Rio de Janeiro was based on epidemiological, clinical, anatomical and pathological findings, and was confirmed by the presence of salinomycin in the feed given to the rabbits. The amount of salinomycin detected in the feed submitted for analysis was 26.9ppm. Even though we were unable to experimentally reproduce the poisoning using similar amounts of salinomycin, the evidence indicates that the outbreak occurred due to improper mixing of the drug with the feed, as frequently described (Whitlock et al. 1978, Rollinson et al. 1987, Novilla 1992). The possible presence of an ionophore antibiotic potentiator in the feed cannot, however, be excluded. According to the National Research Council Committee on Animal, feed containing salinomycin doses above 50ppm can cause negative effects on performance, such as growth depression and decrease in food ingestion. Salinomycin at a concentration of 50ppm in the feed did not cause the death of the animals in group III; on the other hand, it caused only mild anorexia and apathy. This reinforces the possibility that the poisoning outbreak occurred due to the presence of non-homogeneous concentrations of the drug in the feed as a

consequence of improper feed mixing. According to the information obtained from the multinational company that manufactures the coccidiostatic, the use of salinomycin for rabbits was not recommended at the time the outbreak occurred. The comparison of our data with those from studies in cattle (Gava et al. 1997) indicates that rabbits are more resistant to salinomycin. Piglets, adult swine and broiler chickens can also tolerate up to 60, 70 and 30ppm of salinomycin, respectively (Laczay et al. 1989, Ganter et al. 1995). The clinical picture of the animals was, basically, a consequence of the locomotor disorder caused by the lesions in the skeletal musculature, similarly to that reported for poisoning by narasin in rabbits (Salles et al. 1994) and swine (Armién et al. 1997), and salinomycin in swine (Miller et al. 1986, Ganter et al. 1995), horses (Rollinson et al. 1987), turkeys (Harries & Hanson 1991, Andreasen & Schleifer 1995, Assen 2006), cattle (Bastianello et al. 1996, Gava et al. 1997) and cats (Van der Linde-Sipman 1999). Diarrhea has been observed subsequent to consumption of salinomycinin by horses (Rollinson et al. 1987) and cattle (Gava et al. 1997), and after narasin poisoning in rabbits (Salles et al. 1994), but its pathogenesis is unknown. Diarrhea has been suggested to be resultant from the ionic alteration caused by the drug (Bergen & Bates 1984), and Schafer et al. (1984) observed that salinomycin profoundly affects electrolyte transport in the distal colon of rabbits. Ataxia is one of the clinical signs of nervous system diseases with impairment of cerebellar and spinal cord proprioceptive functions (Mayhew 1989, Riet-Correa et al. 2002). Although the clinical signs of ataxia observed during the course of experimental poisoning by monensin in swine have been proposed to be caused by interference of the ionophore with the peripheral neurotransmission (Van Vleet et al. 1987), we believe that the ataxia observed in the rabbits in this study was due to the weakness caused by primary lesions in the skeletal musculature, as postulated by Salles et al. (1994). One animal exhibited dark urine. Myoglobinuria is indicative of severe muscular damage (Hulland 1985), and has been observed in cases of poisoning in several species. In the rabbits poisoned by salinomycin, the lesions were most prominent in the thigh, cervical, longissimus dorsi and abdominal muscles. Lesions in the smooth musculature were not found, probably because ionophore antibiotics compromise energy production by the cells, and the metabolism of cardiac and skeletal muscles is more dependent on energy than that of smooth muscles. We believe that respiratory failure caused by lesions in the intercostal muscles and in the diaphragm was the cause of death of the animals. Lesions in the myocardium were mild or absent in rabbits poisoned by narasin (Salles et al. 1994). In this study, some rabbits showed signs of heart failure such as pulmonary edema, congestion of the kidneys, spleen and lungs, and livers with a nutmeg appearance, in spite of the absence of gross or microscopic alterations in the myocardium. It is possible that functional or even ultramicroscopic alterations are involved in the etiology of heart failure (Mitani et al. 1976). These may include hyperkalemia (Nuytten et al. 1981) or increased sarcoplasmic calcium levels in the myofibers (Safran et al. 1996). Signs of regeneration as denoted by satellite cell proliferation were present in the thigh musculature in this study, and have been observed by others during ionophore antibiotic poisoning in swine (Van Vleet & Ferrans 1984). The differential diagnosis of ionophore antibiotic toxicosis in rabbits should include vitamin E / selenium deficiency as a key factor. This deficiency generally affects young rabbits, most of times during lactation, months after the introduction of the deficient feed (Ringler & Abrams 1970); in addition, pansteatitis is possibly associated with this condition (Jones et al. 2000). In this study, the only source of selenium for the rabbits was the commercial feed; notwithstanding, none of the animals that died exhibited pansteatitis. Although animals of all ages died during the poisoning outbreak, the number of deaths was higher in lactating rabbits, suggesting that these either ingested higher amounts of salinomycin as they double feed ingestion during lactation, or are more sensitive to this antibiotic. On the other hand, the mortality rate decreased to baseline levels as promptly as salinomycin was withdrawn from the rabbit feed. The best thing is to prevent toxicosis and, in order to do it, care should be taken to establish the correct dosages for each species, distribute the ionophore in the feed in a homogeneous way, and check for the concomitant use of other drugs that may act as potentiators.

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