Food and Chemical Toxicology 68 (2014) 283–289

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Acute maduramicin toxicosis in pregnant gilts

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ARTICLE INFO

Article history:
Received 23 August 2013
Accepted 25 March 2014
Available online 3 April 2014

Keywords:
Gilts
Pigs
Maduramicin
Toxicosis

ABSTRACT

Ionophores are used as feed additives for the control of coccidiosis and growth promotion in farm animals. Reports of maduramicin toxicosis in farm animals are scarce. The present work describes an acute maduramicin toxicosis affecting 22 pregnant gilts, 2 pregnant sows and 2 boars, resulting in a total mortality of 65% within 2 days. The clinical and histopathological findings observed shared similar characteristics to acute ionophore toxicosis in pigs, being characterized by severe myodegeneration in skeletal muscle and degenerative changes in the myocardium. Important clinical pathology indices found were elevated levels of CPK and ALT. In contrast to the pregnant gilts, the two pregnant sows completely recovered after 1 month and farrowed 2 months after the intoxication event healthy piglets. The lack of effect of maduramicin on the fetuses might be indicative of poor placental penetration of maduramicin. Moreover, the present work reports for the first time maduramicin levels in livers (0.5 mg/kg) of gilts exposed to lethal concentrations of maduramicin (18.5 mg/kg) in the feed. As the average feed intake of the gilts was estimated to be 3.5 kg feed/day, the mean maduramicin intake leading to the observed high mortality rate was 0.4 mg/kg body weight/day.

1. Introduction

Carboxylic polyether ionophores are extensively used worldide as feed additives for the control of coccidiosis and growth promotion in farm animals (Dorne et al., 2013; Novilla, 1992). In contrast to the European Union, in which ionophores are authorized solely for use in poultry (except salinomycin being also authorized for rabbits), in the USA lasalosid, laidlomycin, monensin and semduramicin are also approved in cattle for enhanced feed efficiency and growth (Dorne et al., 2013; EFSA, 2006, 2007a, 2007b, 2008a, 2008b, 2008c, 2008d). Approved ionophores in Israel are listed in Table 1 together with their recommended maximum levels in complete animal feed and the animal species for which the use of the ionophores is authorized (The Israeli Drug Registry). The coccidiostatic action of the ionophores is primarily associated with their ability to form lipid soluble zwitterionic complexes with cations (Na^+, K^+, Ca^{2+}) thereby promoting their transport across the cell membrane (Novilla, 2012). The ionophoric activity results in altered ionic concentration gradients across the cell membrane, calcium overload, intracellular pH alteration, enhanced lipid peroxidation, eventually leading to cell death (Novilla, 1992, 2012). At the recommended dosage in complete animal feed, the ionophores primarily affect protozoan parasites and bacteria; however at higher dosages the host becomes highly vulnerable towards hazardous adverse effects, due to the low safety margin of some ionophores in sensitive species (Dorne et al., 2013). The main target organs injured by toxic doses of carboxylic ionophores are the heart and skeletal muscles in all species studied (EFSA, 2006, 2007a, 2007b, 2008a, 2008b, 2008c, 2008d).

Maduramicin is approved in Israel, the USA and the European Union as a coccidiostat for broiler chickens and turkeys with a maximum level of 5 mg/kg in feed (EFSA, 2008d; Table 1). Case

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http://dx.doi.org/10.1016/j.fct.2014.03.034
0278-6915/

Table 1
List of approved ionophores in Israel[a] and their maximum recommended content in complete farm animal feed.

Coccidiostats	Target animal	Maximum final concentration in feed (mg/kg)
Maduramicin ammonium	Broiler chickens	5
	Turkeys (up to 16 weeks)	5
Salinomycin sodium	Broiler chickens	60
Monensin sodium	Broiler chickens	125
	Chickens reared for laying (up to 16 weeks)	120
	Turkeys (up to 16 weeks)	100
	Cattle	45
Lasalocid sodium	Broiler chickens	125
	Turkeys (up to 12 weeks)	125
	Cattle and Sheep	35
Narasin	Broiler chickens	50

[a] The Israeli Drug Registry.

reports of maduramicin toxicosis in farm animals, especially in pigs, are scarce in comparison to other ionophores such as monensin or salinomycin (Miskiminns et al., 1996; Plumlee et al., 1995; Sanford and McNaughton, 1991; Shlosberg et al., 1986, 1992, 1997). In Israel, poultry litter from maduramicin-treated broilers has been fed to beef cattle as rich source of protein and minerals, resulting in the past in maduramicin toxicosis (Shlosberg et al., 1992, 1997). Several case reports described the deleterious effects of maduramicin on cattle fed on poultry litter, containing maduramicin even at a final concentration as low as 2.5 mg/kg (Fourie et al., 1991). Additional unintentional sources of maduramicin contamination in animal feed may stem from cross-contamination during the production in a feed mill of different feeds when switching over from one product to another, or as a result of accidental incorporation into non-target animal feed (EFSA, 2008d). In several reported cases, toxic levels of maduramicin in animal feed were found to be directly implicated in the cause of death of various animal species, including cattle, pig and sheep, mainly as a result of cardiac and/or respiratory failure (Fourie et al., 1991; Sanford and McNaughton, 1991; Shlosberg et al., 1992, 1997). Clinical signs of maduramicin toxicosis are similar to other ionophore toxicoses and may include feed refusal, anorexia, respiratory distress, lethargy, ataxia, recumbency, and sudden death (Sanford and McNaughton, 1991; Shlosberg et al., 1992, 1997; Van Vleet et al., 1982). Histologically, degenerative myopathy involving skeletal and cardiac muscles is invariably encountered if not peracute in nature (Bastianello et al., 1995; Shlosberg et al., 1997). Activity of the muscle-derived enzymes aspartate aminotransferase (AST), lactic dehydrogenase (LDH) and creatinine kinase (CPK) were often elevated in intoxicated species (Fourie et al., 1991; Sanford and McNaughton, 1991; Shlosberg et al., 1992, 1997).

Between April 9 and 10, 2013, 65% mortality occurred in a pig farm located in the southern district of Israel, involving 15 pregnant gilts and two young boars out of 26 pigs following ingestion of feed contaminated with maduramicin. The aim of the present work is to provide a full description of acute maduramicin toxicosis in pigs including clinical signs, gross pathology and histopathology in association with the corresponding maduramicin levels found in the feed and the livers of the dead pigs.

Table 2
Nutritional composition of complete feed.

Raw material	% Ingredients of complete feed
Wheat	20.0
Barley	10.0
Corn	35.0
De-hulled soybeans	3.0
Dried Distillers Grains Golden south	8.0
Sunflower meal	9.0
Wheat bran	3.7
Calcium carbonate	1.5
Monocalcium phosphate	0.4
Sodium chloride	0.3
Sepiolite clay mineral	2.0
Feather meal	2.0
Poultry meat and bone meal	3.0
Toxibond® pro[a]	0.1
Choline chloride 75% liquid	0.02
Lysine	0.3
Premix[b]	0.3

[a] Mix of thermically activated and hydrated sodium and calcium aluminum silicates, mannan oligo saccharides, beta glucans, fructo oligo saccharides, enzymes, probiotics and prebiotics.

[b] Contains: dicalcium phosphate, seashell flour, kelp meal, sodium chloride, vitamin A supplement, vitamin D_3, vitamin E, riboflavin supplement, D-calcium pantothenate, niacin supplement, choline chloride, vitamin B12 supplement, folic acid, thiamine hydrochoride, pyridoxine hydrochloride, biotin, manganous oxide, ferrous sulfate, monohydrate, calcium iodate, copper sulfate, zinc sulfate, sodium selenite.

2. Materials and methods

2.1. Toxicosis event

History of the herd: the affected pigs consisted of 22 pregnant gilts (1 month pregnancy, 8–8.5 months old, weighting 150–170 kg), 2 pregnant sows (1 month pregnancy, 2 years old, weighting 220–230 kg) and two young boars (1 years old, weighting 230–230 kg), which were held at a separated confinement. The gilts and sows were housed in three different confinements within a separated facility from the other breeders, which were also grouped and housed according to their age and reproductive stage. The pigs within the farm (1150 sows and 12,500 pigs at all age groups) are mainly raised for meat production and to a minor extent for medical research. The animals were housed in semi-open covered barns with pens, on concrete slatted floors. At the time of toxicosis, this group of pregnant gilts and sows, as well as the two young boars, were fed the same diet batch, a nutritionally balanced feed (Table 2), while the rest of the breeders and fattening pigs within the farm were fed other batches of varying compositions according to their age, reproductive stage or fattening status. The feed ingredients were mixed and supplied by a local feed mill. Before artificial insemination, gilts were vaccinated against Porcine Parvovirus (PPV), *Erysipelothrix rhusiopathiae* (Ery) and *Escherichia coli*, and boosted against *Mycoplasma hyopneumonia*e and Porcine Circovirus type 2 (PCV2). At the time of the toxicosis event the farm was serologically free from Aujeszky Disease (AD), Porcine Reproductive and Respiratory Syndrome (PRRS), subtypes H1N1, H1N2 and H3N2 of Swine Influenza Virus (SIV). The serological tests were performed on a regular basis three times a year by the Italian Veterinary Institute (IZS-LER) in Brescia, Italy. After 7 days of feed consumption of the same batch of feed, 10 gilts were found dead on the eighth day early in the morning as well as two young boars held at an adjacent confinement, which were given the same feed as the pregnant gilts and sows (Table 3). The sudden deaths occurred without any previously noticeable clinical signs. On the following day, 5 more gilts were found dead, while the remaining pregnant gilts displayed characteristic clinical signs of severe ionophore toxicosis (Miskiminns et al., 1996; Plumlee et al., 1995; Sanford and McNaughton, 1991). The suspected contaminated feed was immediately removed on the first day and replaced by a complete feed supplied by a different feed mill manufacturer. As the clinical status of the remaining gilts ($n = 7$) deteriorated over the next 3–4 days, they were sent to the slaughterhouse located within the farm facility for euthanasia and discarded. However, the remaining two pregnant sows displayed moderate clinical signs of toxicosis, followed by marked improvement over the next days and completely recovered after a month, displaying no signs of intoxication. The two sows eventually gave birth to healthy piglets. An overall mortality of 65% was recorded by the end of the event (excluding the seven euthanized gilts).

2.2. Laboratory investigations

2.2.1. Feed analysis

Samples of the suspected feed from the intoxication event were analyzed for doxycycline, chlortetracycline and oxytetracycline as well as for the ionophores monensin, lasalocid, salinomycin, maduramicin, semduramicin and narasin by liquid chromatography tandem mass spectrometry (LC/MS/MS).

The feed was analyzed for the elements As, Cd, Co, Zn, Cu, Fe, Pb, Mn, Hg, Mo, Se, Tl and Zn by utilizing ICP-AES (model ARCOS from Spectro GMBH, Germany) according to the EPA method 6010B. Moreover, a wide range of pesticides, including