Acute Salinomycin and Maduramicin Toxicosis in Lactating Sows

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ABSTRACT

The epidemiological investigation of an incident that killed 98 sows (170-220 kg b.w.) out of a total of 180 sows was described. The sows were at the beginning (1-2 weeks) of their lactation period. The incident started with the sudden death of 8 sows and lasted 20 days. No clinical signs of disease were observed prior to the death of the first animals while the remaining sows displayed clinical signs of ionophore toxicosis, including ataxia, recumbency, respiratory distress, lethargy and partial anorexia. The investigation consisted of clinical examinations, autopsy, blood and serum clinical chemistry, bacteriology, virology combined with chemical analysis of ionophores and the presence of pesticides in the feed. Three batches of identical feed were consumed by the affected sows. Lesions and degenerative changes were found only in the skeletal muscles and myocardium. Microscopically, degenerative changes in the skeletal muscle were characterized by hypereosinophilia, loss of cross-striation and fragmentation. Gross increase in liver enzyme activities and elevation of serum urea and creatinine values were observed. High levels of maduramicin (7.0 to 25.2 mg/kg) in two batches and salinomycin (2.0 to 42.1 mg/kg) in all three batches of feed were found. The results of the investigation indicated simultaneous ionophore poisoning by two agents, maduramicin and salinomycin, in a non-target species.

Keywords: Sow; Toxicosis; Maduramicin; Salinomycin; Feed.

INTRODUCTION

Non-target animal feed cross-contamination as well as accidental incorporation of polyether ionophores into non-target feed are common sources for ionophore toxicosis in farm animals (1, 2, 3, 4). Ionophores are commonly used as feed additives for the control of coccidiosis and growth promotion in farm animals (5, 6). Coccidiostats are authorized for use solely in poultry or, like the ionophores monensin and lasalocid, they are also approved in cattle for medical use or for enhanced feed efficiency and growth (6, 7, 8, 9, 10, 11). The coccidiostatic action of ionophores is associated with their ability to form lipid soluble zwitterionic complexes with cations (Na^+, K^+, Ca^{2+}) thereby promoting their transport across the cell membrane, eventually leading to cell death (12). Protozoa parasites in the gastro-intestinal (GI) tract are the main target of ionophores at the recommended dosage; however at higher concentrations, due to the low safety margin of the ionophores, animals are highly susceptible to

serious adverse effects (6). The most vulnerable organs to ionophore injury are the heart and skeletal muscles in all species studied (7, 8, 9, 10, 11).

Case reports of ionophore toxicosis in pigs are relatively scarce as compared to other animal species such as poultry and cattle (1, 2, 3, 4). The aim of the present case report is to describe the acute maduramicin and salinomycin toxicosis in sows comprehensively in terms of clinical signs, gross pathology and histopathology in correlation with the corresponding ionophore levels found in the feed.

MATERIALS AND METHODS

Between March 8 to 28, 2013, an unprecedentedly high mortality rate in lactating sows occurred in a pig farm located in the southern district of Israel. The sows died over a time course of 20 days following ingestion of 3 different batches of complete feed contaminated with maduramicin and salinomycin consumed over a time period of 8 days.

HISTORY OF THE HERD

The affected group consisted of 180 lactating sows (7-14 days of lactation) aged 2-4 years, weighing 170-220 kg. The overall breeder population in the farm was 1,150 heads, including pregnant gilts. The lactating sows were housed in a separated confinement from the other pigs, which were also grouped and housed according to age and weight. The pigs within the farm (12,500 pigs in all age groups) were mainly used 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 the incident, all the sows in lactation consumed consecutively three different batches of the food containing the same ingredients but manufactured on different dates: nutritionally balanced total mixed ration consisting of (on a dry weight basis) 25% wheat, 10% barley, 31% corn, 8% dried distillers grain, 5% sunflower meal, 3.3% wheat bran, 8.7% soy as well as 9% vitamins, minerals and other feed additives, as detailed in Table 1.The feed ingredients were mixed and supplied by a local feed mill located in the south of the country.

The sows received a booster vaccination against *E.coli*, *Mycoplasma hyopneumonia*e and Porcine Circovirus type 2 (PCV2) before farrowing; then, during lactation, a booster vaccination against Porcine ParvoVirus (PPV) and *Erysipelothrix rhusiopathiae* (Ery). The farm, as all other pig farms in Israel, was free from Aujeszky Disease (ADV), Porcine Reproductive and Respiratory Syndrome (PRRS), subtypes H1N1, H1N2 and H3N2 of Swine Influenza Virus (SIV).

On March 8, 2013, eight sows were found dead without any preceding clinical signs, while the remaining sows displayed clinical signs of ataxia, recumbency, respiratory distress, lethargy and partial feed refusal. On the next day, five more sows were found dead early in the morning. None of the suckling piglets showed any signs for illness and seemed to be healthy during the course of the event. Given the epidemiological background of the herd, the absence of systemic diseases with ability to induce quick and sudden death in adult animals, the absence of clinical signs in other animals (growing pigs; pregnant sows), suspect were focused about poisoning and/or intoxication via the feed destined to the affected group of animals. Therefore, feed

Table 1: Nutritional composition of total mixed ration (TMR)

% of TMR	Raw Material
Wheat	25
Barley	10
Corn	30.654
De-Hulled Soybeans	8.7
Dried Distillers Grains	8
Sunflower Meal	5
Wheat Bran	3.3
Oil	1.8
Calcium Carbonate	1.7
Monocalcium Phosphate	0.85
Sodium Chloride	0.28
Sepiolite Clay Mineral	2
Feather Meal	2
Toxibond® Pro[1]	0.1
Premix[2]	0.3
Choline Chloride 75 Ml	0.02
Biofeed Wheat	0.02
Lysine	0.276

[1] Mix of thermically activated and hydrated sodium and calcium aluminum silicates, mannan oligo saccharides, beta glucans, fructo oligo saccharides, enzymes, probiotics and prebiotics.
[2] Contains: dicalcium phosphate, seashell flour, kelp meal, sodium chloride, vitamin A supplement, vitamin D_3, vitamin E, riboflavin supplement, D-calcium pPantothenate, 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.

batches were suspected to be the cause of the intoxication; these were immediately removed from the sows' diet and replaced by a complete total mixed ration from a different local feed manufacturer containing the same composition as detailed in Table 1. Despite this change, the clinical status of some of the sows deteriorated over the next couple of days; these were euthanized due to animal welfare concerns and discarded.

LABORATORY INVESTIGATIONS

Feed analysis

Samples of the suspected feed from the intoxication event were analyzed at the Residue Control Laboratory of "Kimron" Veterinay Institute, Beit Dagan, Israel for doxycycline, chlortetracycline and oxytetracycline as well as for the ionophores monensin, lasalocid, salinomycin, maduramicin, semduramicin and narasin, by mean of liquid chromatography tandem mass spectrometry (1260 Infinity, Agilent Technologies, CA, USA ; API 4000, AB Applied Biosystems, MSD Sciex, Singapore).

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 (ARCOS, Spectro Analytical Instruments GMBH, Kleve, Germany) according to the EPA method 6010c. (14) Moreover, a wide range of pesticides, including organophosphates, carbamates, pyrethroids and organochlorides were analyzed (Agilent Technologies VLMSD series, Santa Clara, CA, USA) according to a screening method described earlier (15).

Blood biochemistry

Blood samples were collected from the jugular vein on the first few days of the illness from 12 sows, belonging to same group of dead sows. The serum was assayed for basic enzymatic test panel: creatine phosphokinase (CPK), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), creatinine and urea; additional test panel: sodum, chlorine, potassium, calcium, total bicarbonate. Assays were performed by Cobas Integra 400, Roche Diagnostics (Rotkreuz, Switzerland), according to materials and methods supplied by Roche Diagnostics GmbH (Mannheim, Germany), as in use at Laboratory of The Veterinary Hospital of "Kimron" Veterinary School, Beit Dagan.

Hematology

Whole blood was examined to determine the values of the red blood-cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell count (WBC) and differential white blood-cell count. Tests were performed according the methods in use at The Veterinary Hospital of "Kimron" Veterinary School, Beit Dagan, (ADVIA 120 Hematology, Siemens, Munich, Germany)

Necropsy

Two recently dead sows were submitted to the Kimron Veterinary Institute for full necropsy 2 days after the mortalities of 5 sows.

Bacteriology and Virology

Bacteriological culture was performed from organs of necropsied sows (lungs, spleen, liver, kidney and intestine). Samples were inoculated in nutrient agar; MacConkey agar and 5% sheep blood agar plates; incubated at 37 C; examined at 24 and 48 h. Additional blood agar plates were incubated for 48 h in anaerobic conditions. Biologic test for Botulism was also performed.

ELISA-Ab was applied to screen against the following viral diseases in serum of sampled sows: Transmissible Gastro Enteritis (TGE) and Porcine Corona Respiratory Virus (TGE/Respiratory Corona differentiating test; Svanova, Uppsala, SW); Porcine Circovirus type 2 (PCV2) (PCV2-Ab; Synbiotics, Lyon, F); Classical Swine Fever (CSF) (CFS-Ab PrioCHECK, Prionics, Lelystad, NL); Swine Influenza (SI) (LSI-Ab, Lissieau, France) and Porcine Reproductive and Respiratory Syndrome (PRRS) (PRRS-Ab PrioCHECK, Prionics, Lelystad, NL).

Reverse transcriptase PCR on RNA from whole blood samples was used to screen against CSF and SI viruses, according to methods in use at "Kimron Veterinary Institute", Beit Dagan, as previously described (16).

Histopathology

Tissues samples collected for histopathological examination included the heart, skeletal muscles (diaphragm, intercostal, triceps, *vastus lateralis*, *longissimus dorsi*), lung, liver, kidney, spleen, brain and small intestine. The tissue samples were fixed in 10% neutral buffered formalin. Paraffin blocks were made and sectioned at 3-4μm and stained with haemtoxylin and eosin.

RESULTS

Bacteriology, Virology

Examined samples were negative to aerobic and anaerobic cultures, to biologic test for Botulism, to ELISA-Ag and PCR test for PCV2, CSF and SI. ELISA-Ab resulted positive for PCV2, inline with the epidemiological situation in Israel (17).

Feed analyses

The analysis of the 3 feed batches consumed for 8 days by all the sows prior to the first mortalities, revealed a combination of high levels of maduramicin and salinomycin with values ranging between 7 to 25 and 2 to 42 mg/kg, respectively (Table 2).The concentration of both ionophores were analysed by use of an LC/MS/MS method (13), according to methods in use at The Residues Control Laboratory of the "Kimron" Veterinary Institute, Beit Dagan (1260 Infinity, Agilent Technologies, CA, USA ; API 4000, AB Applied Biosystems, MSD Sciex, (Singapore).

Due to high rotation-replacement of feed (in average 2 to 3 feed batches per week), the total individual exposure to maduramicin and salinomycin by each individual animal over 8 days could only be estimated as an average intake in relation to total quantity of feed supplied to lactating sows: maduramicin 0.20 to 0.44 mg/kg/bw/day; salinomycin 0.07-1.22 mg/kg/bw/day.

Table 2: Ionophore levels in three feed batches listed according to their manufacturing date. The feeds were consumed over a time period of 8 consecutive days before the first mortality event occurred.

Manufacturing date of TMR	Mean ionophore level (mg/kg)
21.2.2013	Maduramicin: 7 Salinomycin: 2
27.2.2013	Salinomycin: 42.1
3.3.2013	Maduramicin: 25.2 Salinomycin: 6 Monensin: 14.3

Moreover, clinically insignificant levels of monensin were found in the third batch at a level of 14.3 mg/kg (Table 3). The antibiotics doxycycline, chlortetracycline and oxytetracycline were below the detection limits in both total mixed rations analyzed (data not shown). Both feed batches were also analyzed as described in (15) and were found to be free of pesticide's contamination.

Although the contaminated batches were replaced by a

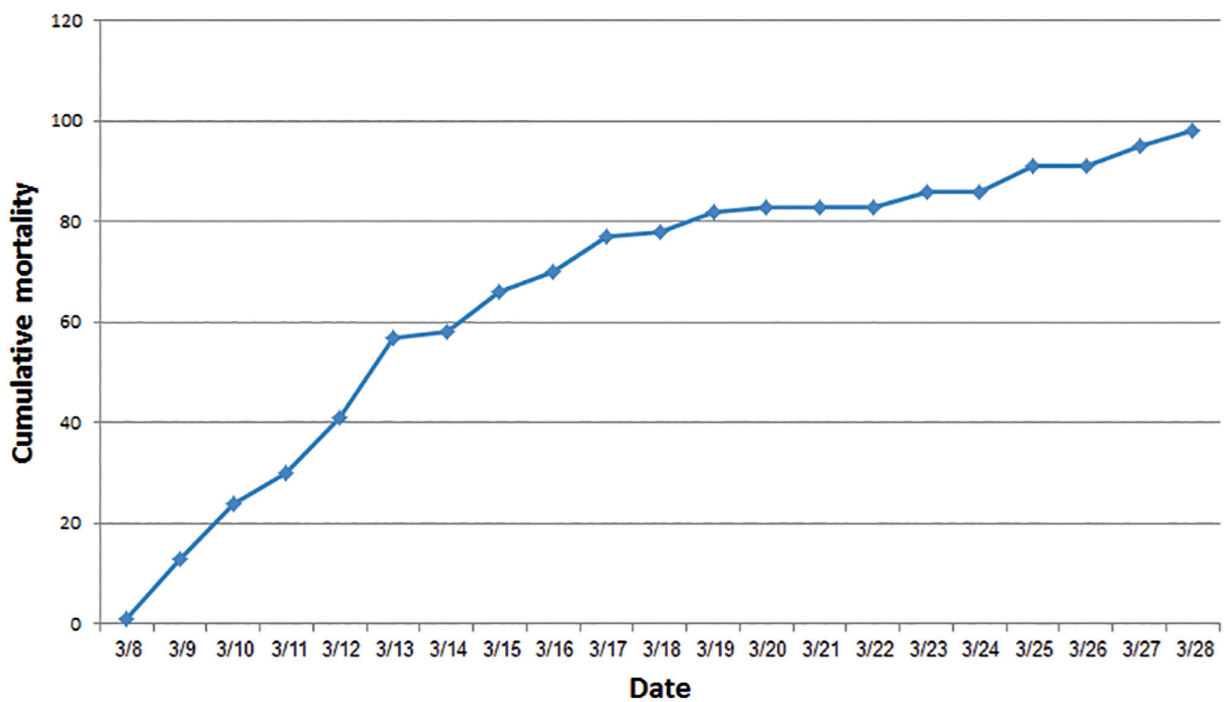

Figure 1: Cumulative mortality incidence following lethal consumption of 3 different feed batches contaminated with ionophores over a time period of 8 days.

Table 3: Mean levels of creatinine, urea and serum activity of creatine phosphokinase (CPK), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) in healthy and in ionophore intoxicated sows.

Biochemical marker	Average enzyme activity in healthy sows (Range)[a]	Average enzyme activity and creatinine and urea levels in intoxicated sows[b] ± SE (range)
CPK	47 U/L (0-130)	>10^6 U/L
ALT	45 U/L (15-52)	760 ± 266.5 U/L (494-1027)
ALP	65 U/L (20-70)	109 ± 67.9 U/L (41-177)
AST	30 U/L (14-42)	9062 ± 28* (6300-11824)
Creatinine	0.4-1.2 mg/dL	4.0 ± 0.2 mg/dL (3.88-4.2)
Urea	15-30 mg/dL	65.8 ± 6.8 mg/dL (59.7-72.6)

[a] Reference values of healthy sows obtained from Veterinarian Breeding Service, Development Agriculture Projects, Kibutz Lahav, Israel; unpublished data.
[b] Data from 12 sows, 1-4 years of age, weighting 170-220 kg, at 7-14 days of lactation.

new complete feed free of ionophore residues within very few days after first signs of the intoxication event, the clinical signs persisted during the next three weeks. Since the surviving eighty two sows remained thriftless, they were euthanized and destroyed to prevent human consumption. During a time period of 20 days, 98 sows died, constituting of an overall mortality of 54% (excluding the euthanized sows). Figure 1 illustrates the pattern of mortality in the herd.

Necropsy

The 2 dead sows submitted to Kimron Veterinary Institute for necropsy were in good physical condition. During post mortem examination pale areas or white streaks in skeletal muscles such as legs, *longissimus dorsi, psoas*, diaphragm were seen. Some pale areas on the left ventricle and septum were also observed.

Histopathology

Pronounced degenerative changes were observed in the skeletal muscle, which included swelling of myofibers, loss of striations, coagulation necrosis and fragmentation (Figure 2). Myocardial damage was characterized by acute multifocal myocyte necrosis.

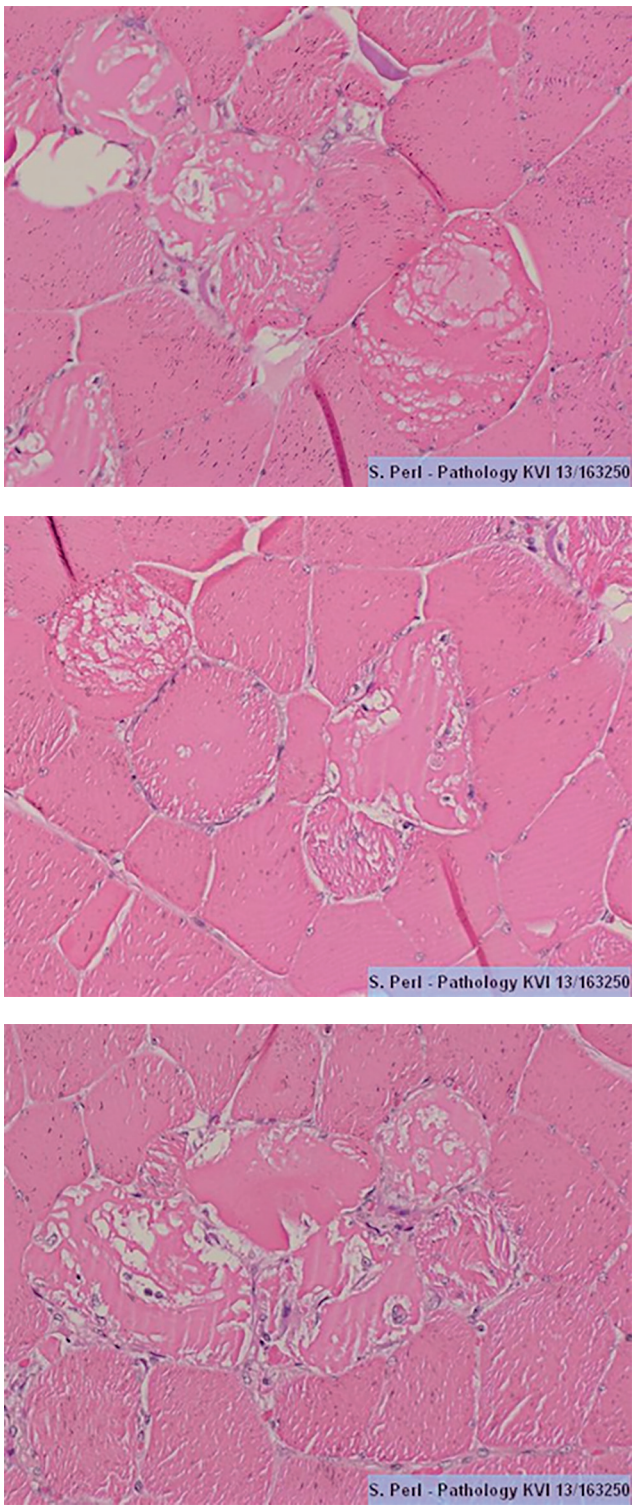

Figure 2: Skeletal muscle histo-section from a sow, 7-14 days in lactation, that died 8 days after ingestion of feed contaminated with ionophores. Necrotic myocytes characterized by, hyalinization and fragmentation. Within and adjacent to the necrotic myocytes there are a few lymphocytes and neutrophils. H&E X 200

Blood analysis

Blood samples from 12 intoxicated sows revealed significant increased activities of aspartate aminotransferase (AST), creatine kinase (CPK) and alanine aminotransferase (ALT), and moderate increase of urea and creatinine levels in intoxicated sows compared to normal healthy pigs. Alkaline phospatase (ALP) did not show any significantly difference from normal values (Table 3).

Blood haemoglobin, haematocrit, red blood-cell count (RBC), packed cell volume (PCV) and white blood cell count (WBC) were all within the normal limits. No significant alterations were detected in serum concentrations of sodium, chloride, potassium, calcium and HCO_3 (data not shown). Although histologically no kidney damage was observed, the high urea and creatinine levels found in both sows could be indicative of acute renal failure (Table 3).

DISCUSSION

Case reports on salinomycin and maduramicin toxicosis in pigs are relatively scarce in comparison to other coccidiostat intoxications, since maduramicin is authorized in the USA and Europe only for poultry (at 5 mg/kg), while salinomycin is approved for poultry and rabbits (at 50-70 mg/kg and 25 mg/kg, respectively) (10,11). To the best of the authors' knowledge, only 3 reports of mass maduramicin toxicosis in pigs have been published. The first two briefly described the epidemiology, clinical signs and clinical pathology of growing pigs, 6 to16 week old (2, 3), and the last described an acute toxicosis in pregnant gilts, by our group (13).

The clinical and histopathological findings observed in the present event shared similar characteristics to acute monensin and salinomycin toxicosis in pigs reported previously (3, 4). The lesions and degenerative changes were also found only in the skeletal muscles and myocardium. Severe myodegeneration was more pronounced in skeletal muscle than in the myocardium, as reported for monensin and salinomycin toxicosis (3, 4). Microscopically, degenerative changes in the skeletal muscle were characterized by hypereosinophilia, loss of cross-striation and fragmentation. The atrial myocardial lesions were more difficult to detect and could be characterized as mild multifocal myocardial necrotic fibers and fragmentation.

No gross clinical and histological signs of renal injury were present, therefore increased creatinine levels were probably the result of rhabdomyolysis. Destruction of myofibers may also cause renal failure and therefore be responsible of increased blood urea levels. In the present investigation, the most important clinical pathology indices characteristic to rhabdomyolysis were the highly elevated levels of AST, CPK and ALT (Table 3), as a consequence of the above mentioned severe myodegeneration observed in both in skeletal and myocardium.

It has been observed that maduramicin can cause skeletal muscle and heart cell damage, resulting in skeletal muscle degeneration, heart failure, (18) inhibiting myo-cells proliferation and inducing cell death (18). Ataxia and recumbency were also the main symptoms observed in course of salinomycin intoxication, and attributed to acute skeletal muscle necrosis, as estimated by muscle enzyme activities increase (CPK, AST) and histopathological examination (19).

Rear limb trembling, lethargy, reluctance to stand were observed in salinomycin intoxication of 150 pigs at 441-720 ppm, with 17% mortality within 24 hours (3). In that case, high levels intoxications caused a quick mortality which probably did not allow the development of typical myodegenerative lesions. In our case, clinical signs, high mortality levels, occurrence of histological lesions at both skeletal and heart muscles, were all present.

In contrast to monensin and salinomycin toxicosis reported previously in pigs, diarrhea and dark-reddish urine were not observed (3, 4) and normal body temperature of ~38°C was recorded.

The epidemiology, clinical signs, pathological lesions and the high maduramicin and salinomycin levels found in the complete feed, together with the absence of any virological and bacteriological involvement, the exclusion of other plausible causes of similar syndromes (such as toxic levels of doxycycline, low Se levels, ingestion of cardiotoxic plants), provide strong evidence that the ionophores maduramicin and salinomycin were the principle toxic agents. Monensin levels found in the total mixed ration of the third batch (Table 3) were below the toxic levels determined in numerous studies for a wide range of farm and laboratory animals including pig, cattle, sheep and poultry (15). Notwithstanding, an additive/synergistic contribution of monensin to the toxic effect exerted by maduramicin and salinomycin could not be ruled out. Moreover, at present there are no studies available that describe the potential interaction of two or more ionophores in target and non-target animals. Therefore, studies

on additive/synergistic interaction of different ionophores on target and non-target animals are warranted.

Following an investigation to determine the source for the ionophore contamination, it was concluded that a premix containing maduramicin and salinomycin was accidently added to the feed intended for the sows. Numerous case reports were published in recent years regarding ionophore toxicosis, due to accidental incorporation of ionophores into non-target animal feed, indicating that cross-contamination of these compounds is still a challenge to be dealt with.

The case study described herein, is the first to report simultaneous maduramicin and salinomycin toxicosis in lactating sows. Interestingly, none of the suckling piglets displayed clinical signs of intoxication, suggesting insignificant carry-over of the ionophores into the sow milk. During the course of the intoxication, the suckling piglets were separated from their mothers and relocated to a separate confinement or fostered to healthy sows.

Peculiar in our case were the presence of clinical signs, high mortality levels, the occurrence of histological lesions at both skeletal and heart muscles.

In conclusion, a direct link was established between the presence of maduramicin and salinomycin ionophores simultaneously in feed and fatal intoxication resulting in 54% mortality of sows.

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