Acute maduramicin toxicosis in pregnant gilts

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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.

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1. Introduction

Carboxylic polyether ionophores are extensively used worldwide 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, lailomycin, monensin and senduramicin 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+, Ca2+) 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
reports of maduramicin toxicosis in farm animals, especially in pigs, are scarce in comparison to other ionophores such as monensin or salinomycin (Miskimins et al., 1996; Plumlee et al., 1985; 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., 1991). 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.

### 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 year old, weighting 230–230 kg), which were held at a separated confinement. The gilts and sows 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. The pigs within the farm (1150 sows and 12,500 pigs) were held at a separated confinement. The gilts were 4 months old, weighting 220–230 kg, two young boars held at a separated 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 (Miskimins 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 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 dosycycline, chlorotetracycline 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, Ti 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...
organophosphates, carbamates, pyrethroids and organochlorines were analyzed by GC/MS (7890A gas chromatograph, Agilent Technologies, Santa Clara, USA), according to a screening method published by Shimshoni et al. (2012).

2.2.2. Liver samples

Liver samples from 7 dead gilts were analyzed for the ionophores monensin, lasalocid, salinomycin, maduramicin, semduramicin and narasin by LC/MS/MS.

2.2.3. LC/MS/MS analysis of ionophores in animal feed and liver

The ionophores were analyzed by an in-house validated multi-residue method according to the guidelines published in SANCO/12495/2011. Briefly, 5 g of the ground test sample (animal feed) or 5 g liver were homogenized in 10 mL acetonitrile for 1 min and further diluted with additional 10 mL acetonitrile (30 mL for feed). The diluted samples were vortexed for 1 min and centrifuged at 600g for 5 min. Then, 3 mL of the supernatant (1 mL for feed) was transferred to a clean glass test tube and evaporated to dryness at 60 °C (the remaining supernatant was used for analysis of tetracyclines, see Section 2.2.4). The residue was dissolved in 4 mL hexane and a sample clean-up procedure was applied utilizing silica solid phase extraction columns. The columns were preconditioned with 3 mL hexane followed by loading the reconstituted sample on the columns. The ionophores were eluted with 5 mL methanol into a new glass test tube and evaporated to dryness at 60 °C. The sample was eventually reconstituted with 0.25 mL methanol (1 mL for feed) and injected to LC/MS/MS. The method utilized an Agilent 1100 (Agilent Technologies, Germany) liquid chromatography system (equipped with binary pump, degasser, column compartment and autosampler) combined with an Applied Biosystems ABI 3200 QTrap (Applied Biosystems, Toronto, Canada) mass spectrometer. LC separation was performed using a C18 Hypersil Gold column (3 μm, 100 x 2.1 mm, Thermo Electron Corporation, Bellefonte, PA, USA). The mobile phase consisted of 0.01 M ammonium formate pH 3.5 (Sigma–Aldrich, USA) and acetonitrile (J.T. Baker, Deventer, Netherlands). The ionophores were separated using isocratic condition consisting of 10% buffer and 90% acetonitrile at a flow rate of 0.5 mL/min. The column compartment was kept at 40 °C and the injection volume was 5 μL. Turbo ion spray for ESI/MS/MS in positive ion mode was operated at a temperature of 500 °C. Multiple reaction monitoring (MRM) was applied. Precursor ions and product ions of each ionophore were selected as listed in Supplemental Table S1 together with their corresponding lower limit of quantification (LOQ).

2.2.4. LC/MS/MS analysis of doxycycline, chlortetracycline and oxytetracycline in animal feed

An aliquot of 1 mL of supernatant (of Section 2.2.3) was vortex-mixed with 1 mL of hexane and centrifuged 5 min at 10,000g. The hexane layer was removed and the acetonitrile (lower) phase was evaporated to dryness at 60 °C. The sample was re-dissolved in 5 mL 0.2% formic acid (Sigma–Aldrich, USA). Clean-up was performed on the farm by the Veterinary Officer for Swine Diseases and the Animal Disease Order, Article 11, 1985.

2.4. Statistical analysis

The results of the blood tests were analyzed for differences between healthy (standard reference values, Table 4) and intoxicated sows by student t-test, with a level of significance of p < 0.05. The biochemical values of the healthy gilts were provided by the Veterinarian Breeding Service, Development Agriculture Projects, Kibutz Lahav, Israel. The biochemical reference values were determined from dozens of healthy pregnant gilts over a time period of five years, weighing 140–180 kg.

2.2.6. Bacteriology and virology

Fresh samples of lung, intestine, colon, tonsils, mesenteric and inguinal lymph nodes, liver, spleen and kidney from 7 dead gilts were examined for evidence of bacteriological and viral infections. In addition, fresh blood and serum samples were collected in potassium EDTA tubes from the jugular vein on the 8th day of the intoxication event from 10 randomly chosen live intoxicated gilts. Bacteriological culture was performed from internal organs of dead gilts (lungs, spleen, liver and kidney). Samples were inoculated onto nutrient agar, MacConkey agar and 5% sheep blood agar plates, incubated at 37 °C and examined after 24 and 48 h. An additional blood agar plate was incubated for 48 h in anaerobic conditions (Pack Anaero, Mitsubishi, USA). Intestinal contents were enriched for salmonella isolation in tetrathionate broth at 37 °C for 24 h and then inoculated onto MacConkey and brilliant green agar plates. The plates were examined after further incubation for 24 h at 37 °C.

ELISA-Ab was applied to screen against the following viruses in fresh gilt serum: Transmissible Gastro Enteritis (TGE), Porcine Corona Respiratory Virus, Porcine Circovirus type 2 (PCV2), Classical Swine Fever Virus (CSF), Swine Influenza Virus (SIV) and Porcine Reproductive and Respiratory Syndrome (PRRS). ELISA-Ag was used to screen against antigen of PCV2 and CSF. Immunohistochemistry from frozen tissues of 5 g from 2 dead gilts was performed on the farm by the Veterinary Officer for Swine Diseases and the Animal Disease Order, Article 11, 1985.

2.2.7. Necropsy and gross pathology

A total of 5 gilts were submitted to the Kimron Veterinary Institute for full necropsy on the first day of the intoxication event. In addition, necropsy in 2 dead gilts was performed on the farm by the Veterinary Officer for Swine Diseases and the Animal Disease Order, Article 11, 1985.

2.2.8. Histopathology

Tissues collected for histopathological examination from 7 gilts included the entire heart, skeletal muscles (diaphragm, intercostal, triceps, vastus lateralis, longissimus dorsi, intermusculus, semimembranosus, semitendinosus, and biceps femoris), lung, liver, kidney, spleen, brain, both ventricular walls and the interventricular septum, and small intestine and were preserved in 10% buffered formalin. Tissues were embedded in paraffin, sectioned at a 5 μm thickness and stained with haematoxylin and eosin.

2.3. Ethical animal use

Euthanasia was justified by the poor health conditions of the gilts, characterized by severe respiratory distress and complete reluctance to stand, according to the guidelines specified in the Guidelines for Pig Rearing, Article 5e, 2012. Euthanasia was executed by a qualified person utilizing electrical stunning under the supervision of the farm veterinarian in compliance with the Guidelines for Pig Rearing, Appendix A4, 2012, as well as in compliance with the COUNCIL DIRECTIVE 91/49/EC (Commission of the European Communities, 1993). Blood sampling for diagnostic purpose was performed by the farm veterinarian in compliance with the Animal Welfare Order, Article 11, 1985.
3.3. Histopathology

Microscopic examination of the skeletal muscle revealed multifocal loss and injury of up to 30% of myofibers with variable levels of injury (Fig. 1). There was mild to moderate variation in myofiber size. The muscle injury was acute and less than 10% of the myofibers within the affected regions were effaced and replaced by small amounts of fibroplasia and fibrosis. Some of the affected myofibers were swollen with hyper eosinophilic, often vacuolated cytoplasm and were interpreted as degenerating myofibers. Other myofibers were fragmented, hyper eosinophilic, exhibiting loss of stria and were interpreted as necrotic myofibers (rhabdomyolysis). These necrotic myofibers often contained small hyperbasophilic, and often fragmented nuclei (nuclear pyknosis and karyorrhexis respectively). Rarely myofibers were replaced by deeply basophilic granular to amorphous flocculent material interpreted as mineralization. No evidence of myofiber regeneration (such as increased number of nuclei, internal nuclear rowing, and karyomegaly) was observed. Multifocally expanding the endomysium and perimysium and surrounding necrotic myofibers, there were small to moderate numbers of macrophages, neutrophils, lymphocytes, admixed with cellular and karyorrhectic debris.

Microscopic examination of the heart exhibited mild multifocal myocardial necrotic fibers exhibiting fragmentation with nuclear karyorrhexis and karyolysis (Fig. 2). The necrotic and degenerating cardiac myofibers were multifocally randomly distributed, mostly within the left ventricular myocardium and the interventricular septum (both equally affected) and to a lesser extent within the right ventricular wall. Both atria had only minimal injury. No mineralization or inflammatory infiltrate were observed within the heart.

In the kidneys multiple dilated tubules were observed. The affected tubules were lined by variably injured cells. Some of the injured cells were shrunk displaying hyper eosinophilic cytoplasm and karyolytic nuclei and were interpreted as necrotic tubular epithelial cells. Other cells within the affected tubules were swollen, had vacuolated eosinophilic granular cytoplasm with faded nuclei, and were interpreted as degenerating tubular epithelial cells.

3.4. Blood analysis

The blood samples revealed significantly high activities of CPK and ALT, whereas none of the other measured blood parameters

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Table 4

Mean levels of creatinine, urea and blood activities of creatine phosphokinase (CPK), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in healthy and in maduramicin intoxicated pregnant gilts.

<table>
<thead>
<tr>
<th>Biochemical marker</th>
<th>Biochemical blood parameters in intoxicated in healthy gilts (range)</th>
<th>Biochemical blood parameters in intoxicated gilts* ± SE (range)</th>
<th>n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPK</td>
<td>47 U/L (0–130)</td>
<td>86348.17 ± 19933.2 U/L (1890–215,315)</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>45 U/L (15–52)</td>
<td>292.2 ± 76.8 U/L (25–1018)</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>65 U/L (20–70)</td>
<td>82.0 ± 8.5 U/L (28–140)</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.4–1.2 mg/dL</td>
<td>2.0 ± 0.2 mg/dL (1.4–3.3)</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>15–30 mg/dL</td>
<td>35.4 ± 1.9 mg/dL (23–48)</td>
<td></td>
</tr>
</tbody>
</table>

ND = Not determined.
SE = standard error.
* Reference values for healthy gilts were obtained from the Veterinarian Breeding Service, Development Agriculture Projects, Kibbutz Lahav, Israel.

The pregnant gilts were 8–8.5 months old, weighing 150–170 kg and up to one month of pregnancy.

Mean enzyme activity value of healthy vs. intoxicated gilts are significantly different according to student t-test (p < 0.05).
showed any significantly consistent departures from normal values (Table 4). Blood hemoglobin, hematocrit, red blood-cell count (RBC), packed cell volume (PCV) and white blood cell count (WBC) were within normal limits. No significant alterations were detected in serum concentrations of Na, Cl, K, Ca and phosphate (Table S3).

3.5. Feed analysis

The analysis of the complete feed revealed very high levels of maduramicin with a mean value of 18.5 mg/kg (range: 17–20 mg/kg) as well as clinically non-significant mean level of 3.0 mg/kg salinomycin (range: 2.6–3.7 mg/kg). The antibiotics doxycycline, chlortetracycline and oxytetracycline were below the detection limits of 60 μg/kg in the analyzed feed. The elements analyzed in the contaminated feed by ICP-AES (As, Cd, Co, Zn, Cu, Fe, Pb, Mn, Hg, Mo, Se, Tl and Zn) were within the normal limits (Puls, 1994). The pesticide screen did not reveal any contamination.

3.6. Liver analysis

The livers of 7 gilts revealed very high maduramicin levels with mean value of 0.5 mg/kg (range: 0.05–1.15 mg/kg).

3.7. Bacteriology and virology

No pathogenic bacteria (aerobic, anaerobic and Salmonella) and viruses (TGE, PCV2, CSF, PRRS, SIV subtypes H1N1, H1N2 and H3N2) of epidemiological significance were detected in the tissues examined.

4. Discussion

Case reports on maduramicin toxicosis in mammalian farm animals are relatively scarce in comparison to other coccidiostats intoxications, since maduramicin is worldwide authorized only for poultry as a feed additive at a level of 5 mg/kg (EFSA, 2008d). An acute maduramicin toxicosis in cattle has been reported in Israel and South Africa, due to accidental contamination of feed with maduramicin (up to 6 mg/kg) or as a result of ingestion of poultry litter contaminated with maduramicin at concentrations of 2.5–6 mg/kg (Fourie et al., 1991; Shlosberg et al., 1992, 1997). To the best of our knowledge, only one case report of maduramicin toxicosis in pigs has been published (Sanford and McNaughton, 1991), which briefly described the epidemiology, clinical signs and clinical pathology of 6–12 weeks old weaned pigs on an Ontario pig farm. In the aforementioned toxicosis event, Sanford and McNaughton reported a mortality of 550 pigs following ingestion of feed contaminated with maduramicin at a level of 37.5 mg/kg. The intoxicated pigs displayed clinical signs of anorexia, respiratory distress, lethargy, ataxia, recumbency and sudden death. Histologically, severe muscle myopathy of the limbs, back and diaphragm as well as of the cardiac muscle was observed characterized by degeneration and necrosis (Sanford and McNaughton, 1991). Moreover, high sera values for AST and CK were found. The present case study describes a toxicosis event in 22 pregnant gilts, 2 pregnant sows and 2 young boars exposed to high levels of maduramicin, namely 18.5 mg/kg, in their complete feed for a time period of 7 days. Sudden death as well as the appearance of visible clinical signs occurred 7 days after commencement of feeding the contaminated feed, which is in accordance with epidemiology reports in other animal species intoxicated with maduramicin (Bastianello et al., 1995; Fourie et al., 1991; Shlosberg et al., 1997).

The clinical signs of maduramicin toxicosis in the pregnant gilts, sows and 2 boars included mild feed refusal, respiratory distress, lethargy, ataxia and recumbency. In contrast to monensin and salinomycin toxicosis in pigs, no evidence for diarrhea or dark colored urine indicative of myoglobinuria was present (Miskiminns et al., 1996; Plumlee et al., 1995). On the other hand, the clinical and histopathological findings observed in the intoxication event shared similar characteristics to acute monensin and salinomycin toxicosis in pigs (Miskiminns et al., 1996; Plumlee et al., 1995). The lesions and degenerative changes were concentrated mainly in the skeletal muscles and atrial myocardium (Figs. 1 and 2). As for monensin and salinomycin toxicosis, the severe myodegeneration was more pronounced in skeletal muscle than in the myocardium. The atrial myocardial lesions were more difficult to detect and could be characterized by mild multifocal myocardial necrotic fibers and fragmentation. Similar findings were reported for acute salinomycin toxicosis in pigs (Plumlee et al., 1995). The most important clinical pathology indices found in the present report were highly elevated levels of CPK and ALT, being indicative of severe rhabdomyolysis (Table 4).

The epidemiology, clinical signs, and pathological lesions, together with the high maduramicin levels found both in the complete feed and the livers, in addition to the absence of any bacteriological involvement and exclusion of other plausible causes of similar syndromes (such as toxic levels of doxycycline, chlortetracycline and oxytetracycline, low selenium levels, ingestion of cardiototoxic plants), provide strong evidence that maduramicin was the causative toxic agent. The salinomycin levels found in the contaminated feed (2.6–3.7 mg/kg) were markedly below the toxic levels determined by numerous studies for a wide range of farm animals including pigs, cattle, sheep and poultry (EFSA, 2008a). Notwithstanding, an additive/synergistic contribution of salinomycin to the toxic effect exerted by maduramicin cannot be ruled out, although the low level of salinomycin and exceptionally high maduramicin concentration in the complete feed and liver samples, undisputedly point at maduramicin as the causative toxic agent.

Following an investigation to determine the source of the maduramicin contamination, it was concluded that a premix containing maduramicin was accidently added to the feed intended for the gilts. Since numerous case reports have been published in recent years regarding ionophore toxicosis due to accidental incorporation of ionophores into non-target animal feed, strict monitoring should be implemented by in-house and Feed Regulatory Services to prevent similar toxicoses in the future (Baird et al., 1997; Chalmers, 1988; Fourie et al., 1991; Martino et al., 2009; Miskiminns and Neiger, 1996; Muylle et al., 1981; Novilla, 1992;
K. Plumlee et al., 1995; Shlosberg et al., 1997). To better understand the effect of maduramicin on pregnant sows within their first trimester. The lack of effect of maduramicin on the fetuses at the present case report might be indicative of poor placental penetration of maduramicin, which could be associated with its high molecular weight (917.13 g/mol) and the presence of charged carboxylic group at physiological pH (calculated pKa value of maduramicin 4.01), concerning maduramicin hydrophilic properties (Eshkoli et al., 2011; Human Metabolome Database). The relatively fast recovery of the older pregnant sows in contrast to the young pregnant gilts was remarkable, although no general conclusions can be drawn due to the low number of affected sows. Shlosberg et al. demonstrated that age differences as well as body weight have pronounced effect on maduramicin susceptibility in calves (Shlosberg et al., 1997). The younger calves had a higher metabolic rate and therefore ingested more contaminated feed on a body weight (BW) basis than the older calves. This phenomenon might explain the survival and fast recovery of the two pregnant older sows, which most likely ingested less maduramicin contaminated feed. The case study described herein, reports for the first time maduramicin levels in livers of gilts exposed to deadly concentrations of maduramicin in the feed. The average feed intake of 8–8.5 months old gilts weighting 160 kg was estimated to be 3.5 kg feed/day, hence 22 g feed/kg BW; consequently at an average maduramicin level of 18.5 mg/kg feed, the mean maduramicin intake leading to the observed high mortality rate and clinical and pathological signs was 0.41 mg/kg BW/day. The estimated maduramicin intake value of 0.41 mg/kg BW/day yielded a mean liver concentration of 0.5 mg/kg liver. Assuming a linear relationship between the oral dosage of maduramicin and the corresponding liver concentration, a rough estimation can be drawn regarding the maximum daily oral dosage in pigs above which the non-compliant level of 2 μg/kg animal tissue will be exceeded (Commission Regulation No 610/2012; Dorne et al., 2013). Hence, in order to comply with the residual level of 2 μg/kg animal tissue, the oral intake of maduramicin should not exceed 0.0016 mg/kg BW/day in adult sows. Accordingly, the maximum level of maduramicin in complete animal feed should not exceed 0.07 mg/kg feed/day, which applies to cross-contamination of feed at a level of 1.4% of the maximum authorized maduramicin concentration for target animal species. In light of the results described in the present case report, thorough studies in various non-target animal species intended for human consumption are required in order to determine maximum levels of maduramicin in complete animal feed, below which the non-compliant level of 2 μg/kg animal tissue will not be exceeded. Moreover, appropriate maximum levels of cross-contamination needs to be empirically defined, in order to comply with the requirements set by the Regulatory Authorities for food and feed safety.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgment

The authors gratefully thank Mr. Yossi Hofi, Mr. Tzviel Moskovich and Dr. Oran Erster for their excellent technical assistance.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.fct.2014.03.034.

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