

TOXICITY OF THE CHICKEN ANTICOCCIDIAL MADURAMICIN IN RABBITS : CLINICAL MANIFESTATIONS AND PATHOLOGICAL FINDINGS

J. E. Peeters¹, R. Wyffels², R. Ducatelle³, R. Geeroms¹ and D. Vandergheynst¹

¹ National Institute of Veterinary Research
Groeselenberg 99, B 1180 Brussels (Belgium)

² Provincial Laboratory of Animal Health
Industrielaan 15, B 8820 Torhout (Belgium)

³ Faculty of Veterinary Medicine, State University of Ghent
Casinoplein 24, B 9000 Gent (Belgium)

Introduction

Ionophores are broad spectrum anticoccidials which have enjoyed considerable commercial success in chickens since the early seventies. Yet, they are toxic products with a narrow therapeutic index. Monensin is known to cause growth retardation in chickens when it is included at a level 50 % higher than the recommended dose (Macpherson, 1978). In horses monensin induces cardiac failure and high mortality (Matsuoka, 1976, Ordidge et al., 1979). Similar observations have been done in rabbits after feeding diets containing 50-100 ppm of salinomycin or 10-50 ppm of narasin (Peeters et al., 1981, 1982, Morisse et al., 1986) : intoxication was associated with acute mortality within 5 days, with heart dilatation, liver and kidney congestion and pulmonary oedema. Reproduction stock and rabbits near marketing age were most severely affected. The inexperienced observer may easily confuse these features with those of the viral haemorrhagic disease (VHD).

Maduramicin (Cygro®, Cyanamid) is also a member of the group of ionophores. The drug is not registered for rabbits and is consequently not allowed in rabbit feeds. Until now no documented evidence of toxicity for rabbits has been published in the international literature. The present study will report on accidental maduramicin intoxication in 73 Belgian commercial and domestic rabbitries and on the pathology observed after experimental feeding of rabbits with maduramicin supplemented feed.

Materials and methods

Necropsy, histology and microbiology

Between October 15th and November 1st 1991 weaned rabbits and reproduction stock issued from 39 commercial and domestic rabbitries were necropsied by the Provincial Laboratory of Torhout and from 25 rabbitries by the Faculty of Veterinary Medicine of Ghent. Rabbits from another 9 large commercial rabbitries were necropsied at the National Institute of Veterinary Research. Most rabbits belonged to a mixed breed of New Zealand White and Dendermonde White. The reproduction system used was intensive to semi-intensive and rabbits were weaned between 4 and 5 weeks of age. All animals were fed the same batch of a commercial pelleted ration *ad libitum*. By a mistake in the production hall, this batch contained 10 ppm of maduramicin (Cygro®, Cyanamid), the double of the recommended dose level for broilers, instead of 66 ppm of robenidine (Cycostat®, Cyanamid).

Immediately after necropsy, portions of lungs, liver and kidney and in some instances also of thymus, trachea and brain were fixed in 10 % (v/v) formaline in phosphate buffered saline - pH 7.2 (PBS) for paraffin sections. They were embedded in paraplast-plus and cut at 5 μ m. Sections were stained with haematoxylin and eosin and examined by light microscopy. Portions of liver were stored at -25 °C until tested for presence of VHD-virus by the haemagglutination test (HA). Presence of facultative aerobic bacteria in duodenum, jejunum, ileum, caecum, liver and lung were evaluated according to standard procedures. The presence of *Clostridium spiroforme* was researched after staining caecal smears according to Gram. Samples of caecal content were taken for parasitology and virology.

Haemagglutination test

Human type O red blood cells (RBCs) were washed three times in PBS containing 0.5 % (w/v) of bovine albumine (fraction V) and suspended at 0,75 % (v/v) concentration in PBS. Two-fold dilutions of clarified supernatant of a 10 % (w/v) homogenate of liver in PBS was incubated with an equal volume of washed RBCs in microtiter plates at 25 °C. After an incubation of one hour, agglutination at titers greater than 10×2^4 was considered positive. Specificity of end point dilutions between 10×2^2 and 10×2^6 was tested by inhibition of the haemagglutination with hyperimmune anti-VHD rabbit sera.

Laboratory experiments

Experiment 1. A total of 28 twelve-week-old New-Zealand White rabbits were housed individually in heat sterilized wire-floored metal cages and received a standard commercial pelleted ration *ad libitum*. The rabbits were inoculated two by two with 14 different clarified supernatants of 20 % (w/v) liver homogenates in PBS. Animals received 2 ml by the subcutaneous route. Homogenate A contained the NIDO-reference strain of VHD 90/97/1 used for vaccine evaluation (Peeters et al., 1991). Homogenates B and C originated from two recent Belgian field cases of VHD and the livers tested established haemagglutination titers of 10×2^{20} and 10×2^{18} respectively. Homogenates D to N originated from rabbitries showing high mortality 3 to 6 days after the introduction of a new batch of feed. With the exception of homogenates D, E and F which showed HA-titers of 10×2^4 , they all showed a negative HA-reaction. Animals were checked daily for clinical signs or mortality during the first week after inoculation. Rectal body temperature was measured daily at 9 a.m. Also the individual weight gain and feed consumption were determined. Then surviving rabbits were divided into two groups, each containing one animal of each inoculation-pair. One group was maintained on the original standard diet, whereas the other group received a pelleted feed containing 10 ppm of maduramicin. The animals were further checked as described above during 3 weeks. Dead animals were necropsied as described above.

Experiment 2. A total of 30 eleven-week-old New-Zealand White rabbits were housed individually in heat sterilized wire-floored metal cages. They were separated into two groups with similar average weight. One group comprising ten rabbits served as control group and received a standard commercial pelleted ration *ad libitum*, whereas the other group of twenty rabbits received a diet containing 10 ppm of maduramicin during one week. Then the maduramicin-containing feed was withdrawn and replaced by the feed used in the control group. Animals were checked daily for clinical signs or mortality during three weeks. Also the individual weight gain and feed consumption were determined and dead animals were necropsied as described above.

Results

Case history and pathological findings

Case history. Acute mortality was reported to our Institute from 73 domestic and commercial rabbitries. In all rabbitries mortality started within 3 to 6 days after administering a new batch of commercial pelleted feed. This batch was shown to contain a mean concentration of 10 ppm of maduramicin. At first, rabbits at slaughter age (2.000-2.500 kg), replacement does and lactating does at the end of gestation were most severely affected. Later on, also other reproduction animals became affected. No difference in clinical signs was observed between does and bucks. At the start of the outbreaks necropsy was characterized by congested enlarged thymus and by pulmonary and hepatic congestion. As numerous confirmed cases of VHD were reported in the region during the same period, the local veterinary surgeons initially interpreted the clinical signs and lesions as VHD and advised their clients to sell all marketable meat rabbits as quickly as possible and to vaccinate subsequently the complete reproduction stock and fattening rabbits exceeding 9 weeks of age. This made correct evaluation of mortality in broiler rabbits impossible. In reproduction animals mortality varied between 30 and 80 %, surviving animals showed abortion and there was increased neonatal mortality. The animals became emaciated and showed anorexia, apathy, prostration, rough coat and cries before death. Moreover, they adopted a specific attitude with the head bent between the forelegs and stayed so without moving nor eating until death 24 to 72 hours later. Later on also young rabbits died, from the age of 6 weeks onwards. As vaccination against VHD was not followed by a significant improvement of the situation and as laboratory analysis did not confirm the clinical diagnosis of VHD, the feed became more and more suspected as a possible source of problems. Also the fact that mortality was only associated with one kind of feed in rabbit units using two different batches of feed further supported this hypothesis. As a consequence, most breeders withdrew the suspected feed. When the feed was withdrawn within one week, most animals recovered within 2 weeks. When the animals took the feed for more than 2 weeks, withdrawal was followed by reduced mortality, although 15 to 30 % of breeding animals remained apathic and cachectic.

Necropsy. The image at necropsy was basically the same in all observed cases. The thymus showed varying degrees of enlargement and congested vessels, the lungs were oedematous and congested with sometimes petechial haemorrhages and so were heart, liver and kidneys. Also the trachea showed hyperaemic reddening of the mucosa. Later on animals became cachectic, the heart was pale and enlarged, the liver showed a marked lobular pattern and there was excess fluid in the pericardium and thoracic and abdominal cavity, often containing transparent gelatinous clots.

Histopathology. Lesions of brain, thymus, trachea, lungs, heart, liver and kidney were mainly of the hyperaemic-oedematous type. The heart muscle lost striation and showed oedema, while the fibres showed a granular structure. In animals surviving over a fortnight, myocardial fibrosis and infiltration of macrophages could be seen in the heart muscle. Besides the heart, the liver was the most severely affected organ. At first the liver showed congestion of the central vein and dilatation of the associated sinusoids, later on centrolobular and midzonal hepatocytes developed fatty degeneration to necrosis, whereas *the hepatocytes at the periphery of the lobules remained almost unaffected.*

HA-test. Only 6 liver extracts from rabbits from 4 different rabbitries showed HA-titers of 10×2^4 . These reactions were not inhibited by hyperimmune anti-VHD rabbit sera and were considered to be aspecific. All other extracts remained negative.

Parasitology, bacteriology and virology. In most rabbits one or more of the following pathological agents were detected : atypical myxomatosis, rotavirus-infection, *Cryptosporidium parvum*, *Eimeria spp.*, enteropathogenic *Escherichia coli*, *Clostridium spiroforme* and *Pasteurella*

multocida, but none of these agents could be related with the observed mortality and lesions. In all instances, microbiology of heart and liver showed negative results.

Fig. 1. Exp. 1. Mean daily feed intake after treatment of rabbits with 10 ppm of maduramicin during 3 weeks

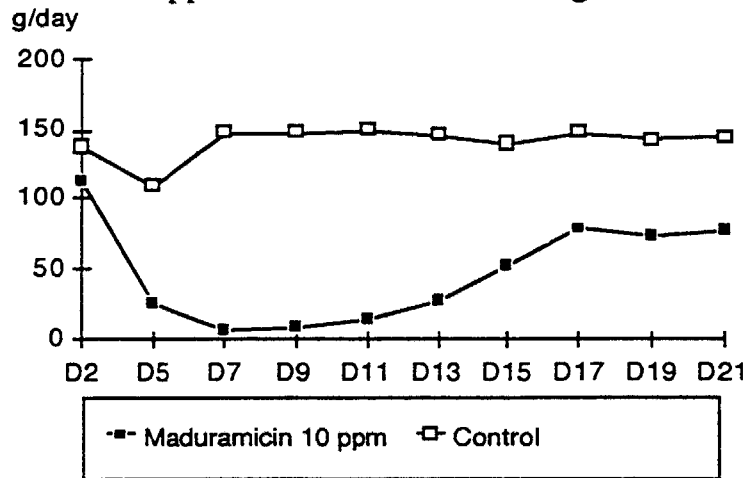
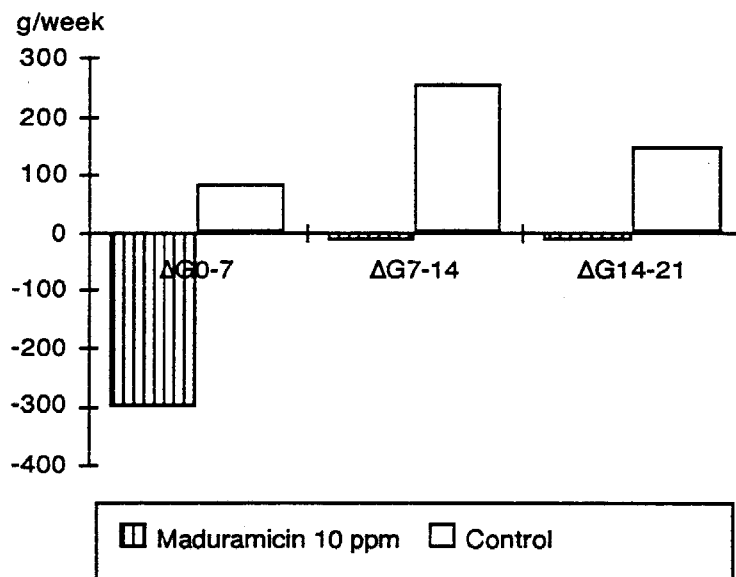


Fig. 2. Exp. 1. Mean weight gain after treatment of rabbits with 10 ppm of maduramicin during 3 weeks



Experiment 1

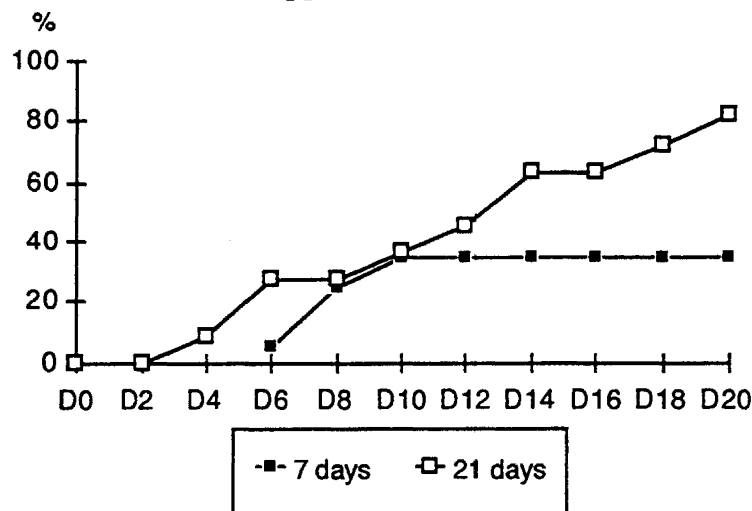
To exclude possible involvement of VHD in the rabbitries showing high mortality after changing feed, 12-week-old rabbits were inoculated with liver extracts of suspected rabbits. Rabbits inoculated with the NIDO-VHD reference strain 90/97/1 showed anorexia and rise of body temperature with 1.0 to 1.8 °C within 48 hours after inoculation. Both animals died three days after inoculation without apparent clinical signs. Similar observations were made in rabbits inoculated with homogenates B and C issued from field cases of VHD. Necropsy revealed typical lesions of VHD : marked hypertrophy of the thymus with numerous petechiae, haemorrhagic pneumotracheitis as well as hypertrophy and degeneration of the liver. Excess fluid in thoracic or abdominal cavity was not present. Histopathology mainly showed lesions of necrotic hepatitis, characterized by haemorrhage, acidophilic degeneration and necrotic foci, which sometimes became

confluent and formed extensive local areas, mainly *at the periphery of the lobules*. HA-titers of liver extracts exceeded 10×2^{15} .

A total of 9 out of 22 rabbits inoculated with homogenates D to N showed slight transient hyperthermia (0.5-0.7 °C) during 2 to 3 days. No mortality nor other clinical signs were observed during the first week after inoculation. Feed intake and weight gain were not influenced.

One week after inoculation the surviving animals were divided into two groups of 11 : one group was maintained on the original diet, whereas the other group received a feed supplemented with 10 ppm of maduramicin. The first two days feed intake of both groups was almost identical, but then the feed intake of maduramicin-supplemented animals dropped spectacularly (Fig. 1). Between 5 and 11 days after changing food the medicated group stopped feed intake almost completely. Afterwards feed intake started again and reached 54 % of normal intake after 3 weeks. Animals lost 303 g of body weight during the first week and 14 and 15 g resp. during the 2nd and 3rd week (Fig. 2). Only 2 out of 11 animals survived 3 weeks of maduramicin-supplementation. Mortality started already 4 days after changing to the test diet and then progressed continuously (Fig. 3). Clinical signs, macroscopic and microscopic lesions at necropsy were identical to those observed in the field.

Fig. 3. Cumulative mortality after 7 or 21 days of treatment with 10 ppm of maduramicin



Experiment 2

During the first week of maduramicin supplementation animal behaviour was similar to the previous experiment : feed intake was hardly influenced during the first 3 days and then dropped to 35 % of the feed intake of control animals at day + 7 (Fig. 4). Mortality started 6 days after starting treatment.

When the maduramicin-supplemented feed was withdrawn on day +7, animals almost immediately started eating again. Feed intake reached normal levels 12 days after withdrawing (Fig. 4). This was associated with increased body weight and even with compensatory weight gain during the second week post-withdrawing (Fig. 5). Nevertheless, mortality continued until day 3 post-withdrawing (Fig. 3). Necropsy showed exactly the same macroscopic and microscopic lesions as those observed in the field and during experiment 1, although clinical signs were less pronounced. Nervous symptoms were not observed. Necropsy and histopathology of animals killed 2 weeks after withdrawing the drug did not reveal any significant lesion.

Fig. 4. Exp. 2. Mean daily feed intake after treatment of rabbits with 10 ppm of maduramicin during 1 week

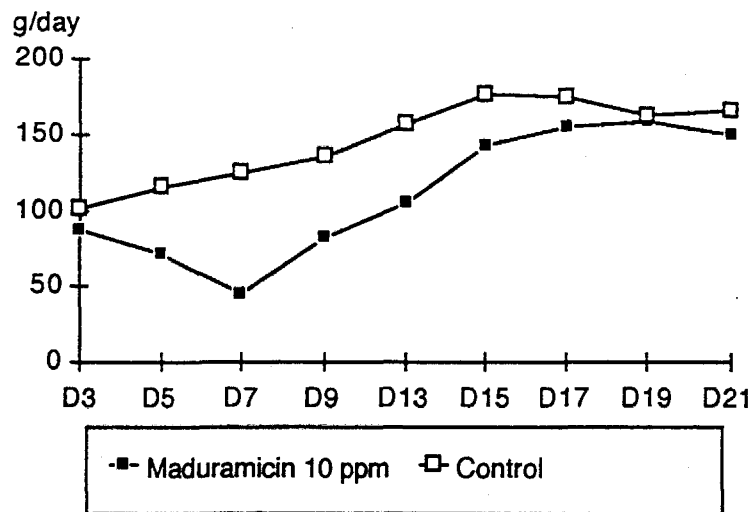
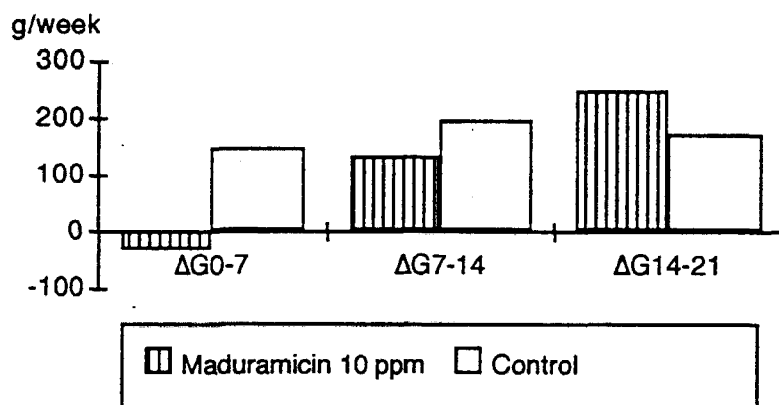


Fig. 5. Exp. 2. Mean weight gain after treatment of rabbits with 10 ppm of maduramicin during 1 week



Discussion

The data from the field and the laboratory indicate that maduramicin is highly toxic for rabbits at 10 ppm. The clinical evolution of intoxication is quite similar to the situation after monensin, narasin and salinomycin intoxication : other authors as well reported that intoxication affected mainly breeding stock, probably because of the higher feed intake. However, in contrast with the latter ionophores which induce reduced feed intake from the first moment of administration, maduramicin fed rabbits showed normal feed intake during the first 2-3 days of administration, which of course increases the risk of intoxication.

Polyether monocarboxylic acid ionophores as monensin and narasin interfere with the cellular potassium metabolism (Pressman, 1976). A chemically related compound, A204, causes intracellular and mitochondrial depletion of potassium in rats and increased urinary excretion of the ion (Meyers et al., 1971). The release of potassium from mitochondria is attributed to the inhibition of the energy-linked mitochondrial ion pump (Pressman, 1976). The anticoccidials A204

(Wong et al., 1971), monensin and narasin (Wong et al., 1977) were shown to have an inhibitory effect on the mitochondrial ion-dependent adenosine triphosphatase. Decreased intracellular potassium explains the cardiac failure in narasin and salinomycin medicated rabbits (Peeters et al., 1981, 1982). As maduramicin also causes heart failure in rabbits, characterized by enlarged pale hearts, generalised congestion of internal organs, hydrothorax and ascites, it is not excluded that the drug also interferes with cellular potassium metabolism. This should be confirmed by further experiments.

In dogs and cats it has been shown that the toxic effects of the related ionophore A204 on abdominal and heart muscles and on the diaphragm are reversible (Todd et al., 1971). According to evidence from the field and to the results of experiment 2, this seems also the case in maduramicin medicated rabbits. This favours prognosis in rabbits after short exposure to the drug. Nevertheless, our field data suggest that prolonged exposure of rabbits to toxic doses of maduramicin leads to irreversible lesions as 15 to 30 % of breeding animals remained cachectic and apathic.

Anyhow, the described erroneous maduramicin administration to rabbits stresses the high responsibility of the feed factories. Extreme care should be taken to identify and handle ionophorous drugs in the production halls correctly. Moreover, as Morisse (1986) reported accidental contamination of rabbit feeds by previously manufactured chicken feeds, the machinery should be cleaned thoroughly before starting production of rabbit feeds after a batch of chicken feed.

Intoxication by ionophores can easily be confused with the viral haemorrhagic disease by the inexperienced observer. Yet, in absence of serological tests both affections can easily be distinguished by macroscopic and microscopic features: VHD rarely affects rabbits younger than 9 weeks and affected rabbits never show enlargement of the heart, nor hydrothorax or ascites. Histopathology of VHD affected livers is characterized by necrosis, mainly at the periphery of the lobules (Marcato et al., 1991). Ionophores cause lesions of the hyperaemic-oedematous type and if necrotic hepatocytes are found, they are mainly situated in the central and midzone.

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Summary

Erroneous incorporation of 10 ppm of the chicken ionophorous anticoccidial maduramicin (Cygro[®], Cyanamid) in commercial rabbit feed caused up to 80 % of mortality in 73 commercial and domestic rabbitries. Reproduction stock and rabbits near marketing age were most severely affected. Clinical signs included anorexia, apathy, prostration, rough coat and cries before death. Emaciated animals adopted a specific attitude with the head bent between the forelegs. Necropsy revealed enlarged thymus, pulmonary oedema and congestion, pale enlarged heart, marked hepatic lobular pattern and excess fluid in pericardium and thoracic and abdominal cavity. Histopathology of the liver showed congestion of the central vein and sinusoids and later on fatty degeneration to necrosis of centrolobular and midzonal hepatocytes. Peripheral hepatocytes remained almost unaffected allowing differential diagnosis with the viral haemorrhagic disease. The heart muscle lost striation and showed oedema. Later on myocardial fibrosis was established. Experimental feeding of 12-week-old rabbits with 10 ppm of maduramicin was followed by 82 % mortality within 3 weeks. Mortality started within 4 to 6 days. Pathology was identical as observed in the field. Most animals did not show any reduction of feed intake during the first 2 to 3 days of medication. Then feed intake dropped spectacularly to increase again after 3 weeks. One week of medication followed by withdrawal of the drug resulted in 35 % of mortality and surviving animals recuperated completely. Nevertheless, prolonged exposure of rabbits to toxic doses of maduramicin may lead to irreversible lesions in 15-30 % of breeding animals as suggested by evidence from the field.