Outbreaks of nutritional cardiomyopathy in pigs in Brazil

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Dilated cardiomyopathy (DCM) is a condition that affects the myocardium, seldom reported in pigs. The DCM is characterized by ventricular dilation, which results in systolic and secondary diastolic dysfunction and can lead to arrhythmia and fatal congestive heart failure. This study described the clinical, pathological, chemical and toxicological findings of nutritional dilated cardiomyopathy (DCM) in nursery pigs through natural and experimental studies. Naturally occurring cases of DCM in three swine farms were investigated through necropsy (fourteen pigs), microscopic, virological, chemical and toxicological exams for the detection of the etiology. The experimental study was conducted with nine 40 days-old piglets, which were divided into three groups of three piglets each. Group 1 was fed with the suspected diet of the naturally occurring cases, Group 2 with half of the suspected diet and half of a control diet, and Group 3 received only the control diet. Clinical signs were recorded. All pigs were submitted of euthanized, necropsie and collection sample for laboratories exams, after 15 days of experiment onset. At the necropsy, all naturally occurring cases had bilateral cardiac dilatation associated to hepatic enhanced lobular pattern (nutmeg liver) and lungs edema. Microscopically, the heart revealed severe hypertrophy and vacuolization of cardiomyocytes, as well as myofiber disarray. Feed analysis revealed low-quality standard soybean meal. After the suspected feed was replaced, clinically ill pigs recovered, and mortality ceased. At the experimental study, two piglets from Group 1 had cough, dyspnea and diarrhea. At the necropsy, these animals had similar gross and microscopic lesions to the natural cases. The nutritional DCM in pigs may be associated to the diet with low-quality soybean meal, as it was further confirmed through an experimental study.

INDEX TERMS: Outbreak, nutritional cardiomyopathy, pigs, Brazil, swine, cardiomyopathy, heart, diet, soybean meal, heart failure.

RESUMO.- [Surtos de cardiomiopatia nutricional em suínos no Brasil.] Cardiomiopatia dilatada (CMD) é uma condição que afeta o miocárdio, raramente relatada em porcos. A DCM é caracterizada por dilatação ventricular, que resulta em disfunção sistólica e disfunção diastólica secundária e pode levar a arritmias e insuficiência cardíaca congestiva fatal. Este estudo descreve os achados clínicos, patológicos, químicos e toxicológicos da CMD em suínos de creche através de estudos naturais e experimentais. Investigaram-se três granjas com surtos de mortalidade por CMD através de exames de necropsia (catorze suínos), microscópicos, virológicos, químicos e toxicológicos para a detecção da etiologia. O estudo experimental foi conduzido com nove leitões de 40 dias de idade, divididos em três grupos de três leitões cada. O grupo 1 foi alimentado com a dieta suspeita dos casos naturais; o 2 com metade da dieta suspeita e metade de dieta controle; e o 3 recebeu apenas
INTRODUCTION

Cardiomyopathy (CM) refers to conditions that cause structural and/or functional abnormalities in the myocardium with concomitant dilatation or hypertrophy of one or both ventricles and possibly all four chambers of the heart (Richardson et al. 1996, Elliott et al. 2008, Harvey & Leinwand 2011). It affects humans and domestic animals and may be categorized into three morphologic forms: dilated (congestive), hypertrophic and restrictive (Richardson et al. 1996, Elliott et al. 2008).

Dilated cardiomyopathy (DCM) is a primary or secondary myocardial disorder of characterized by the reduced contractility and ventricular dilation involving the left or both ventricles (O’Grady & O’Sullivan 2004, Miller & Gal 2017). It is an important cause of congestive heart failure in animals (Czarnecki 1984, Edwards 1987, Lobo & Pereira 2002, O’Grady & O’Sullivan 2004, Miller & Gal 2017, Collins et al. 2015).

DCM has been studied in many species, but it has been extensively studied in dogs (Sisson et al. 1999, Lobo & Pereira 2002, O’Grady & O’Sullivan 2004). One form of DCM known as “round heart disease” has been discovered in turkeys, and this form of DCM does not have a well-defined etiopathogenesis (Czarnecki 1984, Stenzel et al. 2008). In cattle, DCM with a familial genetic origin has been determined to be an autosomal recessive inherited disease (Miller & Gal 2017). In swine, DCM has been associated with gossypol poisoning, fumonisin toxicity and cardiomyopathy with an undetermined origin (Loyanchan 2012, Sobestiansky 2012, Collins et al. 2015).

DCM causes ventricular dilation, systolic dysfunction, and secondary diastolic dysfunction, which may progress to congestive heart failure, arrhythmias, and eventually lead to death (Sisson et al. 1999, Sisson & Thomas 1999). Morphologically, myocardium is thinner, with dilated and flaccid cardiac chambers and, thus is weakened and unable to pump blood efficiently (Edwards 1987, Keene et al. 1994, O’Grady & O’Sullivan 2004).

The aim of this study was to describe the clinical, pathological, chemical and toxicological findings of nutritional DCM in nursery pigs through natural and experimental studies.

MATERIALS AND METHODS

Natural cases: clinical, epidemiological and histopathological features. From 2011 to 2014 eight porcine samples of multiple organs from three farms in Southern Brazil with high mortality rates of nursery-growing pigs Paraná (A), Rio Grande do Sul (B), and Mato Grosso do Sul (C) had microscopical lesions consistent with congestive heart failure. On-site visits to the three farms were performed and data regarding the clinical signs and epidemiology were obtained with the clinician veterinarians. Fourteen piglets with clinical signs of cough and dyspnea from A (4 piglets), B (6) and farm C (4) were subjected to euthanasia followed by necropsy. Multiple tissue samples were collected, fixed in 10% neutral buffered formalin, processed for histopathology, and stained with hematoxylin and eosin (HE). Heart sections were also stained with periodic acid-Schiff (PAS) and Masson’s trichrome (MT) to characterize morphologically the cardiac injury. Lung sections were also stained with Perl’s Prussian blue stain to highlight the congestive heart failure lesions (heart failure cells).

Experimental study. The experimental study was conducted during 15 days with nine 40-day-old piglets (15kg of weight), which were further divided into three groups: 1 (suspected feed), 2 (50% suspected feed + 50% control feed) and 3 (100% control feed). The suspected feed was collected at the farm B, and was formulated with corn, soybean meal and commercial premix. The animals received their respective diet and water ad libitum for the experimental period, were daily monitored and weekly weighed.

All nine pigs were humanely euthanized as required by current legislation under the approval of the UFRGS Ethics and Animal Experimentation Committee (approval protocol number 29467). The carcasses were weighed and at necropsy multiple tissues, were collected and fixed in 10% buffered formalin. The whole heart from each pig was collected and weighed after the blood was drained. The heart weight/body weight (HW:BW) coefficient was then determined (Turk 1983, Richardson et al. 1996).

Chemical and toxicological analysis. Liver samples collected from the necropsied pigs of farm B and from the experimental study were frozen at -20°C to evaluate vitamin E and selenium levels. Liver and heart samples previously fixed in 10% formalin from piglets of farms B and C and from the experimental groups were used to determine the levels of other minerals. Feed samples from farms A and B were collected for chemical and toxicological analyses according to the substances and methodologies shown in Table 1.

A protein analysis (proteinogram) was performed on the feed sample from farm B, with the dosage of the following amino acids: alanine, arginine, aspartic acid, glycine, isoleucine, leucine, glutamic acid, lysine, cysteine, methionine, phenylalanine, tyrosine, threonine, tryptophan, proline, valine, histidine, and serine.

Molecular analysis. Fixed and paraffin-embedded myocardium samples of two pigs from each outbreak and from the experiment were subjected to molecular analysis for the detection of the major viral agents responsible for heart lesions in pigs: porcine circovirus, porcine parovirus, influenza A, pestivirus and enterovirus, according to previously described protocols (Opriessnig et al. 2003, Vilcek et al. 2003, Vecchia et al. 2012).

RESULTS

Natural cases: clinical features and gross findings

Three outbreaks of acute respiratory failure, with dyspnea, cough, fatigue, and anorexia, followed by death within days in nursery-growing pigs (30-70 days-old) were observed...
Outbreaks of nutritional cardiomyopathy in pigs in Brazil

Table 1. Chemical and toxicological dosages in liver, heart, soybean meal and feed samples of the piglets with dilated cardiomyopathy

<table>
<thead>
<tr>
<th>Elements</th>
<th>Samples</th>
<th>Technique</th>
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<tbody>
<tr>
<td>Selenium</td>
<td>Liverb,e</td>
<td>EAA</td>
</tr>
<tr>
<td>Gossypol</td>
<td>Soybean meal e feedb,e</td>
<td>HPLC</td>
</tr>
<tr>
<td>Ionophores</td>
<td>Feedb</td>
<td>HPLC</td>
</tr>
<tr>
<td>Monocrotaline</td>
<td>Soybean meal e feedb</td>
<td>UPLC-MS/MS</td>
</tr>
<tr>
<td>Minerals (Fe, Cu, Co, Zn, Ca, Mg, K e Na)</td>
<td>Liver and heartb,c,e</td>
<td>EAA</td>
</tr>
<tr>
<td>Toxic components</td>
<td>Soybean mealb,c and feedb</td>
<td>GC/MS</td>
</tr>
<tr>
<td>Mycotoxins (AFB)</td>
<td>Feedb</td>
<td>HPLC/FLD</td>
</tr>
<tr>
<td>Mycotoxins (FB e T-2)</td>
<td>Feedb</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Liverb</td>
<td>HPLC</td>
</tr>
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EAA = atomic absorption spectrometry, HPLC = high performance liquid chromatography, UPLC-MS/MS = ultra-high performance liquid chromatography-mass spectrometry, GC/MS = gas chromatography/mass spectroscopy, HPLC/FLD = high performance liquid chromatography with fluorescence detection, LC-MS/MS = liquid chromatography-mass spectrometry, AFB = aflatoxin B1, FB = fumonisin B, T-2 = toxin T-2; b farm A, c farm B, e farm C, experimental study.

Natural cases and experimental study: histological findings

The histological examination of the myocardium in all the pigs of natural cases revealed marked cardiomyocyte hypertrophy and attenuated wavy fibers in the myocardium, shown in Figure 2A, and the heart from a control pig is shown in Figure 2B. Additionally, in two pigs (farms A and B), multifocal fibrinoid necrosis of blood vessel walls, and a mild multifocal infiltrate of lymphocytes in the epicardium and myocardium were observed. The liver had moderate to marked congestion, necrosis, and degeneration of the centrilobular hepatocytes (nutmeg liver) (Fig.2C,D). The lungs revealed moderate interlobular septal edema (Fig.2E) and a moderate number of hemosiderin-laden macrophages (heart failure cells) within the alveoli (Fig.2F), which were highlighted through Perl’s Prussian blue stain. Fibrinoid necrosis of the vessel walls was highlighted through PAS stain, which also revealed lesions in the lymph nodes in one pig at farm A, and in the spleen and brain in two pigs at farm B.

Only two piglets from Group 1 that displayed clinical signs and gross lesions of DCM had microscopic abnormalities. These were similar to those observed in natural cases. Piglets from Group 2 and 3 did not present any microscopic lesions.

Chemical and toxicological analyses

Selenium values were below 2.16μg/kg (RV: 0.1 to 2.9μg/kg) (Pallarés et al. 2002) in the analyzed samples of all the pigs for the natural and experimental cases. Vitamin E values in the liver of the pigs both from natural and experimental cases were below 8.55μg/kg in all pigs (RV: 3.8-10μg/kg), except for one pig in Group 1 (14.29μg/kg). Proteinogram analysis had values within the reference range.

All of the piglets with DCM in the natural and experimental cases had high magnesium levels (600-800μg/kg) in the myocardium compared to the control animals (300-400μg/kg). The iron levels in the liver were lower in affected animals from the Experimental Group (113-120μg/kg) than in animals in the Control Group (145-150μg/kg).

Gossypol, monocrotaline and ionophores were not detected in feed samples from B and C. Additionally, mycotoxin levels in the feed were below the toxic threshold (<10mg/kg for fumonisins and <50μg/kg for aflatoxin) (Sobestiansky 2012)
Molecular analysis
Porcine circovirus, porcine parvovirus, influenza A, pestivirus, and enterovirus molecular analysis from heart samples of natural and experimental cases yielded negative results.

DISCUSSION
The diagnosis of congestive heart failure by nutritional DCM in pigs was obtained by the association of the pathological, epidemiological, and experimental findings. DCM in pigs has been linked to poisoning by gossypol, fumonisin toxicosis and unknown causes (Loynachan 2012, Sobestiansky 2012, Collins et al. 2015, Miller & Gal 2017). Which were ruled out in this study through the chemical and toxicological tests of the feeds from B and C. Magnesium levels in the heart were higher in piglets with DCM (both natural and experimental pigs) than in the Control Group, which may be related to the occurrence of cardiac alterations (Korpela 1991). However,

Fig. 1. Gross features of nutritional dilated cardiomyopathy in pigs in Brazil. (A) The heart was markedly enlarged and occupied most of the thoracic cavity. There was also moderate ascites and a severe hepatomegaly. (B) The abdominal cavity was filled by abundant amount of translucent fluid (ascites). (C) The enlarged liver had an enhanced lobular pattern at the cut surface. (D) Natural cases had a severely enlarged heart with a globular appearance. (E) At the cut surface, there was severe bilateral ventricular dilation and intraventricular clotted blood. (F) Transversal sections of the heart of one piglet from the experimental cases displayed severe bilateral ventricular dilatation (above) and the heart of a normal control pig is presented (below).
More studies are needed for clarification of this relationship in natural conditions. Additionally, high levels of iron were observed in the liver samples of the piglets with DCM, which likely occurred due to congestive heart failure and associated marked hepatic congestion.

In the outbreaks, the affected animals were mainly nursery piglets, which have a high energy requirement. External factors such as temperature, humidity, and facilities conditions, as well as factors related to diet, may interfere with the intake and utilization of food (Parsons et al. 1991, Berthol et al. 2001, Bunzen et al. 2008, Ramirez & Karriker 2012). In two of the farms (B and C), the soybean meal had poor quality, with low urea activity and low solubility. A low solubility indicates that the soybean meal did not have the recommended water content, and it has possibly undergone a process called “over toasting” (Parsons et al. 1991, Berthol et al. 2001). The consumption of low solubility soybean meal by the nursery pigs probably resulted in decreased digestibility rates and a decrease in

![Fig.2. Histological features of nutritional dilated cardiomyopathy in pigs in Brazil. (A) Cardiomyocyte hypertrophy and attenuated wavy fibers in the myocardium. HE, obj.10x. (B) Cardiomyocytes of a control pig are arranged in homogenous parallel bundles. HE, obj.10x. (C) Moderate to marked hepatic congestion, degeneration and necrosis of the centrilobular hepatocytes (nutmeg liver). HE, obj.10x. (D) Moderate to marked centrilobular congestion (nutmeg liver). Masson's Trichrome, obj.10x. (E) Moderate interlobular pulmonary edema. HE, obj.10x. (F) Moderate numbers of macrophages with intracytoplasmic hemosiderin (heart failure cells) were evidenced within the alveoli. Perl's Prussian blue, obj.40x.](image-url)
lysine availability, which most likely led to reduced weight gain (Parsons et al. 1991). However, the proteinogram of the low-solubility soybean meal, did not detect any abnormalities in the levels of amino acids. An hypothesis that these nutrients were absorbed in smaller amounts cannot be ruled out, since the nutritional value of protein in food does not solely depend on amino acids composition, but also depends on digestibility and availability factors, which were not analyzed in this study (Bunzen et al. 2008).

HW:BW coefficient of the two piglets from the experimental study that had clinical signs and gross lesions were 0.86% and 0.73%, which are almost twice the estimated mean value of pigs (0.40%). In DCM, an increase of the heart chambers (mainly of the ventricles) is observed grossly, which acquire a rounded shape, with a flaccid appearance and a weight 20 to 50% above the normal range, as well as an increase in the HW:BW coefficient (O’Grady & O’Sullivan 2004, Robinson & Robinson 2016). In this study, dilation was observed in all heart chambers, which is similar to that described in dogs and humans (Edwards 1987, Lobo & Pereira 2002, O’Grady & O’Sullivan 2004). While it differs from the previously reported data in cats, mostly involves the left ventricle (Turk 1983).

The gross findings observed in piglets with nutritional DCM in this study are similar to the features of cardiomyopathy caused by tauire deficiency in cats, genetic cardiomyopathy in dogs and humans, gossypol toxicity in pigs and “round heart disease” in turkeys (Edwards 1987, Sisson & Thomas 1999, O’Grady & O’Sullivan 2004, Stenzel et al. 2008, Sobestiansky 2012). The histological lesions observed both in the natural and experimental cases are characteristic of a congestive heart failure due to dilated cardiomyopathy, with wavy fibers in the myocardium. These histopathological features are consistent with the pattern of lesions observed in DCM in dogs, humans, turkeys, and pigs (Edwards 1987, Tidholm & Jönsson 2005, Stenzel et al. 2008, Collins et al. 2015).

There are few reports of DCM in pigs compared to humans and dogs, in which DCM is the main type of cardiomyopathy diagnosed (Sisson et al. 1999a, Lobo & Pereira 2002, O’Grady & O’Sullivan 2004, Elliott et al. 2008, Shen et al. 2011). Similar to the present study, the “round heart disease” in turkeys has an unknown etiology and pathogenesis (Stenzel et al. 2008); additionally, mulberry heart disease, which is an important heart disease that affects nursery pigs and does not have a defined etiology. However macroscopic and microscopic lesions are different from these cases (Shen et al. 2011).

CONCLUSION

Clinical history, pathological features, laboratory tests, and the results of the experimental study demonstrated the outbreaks of congestive heart failure by nutritional DCM in nursery pigs. This study suggests that the condition is related to the consumption of low-quality soybean meals. However, the specific cause is yet unknown, and further research on swine nutritional DCM is necessary since pigs may contribute as experimental models in human heart diseases.

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Outbreaks of nutritional cardiomyopathy in pigs in Brazil


