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Original article

Fibromuscular dysplasia in arteries and in a vein in broiler chickens

M. Gesek¹, K. Paździor¹, I. Otrocka-Domagala¹, T. Rotkiewicz¹, J. Szarek²

¹ Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-719 Olsztyn, Poland

² Chair of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-719 Olsztyn, Poland

Abstract

Fibromuscular dysplasia (FMD) is a group of nonatherosclerotic, noninflammatory diseases of blood vessels with unknown aetiology. In our study, FMD was diagnosed in blood vessels in samples taken from kidneys, liver and lung of broiler chickens. The FMD occurred during rearing in 8 of 108 broiler chickens examined for the effects of intensive rearing on the internal organs. Histopathological and immunohistochemical examinations revealed medial subtypes of FMD, medial fibromuscular dysplasia and medial fibromuscular stenosis. The first subtype presented as plugs in vessel lumens consisting of smooth muscle and fibrous connective tissue originating from the tunica media. The second subtype presented as a proliferation of smooth muscle cells and fibroblasts originating from the media and leading to lumen stenosis. The aetiology of FMD is still unknown. Thus, genetic factors are suspected as a cause of the disease. This is the first report of FMD in a vein of an animal species.

Key words: fibromuscular dysplasia, medial fibromuscular stenosis, broiler chickens

Introduction

Fibromuscular dysplasia (FMD) is an idiopathic, noninflammatory, nonatherosclerotic disease of arteries and veins, where the lumen is obstructed by a plug originating from the media of the vessel or reduced by cells proliferating from different layers of the vessel wall, leading to lumen stenosis. In humans, FMD most commonly affects the renal and carotid arteries; however, lesions have also been described in veins. The first case of FMD was described in 1938 by Leadbetter and Burkland in a child, and lesions occurred as plugs of smooth muscle in branches of the renal

artery. FMD is classified by three types: intimal, medial and adventitial, depending on the affected vessel wall layer (Perdu et al. 2007, Plouin et al. 2007). Intimal FMD is a rare form characterised by reduction of the vessel lumen by proliferating mesenchymal cells irregularly distributed within a loose matrix of the subendothelial connective tissue. This form accounts for 10% of the human FMD cases in the renal artery (Persu et al. 2011). The medial form is most prevalent and constitutes 80-90% of human FMD cases in the renal artery. This FMD form presents as stenosis of the vessel lumen (“string-of-beads” in humans) or as plugs that can obstruct the vessel. In both cases the

Correspondence to: M. Gesek, e-mail: michal.gesek@uwm.edu.pl, tel.: +48 89 524 61 41

tissue forming the changes originates from the media of the vessel wall. Furthermore, Braga et al. (1996) described two medial subtypes in Japanese quail: medial hyperplasia and medial fibroplasia. The medial hyperplasia was described as a plug in the vessel lumen originating from the media and composed of smooth muscle. The medial fibroplasia was described as lumen stenosis caused by proliferation of the connective tissue derived from the vessel middle layer. In addition, Julian (1980) distinguished other subtypes of medial FMD, i.e., fibromuscular hyperplasia, medial fibrodysplasia and medial fibromuscular stenosis. The adventitial form of FMD is very rare and is caused by proliferation of the connective tissue between the media and adventitia. This form accounts for less than 5% of human FMD cases in the renal artery (Persu et al. 2011).

In animals, FMD was described in domestic turkeys (Julian 1980), Japanese quail (Braga et al. 1996) and dogs (Falk and Jonsson 2000, Mete and McDonough 2011). Fletcher and Abdul-Aziz (2008) suggested that FMD is more commonly found in turkeys than in chickens, although there is no available literature describing FMD in chickens. Furthermore, to date, FMD has not, to the best of our knowledge, been diagnosed in the veins of any animal species, other than man.

The main cause of FMD is still unknown but the disease is likely multifactorial in nature. In humans, the most important factors are thought to be genetic abnormalities, inheritance, hormones affecting the blood vessels, infection with rubella virus, damage of the vessel walls or ischemic stress. In animals, herpes viral infections, angiopathy, endotoxin administration and inherited myotonic muscular dystrophy have all been proposed as causes of FMD (Julian 1980, Braga et al. 1996).

The scientific aims of the study were to investigate FMD cases in the blood vessels of chickens and to determine the morphological changes, classification, aetiology and proliferative activity of the cells forming the lesions.

Materials and Methods

The study was performed on 108 broiler chickens obtained from three commercial broiler flocks and representing three genetic lines (Cobb 500, Ross 308, Hubbard F15). From each genetic line, six randomly selected broiler chickens with no signs of disease were taken for morphological examination on the 3rd, 10th, 17th, 24th, 31st and 38th day of life. The birds were weighed and slaughtered (decision of the Local Ethics Committee in Olsztyn, No. 3/N dated 22.01.2008).

During necropsy, samples of the liver, lungs, kidneys, heart, bursa of Fabricius, intestine, gizzard and spleen were collected, fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections (5 µm) were routinely stained with haematoxylin and eosin (HE). The tissue sections diagnosed with FMD lesions by HE staining were additionally stained with the Masson Trichrome method for smooth muscle (Masson Trichrome kit, Bio-Optica, Italy) and the Verhoeff method for elastic fibres (Verhoeff kit, Bio-Optica, Italy). Additionally, the sections were analysed by immunohistochemistry (IHC).

The immunohistochemical examination was performed using anti-smooth muscle actin (SMA) (1:50 dilution, clone 1A4, Denmark), anti-proliferating cell nuclear antigen (PCNA) (1:200 dilution, clone PC10, Dako, Denmark), and anti-desmin (1:50 dilution, clone D33, Dako, Denmark) monoclonal mouse anti-human antibodies. The antigens were retrieved by microwaving (650 W) in citrate buffer, pH = 6 (for PCNA) and in Tris EDTA buffer, pH = 9 (for SMA and desmin), twice for 3 minutes each. The primary antibody was detected using a system based on an HRP (horseradish peroxidase)-labelled polymer conjugated with secondary antibodies (Envision Plus DAB, Dako, Denmark). The antigen-antibody complexes were visualised using 3,3-diaminobenzidine (DAB) (Envision Plus DAB, Dako, Denmark). For the negative control, the primary antibody was replaced with Dako Mouse IgG2a antibodies at an appropriate dilution (Dako, Denmark). The appropriate normal chicken tissue sections were used as positive controls. Each section was imaged using a Panoramic Scanner MIDI 3DHISTECH (Hungary). The photographs and measurements data of the plugs were prepared using Panoramic Viewer software (3DHISTECH, Hungary).

Results

Among 108 broiler chickens examined, 12 cases of FMD were diagnosed in HE-stained tissue samples. FMD was detected in arteries and a vein in the kidneys and in arteries of the liver and lung in all three genetic lines of broiler chickens (Cobb – 3 birds, Ross – 3 birds, Hubbard – 2 birds). The length and the width of the plugs inside the vessel lumens are illustrated in Table 1. The third measurement was difficult to acquire.

The first FMD cases were detected on the 3rd day of life in two birds, case A and case B, in branches of the renal arteries. The lesions occurred as plugs originating from the media of the vessel and obstructing the lumen of the vessels. In both cases, the point of

Table 1. The length and width of the plugs in all FMD cases.

Case	Age (days)	Length of plug (µm)	Width of plug (µm)	Area of plug (µm ²)	Area of vessel lumen (µm ²)	Genetic line of broiler chickens
A	3 rd	176	77	11 131	18 320	Cobb
B	3 rd	80	45	3 309	22 687	Hubbard
C	10 th	62	35	1 656	3 680	Cobb
D*	17 th	840	330	209 362	290 573	Ross
E*	17 th	215	84	12 702	18 972	Ross
F*	17 th	–	–	–	871	Ross
G**	24 th	173	94	13 187	18 461	Ross
H**	24 th	212	113	12 398	23 080	Ross
I	31 st	143	86	8 047	12 014	Cobb
J***	38 th	213	49	6 573	10 785	Hubbard
K***	38 th	36	31	812	2 150	Hubbard
L	38 th	809	378	241 747	383 090	Ross

* – one bird, 17 days old; ** – one bird, 24 days old; *** – one bird, 38 days old.

attachment was visible (Case A, Fig. 1). A third case of FMD (Case C) was found in one bird on the 10th day of life and presented as a plug in the renal arteriole. By HE staining, the lesion was similar to those observed in cases A and B, with a visible point of attachment.

Because of the small size of the plugs in cases A, B and C, it was not possible to perform Masson's trichrome and IHC staining. However, based on the appearance of the lesions following HE staining, the plugs consisted of smooth muscle and connective tissue and were therefore classified as the medial fibromuscular dysplasia subtype.

On the 17th day, three incidents of FMD (cases D, E, F) were found in the renal arteries of one bird. Cases D and E led to obstruction of the vessel and the point of attachment was visible in both cases (Fig. 2). Masson's trichrome stain showed that the plug in case D consisted of smooth muscle and fibrous connective tissue originating from the media of the vessel (Fig. 3). The Verhoeff stain revealed an irregular arrangement of elastic fibres within cells forming the plug (Fig. 4). In case D, myocytes forming the plug stained positively for SMA (Fig. 5) but were negative for desmin. Immunoreactivity with PCNA was strong in the endothelial cells on the external surface of the plug in case D, but the myocytes, fibrocytes and fibroblasts were negative for PCNA (Fig. 6). Myocytes in the tunica media of the artery with FMD were positive for SMA and desmin. On the basis of histopathological and immunohistochemical examinations, cases D and E were classified as the medial fibromuscular dysplasia subtype of FMD.

Case F occurred as a vessel lumen stenosis. Masson's trichrome stain showed smooth muscle and fibrous connective tissue narrowing from the media (Fig. 7, 8), but no fragmentation of the internal elastic lamina was apparent. IHC revealed strong reactivity for SMA, weak PCNA reactivity in myocytes and strong PCNA reactivity in proliferating endothelial cells. All the data allowed the classification of this case as the medial fibromuscular stenosis subtype.

On the 24th day, FMD was detected in an artery (case G) and a vein (case H) of the kidney of one broiler (Fig. 9). Masson's trichrome staining of case H showed that the connective tissue in the middle of the plug was surrounded by smooth muscle cells (Fig. 10). It was not possible to perform IHC staining. Both lesions were classified as the medial fibromuscular dysplasia subtype based on the HE staining and Masson's trichrome stain.

On the 31st day, another case (I) of FMD in the form of a plug occupying the lumen of a liver arteriole was found. Masson's trichrome stain showed smooth muscle and fibrous connective tissue as a component of the plug. In this case, PCNA immunoreactivity was positive only in the endothelial cells on the external surface of the plug but negative in the cells forming the plug. Desmin immunoreactivity was also negative in plug-forming cells (Fig. 11). This case was classified as the medial fibromuscular dysplasia subtype.

On the 38th day, FMD was found in two renal arterioles (cases J and K) of one bird. Lesions occurred as plugs obstructing the lumen of the vessels. On the same day, FMD was diagnosed in an artery of the lungs of another bird as a plug obstructing the artery

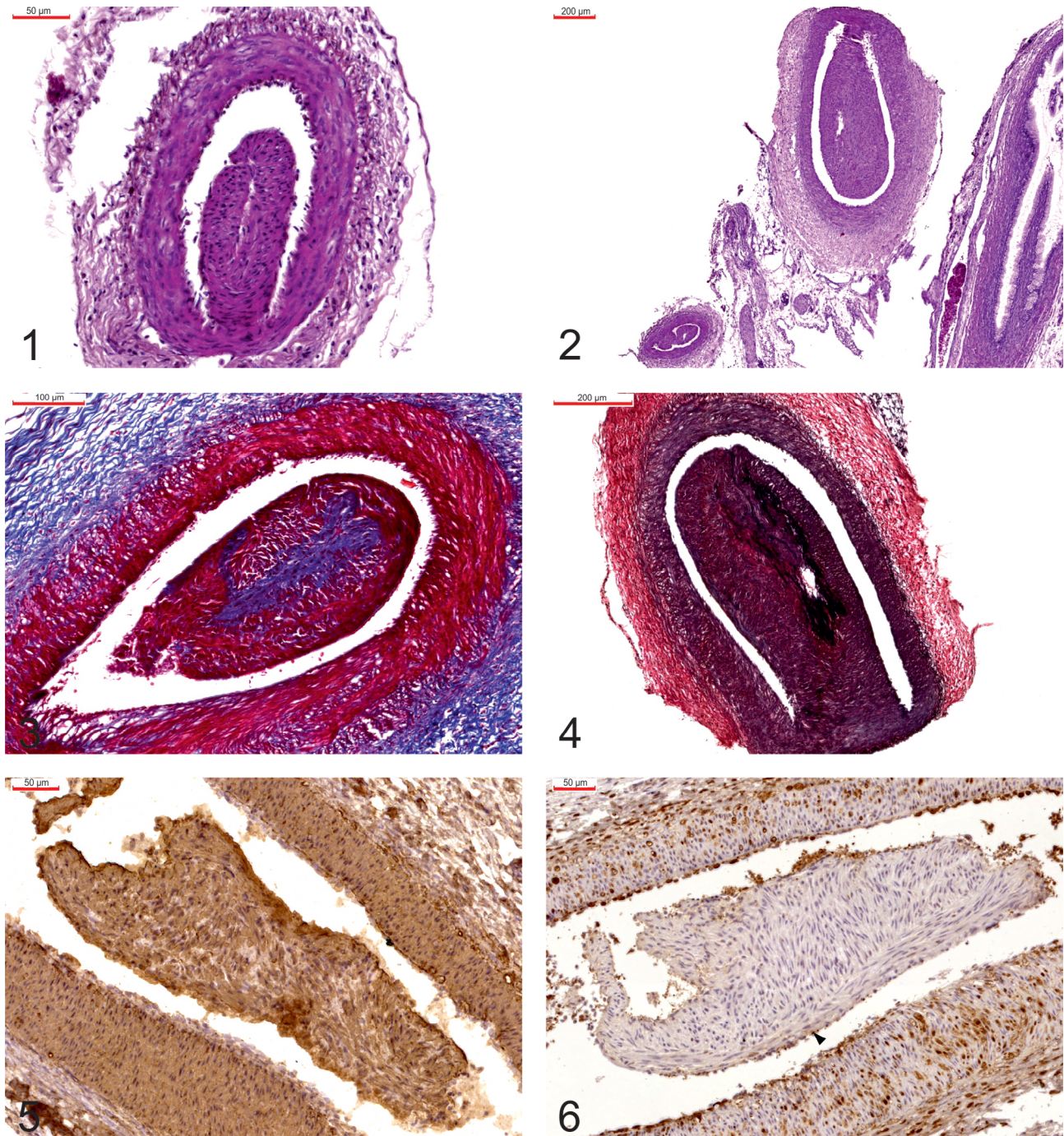


Fig. 1. Case A – artery in kidney – 3 days old; plug narrowing from the media with visible point of attachment. HE, Fig. 2. Case D and E – arteries in kidney – 17 days old; plugs with visible points of attachment. HE, Fig. 3. Case D – artery in kidney – 17 days old; plug consisting of smooth muscle and fibrous connective tissue. Masson's trichrome, Fig. 4. Case D – artery in kidney – 17 days old; irregular arrangement of elastic fibres (black) within FMD cells. Verhoeff stain, Fig. 5. Case D – artery in kidney – 17 days old; positive reaction within FMD cells for smooth muscle actin markers. IHC for SMA, Fig. 6. Case D – artery in kidney – 17 days old; negative reaction for PCNA within FMD cells, positive reaction in endothelium cells on the external surface of the plug (arrow). IHC for PCNA.

lumen, undergoing necrotic changes (case L – Fig. 12). It was not possible to perform Masson's trichrome and IHC staining for cases J, K and L. However, evaluation of HE-stained sections showed that plugs consisted of smooth muscle and connective tissue, and

these cases were classified as the medial fibromuscular dysplasia subtype.

It should be noted that angiopathy, either ischemic necrosis or clots attached to the vessel plugs was not observed in any of the tissues diagnosed with

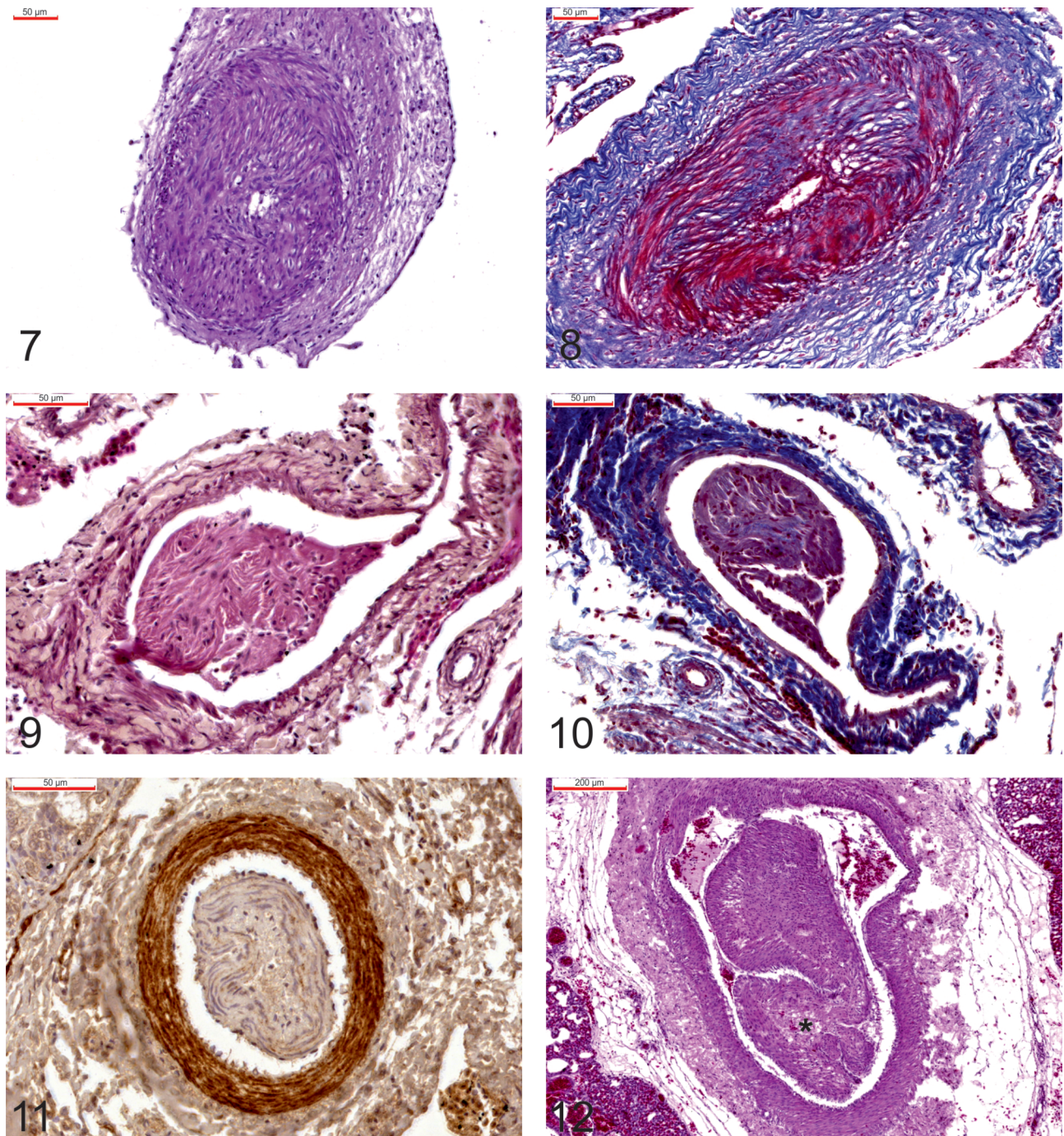


Fig. 7. Case F – artery in kidney – 17 days old; medial fibromuscular stenosis subtype. HE, Fig. 8. Case F – artery in kidney – 17 days old; lumen of artery is reduced by proliferating smooth muscle and fibrous connective tissue from media. Masson's trichrome, Fig. 9. Case H – vein in kidney – 24 days old; medial fibromuscular subtype. HE, Fig. 10. Case H – vein in kidney – 24 days old; plug consisting of smooth muscle and fibrous connective tissue. Masson's trichrome, Fig. 11. Case I – artery in liver – 31 days old; negative reaction for desmin markers within FMD cells. IHC for desmin, Fig. 12. Case L – artery in lung – 38 days old; visible necrosis inside of the plug (asterisk). HE.

FMD. In the kidneys, parenchymatous and adipose degeneration of the proximal convoluted tubules epithelium, smooth muscle hypertrophy in the media of arteries and proliferative glomerulopathy were observed. The hepatocytes demonstrated cloudy swell-

ing, smooth muscle hypertrophy in the media of arteries and proliferation of bile ductules (Gesek et al. 2010, 2013). In the lungs, an increased number of cartilaginous nodules and bone spicules were observed. In addition, pulmonary arterioles showed hypertrophy

of the endothelial cells and hyperplasia of the smooth muscle in the media, with subsequently decreased lumen size within the small blood vessels. This reduction in the lumen size of small blood vessels, together with dilation of the right ventricle of the heart, is thought to constitute the initial stages of pulmonary hypertension syndrome (Gesek et al. unpublished data).

Discussion

In our study, 11 of 12 cases were classified as the medial fibromuscular dysplasia subtype of FMD. One case of the medial fibromuscular stenosis form corresponded to that observed by Mete and McDonough (2011). They reported FMD in a 4-year-old Shih Tzu, in which proliferating smooth muscle cells and myofibroblasts from the media disrupted the internal elastic lamina and caused stenosis of the coronary artery lumen. However, we did not confirm disruption of the internal elastic lamina in our case.

The changes mostly affected arteries but also affected a venous vessel in the kidney of one broiler on the 24th day of life and were classified as a medial fibromuscular dysplasia. This is the first reported case of FMD diagnosed in the vein of an animal species. Rosenberger et al. (1976) described FMD in renal veins of humans suffering from hypertension, where reduction of the vessel lumen was caused by proliferating connective tissue in the media. The case reported in our study was classified as medial fibromuscular dysplasia and the plug was composed of both smooth muscle and fibrous connective tissue. Furthermore, the cases of FMD in arteries of the liver and lungs on the 31st and 38th day, respectively, have not, to our knowledge, been previously reported in any animal species.

FMD was observed in the blood vessels during all examined periods of rearing, and the youngest bird was 3-days-old. The FMD cases reported previously in birds described older animals. Julian (1980) noticed FMD in arteries of a skeletal muscle in a 42-day-old turkey. The youngest bird (quail) with previously reported FMD was 10-day-old (Braga et al. 1996). In other animal species, FMD of the coronary artery was found in a 4-year-old Shih Tzu with sudden cardiac death (Mete and McDonough 2011). Falk and Jonsson (2000) also described 7 cases of FMD in dogs but no information was given regarding the age of the animals.

The size of fibromuscular plugs in FMD depended on the diameter of the blood vessel and ranged from 31 to 378 μm . This result corresponds with the

previous report of FMD in the intramuscular arteries and arterioles in turkeys, where the fibromuscular plugs ranged from 44 to 666 μm (Julian 1980).

The FMD lesions in the blood vessels of older birds revealed morphological changes in the plugs. Necrosis was observed in one case on the 38th day of life. Julian (1980) described similar changes, particularly necrosis of the plugs in some cases with thrombus formation in the absence of endothelial cells. The degenerative changes of the FMD plugs are most likely connected with the extended duration of the disease. Furthermore, in all reported cases the presence of the endothelium on the outer surface of the FMD plugs was observed with no thrombus formation. These endothelial cells showed increased proliferation, which has not been reported previously.

The tissues with obstructive FMD in the blood vessels did not show necrotic changes. The main morphological lesions in the surrounding tissues were degenerative changes and remodelling of the blood vessels (Gesek et al. 2010). Braga et al. also did not observe ischemic or necrotic changes in muscles affected by FMD in Japanese Quail, while Julian (1980) reported degenerative changes, but with no relation to the FMD incidence in turkeys. Other authors reported FMD lesions in the epicardial coronary artery in a dog with myocardial infarction (Mete and McDonough 2011). In humans with renal FMD, the most common symptoms are renovascular hypertension and progressive renal atrophy (Plouin et al. 2007).

Despite numerous investigations in humans and animals, the aetiology of FMD remains unclear. Julian (1980) suggested an infectious agent (herpes virus) as the main cause of vascular changes in turkeys. The author found evidence of angiopathy in arteries, i.e., smooth muscle vacuolisation, endothelial hyperplasia, patchy necrosis of the media and endothelial hyperplasia with valve formation in veins. Similar changes were observed in our study in the form of vacuolisation of myocytes in the media, hypertrophy and hyperplasia of the endothelium of the arteries, and thickening of blood vessel walls. However, the vessels with FMD did not exhibit these changes. Therefore, angiopathy as the pathogenesis of FMD should be excluded. Braga et al. (1996) found FMD more frequently in the mutant strains (LWC) of Japanese quail with inherited muscular dystrophy exhibiting myotonia. In this group, FMD was found in *pectoralis major* muscle arteries in 38 of 67 cases, while a standard Japanese quail breeding line displayed only single cases of FMD. The aetiology of changes was related to a genetic muscle disease, which occurs in the LWC line. Mete and

McDonough (2011) reported FMD in a dog as a primary disease with no detected causative agents. Perdu et al. (2007) demonstrated a higher incidence of FMD in the relatives of patients with renal FMD, and therefore the underlying causes of FMD appear to be genetic. The incidence of FMD in our study was not related to bacterial, viral, or fungal infection, or to angiopathy and demonstration of FMD in the early stages of rearing also suggests a possible genetic cause of the disease. Furthermore, the myocytes forming the FMD plugs were PCNA negative in our study. There are no other reports of the PCNA activity in FMD (or any other proliferation marker) in the available literature. It is possible that the myocytes forming the FMD plug at the point of attachment to the vessel wall are PCNA positive, but such samples are highly difficult to obtain due to the specific morphology of the changes.

The FMD aetiology and morphology still require further investigation. It is expected that modern broiler lines grow fast and have good metabolism, high degree of nutrient utilisation, strong skeleton, high survival rate, disease resistance and high adaptive capacity. Unfortunately, modern genetic lines of broilers, compared with slower-growing birds from the fifties of the XX century, are predisposed to pulmonary hypertension syndrome and ascites, sudden death syndrome, and subclinical disease, including fibromuscular dysplasia. In the latter case, our studies, along with previous reports, suggest a genetic aetiology.

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