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Clinical-epidemiological, anatomic-pathological, histochemical and immunohistochemical characterization of renal cystadenocarcinoma-nodular dermatofibrosis syndrome in 11 German Shepherd dogs[1]

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ABSTRACT.- Thompson R.P.M., Lamego E.C., Melo S.M.P., Irigoyen L.F., Fighera R.A. & Kommers G. D. 2019. **Clinical-epidemiological, anatomic-pathological, histochemical and immunohistochemical characterization of renal cystadenocarcinoma-nodular dermatofibrosis syndrome in 11 German Shepherd dogs**. *Pesquisa Veterinária Brasileira 39(7):499-509.* Laboratório de Patologia Veterinária, Departamento de Patologia, Universidade Federal de Santa Maria, Av. Roraima 1000, Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: glaukommers@yahoo.com

Eleven cases of renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND) are described in German Shepherd dogs diagnosed from January 1994 to January 2018 at the Veterinary Pathology Laboratory of the "Universidade Federal de Santa Maria" (LPV-UFSM). The study sample was composed of eight male and three female dogs at a ratio of 2.67:1. Age ranged from six to 12 years (mean=8.7 years). The main clinical signs reported in descending order of frequency were multiple cutaneous nodules (nodular dermatofibrosis), dyspnea, anorexia, weight loss, recurrent hematuria, vomiting, and polydipsia. Results demonstrated that it is not always easy to clinically recognize this syndrome, but its peculiar anatomical-pathological characteristics allow safe diagnosis. Histologically, it was possible to detect all phases (cysts, papillary intratubular hyperplasia, and cystadenomas or cystadenocarcinomas) of a possible pathological *continuum* of the renal lesions. Uterine leiomyomas were observed in only one of the cases. Through histochemical techniques, it was possible to identify the presence of type I collagen in both cutaneous and renal lesions and consider its possible involvement in the pathogenesis of renal cystadenocarcinoma. Immunohistochemistry (IHC) showed partially satisfactory results in the staining of epithelial cells of renal cysts and neoplasms for pan-cytokeratin.

INDEX TERMS: Clinics, epidemiology, anatomic-pathology, histochemistry, immunohistochemistry, renal cystadenocarcinoma, nodular dermatofibrosis, syndrome, German Shepherd dogs, dog diseases, pathology.

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RESUMO.- [Caracterização clínico-epidemiológica, anátomo-patológica, histoquímica e imuno-histoquímica da síndrome cistadenocarcinoma-dermatofibrose nodular em 11 cães Pastor Alemão.] São descritos 11 casos da síndrome cistadenoma/cistadenocarcinoma-dermatofibrose nodular (CR-DN) em cães Pastor Alemão, diagnosticados entre janeiro de 1994 e janeiro de 2018 no Laboratório de Patologia Veterinária da Universidade Federal de Santa Maria

(LPV-UFSM). Os cães afetados foram oito machos e três fêmeas, estabelecendo-se uma relação de 2,67:1. A idade variou de seis a 12 anos, sendo a média de idade de 8,7 anos. Os principais sinais clínicos relatados foram, em ordem decrescente de frequência, múltiplos nódulos cutâneos (dermatofibrose nodular), dispneia, anorexia, emagrecimento, hematúria recorrente, vômito e polidipsia. Este estudo permitiu estabelecer que o reconhecimento clínico da síndrome nem sempre é fácil, porém suas características anátomo-patológicas peculiares permitem um diagnóstico com segurança. Histologicamente, foi possível detectar todas as fases (cistos, hiperplasia intratubular papilífera, cistadenomas ou cistadenocarcinomas) de um possível continuum patológico das lesões renais. Leiomiomas uterinos foram observados somente em um caso. Através das técnicas histoquímicas foi possível estabelecer que o colágeno tipo I está presente em ambas as lesões, cutâneas e renais, e cogitar seu possível envolvimento na patogênese dos cistadenocarcinomas renais. A técnica de IHQ mostrou resultados parcialmente satisfatórios na imunomarcação das células epiteliais dos cistos e dos neoplasmas renais para pancitoceratina.

TERMOS DE INDEXAÇÃO: Clínica, epidemiologia, anátomo-patologia, histoquímica, imuno-histoquímica, síndrome, cistadenocarcinoma, dermatofibrose nodular, Pastor Alemão, doenças de cães, patologia.

INTRODUCTION

Renal cystadenocarcinoma and nodular dermatofibrosis (RCND) is a rare syndrome characterized by bilateral and multifocal renal cystadenoma/cystadenocarcinoma, nodular dermatofibrosis, and uterine leiomyoma that has been reported mainly in German Shepherd dogs (Lium & Moe 1985, Moe & Lium 1997). This syndrome is caused by mutations in the folliculin gene (*FLCN*, previously *BHD*) gene, which is located in chromosome 5 and is a dominantly inherited disease (Lingaas et al. 2003, Bonsdorff et al. 2009, Pressler et al. 2009). Inactivation of this tumor suppressor gene is one of the critical steps in this disease (Bonsdorff et al. 2009). This syndrome has also been sporadically reported in Boxer, crossbred (White et al. 1998), and Golden Retriever dogs (Marks et al. 1993).

Clinical signs vary greatly among dogs depending on age and stage of the disease at examination (Moe & Lium 1997). They usually include numerous firm cutaneous and subcutaneous nodules, abdominal distension, greatly enlarged kidneys (on palpation), anorexia, fatigue, progressive weight loss, polydipsia, vomiting, and constipation or diarrhea (Lium & Moe 1985).

Gross lesions observed at necropsy are multiple firm and spherical skin and subcutaneous nodules. They may be distributed throughout the body, but with marked preference for limbs, head and back (Suter et al. 1983, Lium & Moe 1985, Moe & Lium 1997). In the kidneys, lesions are bilateral, multiple and cystic, containing a gelatinous, limpid or reddish brown fluid, often with areas of necrosis. The cysts can rupture and release their contents into the peritoneal cavity. (Lium & Moe 1985, Meuten & Meuten 2017). Affected females also tend to present several uterine leiomyomas (Cianciolo & Mohr 2016). Histologically, the cutaneous lesion is denominated nodular dermatofibrosis and, cystadenoma or cystadenocarcinoma can be observed in the kidneys (Lium & Moe 1985).

In general, most textbooks of veterinary medicine (Cianciolo & Mohr 2016, Breshears & Confer 2017, Serakides & Silva 2017) contain little, generic information on RCND, and this syndrome is mostly described in the international literature, with little information on its prevalence and presentation characteristics found in the national literature (Langohr et al. 2002, Inkelmann et al. 2012), which is important for the diagnosis and knowledge of the occurrence of this disease in the country.

The main objectives of this retrospective study were to determine the prevalence of RC-ND in the necropsies routine of the LPV-UFSM and to characterize the clinical-epidemiological, anatomical-pathological, histochemical and immunohistochemical aspects of this syndrome in German Shepherd dogs in the central region of Rio Grande do Sul state, Brazil.

MATERIALS AND METHODS

Protocols of dog necropsies conducted from January 1994 to January 2018 at the Laboratory of Veterinary Pathology of the "Universidade Federal de Santa Maria" (LPV-UFSM) were reviewed in search of cases of cutaneous and renal lesions compatible with those described for renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RC-ND) in German Shepherd dogs. The total number of German Shepherd dogs necropsied during this period was also calculated. Cases 1 to 4 and 1 to 6 of RC-ND syndrome included in this survey were part of the studies by Langohr et al. (2002) and Inkelmann et al. (2012) respectively.

From these necropsy protocols, information on the gender and age of the dogs was obtained. As for age, dogs were divided into three categories, as previously reported (Fighera et al. 2008), namely, puppies (<1 year), adults (≥1 to ≤9 years), and elderly (>10 years).

Clinical signs, outcome (spontaneous death or euthanasia), macroscopic findings (pattern of dermatofibrosis lesions, including number, size, distribution and presence of ulceration in cutaneous nodules; size, location, distribution and characteristics of renal lesions and presence of metastases), and microscopic findings were also determined. Other related injuries, such as uterine and extra-renal lesions of uremia, were also recorded.

As for gross lesions, the data were computed from the descriptions present in the medical reports, complemented by observation of the archives case photographs. Regarding histopathological evaluation, the aspects described in the necropsy reports were considered and, depending on the availability of paraffin-embedded tissues on the LPV-UFSM archives, new histological sections (stained with hematoxylin-eosin, HE) were evaluated.

Renal and cutaneous lesions, depending on the availability of paraffin-embedded tissues, were evaluated using the histochemical techniques of Masson's Trichrome (MT, Masson's Trichrome Histokit, EasyPath, EP-11-20013) and Picro-Sirius Red (PR, Picro-Sirius-Hematoxylin Histokit, EasyPath, EP-11-20011) for better collagen evidencing and typification, respectively. Using TM, the collagen areas stain in blue, whereas using PR under polarized light, type 1 collagen is observed as dense, yellow, orange or red fibers and type 3 collagen as fine, green fibers (Whittaker et al. 1994).

Renal lesions were also assessed by immunohistochemistry (IHC) using bovine polyclonal anti-pancytokeratin antibody (Dako Cytomation, code Z-0622) produced in rabbit (1:2,000). Silanized slides with 3µm histological sections were used. After dewaxing and rehydration of the tissues, antigenic recovery with TRIS-EDTA at pH 9.0 was performed, followed by endogenous peroxidase blocking with 3% hydrogen peroxide. Blocking of non-specific reactions

was performed with protein blocker (EasyPath kit, EP-12-20504). Incubation with the primary antibody occurred in oven for 60 min at 37°C in a humid chamber. Easy Link One polymer (EasyPath kit, EP-12-20504) was used as secondary antibody, incubated in oven at 25°C. 3-3'-diaminobenzidine tetrachloride (DAB; EasyPath kit, EP-12-20504) was used as substrate-chromogen. Sections were counterstained with Harris hematoxylin, dehydrated, and mounted using synthetic mounting medium (Entellan, Merck) and coverslips. Bovine alimentary tract squamous cell carcinoma was used as positive control. The same sections to be tested, with replacement of primary antibody with antibody diluent, were used as negative control.

RESULTS

Epidemiological and clinical aspects

A total of 6,231 dogs (2,958 males and 3,271 females) were necropsied during the study period. Of these, 379 were German Shepherd - 208 males (54.89%) and 171 females (45.11%). Of these, 11 (2.9%) German Shepherd dogs were diagnosed with RCND compatible lesions. The cases were chronologically distributed: two cases in 1994, 1996, and 2012; one case in 2005, 2010, 2011, 2015, and 2018. Epidemiological and clinical aspects are detailed in Table 1. Affected dogs were eight males (80%) and three females (30%), establishing a ratio of 2.67:1. Of the three bitches, two were unspayed. The age of the affected dogs ranged from 6 to 12 years. In one case, the age was not reported. Of the 10 dogs whose age was known, 7 (70%) were classified as adults and three (30%) as elderly. Overall, mean age was 8.7 years and median was 8.5 years.

The main clinical signs described in the medical reports in descending order of frequency were multiple cutaneous nodules (4/11, 36.36%), dyspnea (3/11, 27.27%), anorexia (2/11, 18.18%), progressive weight loss (2/11, 18.18%), recurrent hematuria (2/11, 18.18%), and vomiting (2/11, 18.18%). Seven dogs (7/11, 63.63%) died spontaneously and four (4/11, 36.36%) were euthanized.

Macroscopic changes

Necropsy findings usually showed enlarged kidneys and with irregular contour due to presence of firm or floating cysts and/or neoplastic nodular tissue that protruded on the capsular surface (Fig.1A,B). Table 2 shows these aspects in detail for each dog. The kidneys of the eleven dogs were bilaterally affected. In 10 cases (Dogs 1-9 and 11), cysts and neoplasms were observed in both kidneys. In one case (Dog 10), the left kidney had cysts and neoplasm, whereas the right kidney showed only small cysts.

Cut surface of the kidneys revealed cysts of a few millimeters in diameter (usually filled with clear fluid) (Fig.1C) to large cystic cavities (up to 15cm in diameter and containing clear, reddish brown and dark brown liquids, or clots) (Fig.1D), which replaced much of the renal parenchyma, affecting the cortical (mainly), medullary and pelvic (less often) regions. In most cases, the cystic cavities were also filled by scarce or abundant, dark red or dark brown, porous, extremely friable (necrotic aspect) neoplastic tissue (Fig.1E,F). Rarely (Dogs 4, 8, and 11), solid, whitish neoplastic masses were also observed. Cystic cavity capsules were generally fibrous and firm (Fig.1F). In three cases (Dogs 4, 7, and 8), there was rupture of cysts, with extravasation of contents and/or hemorrhage into the abdominal cavity (hemoperitoneum). Peritonitis was also described in Dog 8.

Skin lesions were observed at necropsy in 11 dogs. The gross characteristics of the cutaneous lesions in each case are detailed in Table 3. In nine cases, the lesions were multiple and numerous, affecting more than a single body area. A few nodules were found in one case (Dog 10) and a single nodule was observed in only one situation (Dog 1). Nodular lesions were distributed in descending order of frequency as follows: hind limbs (9/11, 81.81%) (Fig.2A), forelimbs (8/11, 72.72%), head (3/11, 27.27%) (Fig.2B), and trunk (2/11, 18.18%).

These lesions were observed as papules (<1cm) or nodules (>1cm). In several cases, nodular lesions were very

Table 1. Epidemiological and clinical aspects of 11 German Shepherd dogs with renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND)

Case	Gender	Age (years)	Clinical signs	Outcome
1	M	10	Hematuria, urinary retention, rigid and dilated urinary bladder, hind limb edema.	SD
2	M	7	Progressive weight loss (1 month), cough and dyspnea, anorexia (3 days), and vomiting (1 day).	SD
3	M	8	Numerous cutaneous nodules (2 years). Biopsy was performed three times (results not informed) and there were recurrences. No other clinical signs.	EUT
4	M	6	Polydipsia and dyspnea, died after 3 hours	SD
5	F	6	Anorexia, jaundice. Examinations: normal blood count; ALT: rose from 750 to 1195 (in 2 days). X-ray: mass in the renal region.	EUT
6	M	Adult	Cutaneous nodules on the head and limbs	SD
7	M	9	Vomiting; found in decubitus and with dyspnea, small firm nodules on the paws and head. Mass compressing the colon.	SD
8	F	9	Cystitis and hematuria (1 year). Severe weight loss and anemia. US: A mass was observed in the kidney. Anorexia. Found dead.	SD
9	M	12	Skin tumors that recurred constantly (3 years). Incoordination of the hind limbs. Developed hematuria, being diagnosed with hemorrhagic cystitis; no response to treatment.	EUT
10	M	8	Progressive weight loss, polydipsia, elevated urea and creatinine. US: fluid in the abdominal cavity and chest; changes in the left kidney and pleated intestinal segments. Exploratory celiotomy: multiple altered organs and large amount of fluid in the abdomen.	EUT
11	F	12	Found dead.	SD

M = male, SD = spontaneous death, EUT = euthanasia, F = female, ALT = alanina aminotransferase, US = ultrasound.

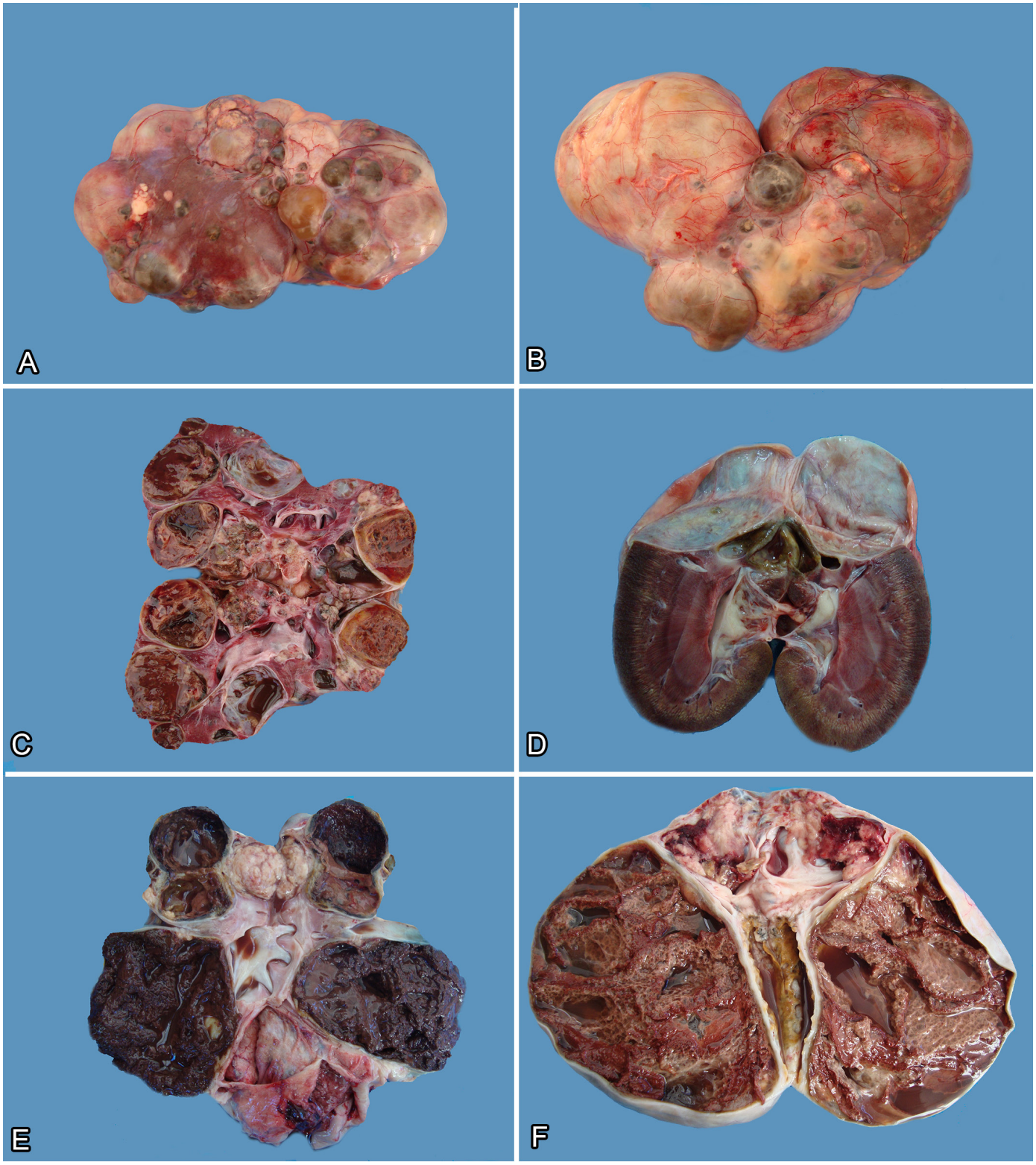

Fig.1. Renal cystadenocarcinoma. (**A**) Kidney of Dog 9 is increased in size, with parenchyma distorted by small subcapsular cystic masses. (**B**) Kidney of Dog 9 increased markedly in size with parenchyma distorted by subcapsular cystic masses of various sizes. The cysts show thick walls. (**C**) Dog 9. At cut, multiple cysts containing neoplastic and sometimes necrotic formations, are observed. There is little renal parenchyma remaining. (**D**) Dog 10. At cut, there is a cyst of approximately 3 cm in diameter located in the corticomedullary region of the cranial pole with fibrous wall and adjacent smaller cystic formations. (**E**) Dog 8. At cut, the kidney shows multiple cystic formations with dark-red, porous and extremely friable content, and some firm, whitish solid portions. (**F**) Dog 8. At cut, the kidney shows multiple cystic formations with brownish, porous and extremely friable content, and some firm, whitish solid portions. A thick, white, fibrous capsule is observed.

Table 2. Macroscopic aspects of renal lesions in 11 German Shepherd dogs with renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND)

Case	Size/weight of kidneys and cystic renal lesions	Content of cysts/cystic cavities
1	RK (30x22x7cm, with cystic cavities from 5 to 15cm in diameter) LK (with nodules of 4x6cm and 2x3cm in the poles)	Reddish-brown liquid
2	RK (16x12cm with cystic cavities) LK (19x12cm with cystic cavities)	Reddish-brown/clear liquid
3	Both (with cysts from 0.1 to 0.5cm in diameter)	Clear liquid
4	RK (with cystic cavities from 0.5 to 1.8cm in diameter) LK (with cystic cavities from 0.3 to 1.5cm in diameter)	Clot
5	RK (3-fold the normal size, with cysts from 3 to 6cm in diameter) LK (with cysts from 1 to 3cm in diameter)	Clot
6	Both (increased volume; with cysts ranging from a few mm to 3cm in diameter)	Viscous and clear liquid
7	Both (with cysts from 0.1 to 2cm in diameter)	Reddish-brown liquid/clot
8	Both (enlarged, with cystic cavities up to 4.5cm in diameter) RK (weight: 1,043g), LK (weight: 1,020g)	Reddish-brown liquid
9	Both (enlarged, with cystic cavities from 3 to 8cm in diameter)	Dark brown liquid
10	RK (with cysts up to 0.2cm in diameter) LK (enlarged, with a few cysts up to 5cm in diameter in the cranial pole)	Clear liquid
11	Both (with cystic cavities from 0.5 to 3 cm in diameter)	Reddish-brown liquid

RK = right kidney, LK = left kidney.

Table 3. Macroscopic characteristics of cutaneous lesions in 11 German Shepherd dogs with renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND)

Case	Number of nodules	Size	Site	Ulceration
1	Single	3cm in diameter	LHL	No
2	Multiple	1cm in diameter	HLs	No
3	Multiple	0.2-2cm in diameter	Head (mentum, face and periocular regions)/FLs /HLs	Yes
4	Multiple	0.3-0.6cm in diameter	RFL (ulnar region)/trunk (scapular region)	Yes
5	Multiple	0.5-1cm in diameter	LHL	No
6	Multiple	From a few mm up to 5cm in diameter	Head/FLs/HLs	No
7	Multiple	0.2-2cm in diameter	FLs/HLs/head/trunk	No
8	Multiple	0.2-0.6cm in diameter	FLs/RHL	No
9	Multiple	0.6-2cm in diameter	RFL/LHL	Yes
10	Two	4x2cm (LFL)/0.5cm in diameter (head)	LFL/head (right ear)	Yes
11	Multiple	4x2x1cm (RFL)/0.5cm in diameter (FL/HL)	FLs/HLs	No

LHL = left hind limb, HLs = hind limbs, FLs = forelimbs, RFL = right forelimb, RHL = right hind limb, LFL = left forelimb.

close to each other, sometimes coalescing and forming larger masses/plaques. The lesions were alopecic or not, sometimes ulcerated (four cases). The epidermal surface was blackened in several cases. At cut, it was observed that the nodular lesions were located in the dermis (Fig.2C) or in the subcutaneous tissue (Fig.2D), and were firm (fibrous) and white in most cases. Occasionally, soft lesions were described.

In Dog 8, there were two protruded nodules in the serosa layer of the uterus (Fig.2E), 0.3 and 0.6 cm in diameter. They were smooth and yellowish. At cut, they were not very firm and homogeneous. Additional findings associated with RCND were firm-elastic, whitish, neoplastic nodules (interpreted as metastatic) in the liver and spleen (Dog 4). In Dog 10, near the stomach (pylorus), there was a fibrous mass in the omentum, which had been transformed into a fibrous capsule that enveloped the entire intestine, causing its pleating and shrinking of the intestinal loops (Fig.2F). Small nodules were also observed in the costal pleura (and pleural effusion). The lungs showed retracted areas in the pleura and the liver had rounded edges (as a result of sharp retraction of the capsule). Extra-renal lesions of uremia were described in Dog 1 (pneumopathy) and Dog 2 (gastropathy, pneumopathy, and parathyroid hyperplasia). In the urinary bladder, urine color was described as chocolate-red (Dog 1) or dark red (Dogs 8 and 9).

Microscopic changes

Renal histological changes were multifocal, affecting the cortical and/or medullary regions, and comprised discrete epithelial cysts (Fig.3A), areas of papillary intratubular hyperplasia (Fig.3A), cystadenomas and/or cystadenocarcinomas (Fig.3B). The presence of these lesions in each case is detailed in Table 4. In the entire study sample, there were multiple small cysts composed of a thin wall and internally lined with a layer of epithelial cells, similar to the normal tubular epithelium, but generally elongated and flat (interpreted as

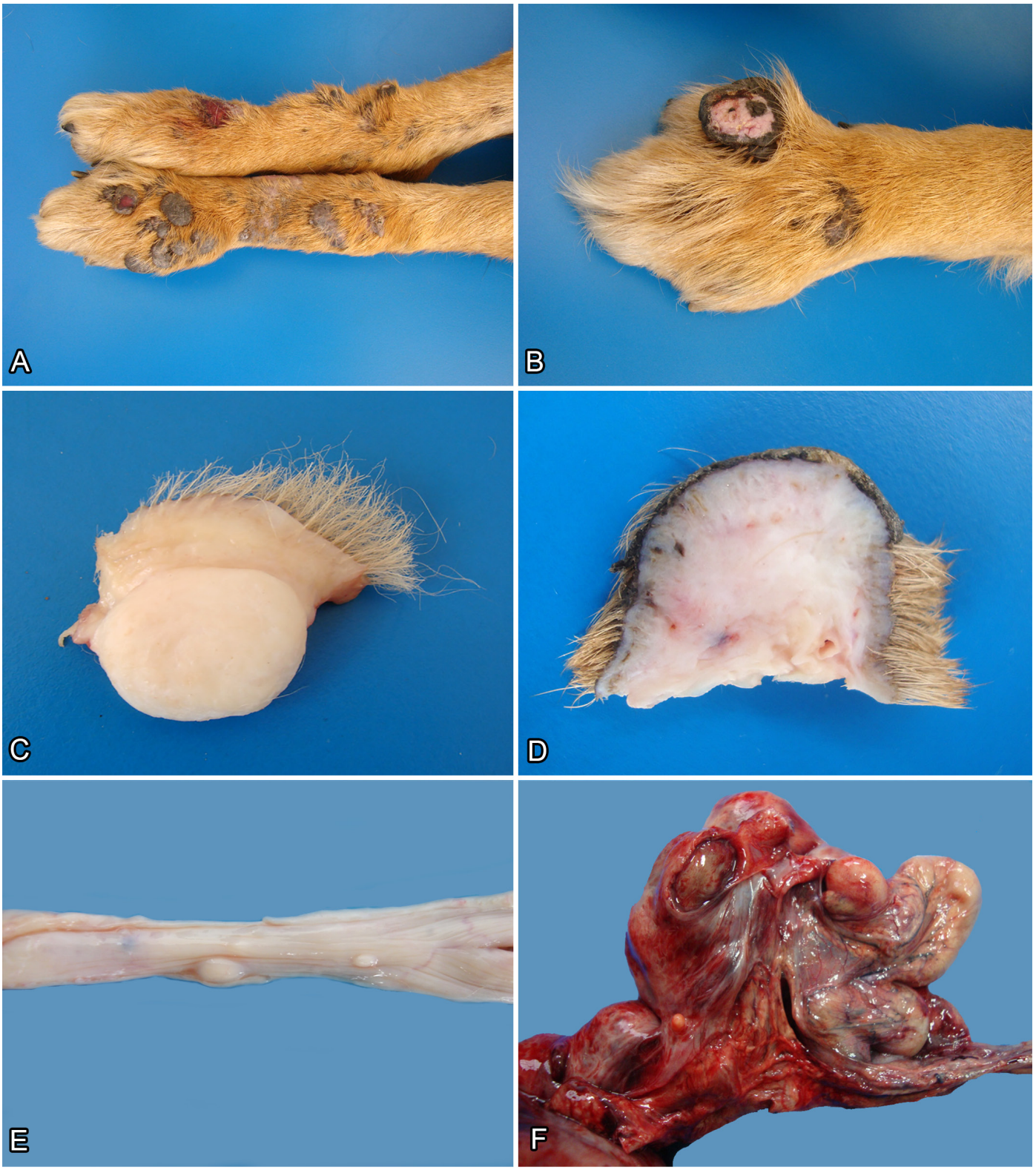

Fig.2. (**A**) Nodular dermatofibrosis in Dog 9. Skin, on the cranial face of the pelvic limbs there are multiple nodules, very close to each other, sometimes coalescing and forming larger masses, occasionally ulcerated. (**B**) Nodular dermatofibrosis in Dog 9. Multiple nodules can be observed in the cranial face of the anterior limb, with the largest nodule showing areas with ulceration and depigmentation. (**C**) Nodular dermatofibrosis in Dog 11. Skin, at cut, a white, firm nodule that extends from the superficial dermis to the deep dermis can be observed. (**D**) Nodular dermatofibrosis in Dog 9. Skin, at cut, the surface shows a firm, whitish nodule that is strictly located in the subcutaneous tissue. (**E**) Uterine leiomyoma in Dog 8. Multiple, smooth, whitish nodules can be observed in the serosa layer of the uterus. (**F**) Intestine of Dog 10. There is a mass consisting of pleated intestine, surrounded by a fibrous capsule, which corresponds to the omentum with metastasis of cystadenocarcinoma.

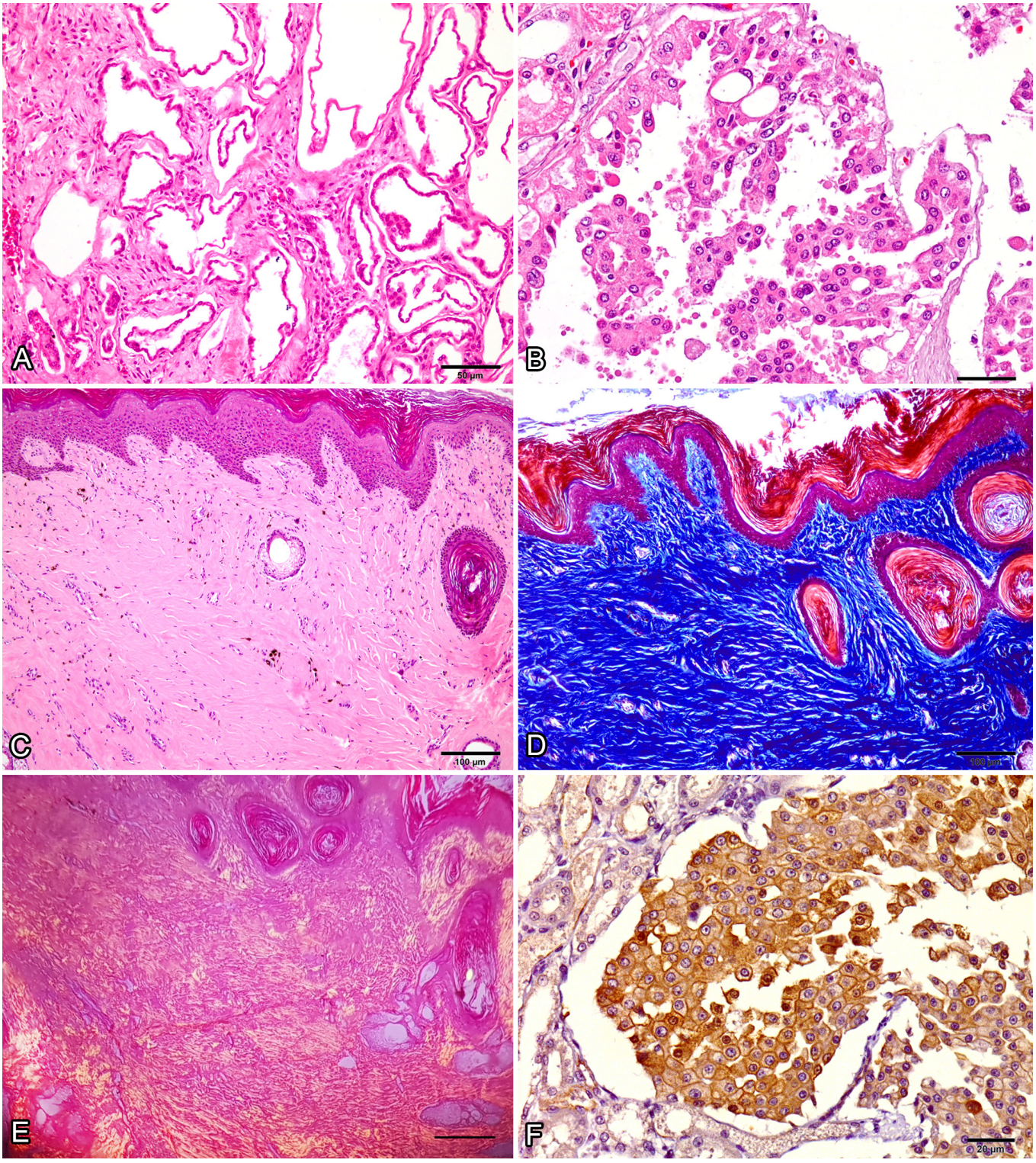

Fig.3. (**A**) Kidney. Focally extensive area with formation of cysts characterized by wall with a single layer of cells. There are two areas with onset of hyperplasia within the cysts. HE, bar=50µm. (**B**) Kidney. Proliferation of neoplastic epithelial cells forming papillary projections. There are binucleated neoplastic cells and a signet-ring cell (vacuolated cytoplasm), characteristic of renal cystadenocarcinoma. HE, bar=20µm. (**C**) Skin. Abundant presence of collagen fibers in the superficial and deep dermis (not shown in the microphotograph) and reduction of skin appendages. Irregular hyperplasia with moderate orthokeratotic hyperkeratosis is observed in the epidermis. HE, bar=100µm. (**D**) Skin. There is proliferation of collagen fibers in the superficial and deep dermis (not shown in the microphotograph) stained in blue by MT. Irregular hyperplasia of the epidermis is observed with moderate orthokeratotic hyperkeratosis and keratosis of the remaining hair follicles. MT, bar=100µm. (**E**) Skin. Same section of Figure 3D, showing thick orange collagen fibers in the dermis, characteristic of type I collagen. PR, bar=100µm. (**F**) Kidney. Positive immunostaining for pancytokeratin of the epithelial cells of a cystadenocarcinoma. IHC HRP-polymer, bar=20µm.

Table 4. Histological aspects of renal lesions in 11 German Shepherd dogs with renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND)

Case	Histological findings of the kidneys
1	Cysts, papillary intratubular hyperplasia, and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hematoidin)
2	Cysts and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hematoidin)
3	Cysts, papillary intratubular hyperplasia, and cystadenoma Peritumoral and/or intratumoral lesions: mild fibrosis
4	Cysts and cystadenocarcinoma Peritumoral and/or intratumoral lesions: mild fibrosis
5	Cysts, papillary intratubular hyperplasia, and cystadenoma Peritumoral and/or intratumoral lesions: mild fibrosis
6	Cysts, papillary intratubular hyperplasia, and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigments (hematoidin and hemosiderin)
7	Cysts, papillary intratubular hyperplasia, cystadenoma, and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigments (hematoidin and hemosiderin)
8	Cysts, papillary intratubular hyperplasia, and cystadenocarcinoma Peritumoral and/or intratumoral lesions: fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hematoidin)
9	Cysts and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hemosiderin)
10	Cysts and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hematoidin)
11	Cysts and cystadenocarcinoma Peritumoral and/or intratumoral lesions: marked fibrosis, necrosis, hemorrhage, cholesterol clefts, mineralization, and pigment (hematoidin)

caused by fluid - not stained - distension of the wall). Six cases presented some cystic formations internally lined with a high cuboidal epithelium, at times with two to three layers of cells, or forming discrete papillary projections (papillary intratubular hyperplasia). In three cases, in the wall of some cystic structures, moderate to marked papillary proliferation of well-differentiated neoplastic epithelial cells was observed, with abundant and eosinophilic cytoplasm. These cells showed single, central nuclei with a single nucleolus (cystadenomas). In general, cystadenocarcinomas were characterized as being part of a larger cystic structure that showed a thick, fibrous wall and was internally lined with neoplastic cuboidal or polyhedral epithelial cells arranged in an irregular papillary pattern. These cells presented abundant cytoplasm and round or oval, vesiculous nuclei with dispersed chromatin. Some cells were binucleated and others had a large cytoplasmic vacuole pushing the nucleus to the periphery (signet-ring cell morphology) (Fig.3B). Mitotic figures were rare. Occasionally, cystic structures filled with neoplastic cells, similar to those described for the papillary areas but arranged in a more solid pattern, were observed. The following features were frequently observed in the cystadenocarcinomas: areas of intense peritumoral (contiguous with the wall of the cystic cavities) and intratumoral (often as connective septa) fibrosis, marked multifocal necrosis sometimes containing cholesterol clefts, areas of focally extensive hemorrhage, mild multifocal mineralization, macrophage infiltrate containing golden-brown granular pigment (hemosiderin) and irregular deposits of golden-yellow crystalloid material (hematoidin).

Metastases of the cystadenocarcinomas were observed in the liver and spleen (Dog 4) and in multiple organs and tissues in Dog 10. In the latter, the fibrous mass in the omentum and the fibrous capsule into which it was transformed (which involved the entire intestine) consisted of abundant fibrous connective tissue (interpreted as a desmoplastic cirrhous reaction), well vascularized, and interspersed with moderately pleomorphic, metastatic epithelial cells, including signet-ring cells. Metastases were also observed in the esophageal serosa, costal pleura, parietal pleura, and liver capsule.

Cutaneous nodules were composed of accumulation of mature collagen, generally arranged in thick fibers and interspersed with a few fibrocytes (Fig.3C). In the dermal nodules, this collagen redundancy was observed in the skin adnexa, which were morphologically normal, or in the presence of dilated hair follicles with keratosis. In the dermis, the limits of these nodules were barely perceptible among the normal adjacent collagen. However, in the subcutaneous tissue, the collagenous nodules were well delimited among the adipose tissue. The cutaneous and subcutaneous nodules were interpreted as nodular dermatofibrosis. The epidermis was acanthotic and with orthokeratotic hyperkeratosis or ulcerated in some nodules. Mononuclear inflammatory infiltrate was occasionally observed.

In the uterus wall of Dog 8, there were well-delimited and non-encapsulated areas of neoplastic proliferation of spindle cells, which were organized in dense bundles; they were well differentiated and similar to smooth muscle cells. The cytoplasm was elongated, abundant, and eosinophilic. The nucleus was elongated and composed of moderately aggregated chromatin. The nucleoli were unique and barely perceptible. The neoplasms were interpreted as leiomyomas.

Histochemistry and immunohistochemistry (IHC)

MT staining provided clearer identification of areas with greater deposition of collagen fibers, mainly those surrounding and interspersing renal cystadenocarcinomas (Dogs 1, 4, 6-8, 10, 11), and those with mild fibrosis surrounding mainly cystadenomas (Dogs 3 and 5). Sequential sections of these cases were PR stained and observed under polarized light. It was observed that areas with fibrosis, regardless of the degree of severity, were composed of type 1 collagen. Likewise, in the areas of nodular dermatofibrosis, collagen fibers were intensely stained in blue under MT staining (Fig.3D) and type 1 collagen was identified under PR staining (Fig.3E).

IHC using the anti-pancytokeratin antibody applied on sections of the kidney showed irregular or absent immunostaining of the epithelial cells of the cysts and neoplasms. The most intensely immunostained cells were observed in areas with cystadenocarcinoma in a more solid pattern in Dog 10 (Fig.3F), as well as in metastasis in the omentum in this case.

DISCUSSION

The age of the 11 affected dogs and their mean age were very similar to those observed in another study conducted with 43 German Shepherd dogs, which is considered as the first detailed pathological description of this hereditary syndrome (Lium & Moe 1985). A male:female ratio of 2.67:1 was established for the 11 dogs of this study. Most reports suggest a predominance of males, in a ratio of 2:1 (Meuten & Meuten 2017). It is worth mentioning that of the 379 German Shepherd dogs necropsied during the study period, 54.89% were male and 45.11% were female.

Clinical recognition of RCND syndrome is not always easy. Based on clinical reports, only two cases had both the observation of urinary tract disease and cutaneous lesions compatible with the syndrome (Lium & Moe 1985). Six cases had signs indicative of urinary tract disease alone (or its consequences) and two dogs showed cutaneous nodules (perhaps renal disease had not yet manifested in these cases). Nonspecific clinical signs, confused with other diseases, may be factors that make clinical suspicion of the RCND syndrome difficult, and the diagnosis is often established only at necropsy and histopathological examination.

Eight dogs had some signs indicative of urinary tract disease (hematuria, urinary retention, azotemia and/or masses in the renal region), but others showed only secondary changes to chronic kidney disease (progressive weight loss, dyspnea, anorexia, vomiting, polydipsia or anemia). Similar clinical signs were observed in a study conducted with 51 dogs with this syndrome, where polydipsia and hematuria (macroscopic) were described in 25% of the cases. Severe clinical signs, including depression, fever and anorexia, were observed in 22% of the dogs. Two-thirds of these animals had peritonitis (sterile) caused by rupture of renal cysts. Pain and dyspnea were due to peritonitis (resulting from cyst rupture) and/or metastases (Moe & Lium 1997).

In only four cases there was description of cutaneous nodules in clinical reports, and in Dog 3, recurrent cutaneous nodules were the reason for the consultation, although they were detected at the necropsy of the 11 dogs studied. Skin lesions were the main reason for owners to take their dog to the clinic in 37% of the cases in a study conducted with 51 dogs with RCND syndrome (Moe & Lium 1997). In the present study, it can be inferred from the description of necropsy that, in some dogs, perhaps because they are small or multiple, but very small in the middle of the long coat, the nodules were not detected or clinically mentioned. Skin lesions were more frequent in the limbs, head and trunk, in descending order of frequency, in this survey. In another study (51 dogs), the most frequent sites of the lesions were limbs and head, and the authors emphasized that the nodules were often difficult to observe (Moe & Lium 1997).

Regarding renal changes at necropsy, the main characteristics were bilateral lesions, presence of multiple cysts of different sizes, and of neoplastic masses contained in cystic cavities, usually necrotic, destructive, and with hemorrhage or, occasionally, as solid masses. Neoplastic cysts and masses often increased kidney size and completely altered its shape. The same characteristics have been described in other studies on this syndrome (Lium & Moe 1985, Moe & Lium 1997) and were considered as indicative of primary multicenter origin (Lium & Moe 1985). The neoplastic lesions observed in the RCND syndrome differ from the sporadic forms of renal carcinomas, which are usually unilateral and single, with occasional metastases to the contralateral kidney (Lucke & Kelly 1976, Baskin & De Paoli 1977, Lium & Moe 1985, Meuten & Meuten 2017).

The simultaneous presence of cysts and the cystic nature of the neoplasms of this syndrome are different from other renal tumors described in dogs (Meuten & Meuten 2017). Renal cysts increase in size with age, and they also seem to present a tendency to malignant transformation of renal lesions with advanced age in dogs predisposed to this syndrome (Lium & Moe 1985). Cysts may be the initial lesion of this disease, and they seem to progress through stages of hyperplasia, adenomas, and adenocarcinomas (Meuten & Meuten 2017). In several cases, the presence of small cysts and the intermediate stages (hyperplasia and/or cystadenoma) of this pathological *continuum* until cystadenocarcinoma were observed. In only two cases the most advanced lesion was described as cystadenoma, without morphological evidence of intratumoral malignancy. Progression of the pathological *continuum* (from cysts to cystadenocarcinoma) would be influenced by genetic factors, with cists of purebred German Shepherd from certain strains prone to malignant transformation more rapidly (White et al. 1998).

According to the histological classification of mesenchymal tumors of the skin and soft tissues in domestic animals of the World Health Organization (Hendrick et al. 1998), nodular dermatofibrosis has been considered as part of a rare syndrome, described mainly in German Shepherd dogs (but may occasionally affect other breeds), where multiple fibrous nodules are observed in the dermis and subcutaneous tissue. According to this classification, these lesions are histologically differentiated from collagenous hamartomas because they are not limited to the superficial dermis and have normal or hyperplastic cutaneous adnexa within the redundant collagen. They are also differentiated from fibromas, which are benign neoplasms of mature fibrocytes producing abundant collagen, in which the fibers are arranged in interlocking fascicles or, less frequently, in swirls. All dogs in this study had predominantly multiple cutaneous nodules in the limbs, as well as in the head or trunk. This distribution has also been observed in studies conducted with larger samples on German Shepherd

dogs (Lium & Moe 1985, Moe & Lium 1997) and with dogs of other breeds (Marks et al. 1993, White et al. 1998, Meuten & Meuten 2017, Serakides & Silva 2017).

Collagenous lesion in the skin and desmoplastic fibrous reaction in renal neoplasms are noteworthy in RCND. In the present study, it was possible to observe that, in both lesions, collagen was dense and showed characteristics indicative of type I staining. This type of collagen is considered mature (Junqueira et al. 1979), and its presence in tumoral desmoplastic tumoral reactions has been studied in other types of carcinomas, including esophageal squamous cell carcinomas (SCC) in cattle (Faccin et al. 2017) and pancreatic ductal adenocarcinomas in humans (Armstrong et al. 2004, Shields et al. 2011). The role attributed to collagen in the desmoplastic reaction has been somewhat contradictory, ranging from acting as a barrier to tumor invasion to an association of its greater expression with a worse prognosis and increased metastatization in human neoplasias (Shields et al. 2011). Type I collagen has been associated with proliferation of neoplastic cells in human pancreatic cancer, and was also observed that desmoplastic reaction, through collagen, promoted a malignant phenotype of the neoplastic cells, resulting in a worse prognosis for the host (Armstrong et al. 2004). The observation of markedly collagenous desmoplastic reaction (caused by type I collagen) in renal cystadenocarcinomas contrasted with the mild fibrous reaction in the cystadenomas of this study, suggesting the need for further studies addressing the existence or not of a prognostic role of this intratumoral reaction in RCND.

Several theories for the pathogenesis of renal and cutaneous lesions in RCND syndrome have been postulated. White et al. (1998), when studying this syndrome in dogs of other breeds, summarized these theories as follows: one hypothesis is that dermatofibrosis is a paraneoplastic syndrome secondary to renal neoplasia; the other theory postulates that they are two different diseases that arise independently and are united by a common hereditary mechanism. Considering the fibrosis observed in the histological evaluation of the kidneys in the cases studied by these authors, they proposed a theory of concomitant onset of fibrosis in the skin and kidney; renal fibrosis would cause obstruction to the tubule flow, with consequent expansion and eventual formation of cysts in the renal tubules. In the cases herein studied, fibrosis seemed to be associated mainly with neoplasms, and may not have been associated with the genesis of cysts through obstructive mechanisms.

Only one of the three bitches in this survey presented uterine leiomyomas. These have also been frequently described in bitches with RCND (Lium & Moe 1985, Moe & Lium 1997); however, a pathogenetic mechanism for this concomitant neoplasm has not yet been proposed.

It is worth noting that in seven cases, almost all of the renal parenchyma was replaced by cystic and neoplastic cystic and necrotic masses; however, only two dogs showed extra-renal lesions of uremia at necropsy; a larger number of dogs with renal insufficiency and, consequently, uremic lesions would be expected. In a study conducted with 43 German Shepherd dogs with RCND, uremia was only observed in two cases (Lium & Moe 1985). The absence of extra-renal lesions of uremia in a larger number of cases is an intriguing finding, considering the marked renal morphological changes observed in the vast majority of the kidneys analyzed in this study.

The literature surveyed showed that a specific immunohistochemical panel has not yet been established for the epithelial cells that comprise the cysts and renal epithelial neoplasms of this syndrome. For renal carcinomas of sporadic occurrence (excluding RCND), it is known that double IHC staining for cytokeratin and vimentin, uromodulin, Pax8, napsin A, and neprilysin confirms the tumor originates from kidney cells (Meuten & Meuten 2017). In the present study, pancytokeratin immunostaining was not constant in all cases. An IHC study conducted with young dogs with early RCND lesions demonstrated strong staining for cytokeratin (broad spectrum) in the epithelium that lined the renal cysts in the dogs assessed, indicating that the epithelium had a distal tubular or collecting ducts origin, because the normal distal epithelial tubule cells presented intense staining for cytokeratin, whereas normal proximal tubular cells showed weak or no staining (Moe et al. 2000).

CONCLUSIONS

This study enabled identification of the prevalence and main clinical-epidemiological and morphological characteristics of renal cystadenoma/cystadenocarcinoma-nodular dermatofibrosis syndrome (RCND).

It also demonstrated that it is not always easy to clinically recognize this syndrome, but its peculiar characteristics (renal neoplasms and nodular dermatofibrosis) observed in the gross and microscopic evaluations allow safe morphological diagnosis. It was possible to detect all phases of a possible pathological *continuum* of the renal lesions.

Through histochemical techniques, it was possible to identify the presence of type I collagen in both cutaneous and renal lesions and consider its possible involvement in the pathogenesis of renal cystadenocarcinomas.

Immunohistochemistry (IHC) showed partially satisfactory results in the staining of epithelial cells of renal cysts and neoplasms for pancytokeratin.

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