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Original Research

Pathologic Lesions of the Budgett Frog (*Lepidobatrachus laevis*), an Emerging Laboratory Animal Model

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***Lepidobatrachus laevis*, commonly called the Budgett frog, is a member of the horned frog family (Ceratophryidae), which has become increasingly popular among amphibian hobbyists. *L. laevis* is also used in biologic research on embryonic development, providing a novel model species for the study of organogenesis, regeneration, evolution, and biologic scaling. However, little scientific literature details disease processes or histologic lesions in this species. Our objective was to describe spontaneous pathologic lesions in *L. laevis* to identify disease phenotypes. We performed a retrospective analysis of 14 captive *L. laevis* frogs (wild-caught and captive-bred), necropsied at the NC State University College of Veterinary Medicine between 2008 and 2018. The majority of frogs exhibited renal changes, including varying combinations of tubular epithelial binucleation, karyomegaly, and cytoplasmic vacuolation; polycystic kidney disease; and renal carcinoma. Many of the renal changes are reminiscent of a condition described in Japanese (*Bufo japonicus*) and Chinese *(Bufo raddei)* toad hybrids that progresses from tubular epithelial atypia and tubular dilation to polycystic kidney disease to renal carcinoma. A second common finding was variably sized, randomly distributed bile duct clusters (biliary proliferation). Other noteworthy findings included regional or generalized edema, intestinal adenocarcinoma, aspiration pneumonia, and parasitism. This retrospective analysis is the first description of histologic lesions identified in captive *L. laevis* populations, providing new insight into spontaneous disease processes occurring in this species for use in disease diagnosis and clinical management.**

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Due to loss of habitat, climate change, and disease, amphibians are becoming increasingly threatened. Current estimates are that one-third of amphibian species (2,030 species) are globally threatened or extinct.[12] Amphibians, especially frogs, are popular in the laboratory, zoos, and private collections, as well as in the exotic pet trade. Increasing our knowledge about diseases and pathologic processes that afflict these species will help veterinarians properly identify predisposing factors, treat disease and enable better management of both captive populations and wild populations.

Lepidobatrachus laevis, commonly called the Budgett frog, hippo frog, or Freddy Krueger frog in the pet trade, is a member of the horned frog family, Ceratophryidae. The natural habitat for *L. laevis* is the semiarid Gran Chaco region of South America.[4] *L. laevis* are popular among amphibian hobbyists and are increasingly being used in laboratory research. The large size and accelerated growth of *L. laevis* embryos has led to their use as a novel model species for the study of organogenesis, regeneration, evolutionary development, and biologic scaling.[2,3,11,31]

Most of our knowledge about *L. laevis* revolves around their use in developmental biology research. *L. laevis* have been studied to determine and refine captive breeding protocols, as well as to investigate gastrointestinal development, developmental evolutionary modifications, the lateral line system in tadpoles, and the biochemical composition of their glycoprotein jelly coat.[6,10,20,21,23,30,31] One report describes chytridiomycosis in a variety of different captive frog species, including *L. laevis*.[29] However, there is minimal scientific literature describing disease processes in this species and no descriptions of histologic lesions. Therefore, our objective in this retrospective study was to describe spontaneous pathologic lesions in *L. laevis*, to identify potential disease phenotypes in this species.

Materials and Methods

We performed a retrospective analysis of 14 captive and wild-caught *L. laevis* frogs that were necropsied at the NC State College of Veterinary Medicine between 2008 and 2018. Thirteen animals were part of a research breeding colony and one was a client-owned animal. The population consisted of 7 males, 6 females, and one of unknown sex. Three individuals were juveniles. The age of many of these animals was unknown; however, adult compared with juvenile was determined based on weight (less than 100 g: Juvenile) or known breeding status or both.

Within the research colony, the frogs were housed individually in clear plastic bins (Spectrum StorPlus) measuring approximately 18 × 26 × 15 in. and holding a minimum of 6 gallons of water. Each tank was set-up with a plastic hut as a hide, a filter and a water heater. The water was treated with 1.89 g of salt and 0.47 mL NovAqua per gallon and changed at least once a week depending on the water quality. Water quality was tested with ammonia and 6-in-1 strips (Tetra) after water changes to

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monitor the pH and levels of ammonia, nitrate, and chlorine. Frogs were fed twice a week with small frozen-thawed neonatal (3 to 4 d old; 3 g) and juvenile (5 to 9 d old; 4.5 g) mice (prorodent.com) and live earthworms (reptilefood.com) that were injected with 0.1 mL of a 23% calcium gluconate solution (Vedco) once a week. Room and water temperatures were set at 70 to 85 and 77 to 82 °F, respectively, and were monitored daily.

In the research colony, 6 frogs were wild-caught, 3 were captive-bred from wild-caught animals at NC State University, 3 were acquired from a commercial breeder (The Frog Ranch Propagation Facility, CA), and one was of unknown parentage. All of the animals in the laboratory breeding colony are routinely tested for chytrid fungus and ranavirus via PCR upon arrival into the facility, and all animals tested negative for these infectious organisms. These animals were used as a breeding colony and were not subject to experimental manipulation. Breeding protocols, housing, and euthanasia methods were approved for use under IACUC no. 19-027-B. The client-owned animal (Case no.1) was acquired from an unknown commercial captive breeder in California in 1990. This frog was housed alone in approximately 10 L of well water for 2 y and dechlorinated Raleigh, NC city water for 15 y. The water was changed weekly, or more frequently if needed, and maintained at approximately 22 °C. The frog was fed live earthworms, Hill's Science Diet canine maintenance diet, and the occasional thawed juvenile mouse.

Thirteen animals were found dead and 1 animal was euthanized. IACUC (19-027-B) approved euthanasia protocol includes 1 mL intracoelomic injection of 5% buffered Tricaine (3-aminobenzoid acid; MS222; pH 7.1 to 7.4) followed by exsanguination and removal of vital organs once unresponsive (absent pain and right reflex). Tissues from necropsied animals were fixed in 10% neutral buffered formalin and routinely processed to generate hematoxylin- and eosin-stained slides (Histology Laboratory, NC State College of Veterinary Medicine). Pathology reports were reviewed to identify the major gross pathologic and histologic findings in each case. Tissues that were generally evaluated histologically include: intestine, stomach, kidney, reproductive organs (ovary or testis), heart, lung, spleen, liver, pancreas, and skin. Histologic slides and reports for each case were reviewed by a single pathologist (HRS) for consistency in lesion identification and description. Each case was numbered 1 to 14 in chronological order based on the date of necropsy. For the frog that was diagnosed with renal carcinoma, four 20 µm paraffin sections from the renal carcinoma were sent to Dr Steven Kubiski at the San Diego Zoo for Ranid herpesvirus testing by PCR analysis.

Results

Common histologic lesions and observations in the kidneys and liver. A summary of all histologic findings, animal source, and signalments is shown in Figure 1. The most common sites with histologic abnormalities in *L. laevis* were the kidneys and the liver. In the kidneys, 11 of 14 frogs exhibited renal tubular epithelial binucleation (Figure 2 A, B) (Case nos 1 to 8, 10 to 12). In addition, a number of these frogs exhibited evidence of tubular epithelial atypia, including karyomegaly (6 of 11 frogs) (Case nos 1, 3, 8, 10 to 12) and cytoplasmic vacuolation (4 of 11 frogs) (Case no. 1, 10 to 12) (Figure 2 A, B). Less often, tubular epithelial cells contained intracytoplasmic brown globular pigment (likely lipofuscin, melanin, or hemosiderin) or eosinophilic globular material (protein) (Figure 2 B). Several frogs also exhibited signs of chronic kidney disease including lymphoplasmacytic or lymphohistiocytic interstitial nephritis (Case no. 10, Case no. 11) or tubulonephropathy (Case no. 13).

In the liver, 9 of 14 frogs exhibited multiple variably sized, randomly distributed bile duct clusters (biliary proliferation) (Figure 2 C, D) (Case nos 1, 3, 5, 7, 10 to 14). In areas of biliary proliferation, bile duct clusters were generally small and lined by a single layer of cuboidal epithelium (Figure 2 C, D); infrequently, proliferative foci were larger and contained more tortuous bile ducts (Figure 2 C). In frogs that exhibited biliary proliferation, there was a subjective increase in the number of hepatic melanomacrophages compared with adult frogs without biliary proliferation (Figure 2 D). The presence of biliary proliferation overlapped with the incidence of renal changes: most frogs with biliary proliferation exhibited one or more of the above-described renal characteristics.

Polycystic kidney disease. Two female frogs (Case no. 1,12) exhibited renal lesions consistent with polycystic kidney disease. Gross lesions included generalized edema of the retrocoelomic space and subcutaneous tissues and marked coelomic effusion. The kidneys contained numerous, variably sized, clear, fluid-filled cavities (Figure 2 E). Histopathologic evaluation revealed severe, widespread regions of tubular dilation and ectasia that replaced normal parenchyma (Figure 2 F). Dilated tubules were lined by a single layer of cuboidal to attenuated epithelial cells. In the most severely affected regions, tubular epithelial cells exhibited moderate anisocytosis and anisokaryosis, frequent binucleation, and occasional karyomegaly, with infrequent formation of small micropapillary fronds projecting into tubular lumens (Figure 2 G). Cytoplasmic vacuolation was frequent among affected tubular epithelial cells, while intracellular accumulations of the granular, globular, brown or eosinophilic material described and interpreted above occurred less frequently. Tubules frequently contained abundant, blue-gray amorphous material (glomerular filtrate, mucous, or mineral) (Figure 2 G). Intratubular accumulations of sloughed epithelial cells, hemorrhage, or amorphous protein casts were also noted. The renal interstitium was variably expanded by edema and regions of fibroplasia. Glomeruli, where identified, were histologically unremarkable.

Spontaneous neoplasia. Renal adenocarcinoma with multifocal metastasis was diagnosed in one female frog (Case no. 8). This frog was directly related to the female frog that developed polycystic kidney disease. At post mortem examination, a mass in the caudal pole of the left kidney and a mass that spanned between the duodenum at the level of the pylorus and the colon were identified (Figure 3 A). Histologically, the renal mass was composed of neoplastic epithelial cells forming variably sized tubules within a loose fibrovascular stroma (Figure 3 B). Neoplastic epithelial cells exhibited abundant amphophilic cytoplasm and large round to oval vesicular nuclei with prominent nucleoli (Figure 3 C). There was marked anisocytosis and anisokaryosis, with frequent, marked cytomegaly, karyomegaly, and multinucleation (up to 7 nuclei) (Figure 3 C). The mitotic rate was mild to moderate, and some large and bizarre mitotic figures were observed. Rarely, neoplastic cell nuclei contained 10 to 15 µm round pale eosinophilic inclusions which were either viral inclusions or cytoplasmic invaginations (Figure 3 C). The liver, duodenum, and colon were multifocally infiltrated and effaced by poorly demarcated, unencapsulated masses composed of a similar neoplastic population as observed in the left kidney. Sections of the renal mass were tested by PCR analysis for Ranid herpesvirus and were negative.

Colonic adenocarcinoma was diagnosed in one female frog (Case no. 1). This frog was a client-owned animal and exhibited concomitant polycystic kidney disease, described above. A colonic mass arising in the wall of the distal colon was identified during postmortem examination. Histologically, the colonic

Case no.	Date	Sex	Age	Weight	Source	Necropsy findings	Cause of death	Miscellaneous notes:
1	08/25/2008	F	18y A	Unk	CO; CB	1) Generalized anasarca, marked coelomic effusion, moderate pericardial effusion 2) Renal: polycystic kidney disease with multifocal tubular epithelial cell atypia (early transition to neoplasia); renal tubular epithelial binucleation, karyomegaly, tubular epithelial vacuolization 3) Stomach and intestines: chronic granulomas with intralesional nematodes 4) Colonic adenocarcinoma 5) Hepatobiliary: bile duct proliferation	Renal failure secondary to polycystic kidney disease +/- colonic adenocarcinoma	
2	11/14/2012	F	A	174g	RBC; WC	1) Coelomic effusion 2) Small intestinal cestodiasis 3) Renal tubular epithelial binucleation	Undetermined	
3	04/21/2014	F	2.5y A	Unk	RBC; WC	1) Coxofemoral joint: femoral head distortion and synovial hyperplasia with periarticular synovial cyst 2) Cardiac: Mild multifocal myocardial necrosis 3) Renal tubular epithelial binucleation and karyomegaly 4) Hepatobiliary: bile duct proliferation	Undetermined; possibilities include sepsis or myocardial necrosis	
4	04/23/3014	M	A	Unk	RBC; Unk	1) Skeletal muscle: marked focal subacute neutrophilic and histiocytic myositis 2) Skin: multifocal acute erosive and ulcerative dermatitis 3) Great vessels: multifocal moderate chronic vascular mineralization 4) Renal tubular epithelial binucleation	Septicemia	
5	10/30/2014	Unk	J	35g	RBC; CB (FR)	1) Marked subcutaneous, coelomic edema and mild coelomic effusion 2) Gall bladder: marked diffuse gall bladder distention with cholelithiasis 3) Pancreatic/bile ducts: moderate ductular fibrosis with intralesional nematode organisms 4) Renal tubular epithelial binucleation 5) Hepatobiliary: bile duct proliferation	Undetermined	
6	01/15/2015	F	J	59g	RBC; WC	1) Marked, focal lateral scoliosis 2) Renal tubular epithelial binucleation	Undetermined	
7	02/04/2015	M	J	77g	RBC; CB (FR)	1) Moderate coelomic effusion 2) Lung: Multifocal, moderate, subacute aspiration pneumonia 3) Intestine: Multifocal, moderate granulocytic enteritis 4) Ilium: focal, cortical incongruity with medullary granulation tissue 5) Renal tubular epithelial binucleation 6) Hepatobiliary: bile duct proliferation	Aspiration pneumonia	
8	03/06/2015	F	A	Unk	RBC; WC	1) Mild coelomic effusion 2) Left kidney, liver, pylorus, intestine: renal adenocarcinoma 3) Liver: marked sinusoidal extramedullary hematopoiesis 4) Renal tubular epithelial binucleation	Renal adenocarcinoma with multifocal metastasis	Liver: Ziehl Neelsen acid fast stain negative Renal adenocarcinoma: Herpesvirus negative by PCR
9	10/19/2015	M	A	Unk	RBC; CB (FR)	Marked postmortem autolysis prevented interpretation	Undetermined	
10	04/25/2016	M	A	138g	RBC; WC	1) Marked subcutaneous edema; marked coelomic and pericardial effusion 2) Heart: Moderate to marked diffuse vacuolar cardiomyopathy with atrial dilation 3) Kidney: Mild to moderate, multifocal, chronic lymphoplasmacytic interstitial nephritis, renal tubular epithelial binucleation, renal epithelial karyomegaly and vacuolation 4) Hepatobiliary: Mild, multifocal, chronic portal lymphocytic hepatitis; bile duct proliferation	Congestive heart failure, presumed	
11	03/01/2017	M	2y A	Unk	RBC; CB (NCSU)	1) Moderate coelomic and pericardial effusion; moderate subcutaneous edema 2) Hepatobiliary: Mild, diffuse, microvesicular lipidosis; bile duct proliferation 3) Stomach, small intestine: Moderate, multifocal lymphangiectasia 4) Kidney: Mild, heterophilic and lymphohistiocytic interstitial nephritis; mild, membranous glomerulopathy, renal tubular epithelial binucleation, renal tubular epithelial karyomegaly and vacuolation	Undetermined; membranous glomerulopathy and lymphangiectasia considered	Kidney: Congo red negative and PAS positive consistent with segmental membranous glomerulonephritis
12	01/09/2018	F	A	Unk	RBC; CB (NCSU)	1) Marked coelomic effusion, anasarca 2) Polycystic kidney disease with tubular epithelial karyomegaly and vacuolation; renal tubular epithelial binucleation 3) Hepatobiliary: bile duct proliferation	Polycystic kidney disease/ renal insufficiency	
13	08/09/2018	M	A	369g	RBC; CB (NCSU)	1) Marked, diffuse, subcutaneous edema and coelomic effusion 2) Hepatobiliary: Moderate to marked, portal, heterophilic, and lymphoplasmacytic hepatitis; bile duct proliferation 3) Kidneys: bilateral, moderate, diffuse tubulointerstitial, lymphoplasmacytic, and histiocytic nephritis with marked tubular ectasia and intralesional cocci 4) Gastrointestinal tract: moderate to marked submucosal edema	Bacterial septicemia	*Citrobacter freundii* cultured from the liver
14	08/13/2018	M	A	206g	RBC; WC	1) Marked, diffuse anasarca, moderate coelomic effusion 2) Kidneys: Severe, chronic, tubulonephropathy 3) Hepatobiliary: moderate, diffuse hepatocellular vacuolation (glycogen- and lipid-type); bile duct proliferation	Chronic renal disease and/or septicemia	*Enterococcus faecium* was cultured from coelomic fluid (in thio only)

Figure 1. (A) and (B) Summary table including case number, date submitted to necropsy, sex (F: female, M: male, Unk: unknown), age (y: year, A: adult, J: Juvenile), weight in grams (g), source (CO: client-owned, CB: captive-bred, RBC: research breeding colony, WC: wild-caught, Unk: unknown; FR: Frog Ranch, NCSU: NC State University), necropsy findings, cause of death, and miscellaneous information including special stains, PCR, and culture findings.

mass was composed of a neoplastic epithelial cell population that expanded and effaced the colonic mucosa and invaded the submucosa along the margins of the mass. Neoplastic cells formed complex papillary and tubular structures supported by a moderate fibrovascular stroma (Figure 3 D). Within tubular and papillary structures, neoplastic cells formed layers of 4 to 5 cells thick and exhibited distinct loss of nuclear polarity. Neoplastic cells exhibited abundant eosinophilic cytoplasm and oval, round, irregularly shaped, granular to vesicular nuclei with one moderately sized nucleolus (Figure 3 D). Numerous neoplastic cells contained an intracytoplasmic vacuole that peripheralized the cytoplasm and nucleus (reminiscent of signet ring morphology), as well as moderate anisocytosis and anisokaryosis, multifocal binucleation or multinucleation, and a high mitotic rate (Figure 3 D).

Relatedness of frogs with renal and hepatic lesions. We sought to determine whether the source of the frogs (wild-caught compared with commercially bred) and the relatedness of the frogs was associated with the development of renal and hepatic lesions. Thirteen of 14 frogs in this retrospective study were part of a research breeding colony, and multiple frogs were related (Figure 4). Six frogs were wild-caught; 3 were the offspring of wild-caught frogs, and 4 frogs were acquired from commercial breeders. The origin of one of the frogs from the research colony (wild-caught, offspring of wild-caught, or commercial breeder) could not be determined. Renal binucleation and bile duct proliferation occurred in frogs from all 3 populations (Figure 4). However, all of the frogs from the research colony with evidence of tubular epithelial atypia (karyomegaly, cytoplasmic vacuolization) were either wild-caught or descended from wild-caught parents (Figure 4).

Other disease processes and histologic lesions. Frogs commonly exhibited abnormal fluid accumulations, including coelomic effusion (10 of 14; Case nos 1, 2, 5, 7, 8, 10 to 14) (Figure 5 A), pericardial effusion (3 of 14; Case nos 1, 10, 11), generalized subcutaneous edema (anasarca) (6 of 14; Case nos 1, 5, 10 to 14),

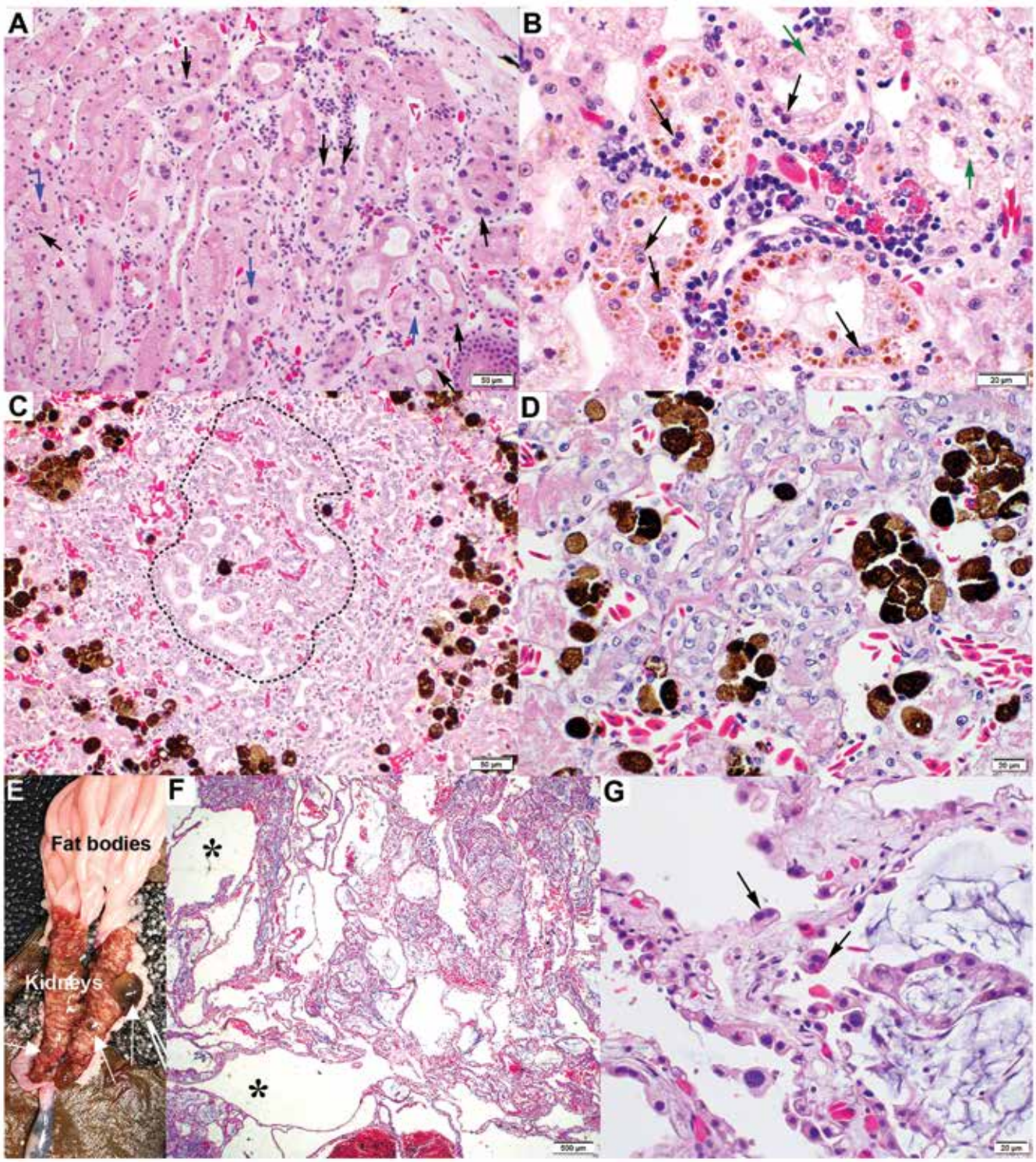

Figure 2. Renal and hepatobiliary abnormalities. A and B. Case no. 11. Kidney. Hematoxylin and eosin [HE]. Renal tubules are lined with epithelial cells displaying binucleation (black arrows) and varying degrees of atypia, including karyomegaly (blue arrows) and cytoplasmic vacuolation (green arrows). C and D. Case no. 3 and no. 10. Liver. HE. Variably sized, randomly distributed bile duct clusters are found throughout the liver. The region of biliary hyperplasia is outlined by a black dotted line. E and G. Polycystic kidney disease. (E) Case no. 12. Gross image of the right and left kidneys with variably sized cystic, clear fluid containing cavities (white arrows). (F) HE. Widespread, severe renal tubular dilation and ectasia (asterisks) causing marked expansion of the renal parenchyma. (G) HE. In regions of marked ectasia, the renal tubules are lined by epithelial cells with moderate anisocytosis, anisokaryosis, occasional karyomegaly and frequent binucleation (arrow). Histologic images were acquired at the following magnifications: A, 200×; B, 400×; C, 200×; D, 400×; F, 20×; G, 400×.

pulmonary edema (Figure 5 B; Case no. 5), and tracheal edema (Case no. 5).

Other disease processes and histologic lesions observed in this frog population included: diffuse vacuolation of ventricular cardiomyocytes (Figure 5 C; Case no. 10); aspiration pneumonia (Figure 5 D; Case no. 7); and endoparasitism of the pancreatic/bile duct, stomach, and intestine (Figure 5 E; Case nos 1, 2, 5).

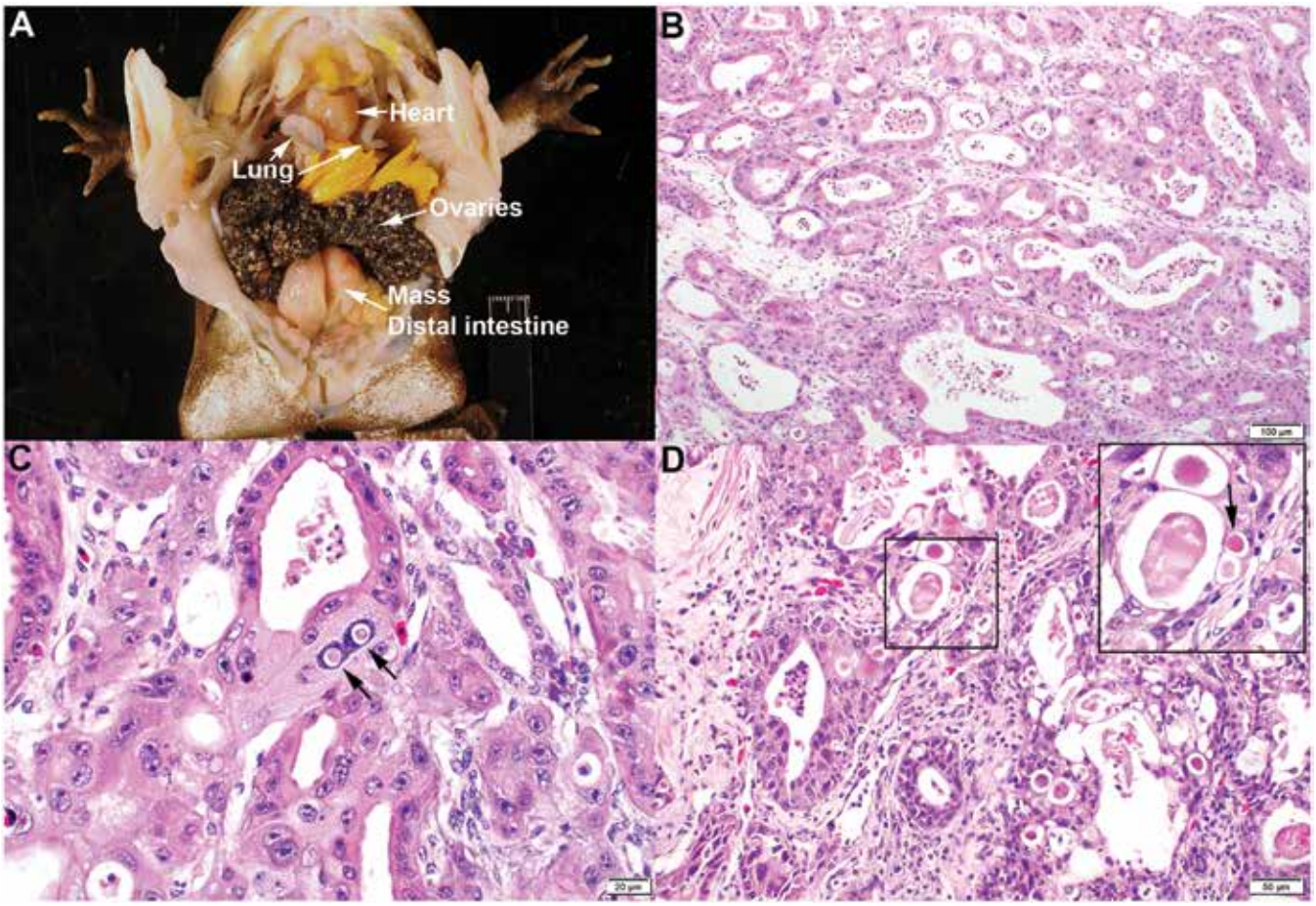

Figure 3. Adenocarcinoma. A and B. Case no. 8. Renal Adenocarcinoma with metastasis to the liver and gastrointestinal tract. (A) Gross image of a 2 cm^3 firm, multilobulated, pink to tan, broad-based mural mass within the distal intestine. (B) Hematoxylin and eosin [HE]. Renal parenchyma is markedly expanded by a neoplastic epithelial cell population forming variably size tubules and supported by a loose fibrovascular stroma. (C) HE. Renal neoplastic cells display marked anisokaryosis and anisocytosis as well as cytomegaly, karyomegaly and multinucleation. Rarely, nuclei contained round pale eosinophilic inclusions (viral inclusion or cytoplasmic invaginations)(arrows). (D) Case no. 1. Intestinal adenocarcinoma. Colon. HE. Colonic mass composed of neoplastic epithelial cells arranged in papillary and tubular structures with a moderate fibrovascular stroma. Cells within the neoplastic population display moderate anisocytosis and anisokaryosis with multifocal binucleation or multinucleation. Tubules frequently contain abundant cellular and necrotic nuclear debris. Numerous neoplastic cells exhibit an intracytoplasmic vacuole that is clear or contains an eosinophilic to amphophilic globule, with peripheralization of the cytoplasm and nucleus (signet ring morphology)(arrow). Histologic images were acquired at the following magnifications: B, 100×; C, 400×; D, 200×.

Discussion

In this retrospective study, we sought to determine histologic lesions and disease processes occurring in Budget's frogs (*L. laevis*). Despite the popularity of *L. laevis* among amphibian hobbyists and the increasing use of this species as an animal model in biologic research, there is minimal scientific literature that describes pathologic examination of healthy or diseased animals. The 14 frogs reported in this study, most of which were derived from a research breeding colony, died or were euthanized and were submitted for postmortem examination. Due to the small size of the research colony at NC State University and the relative difficulty of breeding this species in captivity, our observations are currently limited to unhealthy animals. Since these animals were not admitted as patients to the NC State Veterinary Hospital, there is limited clinical history available to provide insight into clinical conditions that may have preceded death or euthanasia.

In this population, histologic changes were most commonly observed in the kidneys and liver. In the kidney, common findings were renal tubular epithelial binucleation, karyomegaly, and cytoplasmic vacuolization. In the liver, biliary proliferation was common and occurred concurrently with one or more of the described renal findings. We also found 2 cases of polycystic kidney disease and 2 cases of spontaneous neoplasia. The remaining findings were generally uncommon in the total study population.

Renal tubular epithelial binucleation was previously described in a presumably healthy Australian desert frog (*Cyclorana alboguttatus*).[8] *C. alboguttatus* and *L. batrachus* are in the same superfamily of frogs, Hyloidea. Binucleation of renal tubular epithelium could be a common histologic feature in this superfamily of frogs or the Anuran order. We also observed regions of renal tubular epithelial atypia, characterized by karyomegaly and cytoplasmic vacuolation. Renal epithelial cytoplasmic vacuolation may be attributed to intracellular accumulations of lipid or glycogen; however, postmortem swelling of the smooth endoplasmic reticulum cannot be ruled out. Another possibility is that glycogen or lipid accumulation (or both), in addition to karyomegaly, may indicate renal epithelial cell stress or damage. These lesions were observed in wild-caught frogs and their offspring (Figure 4). This could be attributed to the smaller sample size of frogs originating from captive bred animals (n = 3), or it

Case Number	Source *related	Renal binucleation	Karyomegaly	Tubular epithelial vacuolation	Polycystic kidney	Renal carcinoma	Bile duct proliferation
1	Client-Owned	X	X	X	X		X
2	Wild-caught	X					
3	Wild-caught	X	X				X
4	Unknown	X					
5	Frog Ranch	X					X
6	Wild-caught	X					
7	Frog Ranch	X					X
8	Wild-caught*	X	X			X	
9	Frog Ranch						
10	Wild-caught	X	X	X			X
11	Captive-Bred*	X	X	X			X
12	Captive-Bred*	X	X	X	X		X
13	Captive-Bred*						X
14	Wild-caught						X

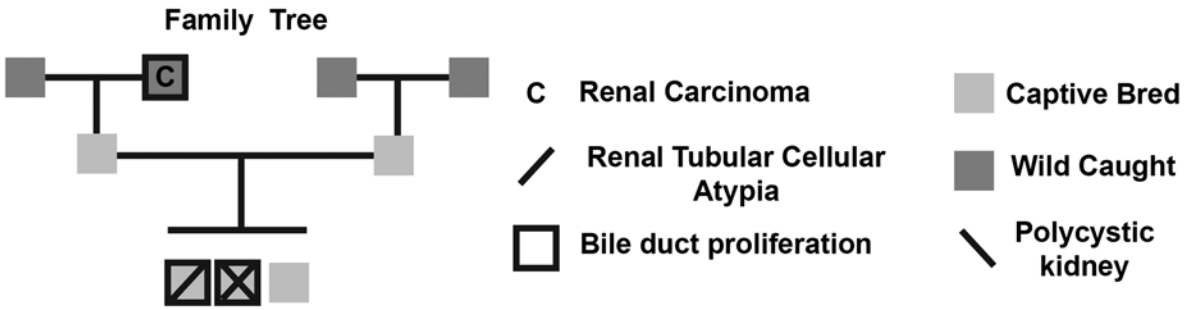

Figure 4. Correlation between renal and hepatobiliary findings and relatedness of individual *L. laevis* frogs. More renal and biliary changes, including renal tubular epithelial karyomegaly, vacuolation, and bile duct proliferation, were found in wild-caught frogs and their offspring as compared with other captive-bred individuals.

could reflect a heritable contribution. Distinguishing between these possibilities will require a larger study population than is currently available.

We identified 2 cases of polycystic kidney disease (PKD) in *L. laevis*. In humans, PKD is an autosomal dominant (ADPKD) or autosomal recessive (ARPKD) inherited disorder characterized by the development of fluid-filled renal cysts leading to progressive renal enlargement and renal insufficiency.[22] In cats, PKD is an autosomal dominant inherited disease affecting mostly Persian breeds.[1] In both humans and cats, ADPKD is associated with mutations in polycystin-1 (PKD1) and polycystin-2 (PKD2).[1,22] Interestingly, a similar condition has been described previously in Japanese (*Bufo japonicus*) and Chinese *(Bufo raddei)* hybrid toads in which PKD has been linked to progression to renal cell carcinoma.[18] The presence of cellular atypia and micropapillary formation in polycystic kidneys from *B. japonicus* and *B. raddei* hybrids, which were also observed in polycystic kidneys from *L. laevis*, were thought to reflect early transition to neoplasia.[18] Due to the small size of the current study population, it is unclear if there is a relationship between renal tubular epithelial atypia, polycystic kidney disease, and renal carcinoma in *L. laevis*. It is possible that *L. laevis* may provide a novel model for studying spontaneous polycystic kidney disease and renal neoplastic transformation. In mammalian species, the coupling of polycystic kidney structures and transition to renal adenocarcinoma has been described in humans in 2 autosomal dominant hereditary conditions and in cats.[1,14]

Biliary duct proliferation was one of the most common abnormalities observed in both captive-bred and wild-caught individuals. Biliary proliferation can occur due to a type of ductal reaction in which hepatic stem cells proliferate with the potential to develop into hepatocytes or biliary epithelium. In humans, bile duct proliferation is a histologic marker of liver disease, particularly cholestatic injury.[5] We found biliary proliferation in 2 animals with hepatic disease (hepatic congestion secondary to heart failure and hepatitis secondary to bacterial septicemia; Case nos 10,13). In these cases, ductal reaction is likely secondary to background injury in which biliary proliferation occurs due to a need to replace missing hepatocellular mass. We also saw a subjective increase in the number of melanomacrophages within the liver of individuals with biliary proliferation, compared with other animals within this study population that did not exhibit biliary proliferation. In other species, melanomacrophages are known to play an immune function and may increase within a background of inflammation. However, we also observed an association between bile duct proliferation and renal abnormalities (Figure 4). In humans with autosomal dominant polycystic kidney disease in which the most common mutation is PKD1, the development of polycystic liver and biliary tract disease is a common extrarenal manifestation.[13] A similar genetic link could potentially account for the common renal and biliary lesions we observed in this *L. laevis* population.

We identified 2 different spontaneously occurring adenocarcinomas (renal and colonic). Within the renal adenocarcinoma, we identified rare, pale, eosinophilic intranuclear inclusion bodies. Inclusion bodies can arise in cells infected with certain viruses, such as herpesviruses, and are composed of aggregated viral

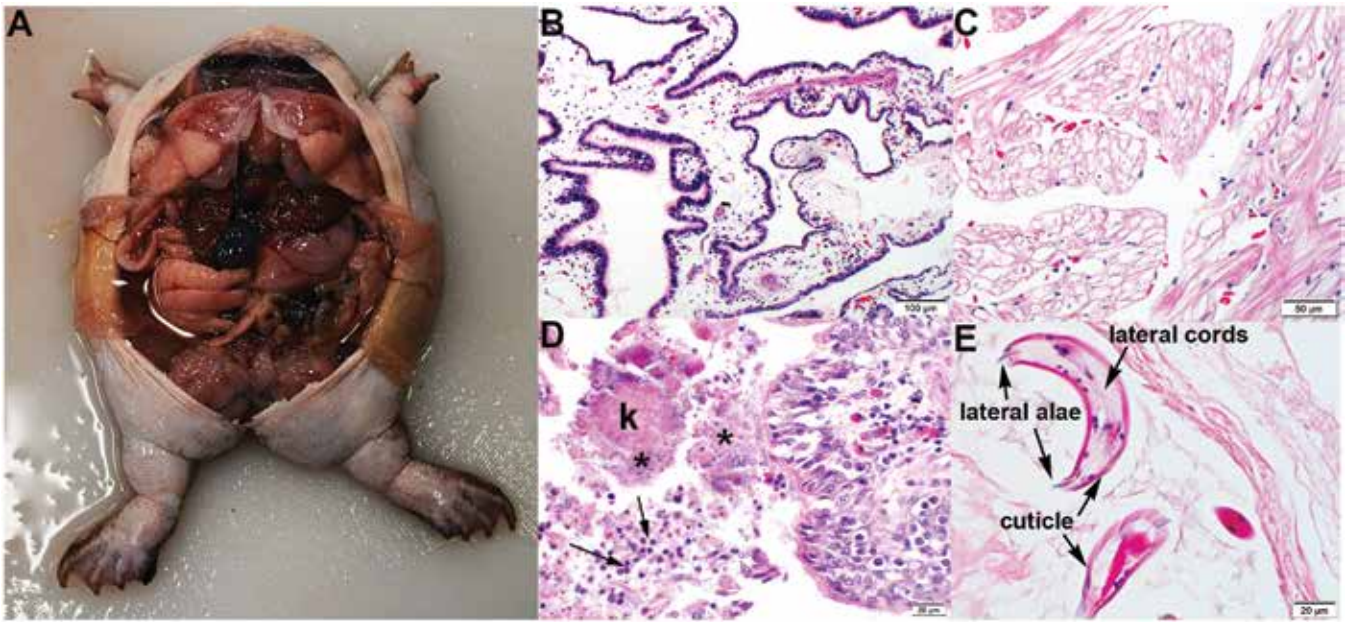

Figure 5. Other contributors to morbidity and mortality. (A) Case no. 13. Subcutaneous edema (anasarca) and coelomic effusion. Gross photograph demonstrating marked, diffuse subcutaneous edema. The coelomic cavity is also markedly distended by approximately 50 mL of clear, watery fluid (effusion). (B) HE. Case no. 5. Lung. Interstitial edema. The pulmonary interstitium is expanded by edema and contains scattered infiltrates of granulocytes. (C) HE. Case no. 10. Heart, ventricle. Cardiomyocyte vacuolation. Diffusely, cardiomyocytes are moderately distended by clear intracytoplasmic vacuoles (glycogen or fluid accumulation). (D) HE. Case no. 7. Lung. Aspiration pneumonia. Falveoli contains abundant mononuclear leukocytes (black arrows), cellular and karyorrhectic debris (k), and myriad bacteria (asterisks) with moderate septal granulocyte infiltrate. (E) HE. Case no. 5. Pancreatic/bile duct. Nematodiasis. The pancreatic/bile duct exhibits moderate periductular fibrosis and contains multiple larval nematodes with a thick eosinophilic cuticle, lateral cords, and lateral alae. Histologic images were acquired at the following magnifications: B, 100×; C, 200×; D, 400×; E, 400×.

proteins. Alternatively, cytoplasmic invaginations, or "pseudoinclusions", can be observed in cell nuclei, and have been described previously in tumors from various animal species, including renal adenocarcinomas.[25,28] In the leopard frog, *Rana pipiens*, renal carcinoma has previously been described in association with Lucke herpesvirus (Ranid herpesvirus-1).[15,16,24] Therefore, we considered the possibility that the inclusion bodies observed in this case were of viral origin. However, PCR results were negative for Ranid herpesvirus-1. We presume that the observed inclusions are cytoplasmic invaginations; however other infectious etiologies cannot be ruled out. Another possibility is that the development of renal adenocarcinoma in this frog represents a progression from polycystic kidney disease, as described above in hybrid toads. However, no evidence of polycystic kidney disease was observed in the nonneoplastic kidney from this frog. Tumors of the gastrointestinal tract in frogs are thought to be rare, with only 4 other cases of intestinal adenocarcinoma reported in the amazon milk frog (*Trachycephalus resinifictrix*), the giant marine toad (*Bufo marinus*), the splendid tree frog (*Litoria splendid*) and the mountain chicken frog (*Leptodactylus fallax*).[17,26]

Coelomic effusion, pericardial effusion, and generalized anasarca were common nonspecific findings in this population of *L. laevis*. Fluid accumulation disorders are common in amphibians, due to their unique anatomy and physiology.[7] Frogs absorb most of their water through their skin where it is stored in large lymph sacs.[7] In addition, amphibian mesonephric kidneys have a special type of coelomostomic nephron that collects fluid directly from the coelomic cavity.[7] Therefore, any disease of the skin, lymphatics, or renal system can cause fluid accumulation.[7] In dendrobatid frogs, fluid accumulation was found most commonly in association with infection, renal disease, and gastrointestinal disease.[7] In this *L. laevis* population, we found abnormal fluid accumulations most commonly associated with renal changes including interstitial nephritis, chronic tubulonephropathy, polycystic kidney disease, renal tubular epithelial karyomegaly and vacuolization, and renal adenocarcinoma. Individuals with more severe kidney changes, especially animals with polycystic kidney disease and those with evidence of chronic kidney disease, exhibited moderate to severe effusions. Effusions and/or anasarca were also noted in individuals with bacteria septicemia and pulmonary, cardiac, or hepatobiliary abnormalities, ranging in severity from mild to marked (data not shown).

Endoparasitism was observed in a small number of individuals (Figure 1). Amphibian species can harbor a variety of endoparasites, including nematodes and cestodes.[32] Common sites for nematodiasis include the lung (often *Rhabdias* sp.) and intestine (often *Strongyloides* sp.), and adult nematodes, as were observed in this case, are typically encountered in these locations.[9,32] The clinical significance of intestinal nematodiasis in amphibians is variable, and is likely to be dependent on factors such as parasite burden and immune status. Cestodiasis is infrequently described in amphibian species, and is thought to be of limited clinical significance in the absence of a heavy parasite burden.[9,32] There are no specific reports of endoparasitism in *L. laevis*, although a survey of helminth parasites in amphibian species in Paraguay reported identification of a cestode (*Ophiotaenia cohospes*) from a related species, *Lepidobatrachus asper*.[19] The low number of parasites observed in frogs in this study suggests that these parasites were unlikely to have been major contributors to morbidity and mortality. Definitive diagnosis of nematode type would have been facilitated by examination of eggs, which were not present in the histologic sections examined.

Due to the small sample population of these relatively rare laboratory animals and the limited background information on

signalment and premortem symptoms, it is difficult to make any definitive clinical correlations. Based on the most common postmortem findings, clinicians may find it helpful to screen for signs of renal and hepatic disease in Budgett frogs, particularly those with fluid accumulations (edema and cavitary effusions). Frequent water quality evaluations may also be helpful, as this species of frogs may be especially sensitive to aqueous toxins, pH, and alkalinity due to a possible predisposition to renal and hepatic disease.

Many of the animals in this retrospective study were of unknown age, and therefore conclusions cannot be related to life-stage. Of the known ages, at least one individual (Case no. 1) was 18 y old. Many of the observed lesions could have been age-related, incidental, and/or unrelated to the cause of death in these frogs. Precise data on the average life span of *L. laevis* is not available, although hobbyists suggest that individuals can live for 15 to 20 y in captivity.[27] No sex predispositions were observed for any of the observed histologic changes.

This report is the first to describe postmortem pathologic lesions in captive *L. laevis* frogs, which are an emerging animal model in biologic research. Our findings provide new insight into the variety of histologic lesions and disease conditions present in captive *L. laevis* populations.

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