LEPTODACTYLUS FALLAX POST MORTEM EXAMINATION

Prosector:…… **Date:…../……/…….** **Path No:…….**

SPECIMEN DETAILS		
Local ID:	GAN:	Sex:
Hatched:…../…../…. Metamorphosed:…./…../….		Age: tadpole / metamorph / juvenile / young adult / adult / old adult
Found dead:…../…../…. Post mortem:…../…../….		State of preservation: good / fair / poor / marked autolysis
Storage since death: fresh / refrigerated / ambient temperature / frozen / fixed with:		

Carefully examine the frog following the steps laid down in this form. At each step, check the last column for samples to be collected.
Whenever *you observe something that you think it* ***may be a lesion or abnormal*** *always: take* ***pictures***, *take a* ***bacteriology swab***, *take samples in* ***formalin*** *and* ***frozen***. Describe the lesion as best as you can, including: Location, size (and numbers or percentage of organ involved), shape, consistency, colour, contents.

1- PREPARATION		Standard pictures to take	**standard samples**
Read microchip. Take morphometric measures.Take two swabs from drink patch for chytrid. Take a ventral and a dorsal picture			**X2 Chytrid swabs**
Give details of circumstances of death and clinical history. Use the back of this sheet if necessary.	**Weight**:……g **Snout-vent length**:……mm **Leg**:……mm		
2- EXTERNAL EXAMINATION			
Examine the skin for wounds, change in colour, ectoparasites, fly eggs or larvae. Manipulate bones to detect fractures. Open the mouth and check its contents, mucosa and tongue, push eyes out and examine them.			X1 3rd digit i n alcohol and digit in formalin Any lesions observed frozen and in formalin Both eyes in formalin.
Is the skin dry and dehydrated? What is the colour of the ventral skin? Are there ulcers on the tip of the toes? Is skin sloughing and how: in large sheaths? or in small, brown bits?. Any other lesions?			

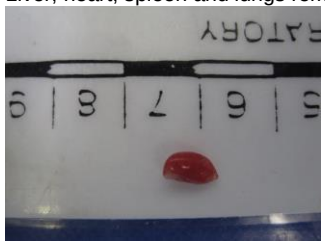
Any lesion or contents in oral cavity? Eyes cloudy?			
3- MUSCLE AND LYMPH SACS			
Using scissors, open ventral skin from most caudal point to tip of mandible and separate it from underlying tissues. Make transversal sections to reflect skin and increase field. Remove skin from ventral thigh area. Take picture. Examine ventral and thigh muscles. Examine lymph sacs.			X1 thigh skin in alcohol, x1 thigh skin in formalin
Give an idea of muscle mass in thighs. Any lesion or change in colour? Acanthocephalan parasites (look like rice grains embedded in muscle)? Any fluid in lymph sac? How much? Describe.			
4- BODY CAVITY			
Open muscle layer with scissor along mid line, making sure not to damage underlying organs. Cut cranially all the way trough sternum and until tip of mandible. Cut transversally to increase field. Then take a picture. Collect any free fluid. Observe the lay out and general aspect of all organs without disturbing normal anatomy. Carefully remove fat bodies.			Free fluid frozen + x2 air dried smears + one bacteriology swab. Fat body frozen and in formalin.
How organs compare in relation to each other? Any abnormally large or small organ? Any lesions observed? Any fluid? How much? Describe. **Locate the urinary bladder**. Is it adhered to intestines? What are its contents like? (normally contains clear urine or nothing). **Check the intestines** are they enlarged? Distended, thin walled and full of pasty contents? Are there fat bodies? How big? Remove and weigh.	Fat bodies weight:…..g		
If urinary bladder and/or intestines are distended they will be very fragile, handle them with care making sure don't break and that the anatomy is preserved.			

5- HEART, LIVER AND SPLEEN

Remove the heart by cutting blood vessels at base. If carcase is fresh, make a blood smear from heart blood.
Separate the liver from the intestines and remove. Find the spleen and remove. Examine each of these organs. Take picture of heart, spleen and liver together.
Take a photo of the coelomic cavity (to include,GIT, lungs, reproductive organs and kidneys). Cut heart in ½ and liver in several small pieces. Examine the cut surface. Open the gall bladder and assess contents.

Liver, heart, spleen and lungs removed

Any lesions in surface of liver or heart? What colour is the liver? What size is the gall bladder and what are its contents like? Is there a sandy content? Is there a large stone?	Gall bladder measurements (diameter) if present: mm



Spleen

Heart

x2 Heart blood smears

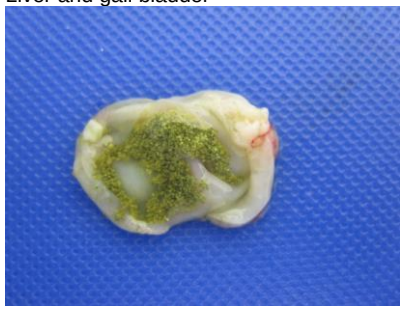
Liver and heart in formalin

Liver and heart frozen

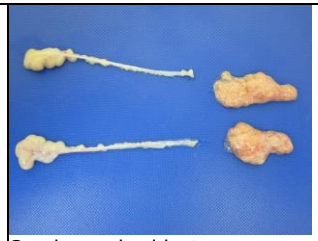
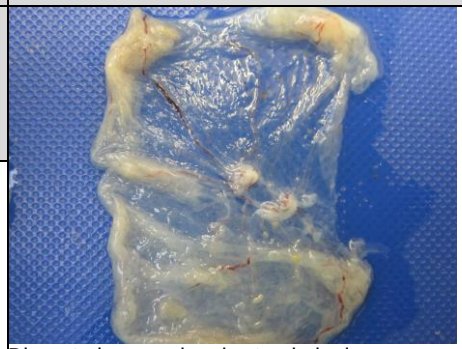
Spleen frozen and in formalin

X1 gall bladder swab and send for bacteriology

Store gall bladder stone if present in plain pot.

		 Liver and gall bladder  Gallbladder contents	
6- LUNGS			
Examine glottis. Cut lungs at bronchus and remove. Check thoroughly one lung for nematodes (either cutting one side and stretching it or by in evaginating it by placing it on finger. Place all nematodes in alcohol. Then cut lung in ½ and place ½ in alcohol and ½ in formalin. Put the second lung whole in alcohol in same pot as nematodes		 Larynx and lungs opened from dorsal aspect	x1 lung + nematodes in alcohol lung frozen lung formalin
Any lesions observed? Any parasites? Describe.			

7- KIDNEYS AND REPRODUCTIVE TRACT			
Gently pull GIT caudally while cutting membranes until gastrointestinal tract is reflected caudally, and the coelomic cavity empty except for kidneys and gonads. Take a picture (including GIT outside of coelom). Remove gonads, cut a section to put in formalin. Remove kidneys, examine.		 Heart, liver, lungs and spleen removed, GIT retracted caudally, notice fat bodies, kidneys and ovaries.	x1 kidney frozen x1 kidney formalin Gonad (section) formalin
What is the size of the gonads? Describe. Is the ovary full of follicles? What is the size and colour of kidneys? Are they symmetric? Haemorrhage? Any round, white mass?			

		 Ovaries and oviducts  Kidneys, testis and Atrophic fat bodies	
8- URINARY BLADDER			
Examine wall of urinary bladder With bone cutters or strong scissors, cut through middle of pubic bone and pull apart to expose the pelvic canal and distal portion of rectum. Follow and check bladder wall until attachment with large intestine. Cut a small section of the bladder wall (over a lesion if there is one) divide in two for freezing and formalin. Access the contents through the whole made. Take a swab for bacteriology, collect contents in bijoux for freezing and all the rest in alcohol. Gently wash inside of bladder with water, examine surface, examine connexion with cloaca			
Are there any lesions on the bladder wall: lumps, thickening, haemorrhage or adhesions to other organs.? (Normally thin, pinkish and transparent) Is the urinary bladder attached to large intestine? If so, is there a fistula connecting both organs? Can you identify the natural opening to the cloaca? Are there any lesions (sweeling, haemorrhage? Etc)		 Dissected, opened and extended urinary bladder	Bacteriology swab fro m urinary bladder contents Bladder contents frozen in bijoux. Rest of contents in alcohol Bladder wall frozen bladder wall in formalin

8- GASTROINTESTINAL TRACT (GIT)

Examine the length GIT. Identify any lesions, and adhesions to other organs. Open gastrointestinal tract longitudinally from oesophagus to cloaca. Take picture. Collect bacteriology swab from large intestine contents. Examine contents Remove and preserve all contents in alcohol. Take picture. Examine mucosa. Examine distal small intestine, large intestine and rectum for lumps, adhesions or fistulae, Take pictures, take small sections of lesion frozen and in formalin. Place parasites in alcohol. Separate GIT from body by cutting around cloacal opening and place whole (including urinary bladder) in a separate, large pot of formalin.

Describe the stomach contents (quantitiy and quality). Describe the GIT wall: Is it thick and fleshy or thin like a membrane? Any parasites? Any areas of haemorrhage? Is the small intestine or large intestine distended? How much (compare to coelomic cavity volume)? Any mass on the intestinal wall? Any adhesions to bladder? Any fistula or rupture?		 Gastrointestinal tract  Empty stomach with small amount of mucus 	GIT + Urinary bladder in formalin. GIT contents in alcohol. LI contents bacteriology swab. From any mass or lesion observed: one piece in formalin and one piece frozen. Any parasites found in a bijoux pot of alcohol.

		Large intestine mucosa after removing content	
9- NERVOUS SYSTEM			
Cut both sciatic plexus and place in formalin. Expose the brain from the ventral aspect of the cranium by removing the thin layer of bone above palate with scissors. Take picture. Gently remove brain and placed in formalin		 Brain	Brain and sciatic nerve in formalin.
Any observations?			
10- FINISH			
Collect section of thigh muscle and place in formalin. Remove one leg, remove muscles and place bones in alcohol, labelled. Double bag carcass remnants and freeze. Label all pots correctly: ID / SAMPLE / DATE / PRESERVATIVE. Use pencil to write labels and protect with layer of cello tape around pot. Send bacteriology samples to lab for analysis. Make sure all waste is incinerated and surfaces disinfected to prevent disease spread. Contact Javier.Lopez@chesterzoo.org or alberto.barbon@durrell.org attaching this form for decision on sample analysis and shipment.			Muscle in formalin Leg in alcohol for skeleton-chronology Carcass double bagged fro freezing.

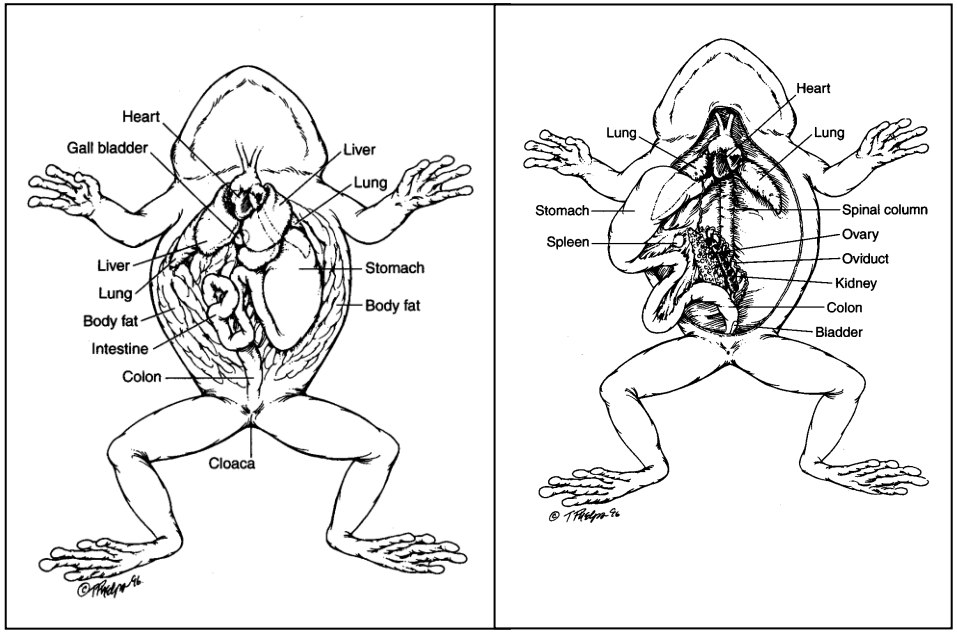
CHECK LIST FOR SAMPLES TO BE COLLECTED DURING POST MORTEM EXAMINATION

(Please tick the boxes to ensure all relevant samples have been collected. Blank cells indicate REQUIRED samples from all post mortems whether lesions are detected or not. Grey boxes indicate that samples are only required if a lesion has been observed. Crossed boxes mean sample not required.

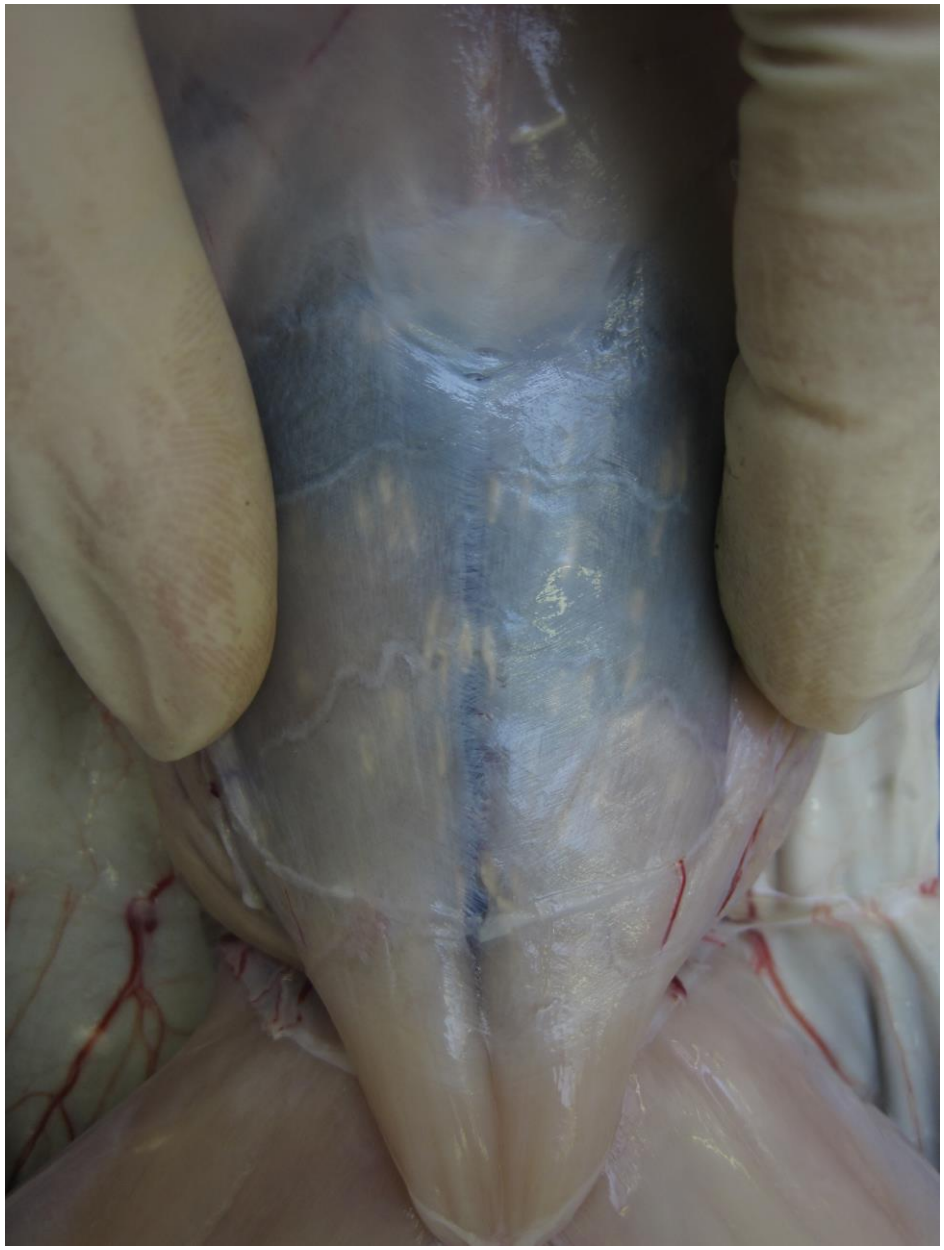
Sample	Frozen	Fixed in formalin	Smear	Fixed in alcohol	Bacteriology swab	
3rd digit						
Skin (drink patch)						
Skin and muscle (for DNA)						
Fat body						
Heart blood						
Heart						
Liver						
Spleen						
Kidney						
Gonad						
Lung						
Lung nematodes						
eyes						
GIT + urinary bladder						
GIT contents						
GIT nematodes						
Brain						
Sciatic plexus						
Thigh muscle						
Leg (skeleto-chronology)						
Carcass						
Other organ with abnormalities / lesions:	**Frozen**	**Fixed in formalin**	**Smear**	**Fixed in alcohol**	**Bact swab**	**PICS**
1 Coelomic fluid						
2 Lymph sac fluid						
3 Urinary bladder contents						
4 GIT mass or other lesion						
5						
6						
7						
8						
9						

Note:

- Frozen samples must be always maintained frozen. Thawing and refreezing will damage them.
- Chytrid swabs are best maintained in fridge but can be transported at room temperature.
- For fixing tissues in formalin or alcohol use a ratio of 10:1 fixative to tissue by volume and ensure all pieces are a maximum of 10mm cube.
- Prior to sending samples in formalin or alcohol, remove as much fixative as possible while maintaining tissue wet, close lid well. Wrap up pot in sufficient tissue paper to absorb any spillage and double bag.



Selected post mortem lesions



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Acantocephala embedded in muscle wall, incidental finding mainly in wild caught animals

Arthrosis, loss of articular cartilage, stifle.

Multiple granulomatous lesions in kidneys.

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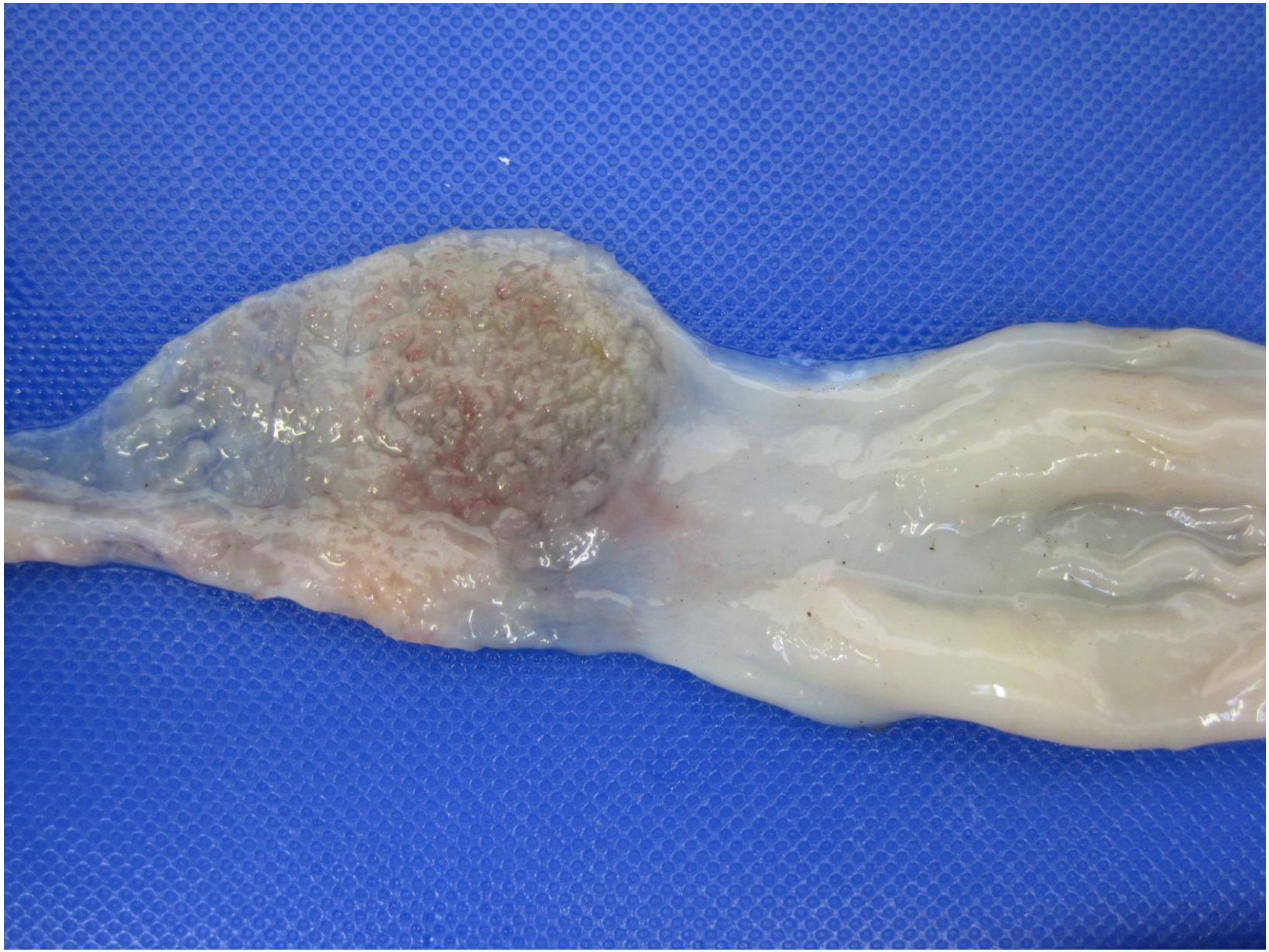

Local ulcerative enteritis, stenosis was suspected due to the large amount of intestinal content proximal to lesion. Histology showed diffuse mucosal ulceration, replaced by necrotic debris, heterophils, free erythrocytes and mixed bacteria, no signs of neoplastic transformation transformed cells in the section.

Severe distension of large intestine due to ingesta accumulation/impaction. Gastrointestinal tract dissected (right)

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Severe timpanism caused by distal intestinal adenocarcinoma, causing adhesions with urinary bladder, notice presence of nematodes is distended portion of the intestine.

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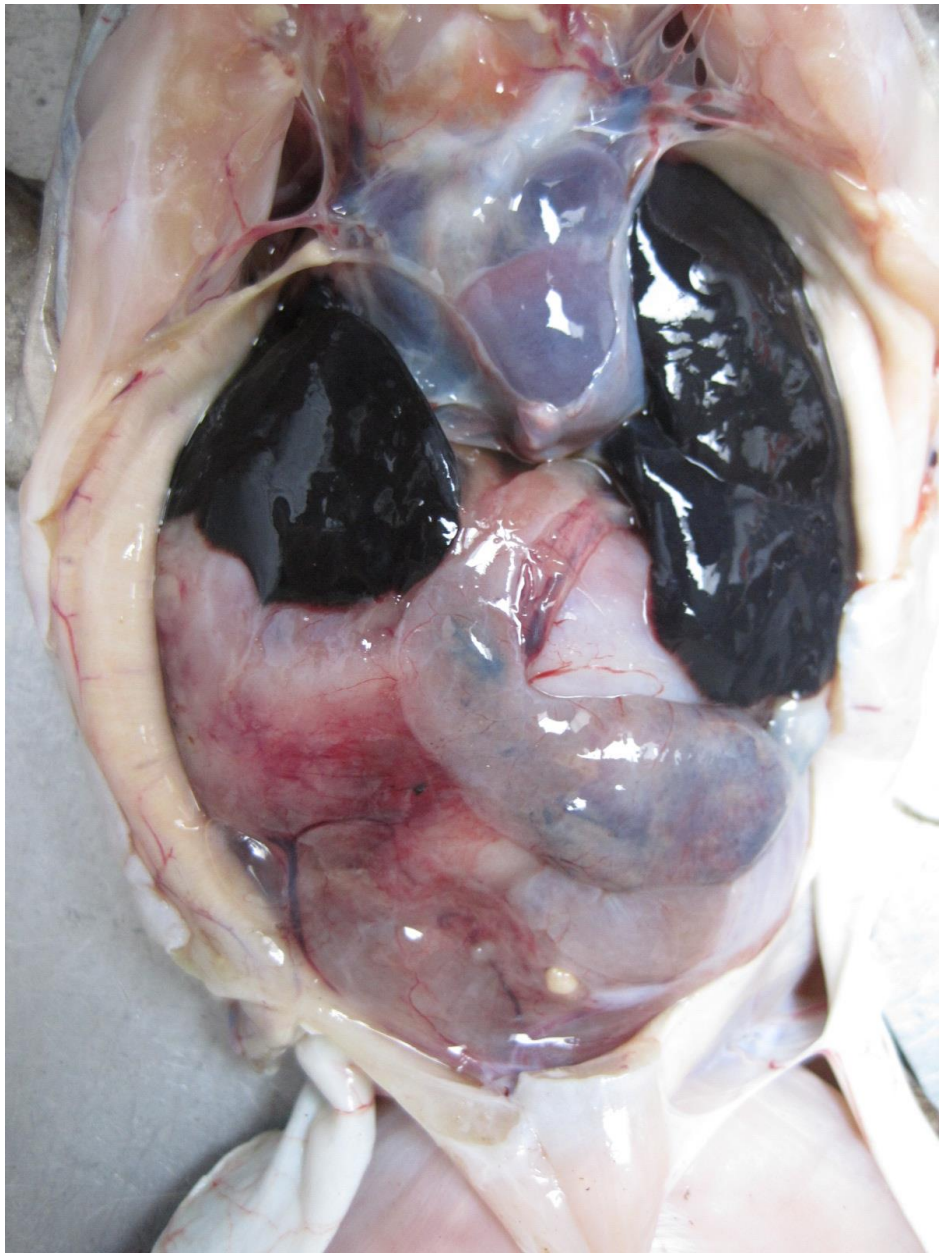

Intestinal adenocarcinoma, adhesions between urinary bladder and intestine.

Dissected gastrointestinal tract and urinary bladder in specimen diagnosed in intestinal adenocarcinoma.

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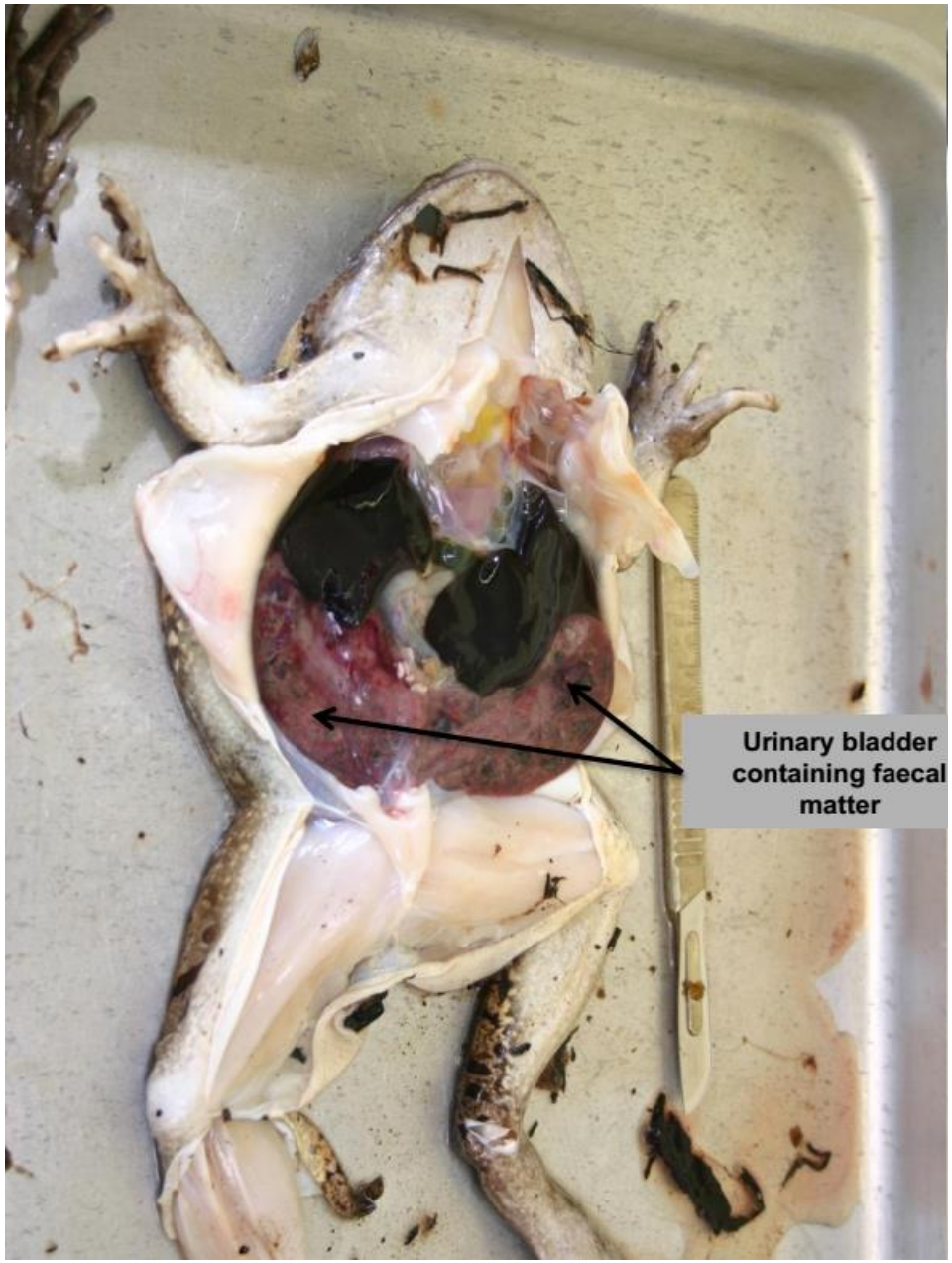
Adhesions between urinary bladder and liver (left), adhesion between bladder and large intestine (right)



Cistitis and urinary bladder filled with faecal material due to fistula between colong and urinary bladder

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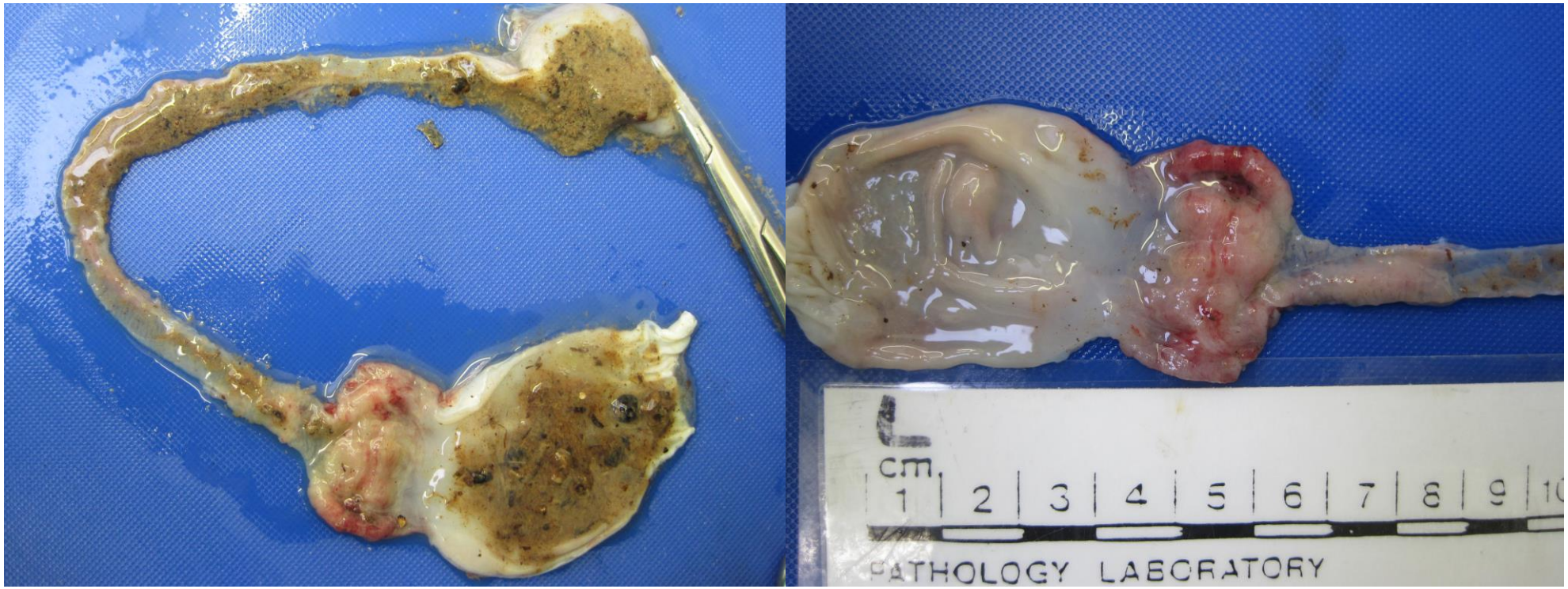
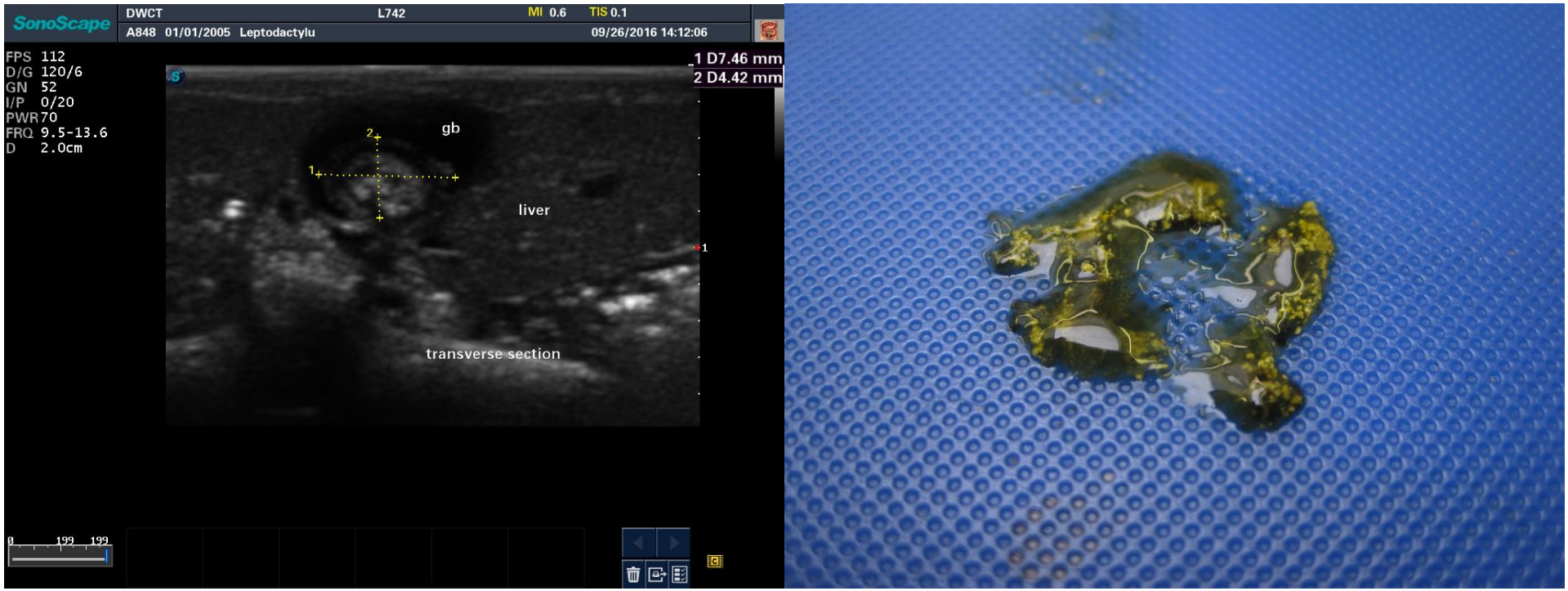


Intestines (cranial section at the tip of forceps). Severe chronic hyperplastic lymphocytic to lymphohistiocytic colitis, with focal intramural fibrosis, diagnosed in histology from grossly abnormal large intestine section, no evidence of neoplasia



Gall bladder content appearance in ultrasound antemortem and gall bladder content appearance at post mortem from the same animal.

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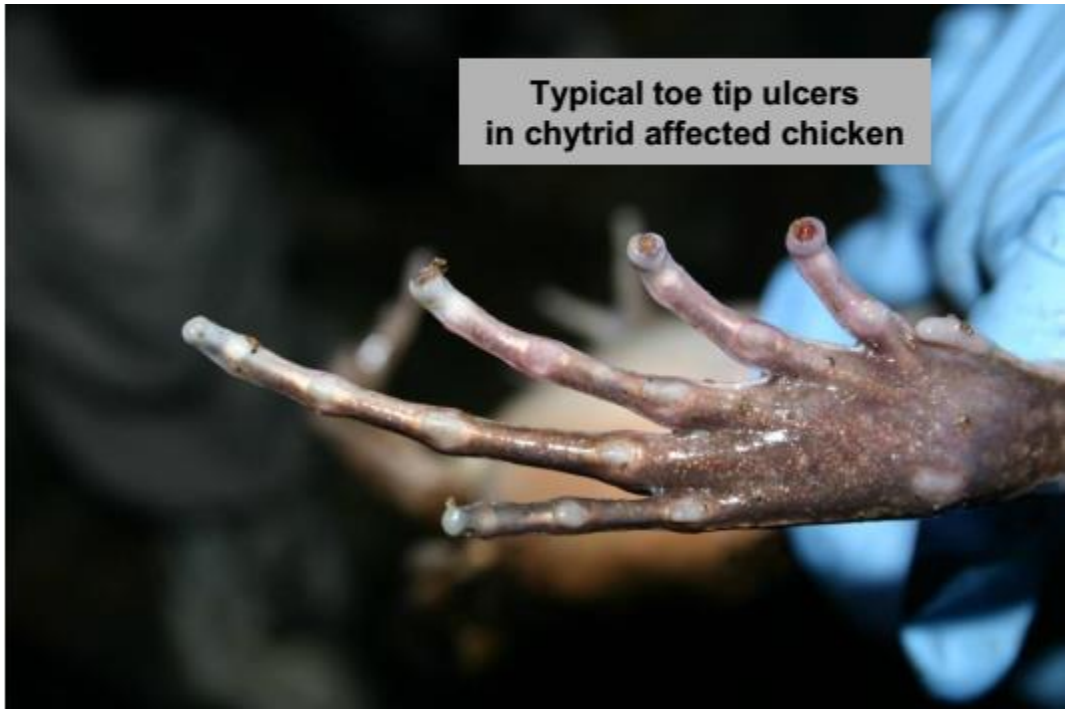

Ulceration in the tip of the digits

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