Pathology Diagnostic Exercise

Training course

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2023-2024

Archives

- Fresh and fixed tissue storage
- Blocks and slides storage
- Digital pathology:
  - Database for scanned slides

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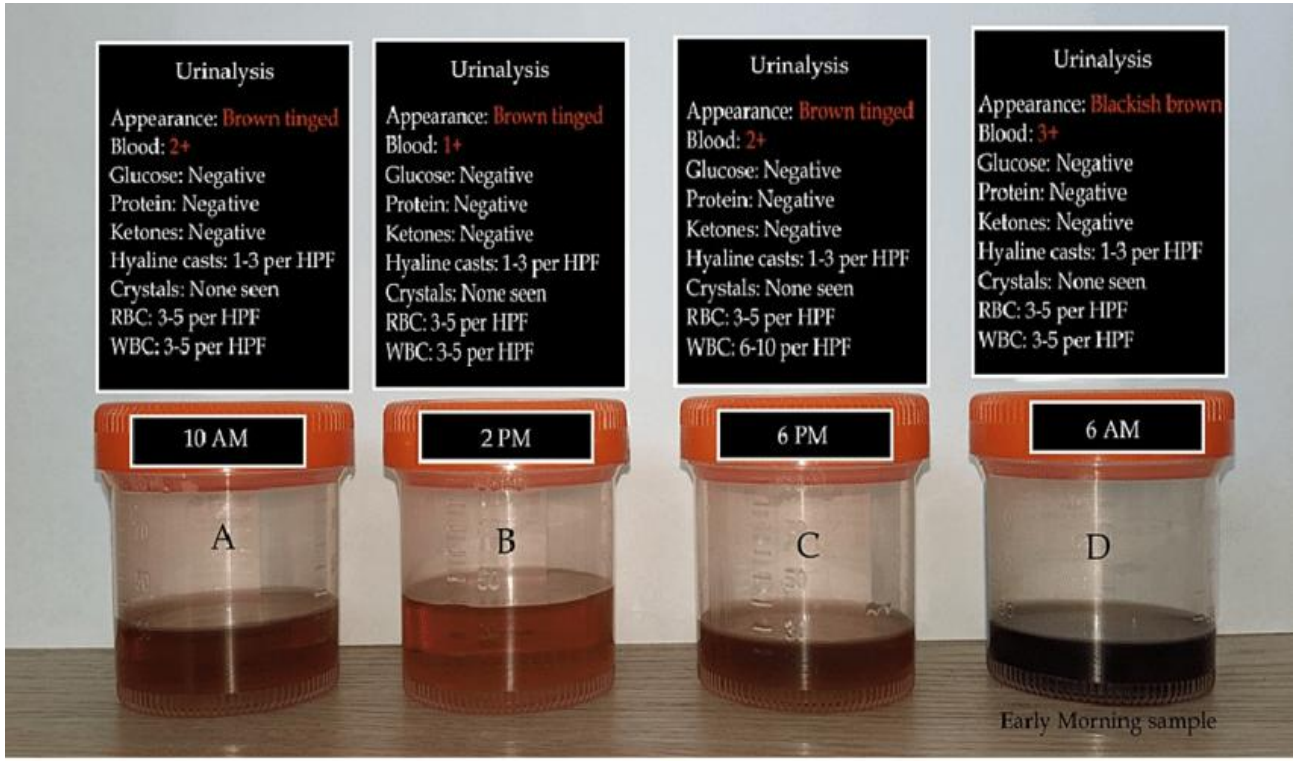
Diagnostic Exercise
From The Davis-Thompson Foundation

Case #: **208**; Month: **March**; Year: **2023**

Morphologic Diagnoses:

- Kidney, hemoglobinuric nephrosis;
- Mucosal and serosal surfaces,
- hemoglobinuric imbibition,
- diffuse.

Urine samples: heamoglobinuria



Typical Gross Findings:

- The gross findings are typical of an **acute intravascular hemolytic** crisis. They include mucosal and serosal hemoglobinuric imbibition, occasionally accompanied by
- **icterus**,
- diffusely dark-red kidneys (**hemoglobinuric nephrosis**) and
- dark-red tinged urine (hemoglobinuria).
- The spleen is generally enlarged, dark red and soft, and
- the liver might be enlarged and diffusely orange due to bilirubin accumulation.

Typical Microscopic Findings: Figure 4

•Diffuse hemoglobinuric nephrosis is histologically characterized by:

➢ acute tubular epithelial degeneration and necrosis; (cells have hypereosinophilic cytoplasm and pyknotic nuclei, karyorrhexis or karyolysis) associated with abundant **intratubular hemoglobin casts** (Figure 4).

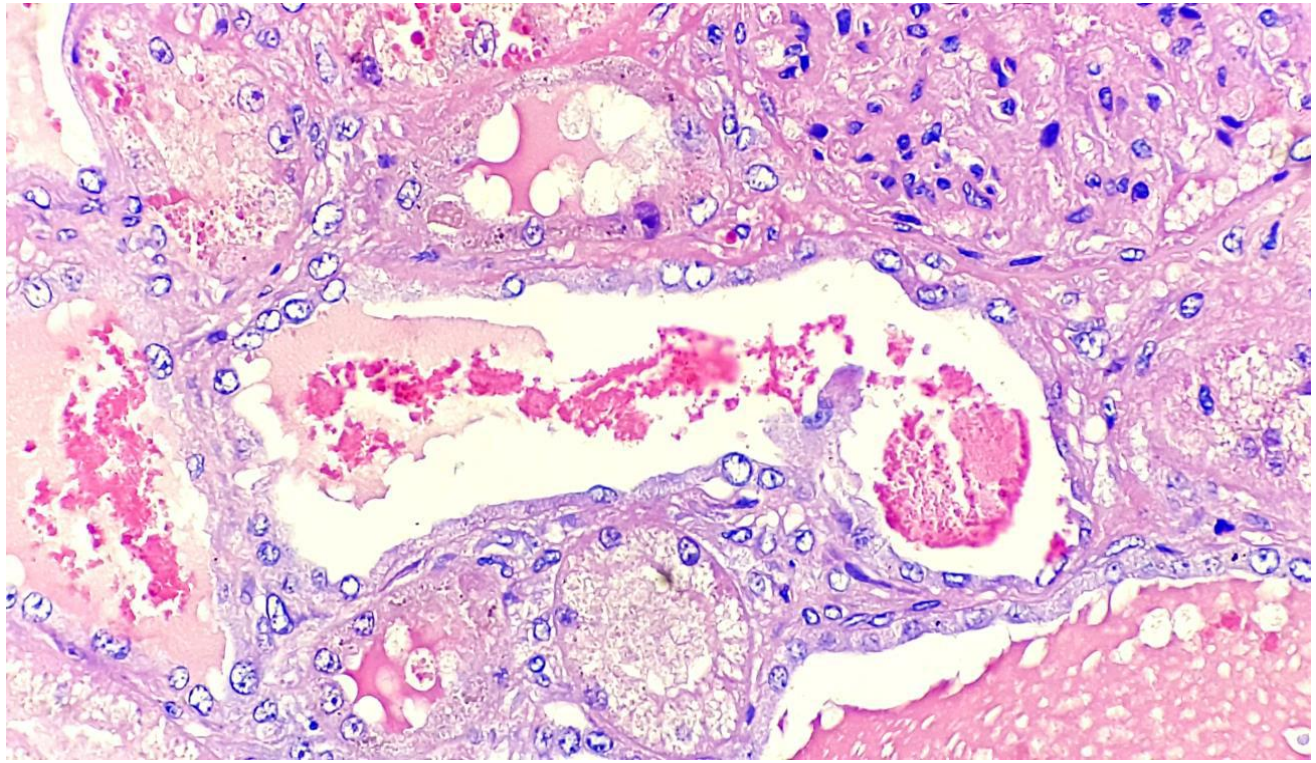


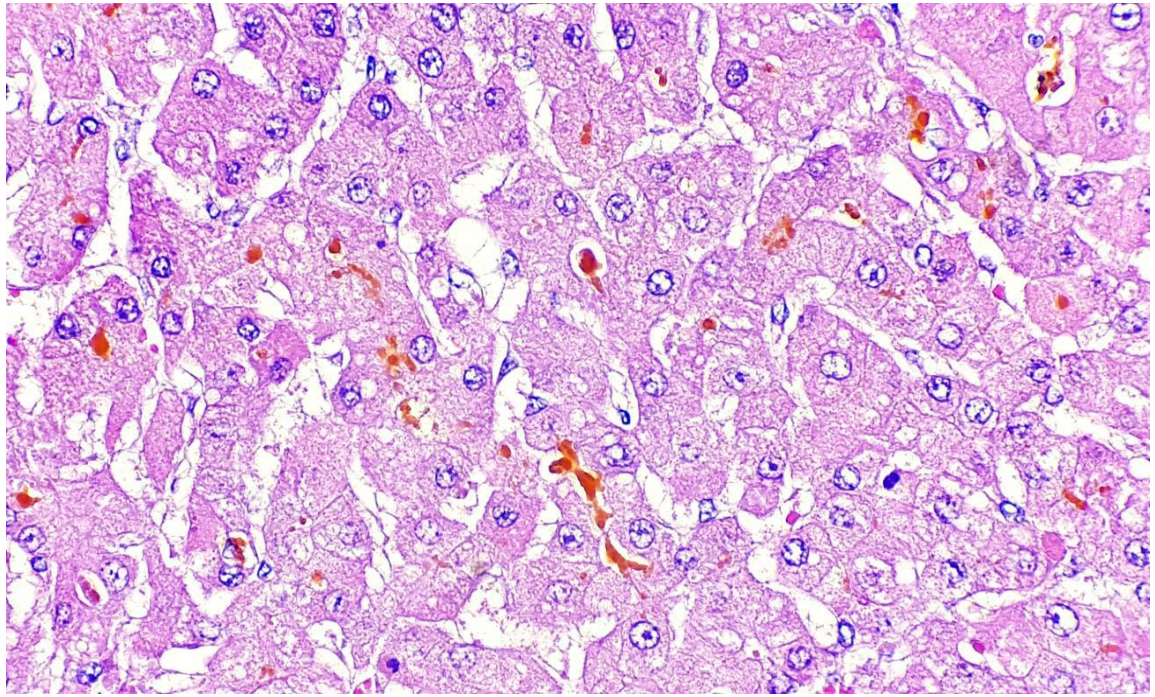
Figure 5

➢Additionally, acute centrilobular necrosis might develop in the liver, mainly due to direct exposure to circulating copper, but also complicated by anemia (hypoxia) and, in some cases, shock.

•Additional findings in the liver might include **accumulation of bilirubin in bile ducts and canaliculi (Figure 5)**

•And in sheep with chronic copper accumulation, infiltration of macrophages with light brown pigment, mainly in the periportal regions.

•The **spleen is diffusely congested** (splenomegaly).



Differential Diagnoses

•The clinical signs (hemoglobinuria), clinical findings (packed cell volume of 4%), and anatomic pathologic findings in this lamb (icterus, splenomegaly, hepatic necrosis, hemoglobinuric nephrosis, and hemoglobinuria), as well as the clinical signs observed in the other lambs involved in this outbreak, were highly suggestive of an intravascular hemolytic process.

The differential diagnoses for intravascular hemolysis in sheep include:

- toxic conditions (copper toxicosis,[8] plant toxicosis such as that caused by onion [*Allicum cepa*] and *Brassicaspp*[1]),
- infectious conditions (leptospirosis,[9] *Clostridium perfringens* type A enterotoxemia [yellow lamb disease],[6] bacillary hemoglobinuria [*Clostridium haemolyticum* hepatitis],[1] eperythrozoonosis [*Mycoplasma ovis*]),[1] and
- parasitic conditions (*Babesia ovis*, *Babesia motasi*).[1]

Differential diagnosis

Diagnostic Exercise: Hemolysis and Sudden Death in Lambs

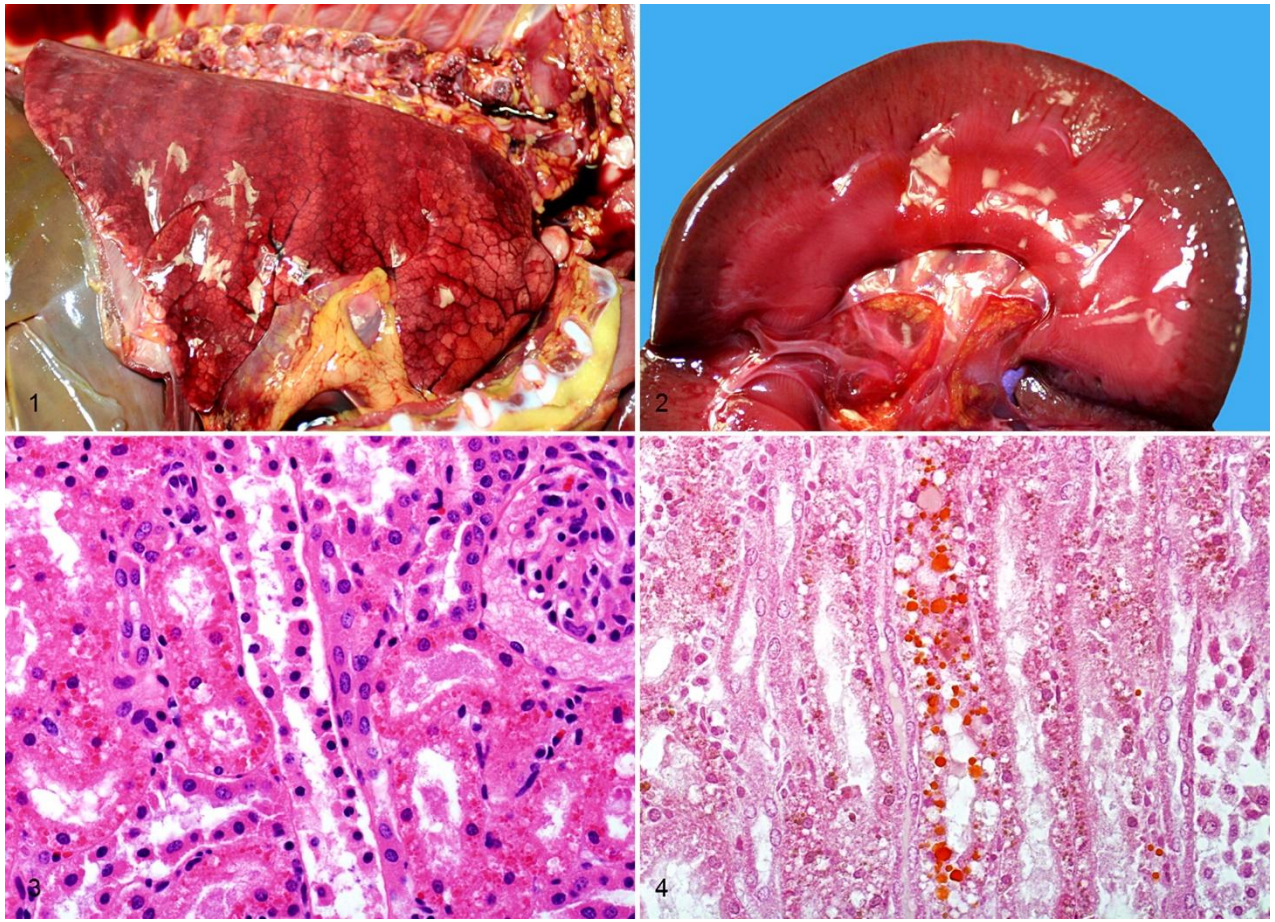


Figure1. Right lung in situ; lamb. Severe diffuse pulmonary edema with expansion of the interlobular septa by dark red fluid. Rib imprints are visible on the pleural surface. Note the yellow discoloration of the subcutaneous tissue of the chest and pericardial adipose tissue and the periosteum of the ribs (icterus).The lung is diffusely reddened, presumably due to hemoglobin imbibition.

Figure 2. Kidney, longitudinal section; lamb. The renal cortex and medulla are diffusely reddened, suggestive of hemoglobinuric nephrosis.

Figure 3. Kidney; lamb. Renal cortical tubular epithelial cells contain numerous, round, variably sized, brightly eosinophilic intracytoplasmic hyaline protein droplets (hemoglobinuricnephrosis). HE.

Figure 4. Kidney; lamb. The intracytoplasmic protein droplets shown in Figure3 stain orange brown with Okajima stain for hemoglobin.

Discussion:

- Based on epidemiologic data, gross and histologic findings, we established a diagnosis of **chronic copper intoxication in this ewe**.
- The **cause of intoxication** was the ingestion of a commercial mineral supplement provided to the cattle on the property and accidently ingested by the sheep. Feed and mineral supplements for cattle contain higher levels of copper than tolerated by sheep and are common sources of intoxication in the latter species (4).
- Although **copper is essential** for several biological processes, it is toxic at excess concentrations.
- In normal conditions, the dietary intake of copper is balanced by copper excretion in the bile (2, 4). When ingested in high levels, however, copper is stored in hepatocytes, culminating in continuous and repetitive cellular damage and death. Sheep have a very limited rate of copper excretion in the bile, which contributes to copper accumulation in the liver (1).

- **Three additional factors** may contribute to copper toxicosis in this species:
  - high levels of copper intake, as seen in this case (which can be present in the water, pasture or feed);
  - low levels of pasture molybdenum (even in the presence of normal copper concentrations), which antagonizes copper uptake; and
  - concomitant exposure to other hepatotoxins, which in turn make the liver more susceptible and less capable of storing excessive copper (1, 2, 4).

- In Brazil, the main hepatotoxin responsible for this third factor are pyrrolizidine alkaloid-containing plants (mainly Senecio) (3, 4).
- In the case reported here, we believe that the high levels of copper intake were the only factor contributing to intoxication, since no hepatic lesions suggesting plant toxicosis were observed.
- Commercial feed containing 15-20 mg/kg of copper are already capable of producing chronic intoxication in sheep (4).
- The technical information in the packaging of the dietary supplement provided to the sheep in this case indicated a minimum of 650 mg of copper per kilogram. Pastures with 15-20 mg/kg of copper and low levels of molybdenum (<0.36 mg/kg) may also cause copper intoxication (4).
- The excess of chronically ingested copper stored in the liver may reach 1000 μg/g without any clinical signs. During this phase, copper is stored within the lysosomes of hepatocyte, causing subclinical cell damage, apoptosis, and increased mitotic rate (1,2).

- Macrophages containing brown granular pigment (copper-containing lipofuscins) appear in the periportal areas (2). Eventually, the liver cannot keep up with the replacement of hepatocytes that can absorb the copper released from dying cells.
- This leads to a sudden increase in plasma copper levels, which causes intravascular hemolysis and centrilobular necrosis as described above in the microscopic findings (1,2).

- Thus, although copper intoxication is chronic, the clinical presentation is acute, and begins when copper is released into the blood stream.
- Sick animals may die within a few hours or days.
- Copper poisoning is a common disease in sheep and should be included as a possible cause of hemoglobinuria and acute death (4).

Title: Sheep, hematopoietic system, intravascular hemolytic crisis

References and Recommended Literature:

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- 2. Cullen J M , Stalker M J. Liver and Biliary System. In: Maxie MG, editor. Jubb, Kennedy & Palmer's Pathology of domestic animals, volume 2. 6 Elsevier; 2016. P.343.
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- *The Diagnostic Exercises are an initiative of the **Latin Comparative Pathology Group (LCPG)**, the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website (http://www.cldavis.org/diagnostic_exercises.html).
- **Associate Editor for this Diagnostic Exercise:** Mariana Flores
- **Editor-in-chief:** Claudio Barros

Diagnostic Exercise
From The Davis-Thompson Foundation*
Case: **215**; Month: **June**; Year: **2023**
Answer sheet

• **Clinical History:** A young male turkey (*Meleagris gallopavo*) was referred to the Veterinary Hospital of the Federal University of Paraíba (UFPB), having multiple skin nodules.

•The owner reported that twenty-four chickens (twelve adult and twelve young) and nine turkeys (four adult and five young) had similar lesions on the same property.

•The animals were raised together, and several birds died, mainly turkeys and chicks.

Clinical Findings:

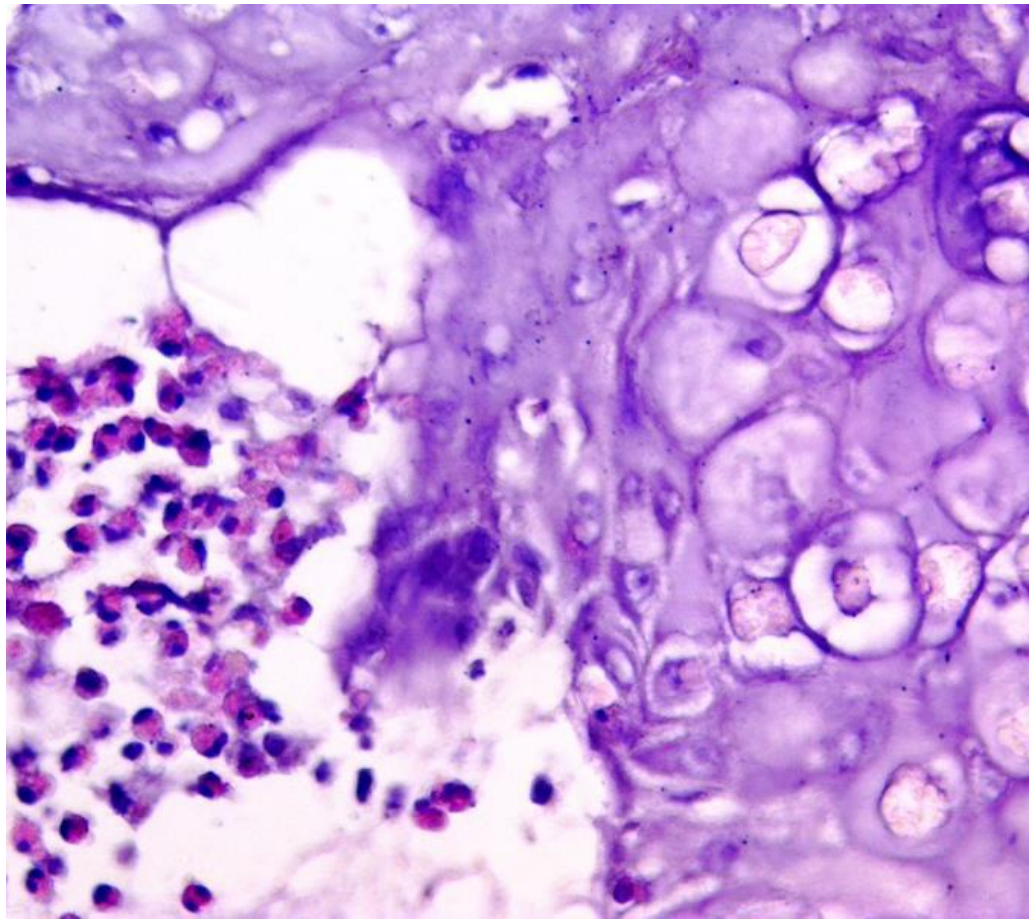
•The turkey was numb, with multifocal to coalescing firm crusted nodules with a depressed center were on the head, neck and limbs, and focally extensive areas of necrosis in the oral cavity.

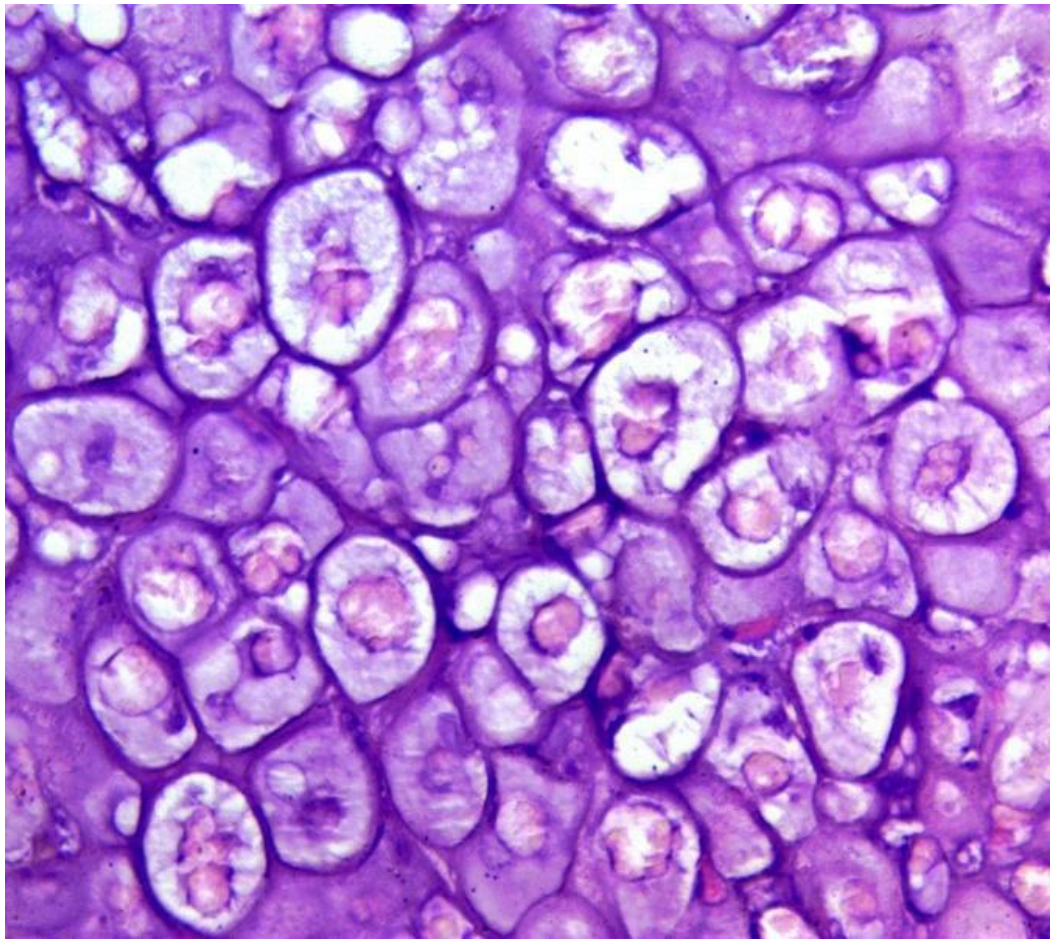
•Fragments from the skin lesions were sampled for biopsy.

Gross Images:

Figure 1: Right lateral (A) and frontal view (B) of the head of a turkey. Multifocal to coalescent, proliferative crusted nodules with a depressed necrotic center

Microscopic Images: Figure 2 Skin.
(A) Epidermal hyperplasia with vacuolar degeneration and heterophilic infiltrate. HE. Obj. 40x.
(B) Ballooning degeneration of the keratinocytes with intracytoplasmic eosinophilic inclusions (Bollinger bodies) that peripheralizes the nuclei. HE. Obj. 60x.





Follow-up questions:

(1) Histological description:

- Feathered skin: The stratum *germinativum* of the epidermis is markedly **hyperplastic** up to twelve times normal thickness and covered by a serocellular crust.
- Keratinocytes are swollen with cytoplasmic vacuolation (**ballooning degeneration**).
- Many keratinocytes were expanded by 15-30 um **eosinophilic intracytoplasmic inclusion bodies** (Bollinger bodies) displacing and distorting the keratinocyte nuclei.
- **Multifocal areas of lytic necrosis** effacing the epidermis and extending into the dermis were observed. Within the areas of necrosis were large amounts of viable and degenerate heterophils admixed with fewer lymphocytes and macrophages.

(2) Name of the condition: Fowl pox.

(3) Etiology: Avian poxivirus.

(4) Forms of clinical presentation:

- Cutaneous (dry pox),
- diphtheritic (wet form) and
- mixed.

(5) Two differential diagnoses:

1. Lymphoproliferative disease of turkeys and
2. vitamin B deficiencies (pantothenic acid and biotin) in young chickens.

Comments:

- In this case, the histopathological confirmation of the avian pox virus infection in the cutaneous lesions **leads to the belief that the lesions** observed in the oral cavity correspond to the diphtheric presentation of the same disease.
- Avian pox virus infection, known as fowl pox, affects domestic and wild birds worldwide (2).
- The virus belongs to the Poxviridae family, which comprises 12 different species causing infection in birds, and its name is defined by the infected bird species, including chickens, turkeys, canaries, sparrows, parrots, pigeons, quails, flamingos, penguins, starlings, mynahs, junco, among others (4).

- Generally, this agent does not cause the death of the individual, but due to the injuries, the risk that the bird suffers a higher risk for secondary bacterial infections, predation or accidents (9).
- In poultry, poxvirus infection can lead to economic losses due to decreased food intake and reproductive rate (3).
- Arthropods serve as mechanical vectors for poxvirus transmission. They feed on the blood of sick birds, become infected, and transmit the virus by inoculating the agent in a healthy bird.
- Direct contact with sick birds through injured skin, ingestion, or inhalation of viral particles are also implicated in the dissemination of poxviruses (2,3).
- The disease can occur in the cutaneous form (dry pox), characterized by proliferative nodular lesions on the skin, mainly in the featherless parts of the body.
- A less common diphtheritic form (wet pox) is characterized by focally extensive proliferative lesions that coalesce in large areas of fibrinonecrotic membranes in the oral cavity and other mucosal surfaces of the upper gastrointestinal and respiratory tract. A mixed form with cutaneous and oral lesions can also occur (3,4).

- Histologically, epithelial hyperplasia occurs with hydropic degeneration (ballooning degeneration) and hyperkeratosis, with characteristic type A eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) in keratinocytes (3,8).
- In the mucous membranes, there is hypertrophy and hyperplasia of mucus-producing cells, followed by an increase in epithelial cells that contain inclusion bodies (8).
- The definitive diagnosis can be obtained through histopathological examination and its association with the clinical history and macroscopic lesions (3,8).
- Other techniques can be used to confirm the diagnosis, including polymerase chain reaction (PCR) and viral isolation (7,10).

Differential diagnoses

- **Differential diagnoses** for the cutaneous form include:
- lymphoproliferative disease of turkeys, caused by an exogenous retrovirus characterized by focal to multifocal tumors (1), and pantothenic acid and biotin deficiencies in young chicks (6).
- In the diphtheritic presentation, the main differential diagnoses include infectious laryngotracheitis of chickens caused by *Gallid alpha herpesvirus 1* (5) and *Trichomonas gallinae*, the latter being more common in columbiforms and raptors (7,8).

Title: Avian poxvirus infection in a turkey

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- 1. Allison AB, Keel MK, Philips JE, Cartoceti AN, Munk BA, Nemeth NM, Welsh T, Thomas JM, Crum JM, Lichtenwalner AB, Fadly AM, Zavala G, Holmes EC, Brown JD. Avian oncogenesis induced by lymphoproliferative disease virus: a neglected or emerging retroviral pathogen? Virol. 2014;450: 2-12.
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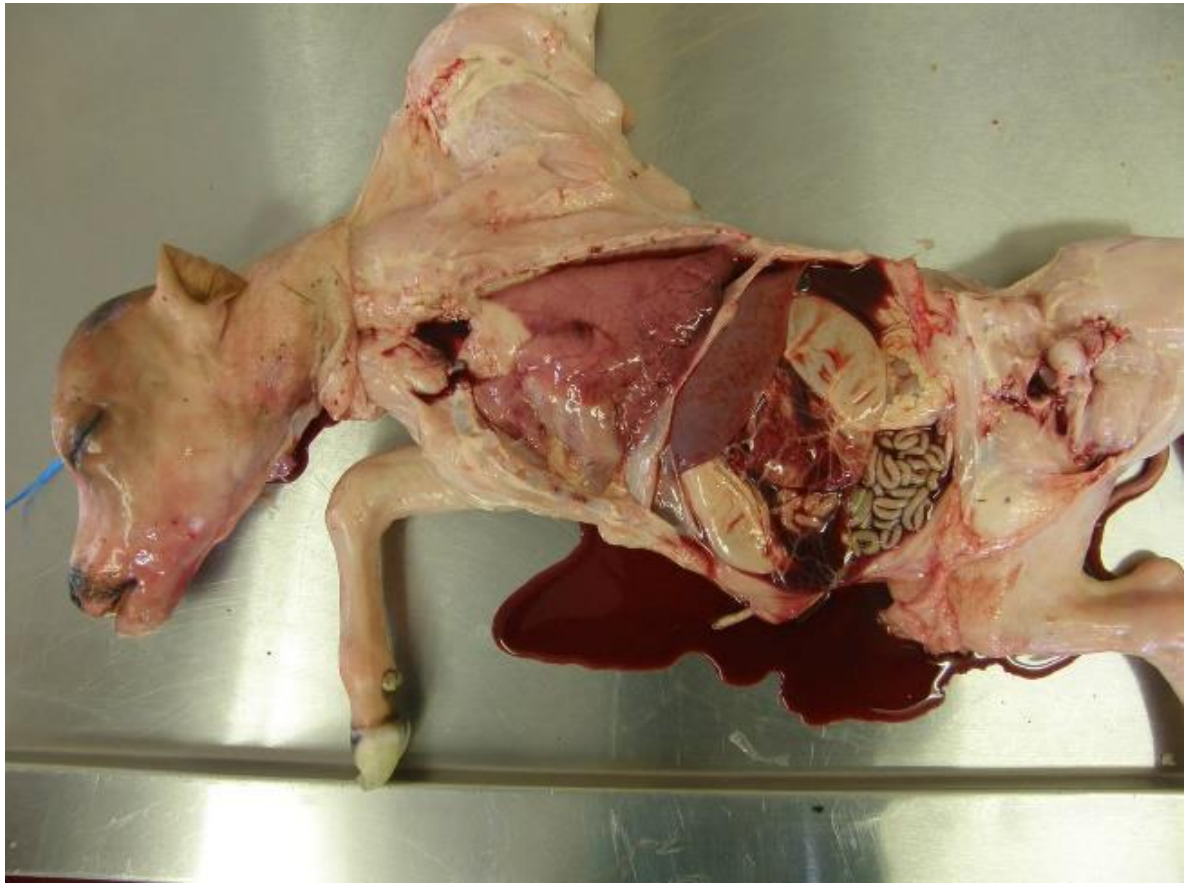
- **Editor-in-chief**: Claudio Barros
- **Associate Editor for this Diagnostic Exercise**: Raquel Rech

Post Mortem Approach to the Liver
Gross Examination
Figure 1: Examine the liver *in situ*



- after opening the abdominal cavity examine the liver in context with other changes in the abdominal cavity.
- Gently wash out any excessive fluid accumulation or haemorrhage in the abdomen to provide a clear in-situ picture of pathology (Figure 1).
- Run water as a gentle soak and **not under pressure** as this may remove and destroy critical lesions such as hematoma, adhesions etc.

Figure 2

•is a case of a ruptured liver in a calf with hematoma and adhesions associated with the rupture site clearly indicating antemortal pathology. If this material had been removed by high pressure washing of the abdomen, it then becomes extremely difficult to distinguish between ante-mortal and post mortal rupture.

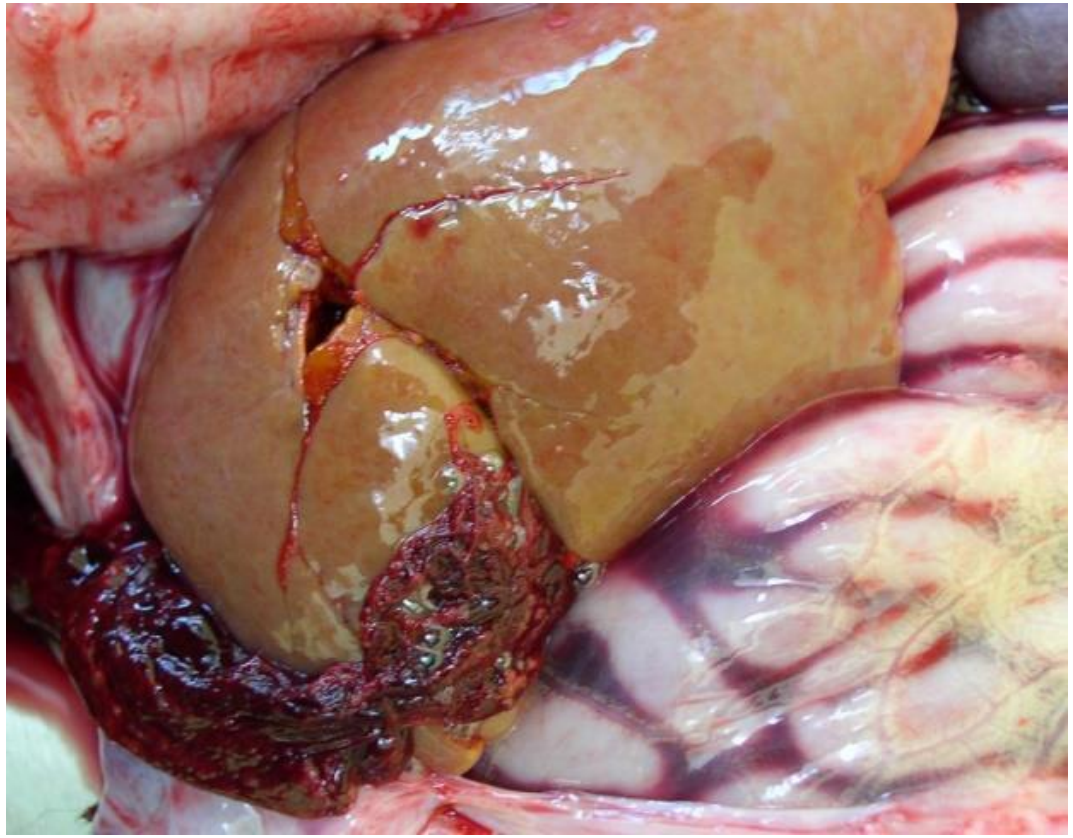
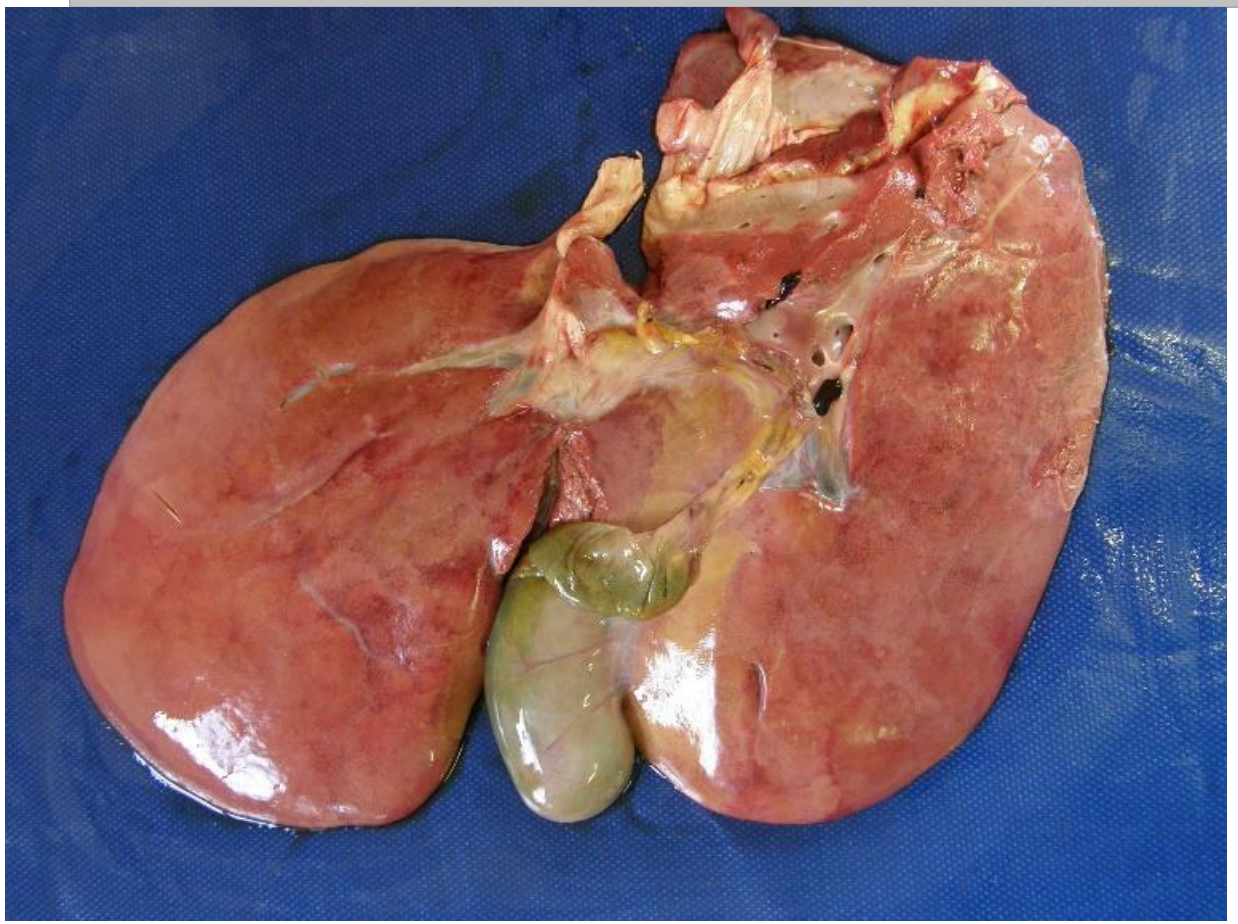
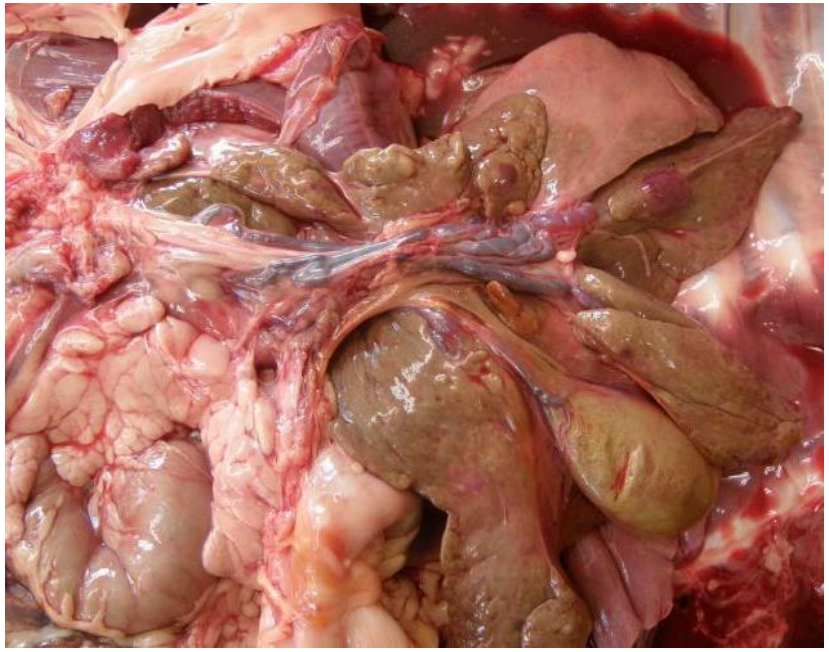


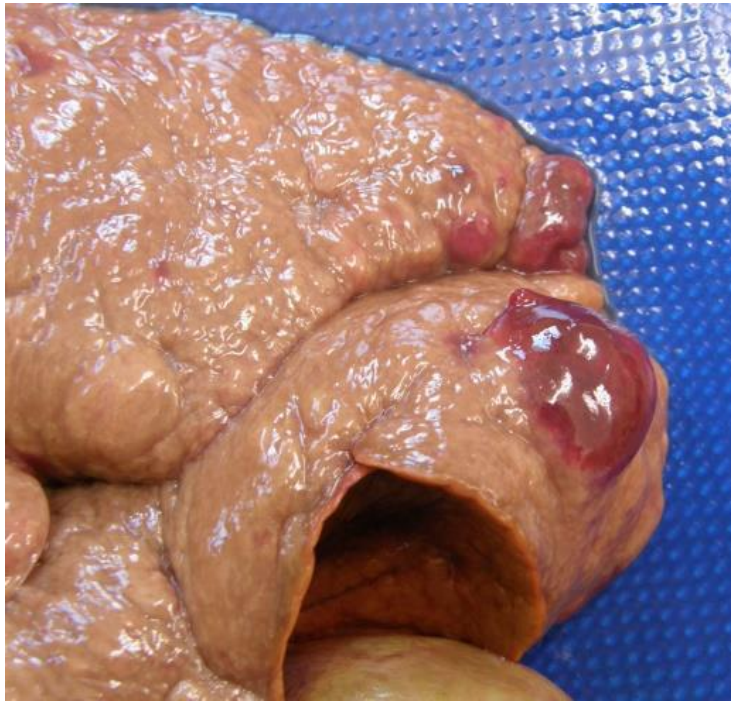
Figure 3:



- **Examine both the diaphragmatic and visceral aspects** – evaluate size, colour, shape, lesion distribution and consistency.
- Since the liver capsule is unable to expand any, enlargement of the liver results in rounding of the liver margins (Figure 3).
- Consistency is evaluated by compressing a 1 cm thick slice of liver between the thumb and index finger.

Figure 4 and Figure 5





Examination of vascular supply to liver – observe the entrance of the portal vasculature into the hepatic parenchyma for any evidence of shunts or other anomalies (Figure-4). In this example of hepatic arterio-venous fistulae, note the aneurismal dilatation of portal veins and tortuous hepatic arteries (Figure-5).

Figure 6

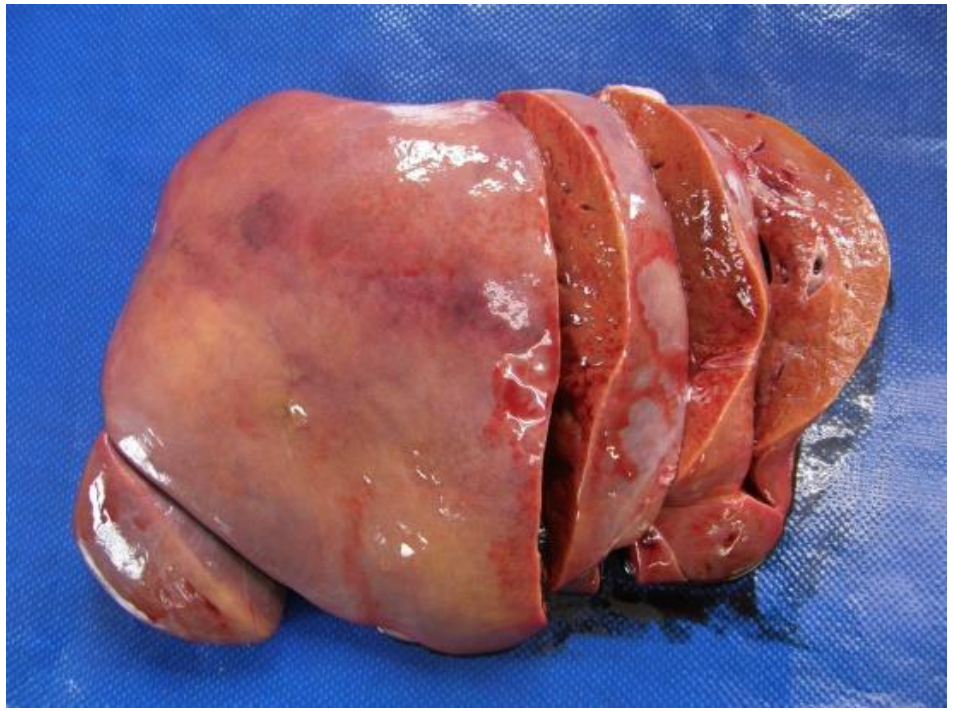
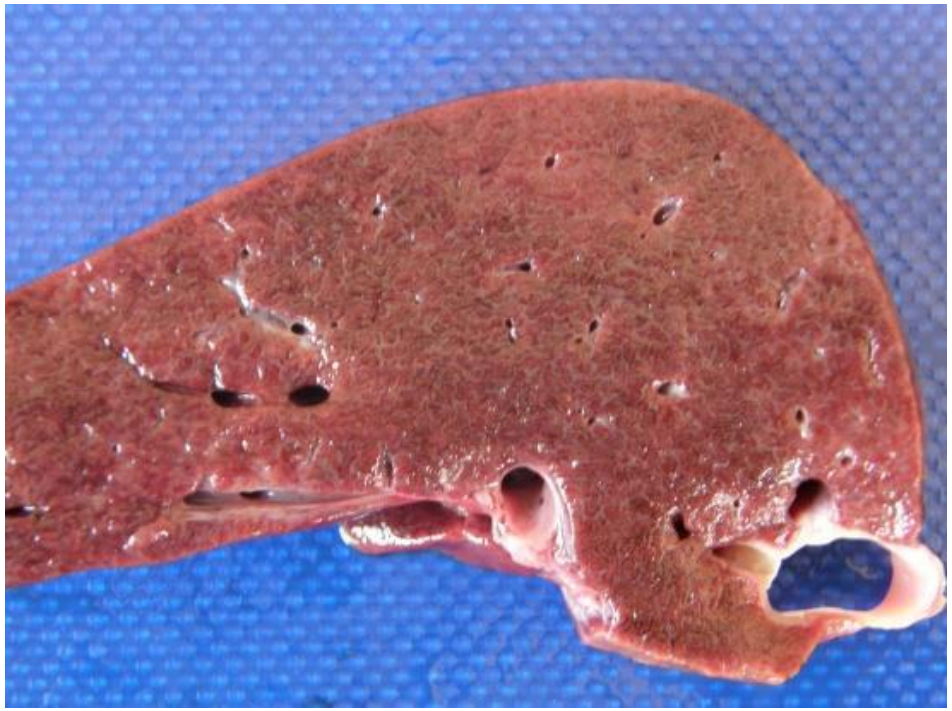


Figure 7



- **Importance of "Bologna Slicing"** – slicing the organ into 1 cm thickness slices;

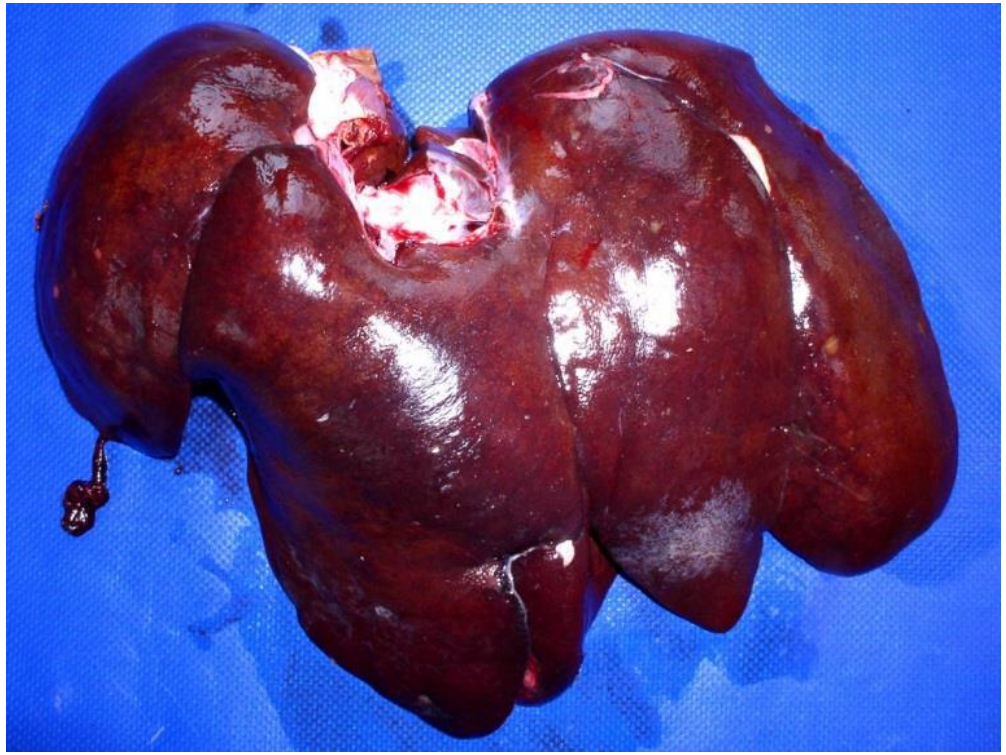
➢ not only enables assessment of consistency but also

➢ allows for evaluation of the cut surface and assists in **determining the distribution of lesions and exact location of focal lesions**.

➢ Slicing also enables assessment of the consistency throughout the organ.

Common Gross Liver Lesions

Figure 8



- **Diffusely enlarged liver (hepatomegaly)**
- May be seen in a variety of conditions where there is either;

➢ diffuse swelling of hepatocytes,

➢ blood pooling or

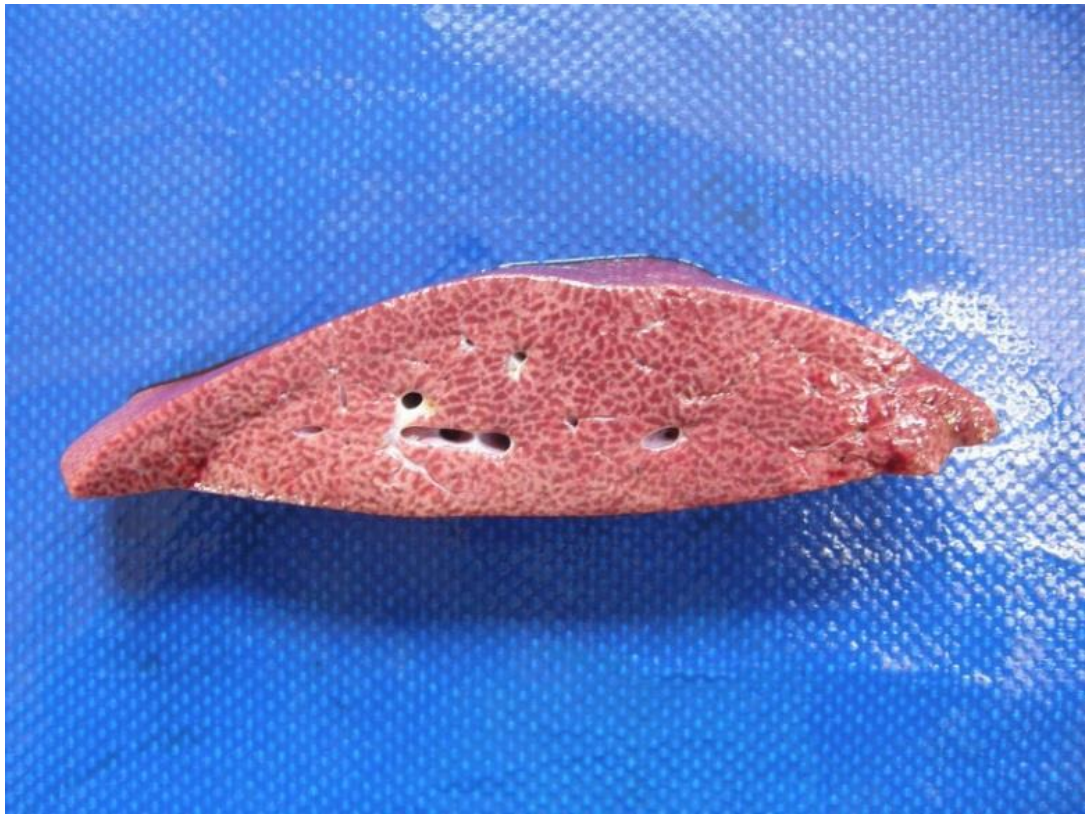
➢ diffuse infiltration by neoplastic cells.

➢ Hepatomegaly with “congestion” is a common post mortal finding due to terminal hypostasis with rigor mortis and intestinal gas pushing mobile blood to the liver from the muscle and bowel (Figure 8).

➢ It is also noted with degenerative hepatopathies (toxic, metabolic) and fatty liver syndromes where there is significant swelling of hepatocytes.

➢ Diffuse neoplastic infiltrates as might be expected with lymphoid, myeloid and mast cell neoplasia.

Figure 9



- **Chronic passive congestion (nutmeg) liver**
- Liver may be slightly enlarged but retains its smooth surface.
- Parenchyma has a mottled dark and light parenchyma due to the blood pooling in centrizonal areas.
- As the lesion progresses colour becomes more uniform with arborizing pale areas in a dark background.
- The cause of this pathology is increased resistance to forward blood flow and is seen with;

➢ chronic heart disease,

➢ lung diseases,

➢ heart abnormalities and

➢ caudal vena caval obstruction (thrombosis, neoplasia). This lesion can be seen in animals of all ages including the fetus.

Figure 10

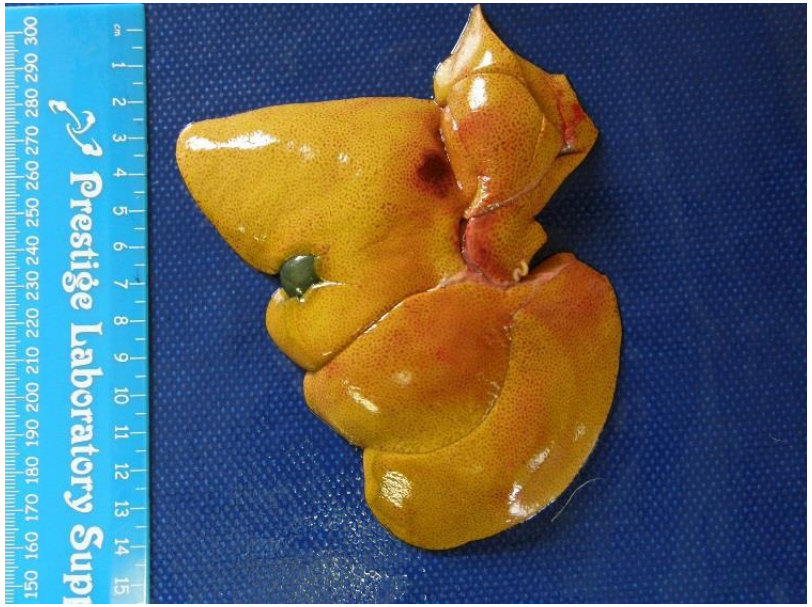
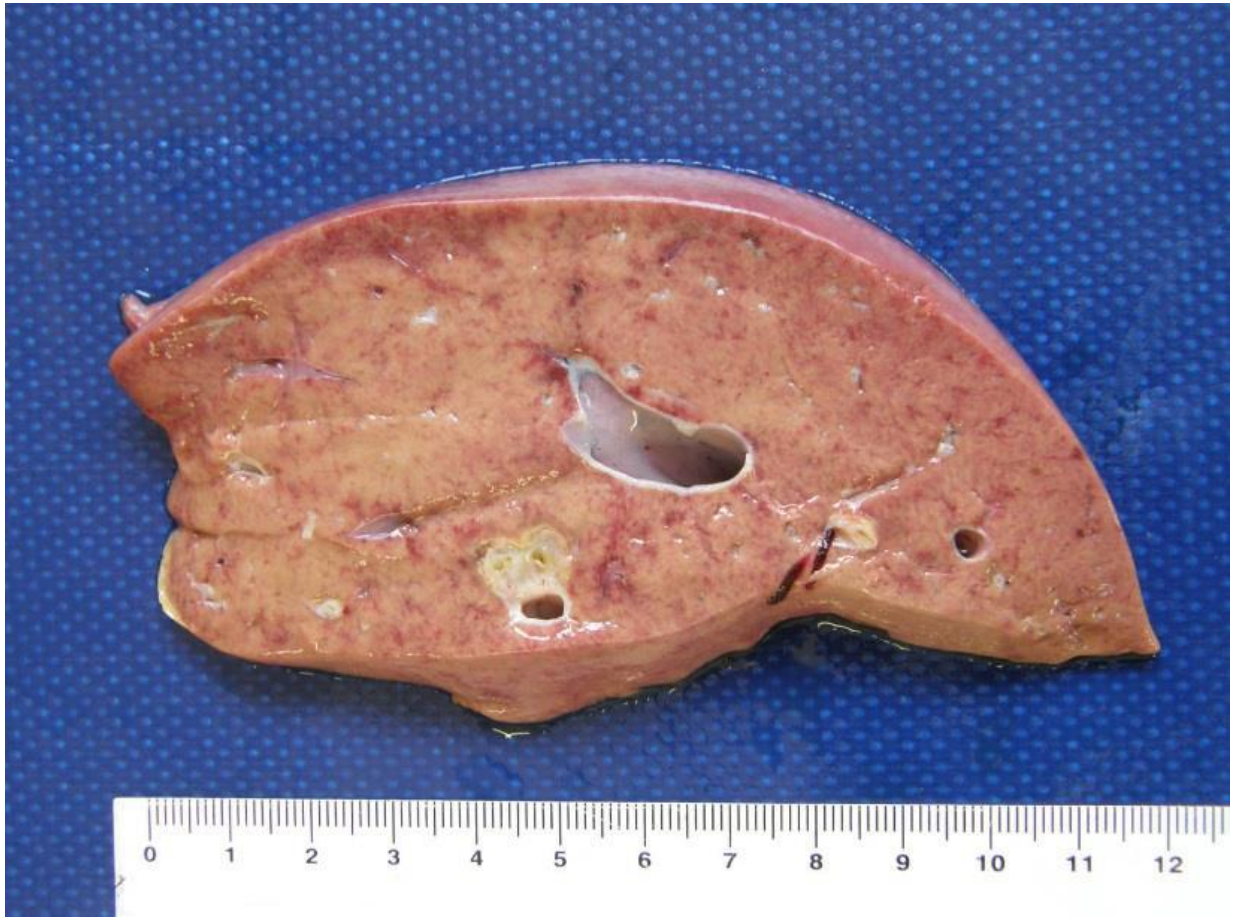


Figure 11

- **Fatty Liver**
- Characterized by a diffusely swollen yellow liver with rounded edges (Figure 10). There is decreased consistency and affected liver biopsies float in water (Figure 11).
- This condition may be seen in all species with acute starvation when there is body fat to mobilize to the liver.
- In chronic starvation, fatty liver is not observed as there is no fat to mobilize. It is also observed with metabolic diseases (negative energy balance, diabetes mellitus, ketosis in cattle, pregnancy toxaemia in sheep), toxic hepatosis (aflatoxins, *Cycad* sp) and hyperlipidaemic syndromes (horses, cats).

Figure 12



- **Extensive (massive / sub-massive) hepatic necrosis**
- Associated with extensive hepatocyte necrosis across all lobules with parenchymal discolouration (necrotic liver tissue paler in colour).
- Consistency is markedly decreased. Most commonly seen with acute toxic hepatosis or viral hepatosis.

Figure 13

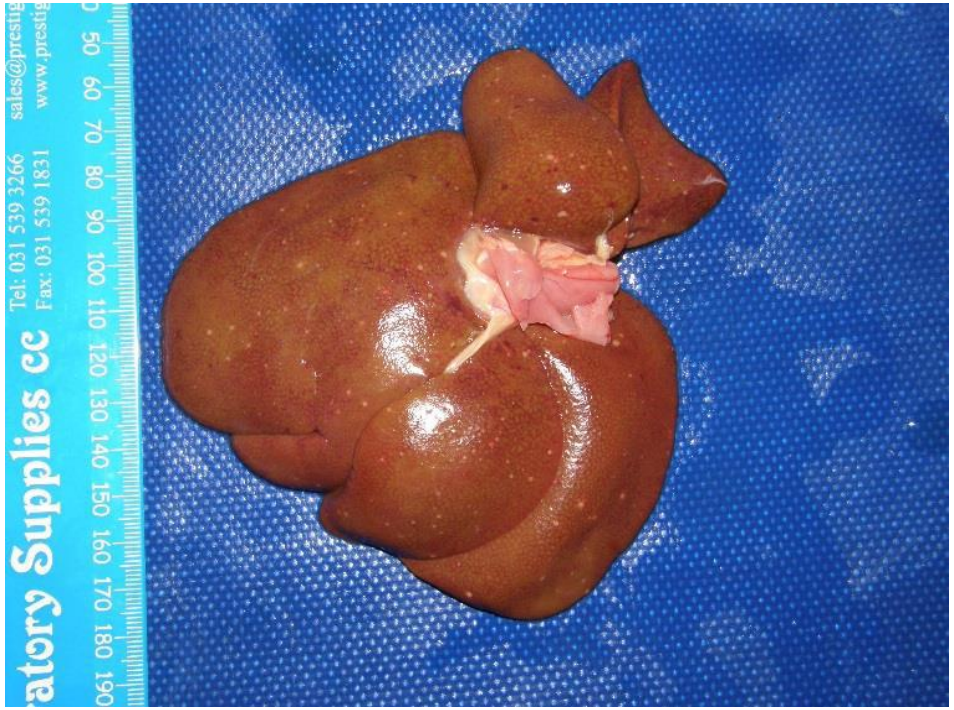
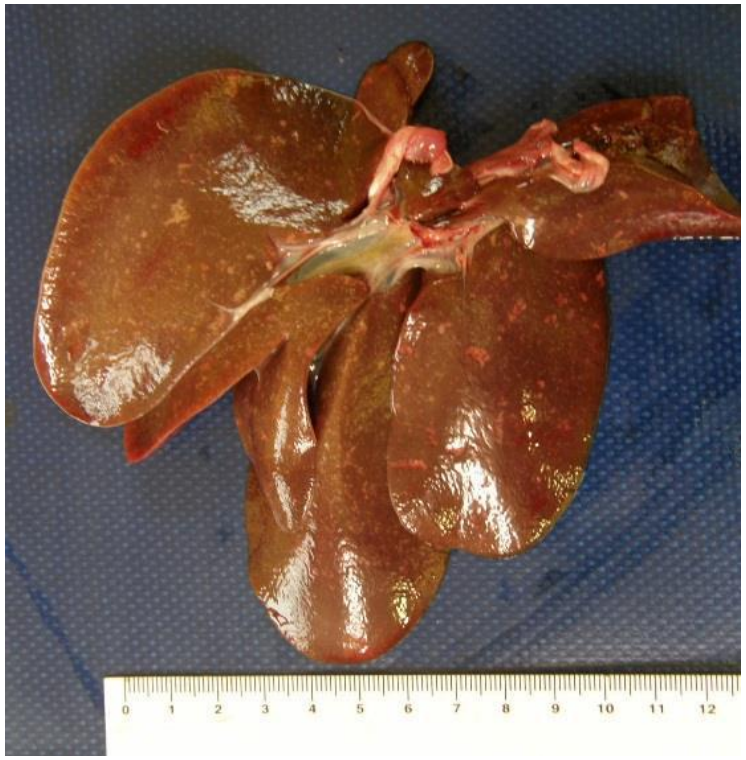


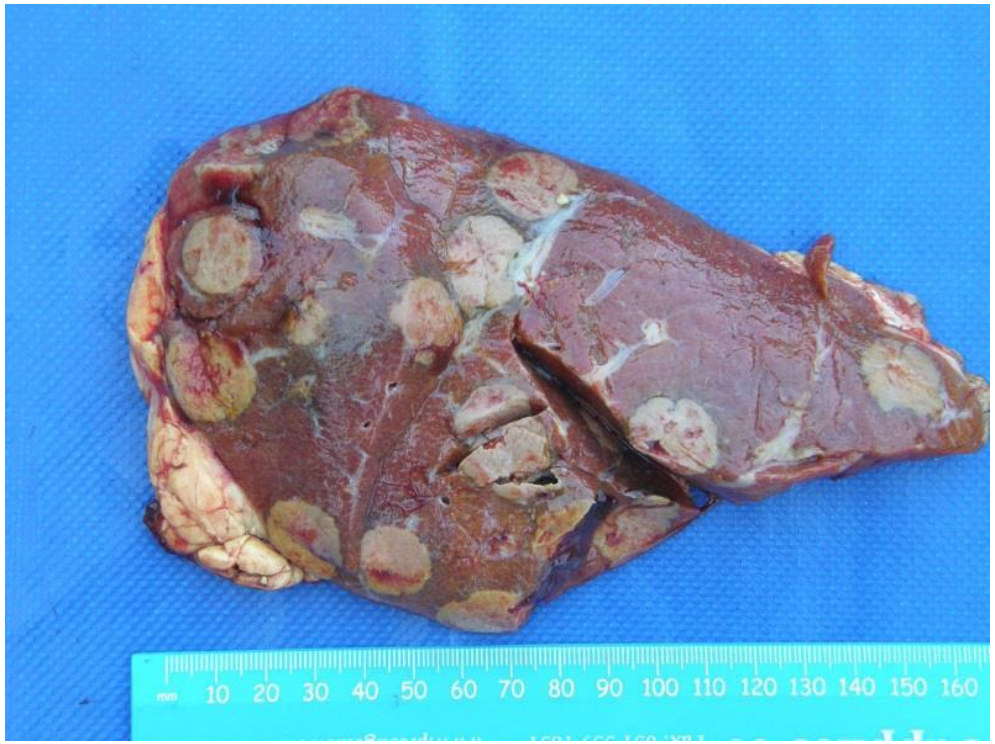
Figure 14



Miliary hepatic necrosis

- This is characterized by small pale foci of necrosis evenly distributed through all lobules of the liver. It is most commonly observed with embolic bacterial infections (Figure 13), as observed in this case of Tyzzer's disease (*Clostridium piliforme*) in a cat, certain protozoal infections (Figure 14) as in this case of toxoplasmosis from a Dassie and some instances of viral hepatitis (Bovine herpes virus abortion).

Figure 15

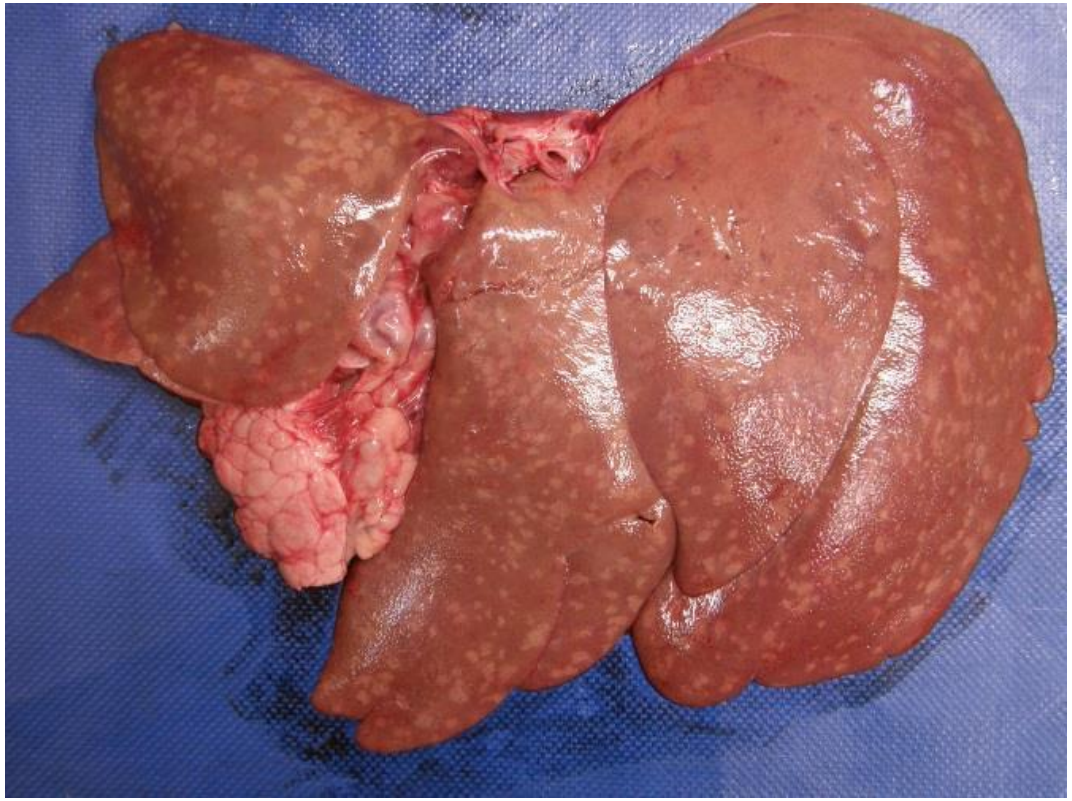


•**Multifocal hepatic necrosis**

•Multifocal areas of necrosis with some lobules affected by a greater or lesser degree.

•This a common feature of necrobacillosis (*Fusobacterium necrophorum*) in cattle (Figure 15) and a feature of many mycotic infections.

Figure 16



•What is important about **putrefactive foci** is that they are **not rimmed by inflammation** (reaction zone).
•Putrefactive liver usually floats in liver due to the post mortal gas production.
•Infectious necrotic hepatitis is also associated with Clostridial bacteria but in the ante-mortal setting.
•Dormant clostridia within the liver undergo proliferation in areas of hepatic necrosis which provide the required anaerobic environment.

Figure 17

•As noted in this case of infectious necrotic hepatitis in a wilde beest (Figure 17) necrotic areas are irregular in shape, are variable in size and rimmed by a red reaction zone of inflammation. In both instances these necrotic / putrefied areas may be associated gas bubbles.

- **Decomposition (putrefaction) vs Infectious necrotic hepatitis**
- Decomposition often characterized by multifocal to miliary discrete pale foci scattered through the liver. Many are associated with the portal areas creating a zonal pattern than can be mis-interpreted as miliary necrosis as evidence by the canine liver in Figure 16.

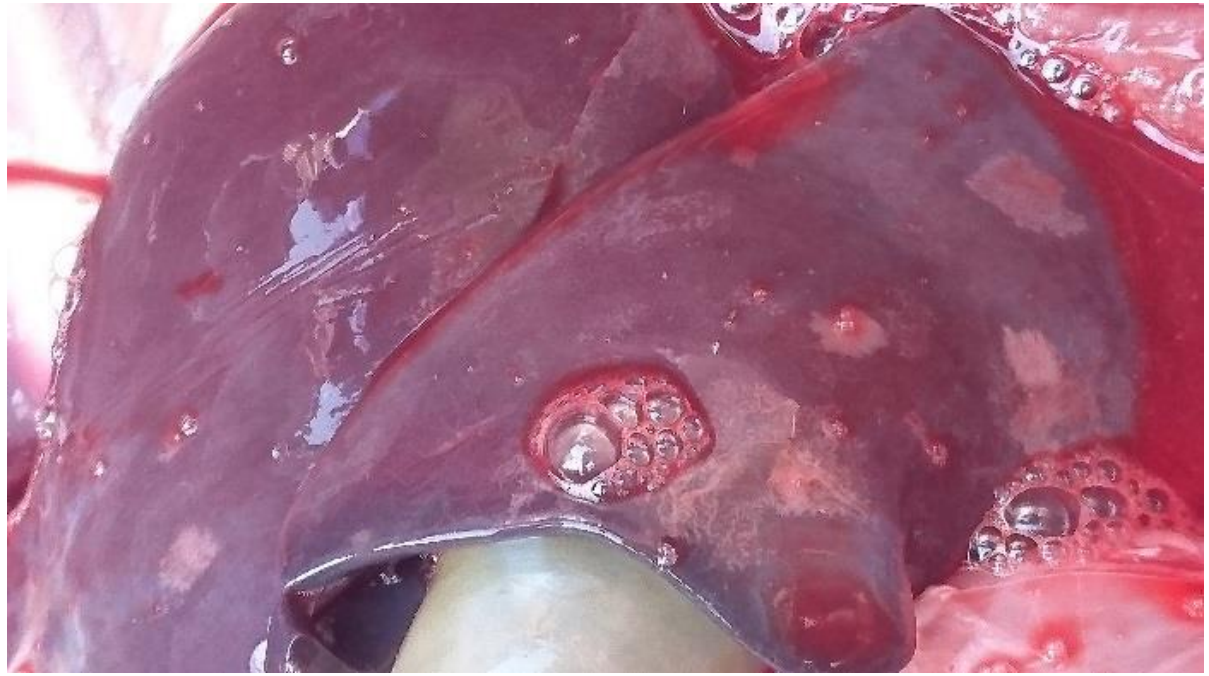


Figure 18

- **Peliosis hepatis /Telangiectasia**
- This characterized by dilated blood filled sinusoidal spaces (Figure 18). This condition has been reported in cattle, dogs, cats and humans, although the pathogenesis remains unknown.
- Two forms of the condition are described either;

➢ from local obstruction of small branches of the portal vein with subsequent hepatic atrophy and sinusoidal dilatation (phlebectatic / telangiectasis) or

➢ from random focal hepatic necrosis (parenchymal type).

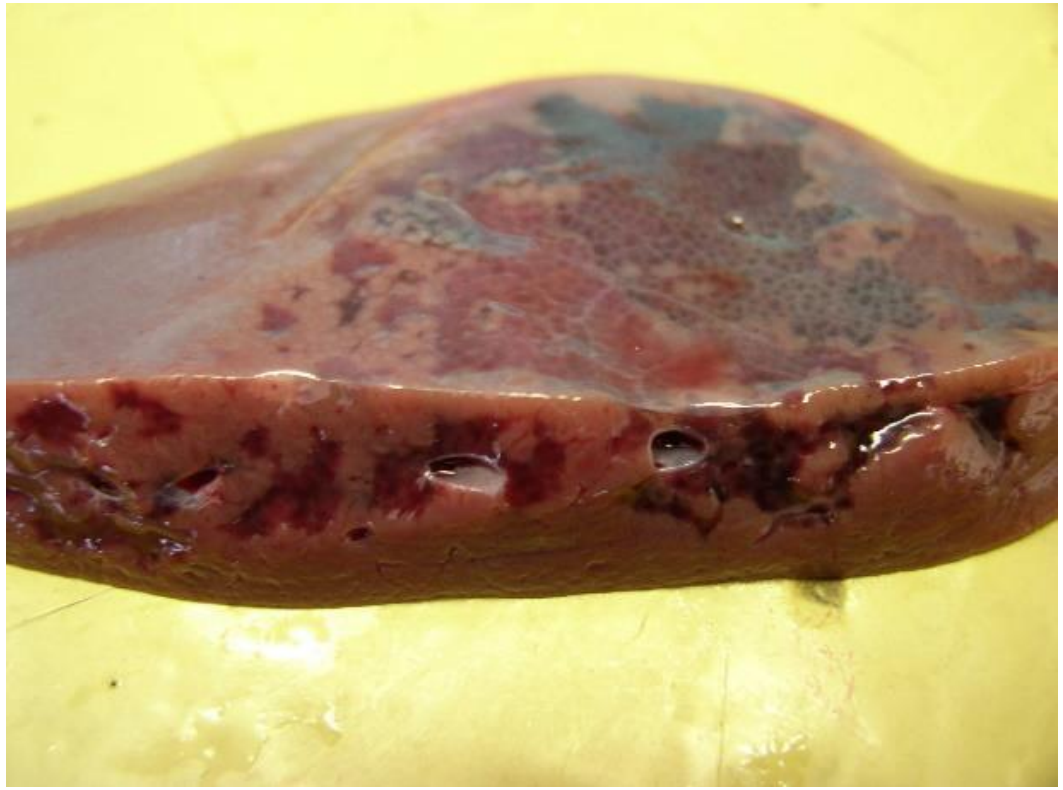


Figure 19

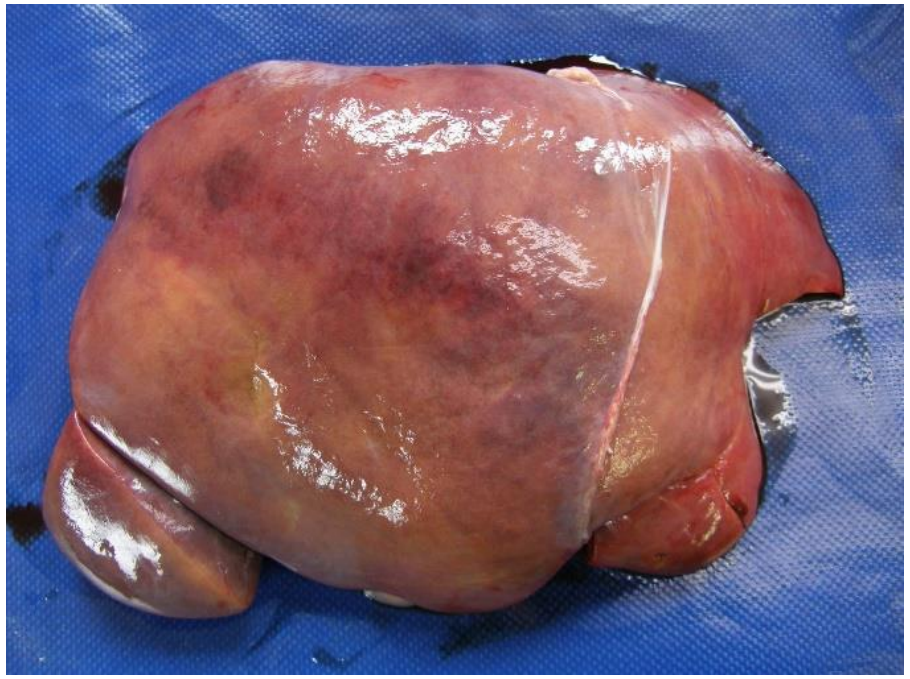
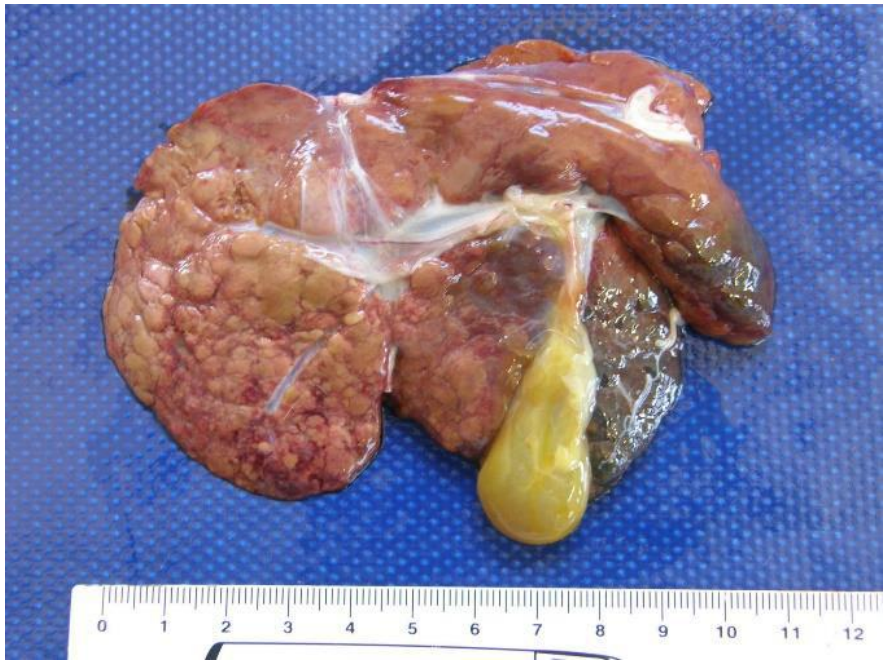
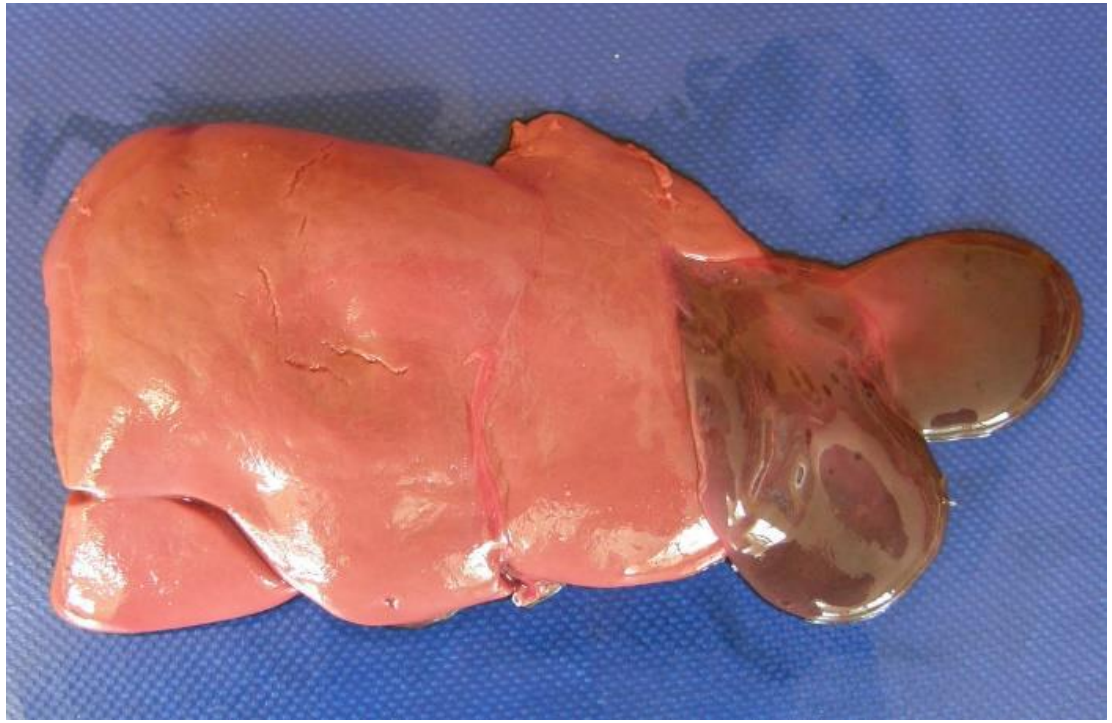


Figure 20



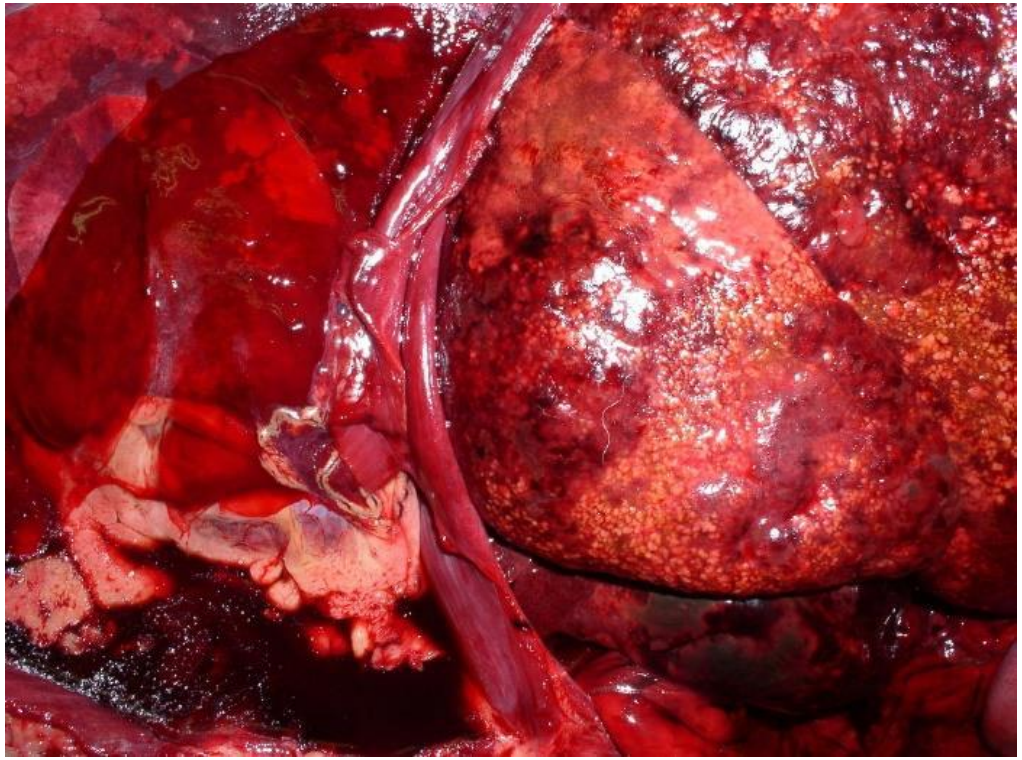
- **Hepatic cirrhosis vs Post necrotic scarring**
- Hepatic cirrhosis is defined as a uniform increase of connective tissue at the same location and severity equally in all lobules due to chronic repetitive damage (cardiogenic, pyorrlizidine alkaloid etc).
- **Grossly** the liver is essentially of normal size or slightly enlarged with normal shape but with increased consistency. **Post necrotic scarring** on the other hand is a condition that develops following acute hepatic necrosis due to a single insult. Areas of necrosis then undergo nodular regeneration.
- Grossly the liver has distorted architecture with a multinodular cobblestoned appearance and has increased consistency.

Figure 21



- **Hepatic cysts**
- Frequently documented in aborted bovine foetuses as demonstrated in Figure 21.
- Parasitic cysts, particularly tapeworm cysts are common particularly in young animals.
- If observed in older animals one must consider biliary tumours particularly if the fluid is green.
- Hepatic cysts are very common in cats and include obstructive biliary dilatation, biliary cystadenomas and cystadenocarcinomas.

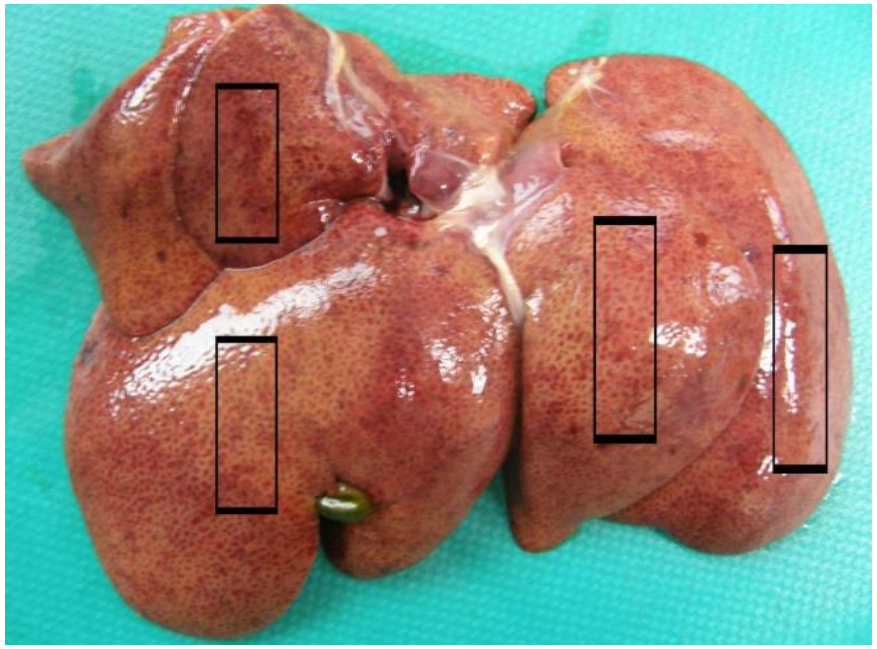
Figure 22

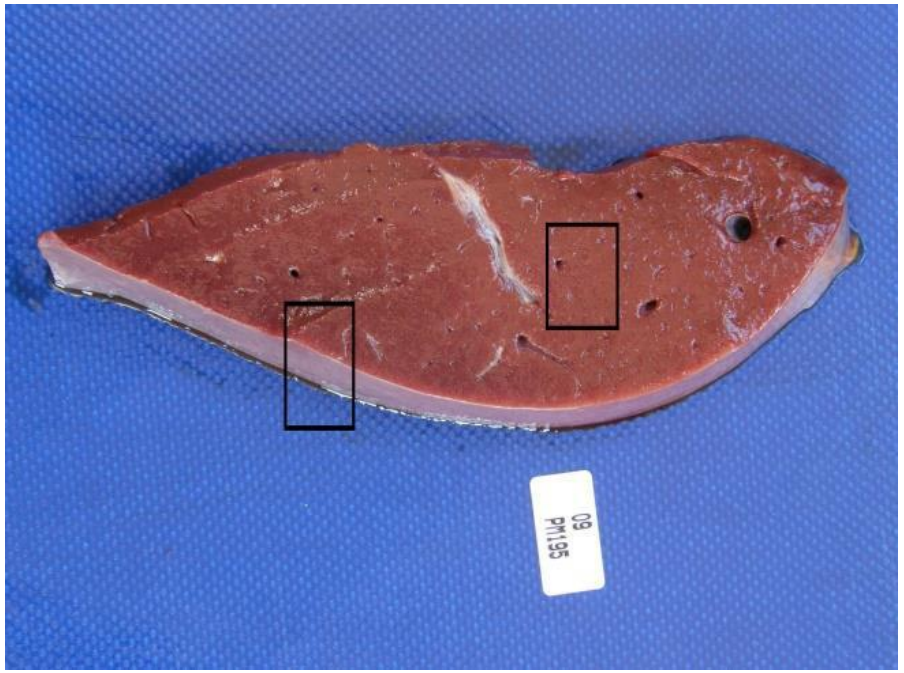


- **Hepatic tumours**
- Primary liver tumours hepatocellular and cholangiocellular are well documented (Figure 22).
- The liver is also a common metastatic location for tumours at other primary abdominal locations (pancreatic, gastrointestinal, splenic).

Sample collection

a. Histopathology



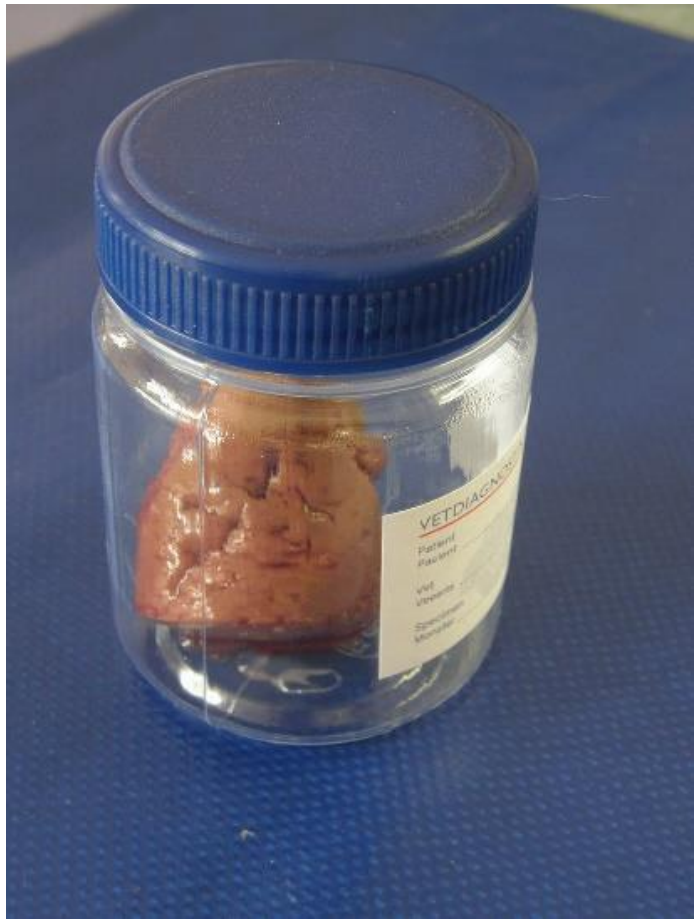


- Figure 23

Figure 24

- Liver tissue slices no thicker than 1cm to enable good fixation should be collected from the left and right lobes of the liver, as well as from any site with grossly visible lesions. Liver sections should include tissues with capsular tissue as well as tissue from at least 1cm below the capsule (Figures 23 and 24).
- Where possible avoid collecting biopsies from liver margins, as they are furthest from the main blood supply and prone to fibrosis. This fibrosis can mask underlying pathology and can be over-interpreted as excessive fibrosis. Large focal lesions should be sampled at the periphery to avoid necrotic centres and evaluate the interaction of the lesion with the normal adjacent tissue.
- Tissue slices are then placed in 10% buffered formalin. Ensure that the formalin jar has adequate formalin and is not overfilled with tissue - work on a ratio of 1 part tissue to 9 parts formalin.

b. Microbiology (Bacterial / Fungal culture, PCR, virus isolation)



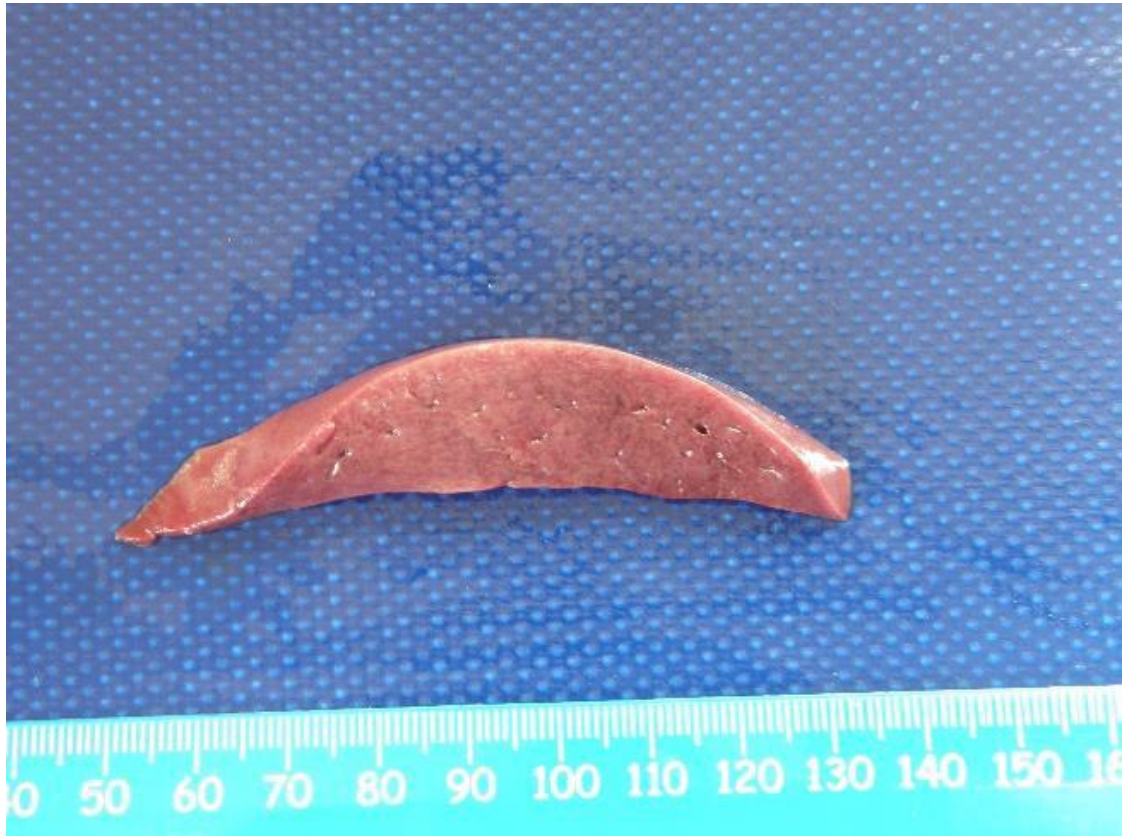
At post mortem, the liver is considered the most important "catch all" organ of choice for culture, as drainage from all abdominal visceral sites passes through the liver. Liver tissue collected for microbiological purposes should be collected immediately after opening the abdominal cavity while all the organs are still *in situ*, to avoid any possible contamination.

A ± 1cm block of tissue (maintains central anaerobic conditions), should be collected aseptically and placed in a sterile container. Do not under any circumstances place multiple tissue types in a single container to create a "tissue pool", as this results in cross contamination and significantly decreases the chances of isolating the primary bacterial pathogen. Alternatively, a charcoal swab can be inserted into the liver tissue and placed in the charcoal gel. Specimens are then transported on ice at 4°C to the laboratory.

c. Mineral Analysis

- The objectives of mineral analysis are
- • To determine if mineral deficiency or excess exists.
- • Access the prevalence of deficiency.
- • Estimate endogenous reserves of trace minerals.

- There are a group of micronutrients which are best described in liver tissue and these include selenium, copper, zinc, manganese, iron and cobalt. Calcium, phosphorus, magnaesium and electolytes are better measured in serum.



Formalin-fixed liver tissue is the preferred sample for mineral analysis, as analyses are validated on fixed liver and formalin-fixation provides significant practical advantages when shipping to the laboratory, as there are no cold chain issues. In addition, archived liver tissue stored in formalin over many years can still be analyse enabling retrospective mineral analysis. A minimum of ± 20 g of liver is required for analysis (Figure 26). For most micronutrients normal physiological levels vary with the age of the animal.

Some complications associated with mineral analysis in serum / plasma include

- Plasma / serum concentrations of selenium, copper and zinc are affected by infection, stress, pregnancy and erythrocyte hemolysis.
- Metalloenzyme activities (eg: glutathione peroxidase used to measure selenium) in plasma are depleted during shipment to the laboratory.
- Zinc contamination by rubber stoppers of blood tubes.

Prior to submission to the laboratory, formalin fixed liver is removed from the fixative, covered with tissue paper and placed in a sealed sample envelope. This facilitates sample transport, as without the risk of formalin leakage, absorbent packing material is not required.

Further Reading

- 1. King J M, Roth L, Dodd D C & Newson M E. 2005. *The Necropsy Book* 4th edn. C.L.Davis, Gurnee.
- 2. Maxie G. 2016. *Jubb, Kennedy & Palmers Pathology of Domestic Animals* 6th edn. Elsevier.
- 3. Schulman F Y. *Veterinarians Guide to Maximising Biopsy Results*. Wiley-Blackwell
- 4. Stromberg P C. 2009. The Principles and practice of Veterinary Surgical Pathology. *Proceedings of the Annual meeting of the American College of Veterinary Pathologists*. Monterey, California.
- 5. Waldner & Blakley. 2014. Evaluating micronutrient concentrations in liver samples from abortions, stillbirths, and neonatal and postnatal losses in beef calves. *Journal of Veterinary Diagnostic Investigation* 26: 376-389
- 6. WSAVA. 2006. *Standards for Clinical and Histological diagnosis of canine and feline liver diseases*. Saunders-Elsevier, Edinburgh.