Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #**258**; Month: **April**; Year:**2025**

Answer sheet

Title: Galloway Hepatic Lipodystrophy

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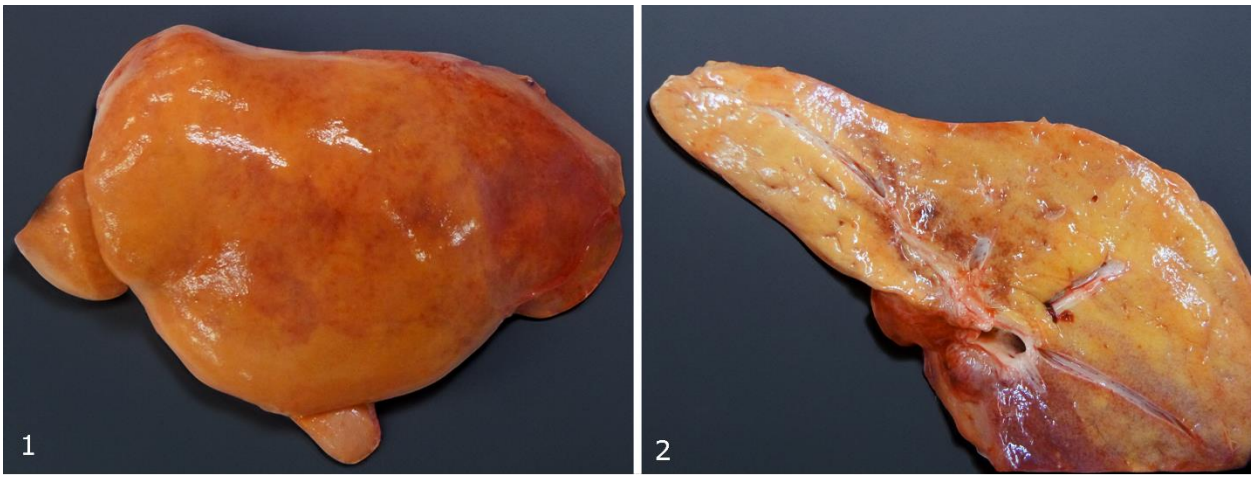
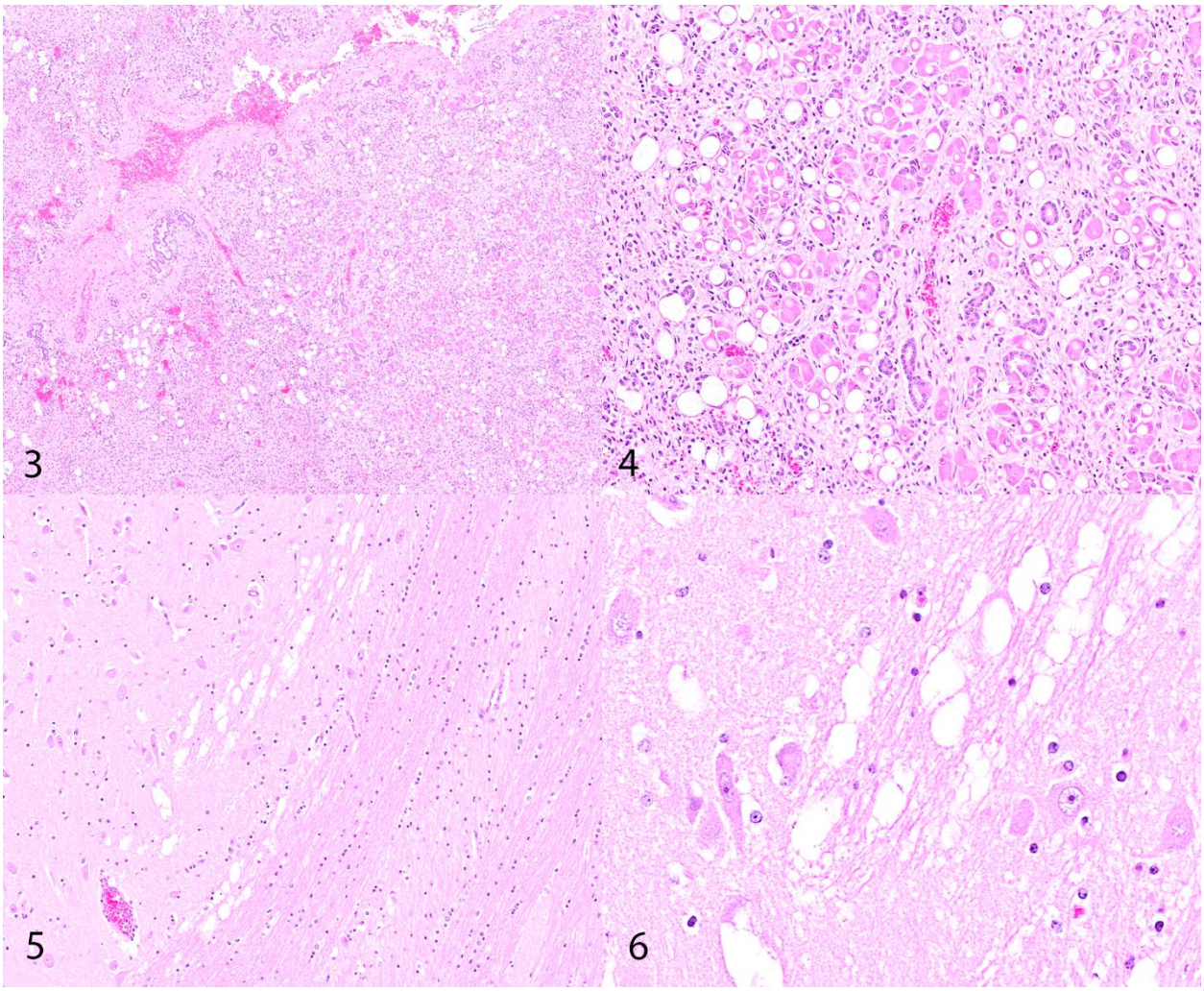
History: A 7-week-old, male, Galloway calf is presented for postmortem. The calf had a lifelong history of yellow pasty diarrhoea and congenital, bilateral contracted forelimbs that was responding to treatment (splinting and high dose, single parenteral oxytetracycline injection). More recently the calf developed significant mentation changes including dullness, not running away when approached, protruding tongue and poor feeding ability. No nystagmus, lameness or joint effusions were noted on physical exam. Another calf presented with acute neurologic deficits and decline at 6 weeks of age 6 months prior although no postmortem examination was performed. The clinician is concerned for viral infection vs congenital abnormality given limb contractures and neurologic signs.

Necropsy findings: Generally, the liver is approx. 1.5-fold enlarged, with rounded edges (hepatomegaly), and diffuse tan to yellow parenchyma (hepatopathy) with multifocal to coalescing areas of red discolouration affecting approx. 85% and 15% of the capsule overlying the left and right lobes, respectively (Figure 1). Upon sectioning, the red discoloration affects approx. 20% of the parenchyma, concentrated around the portal vein (haemorrhage – Figure 2). The abdominal cavity is filled with approximately 250ml of orange-red, clear fluid (ascites).



Figures 1 and 2: Expanded, pale yellow liver *ex-situ* with rounded lobe edges and multifocal-coalescing dark red regions of haemorrhage adjacent portal tracts on cut surface.



Figures 3 and 4: Low and high-power photomicrographs of hepatic parenchyma demonstrating severe fibrosis, biliary hyperplasia and lipidosis of remaining hepatocytes. Figure 5 and 6: Medium and high-power photomicrograph of the cerebral grey-white matter junction showing multifocal-coalescing, well defined clear spaces (spongiosis) displacing the neuropil.

Histologic description:

Liver: Generally, up to 95% of the hepatic parenchyma is lost (necrosis) and replaced by haphazardly arranged, fibrillar, eosinophilic matrix embedded with plump fibroblasts (fibrosis) that completely distorts the hepatic architecture with loss of normal zonal/acinar patterns (Figures 3 and 4). Frequently and multifocally embedded throughout fibrotic regions are tortuous bile duct profiles (ductular reaction – Figure 4). Remaining hepatocytes are clustered into rafts of about 100um in diameter that are separated and isolated from other hepatocytic rafts by fibrosis and bile duct profiles (Figure 4). There is moderate up to 2-fold anisocytosis and marked up to 3-fold anisokaryosis amongst the remaining hepatocyte population. Remaining hepatocytes are frequently up to 5-fold expanded by large up to 20um diameter, clear, round, well demarcated cytoplasmic vacuoles (lipidosis – Figure 4).

Brain: Throughout the brain particularly through the thalamus, hippocampus and cerebellar peduncles are well dispersed up to 2-fold expanded astrocytes with lightly eosinophilic, granular, expanded cytoplasm and an expanded, round nucleus with finely stippled-vesicular chromatin (Alzheimer type II astrocytes – Figure 6). Multifocally and occasionally at the junction of cerebral grey and white matter, the thalamus and the cerebellar peduncles are round-ovoid, well demarcated, up to 50um diameter clear spaces that displace the surrounding neuropil (spongiosis – Figure 5).

Morphologic diagnoses:

Liver: Marked, generalised, chronic fibrosis, ductular reaction, with subacute and active hepatocellular necrosis and lipidosis.

Brain: Mild to moderate, regionally extensive, subacute spongiosis and Alzheimer type II astrocytes.

Ancillary tests:

PCR, bovine viral diarrhoea virus (spleen): Negative

Agar-gel immunodiffusion test, bovine viral diarrhoea virus antibody (blood): Negative

Differential diagnoses:

1. Hepatic lipodystrophy of Galloway calves
2. Aflatoxicosis – milk derived
3. Pyrrolizidine alkaloid toxicosis – milk derived
4. Tetracycline toxicosis

Brief pathogenesis of neurological disease:

- Failure of hepatocytes to convert ammonia to urea within the portal circulation (due to hepatocellular functional impairment, damage or loss) leads to hyperammonaemia.
- Ammonia crosses the blood-brain barrier and is converted to glutamate by astrocytes in the brain.
- Excessive glutamate results in astrocyte degeneration and cell swelling (seen histomorphologically as "Alzheimer type II astrocytes").
- Neuronal degeneration and neurologic impairment is thought to be due to direct neurotoxic effects of ammonia, neurotransmitter imbalances/excitotoxicity and/or brain oedema (seen histomorphologically as spongiosis) due to astrocyte/endothelial degeneration.

Comments: Hepatic lipodystrophy of Galloway calves is a fatal, inherited (presumed genetic) disease of Galloway calves previously described in European and North American populations (6, 7). Histologic findings in these cases include hepatic fibrosis, biliary hyperplasia and hepatic lipidosis morphologically similar to lesions seen in this case. Clinically affected animals generally presented with neurologic signs as a manifestation of hepatic encephalopathy. Serum biochemistry on affected calves reveals marked increased hepatobiliary enzymes that can be used to detect disease antemortem. Affected animals may present from 1w to 5m of age and stillborn/aborted calves have been reported to be affected by the disease as well (6, 7). The cause is unknown however some others suggest that Galloway cattle have a high proportion of selenium-dependent glutathione peroxidase in liver tissue compared to other breeds that result in higher susceptibility to oxidative stress particularly with sub-optimal selenium intake (6, 7). Increased oxidative stress susceptibility could also increase the susceptibility of these animals to environmental hepatotoxins (e.g. pyrrolizidine alkaloids and aflatoxins). Pyrrolizidine alkaloids and aflatoxins were not tested for in this case and so their contribution to disease cannot be completely excluded.

Aflatoxins are mycotoxins produced by specific *Aspergillus* and *Penicillium* fungal species that tend to grow as a mould on stored/unharvested mature grains, legume stubbles or bread. High humidity/moisture and warm temperatures may influence the growth of fungal species on their substrate with grain stored for long periods in moist, warm environments particularly implicated in toxicosis (6, 8). Young animals are particularly susceptible to toxicity which may result in acute hepatic necrosis, generally culminating in sudden death or more subacute-chronic toxicity which may result in reduced growth rates, nonspecific clinical signs and hepatic

encephalopathy in advanced cases. Toxicity results in hepatocellular necrosis, fibrosis, ductular reaction and variation in hepatocellular size and nuclear size similar to megalocytosis seen in pyrrolizidine alkaloid toxicosis (6, 8).

Pyrrolizidine alkaloids are phytotoxins produced by several hundred species of plants worldwide with the plant genera *Senecio, Crotalaria, Heliotropium, Cynoglossum, Amsinckia, Echium* and *Trichodesma* most frequently implicated. Young animals are more susceptible than older animals to toxicosis; however, grazing animals are more likely to be exposed through ingestion of these plants. The toxin causes centrilobular hepatocellular necrosis - due to bioactivation through CYP enzyme system - and with chronicity or repetitive exposure leads to fibrosis and ductular reaction (5, 6). The toxins inhibit DNA synthesis and mitosis in hepatocytes that leads to very large hepatocytes with greatly enlarged nuclei known as megalocytes. In some cases, renal tubular and pulmonary vascular and interstitial lesions are also seen that may induce death due to high expression of CYP enzymes in these tissues also (5, 6).

Both aflatoxins and pyrrolizidine alkaloids are secreted in the milk of cattle ingesting these toxins, however natural cases of solely milk-derived toxicoses in calves have not been reported (5, 8).

Tetracycline derivates are commonly used antibiotics in production animal medicine often for respiratory infections. High doses of oxytetracycline given by slow intravenous infusion has been described in the literature for large animals as being beneficial for the treatment of flexural tendon contractures along with limb splinting (2, 3). Hepatotoxicity as well as nephrotoxicity, myotoxicity and cardiomyotoxicity is well reported with tetracycline overdoses in experimental and observational studies of humans and animals. Hepatic lipidosis is commonly the initial lesion associated with tetracycline hepatotoxicity. Fatal overdoses (from nephrotoxicity) have been reported in heifers given 33mg/kg of intravenous oxytetracycline when animals are otherwise stressed. Clinically healthy heifers developed nephrotoxicity at this same dose rate, but deaths were not recorded (1, 4). The chronicity of hepatic lesions in this case suggests prolonged exposure rather than a single time point exposure as would be expected with tetracycline toxicity in this case. Concurrently the lack of renal and tongue lesions reduces the index of suspicion for tetracycline toxicity, however this cannot be excluded.

To the best of the authors' knowledge this is the first suspected case of hepatic lipodystrophy in Galloway calves reported in Australia.

References:

1. Brihoum M, Amory H, Desmecht D, Cassart D, Deleuze S, Rollin F. Descriptive study of 32 cases of doxycycline-overdosed calves. J Vet Intern Med. 2010;24(5):1203-10.
2. Cihan M, Atalan G, Özba B, Ozaydin I. Treatment of congenital flexural tendon contracture by oxytetracycline administration in calves. The Indian veterinary journal. 2004;81:316-8.

3. Fazili MR, Bhattacharyya HK, Mir MuR, Hafiz A, Tufani NA. Prevalence and effect of oxytetracycline on congenital fetlock knuckling in neonatal dairy calves. 2014. 2014;81(1).
4. Griffin DD, Morter RL, Amstutz HE, Boon GD. Experimental oxytetracycline toxicity in feedlot heifers. The Bovine Practitioner. 1979;1979(14):37-41.
5. Kalač P, Kaltner F. Pyrrolizidine alkaloids of European Senecio/Jacobaea species in forage and their carry-over to milk: A review. Animal Feed Science and Technology. 2021;280:115062.
6. Maxie MG. Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 1: Elsevier Health Sciences; 2015.
7. Wieland M, Mann S, Hafner-Marx A, Ignatius A, Metzner M. Hepatic Lipodystrophy in Galloway Calves. Veterinary Pathology. 2017;54(3):467-74.
8. Zentai A, Jóźwiak Á, Süth M, Farkas Z. Carry-Over of Aflatoxin B1 from Feed to Cow Milk-A Review. Toxins (Basel). 2023;15(3).

*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). 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 DTF website.

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