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SHORT PAPER

Neuroendocrine Carcinoma of the Liver and Gallbladder in a Cow

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Summary

A 15-year-old Limousin-cross cow was presented for examination with neurological signs and serum biochemical changes consistent with liver disease. Necropsy revealed enlargement of the liver with multifocal firm, depressed, pale, circumscribed lesions throughout the parenchyma. Within the gallbladder there were exophytic and villiform mucosal masses. Microscopically, hepatic structure was displaced by neoplastic cells forming trabeculae, nests and rosettes. There was transmural infiltration of the gallbladder by similar cells. The histological pattern of growth of the neoplastic cells, the presence of silver-stained cytoplasmic granules within these cells and the immunohistochemical demonstration of chromogranin A supported the diagnosis of neuroendocrine carcinoma. Bovine liver and gallbladder neuroendocrine carcinomas are rare and this is the first detailed documentation of the disease in the United Kingdom.

Keywords: bovine; gallbladder; liver; neuroendocrine carcinoma

Neuroendocrine tumours arise from the dispersed neuroendocrine system. They are uncommon in men and seldom encountered in domestic animals (Creutzfeldt, 1996). The majority of these tumours in man develop in the intestine (Creutzfeldt, 1996), although primary hepatobiliary and gallbladder neuroendocrine tumours have been reported in human patients (Iwao *et al*., 2001), cats (Patnaik, 1992; Patnaik *et al*., 2005a) and dogs (Patnaik *et al*., 1981, 2005b; Willard *et al*., 1988; Churcher, 1999; Morrell *et al*., 2002). The present case report is the first description of neuroendocrine carcinoma of the bovine liver and gallbladder in the United Kingdom (UK).

A 15-year-old Limousin-cross cow from Wales (UK) reportedly developed mild behavioural changes (altered head carriage, ear twitching and nose licking) as well as tremors and hindlimb ataxia. These neurological signs were of 3–4 weeks' duration and the owner noted that the cow had recently gained weight. Currently in the UK, a report of altered behaviour and neurological signs in an adult cow necessitates destruction of the animal and examination of nervous system tissues to determine whether there are lesions consistent with bovine spongiform encephalopathy (BSE). Clinical examination at the Veterinary Laboratories Agency, Weybridge found that the cow had watery diarrhoea and neurological examination demonstrated restlessness, an exaggerated menace response and over-reactivity to tactile stimuli. Haematological and serum biochemical abnormalities included anaemia, lymphopenia and neutrophilia, hypoalbuminaemia, and increased activity of liver enzymes, including aspartate aminotransferase, glutamate dehydrogenase, and γ-glutamyl transferase (Table 1).

The cow was humanely destroyed by injection of pentobarbital and necropsy was performed. Cerebrospinal fluid was collected and tissue samples (whole brain and sections of the spinal cord, selected lymph nodes, spleen, palatine tonsil, parts of the small and large intestine) were taken for BSE evaluation. Limited examination of other viscera revealed enlargement of the liver, with all lobes containing pale, circumscribed, non-encapsulated depressed foci, 1–4 cm in size. The

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Table 1
Haematological and serum biochemical abnormalities

Parameter	*Reference range*[*]	*Value*
Red blood cells ($\times 10^{12}$/l)	5–10	4.42
Neutrophils ($\times 10^{9}$/l)	0.6–4	7.6
Lymphocytes ($\times 10^{9}$/l)	2.5–7.5	1.7
Glucose (mmol/l)	2.8–3.6	3.84
Aspartate aminotransferase (U/l)	75–130	138
γ-Glutamyl transferase (U/l)	0–30	84
Glutamate dehydrogenase (U/l)	0–25	28
Magnesium (mmol/l)	0.7–1.3	0.61
Glutathione peroxidase (U/ml RBC)	>30	19.3

[*]Provided by the Clinical Pathology Laboratory, VLA, Shrewsbury, UK.

gallbladder was enlarged to nearly twice its normal size and the mucosal surface displayed diffuse, pedunculated, villiform projections 3–4.5 cm long and 2–3 cm wide (Fig. 1). Other gross findings included a single cyst (1 cm in diameter) within the right renal cortex and bilateral enlargement of the adrenal glands, which were approximately twice their normal size.

Histopathological examination revealed that the hepatic parenchyma was infiltrated by neoplastic cells arranged in trabeculae, acini and rosettes separated by variably sized bands of fibrous connective tissue (Fig. 2). Neoplastic cells were polygonal, with indistinct cellular margins and a moderate quantity of eosinophilic granular cytoplasm. The nuclei of these cells were oval, with finely stippled chromatin and multiple distinct nucleoli. There was an average of three mitotic figures per 10 fields examined with a ×40 objective. Multiple blood vessels contained clusters of similar neoplastic cells.

Fig. 1. Opened gallbladder showing mucosal villiform masses projecting into the lumen.

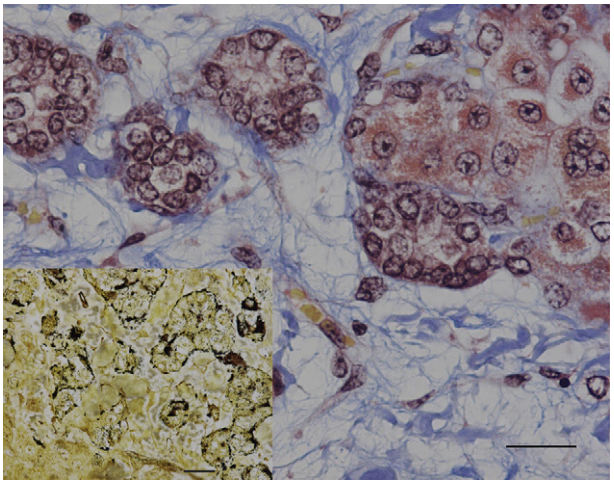

Fig. 2. Section of liver showing rosettes and trabeculae of neoplastic cells surrounded by a loose collagen matrix. Martius Scarlet Blue. Bar, 25 μm. Inset shows argyrophilic tumour acini. Grimelius silver stain. Bar, 20 μm.

The masses projecting from the mucosa of the gallbladder consisted of a stalk of oedematous, loosely arranged connective tissue matrix, lined by ciliated columnar epithelium and incorporating areas of glandular hyperplasia represented by numerous, cystic, mucus-filled submucosal glands. Moderate lymphoplasmacytic inflammation was associated with these regions of glandular hyperplasia. The wall of the gallbladder was further diffusely infiltrated by neoplastic cells as described within the liver. These extended from the serosal to the mucosal surface. Neoplastic cells within the gallbladder frequently formed dense cellular sheets, as well as trabeculae, separated by fibrovascular stroma (Fig. 3). Tumour cells were noted within the lumina of medium-sized blood and lymphatic vessels of the gallbladder wall.

Histochemical stains and immunohistochemical labelling were used to confirm the tentative microscopical diagnosis of hepatobiliary neuroendocrine carcinoma. Sections were stained by the Churukian–Schenck method for the chromaffin reaction, by Grimelius silver stain to demonstrate argyrophilic neurosecretory granules, and by Martius scarlet blue (MSB) to highlight fibrous connective tissue. Immunohistochemistry (IHC) was performed on sections of the liver tumour with antibodies directed against chromogranin A, kermix (AE1/AE3/CK1), cytokeratin (CK) 19 and CK 7 (all from Dako; Carpinteria, CA, USA), vimentin (Ventana; Tucson, AZ, USA), neuron-specific enolase (NSE) and synaptophysin (both from CellMarque; Rocklin, CA, USA) in a standard avidin–biotin–peroxidase conjugate technique.