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# Characterization of cytoplasmic hyaline bodies in a hepatocellular carcinoma of a dog

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ABSTRACT

This report describes the morphological and immunohistochemical features of intracytoplasmic inclusion bodies found in a 13-year-old Yorkshire dog with a hepatocellular carcinoma and referred for anorexia, lethargy and mild polydipsia. Fine-needle aspirates of the large abdominal mass revealed high number of pleomorphic neoplastic hepatocytes, containing round to polygonal, well-demarcated, hyaline bodies. Same findings were histologically confirmed on multiple biopsies. Immunohistochemically, the inclusion bodies were negative for alpha-1-antitrypsin, carcinoembryonary antigen, fibrinogen, IgG, IgM, cytokeratins 7, 8, 18, 19, 20. By transmission electron microscopy, the cytoplasmic inclusions were composed of granular homogeneous or reticulated electrondense matrix, enclosed within dilated rough endoplasmic reticulum or remnants of its membranes, consistent with proteinaceous material accumulated within neoplastic hepatocytes due to aberrant protein secretion or transport. This is the first detailed characterization of hyaline cytoplasmic inclusion bodies in canine hepatocellular carcinoma.



Hepatocellular carcinom as (HCCs) are infrequently seen in dogs as large solitary masses or widespread infiltrations throughout the liver with metastases to regional lymph nodes and occasionally distant sites. Morphologically, neoplastic hepatocytes could be highly variable, ranging from well to poorly differentiated (Cullen and Popp, 2002). In men, different types of intracytoplasmic inclusions, such as hyaline bodies (HBs) (Stumptner et al., 1999), pale bodies (PBs) or ground glass-like bodies (Nakashima et al., 1992), $\alpha_1$-antitrypsin (AAT)-containing globules (Anthony, 1994), and Mallory bodies (MBs) (Denk et al., 2000), each with a specific morphology and chemical composition, have been found in HCC cells.

To the authors' knowledge, this is the first detailed characterization of intracytoplasmic hyaline inclusion bodies in a canine hepatocellular carcinoma.

A 13-year-old male Yorkshire terrier dog was presented with a history of lethargy, anorexia and mild polydipsia. Two years before, the dog was diagnosed with insulin-dependent diabetes and hypothyroidism, being daily treated since then with insulin and L-thyroxin. Clinical examination revealed mild hypotermia (38.2 °C) and keratoconjunctivitis. Haematology and serum biochemistry results are summarized in Table 1. A large abdominal mass involving liver, pancreas and right adrenal gland was detected by ultrasonography. For cytology, an ultrasound-guided fine-needle aspiration of the mass was performed. On May Grünwald–Giemsa-stained slides, moderate number of large epithelial cells, morphologically consistent with hepatocytes and exfoliating as individual cells or in small clusters, occasionally arranged in acini or rows were observed. The cells had large slightly basophilic cytoplasm, filled with large, round and sharply demarcated clear lipid globules. Many cells contained round to polygonal, variably-sized, glassy, slightly basophilic cytoplasmic bodies (Fig. 1A). Nuclei were round to ovoid, with irregular granular chromatin, variable number of prominent nucleoli and occasional intranuclear pseudoinclusions; anisocytosis and anisokaryosis were moderate. Haematoxylin-Eosin (H&E) and PAS (Periodic Acid Schiff) without and with diastase treatment (PAS-D) on alcohol-fixed smears, as well as Oil-Red-O staining on air-dried smears were performed for further characterization. Scattered cells contain moderate amount of PAS-positive intracytoplasmic material, revealed to be negative after diastase treatment and thus consistent with glycogen and some type of glycoproteins. The cytoplasmic inclusions stained intensely eosinophilic with H&E. Oil-Red-O stain confirmed the lipid composition of the clear globules. Cytologically, a hepatocellular carcinoma with unknown cytoplasmic bodies and associated lipidosis was diagnosed.

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**Table 1**
Haematology and serum biochemistry results from a 13-year-old, male Yorkshire terrier dog with hepatocellular carcinoma.

| Selected parameters | Result | Reference interval |
|---|---|---|
| MCV (fl) | 72.2 | 61–72 |
| Hb (g/dl) | 34.6 | 34–38 |
| Hct (%) | 33 | 38.6–54.5 |
| Platelets ($\times 10^3$ cells/μl) | 583 | 160–440 |
| Albumin (g/dl) | 2.4 | 2.6–3.8 |
| AST (U/l) | 211 | 15–40 |
| ALT (U/l) | 564 | 15–65 |
| ALP (U/l) | 552 | 20–120 |
| Amylase (U/l) | 1045 | 350–900 |
| Lipase (U/l) | 937 | 120–630 |
| Cholesterol (mg/dl) | 340 | 120–300 |
| Triglycerides (mg/dl) | 112 | 30–85 |
| BUN (mg/dl) | 97 | 18–43 |
| Creatinine (mg/dl) | 1.66 | 0.7–1.3 |
| Glucose (mg/dl) | 154 | 75–115 |
| Sideremia (μg/dl) | 66 | 81–220 |
| Saturation rate (%) | 15.1 | 25–52 |
| UIBC (μg/dl) | 370 | 150–300 |
| Ferritin (ng/ml) | 205 | 60–190 |
| C-reactive protein (mg/dl) | 4.56 | 0–0.15 |

Parameters are listed in SI units; MCV, mean corpuscular volume; Hb, hemoglobin; Hct, hematocrit; AST, aspartate aminotransferase; ALT, alanine aminostransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; UIBC, unsaturated iron binding capacity.

Computed tomography (CT) confirmed the hepatic origin of the mass with involvement of the caudal lobe. Tissue samples collected by transcutaneous CT-guided biopsy were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned (5 μm) and stained with H&E. The histologic examination revealed the presence of trabeculae of neoplastic hepatocytes, multifocally admixed with irregular vascular lacunae and containing intracytoplasmic round bodies. The latter ones were eosinophilic by H&E, (Fig. 1B), focally PAS-positive and diffusely negative after diastase treatment. A trabecular-type hepatocellular carcinoma with peliosis was confirmed. The mass was surgically excised and multiple samples were processed as previously described. Histopathology of the excised mass confirmed the same findings observed on bioptic samples.

Immunohistochemistry (IHC) was performed with a panel of monoclonal or polyclonal antibodies, specific for the following molecules: alpha-1-antitrypsin (polyclonal), carcinoembryonary antigen (CEA, monoclonal), fibrinogen (polyclonal), IgG (polyclonal), IgM (polyclonal), cytokeratins 7, 8, 18, 19, 20 (polyclonal). Unless otherwise stated, all antibodies were purchased from Dako (Glostrup, Denmark). The inclusion bodies resulted negative for all these markers.

For further characterization, representative 1 $mm^3$ pieces from the mass were fixed in 2.5% gluteraldehyde, post-fixed in osmium tetraoxide 1%, infiltrated with propylene oxide, and embedded in epoxy resin (Durcupan™ ACM Fluka, EMS, Hatfield, PA) for transmission electron microscopy (TEM). Ultrathin sections were stained with uranyl acetate and lead citrate. TEM revealed globular cytoplasmic inclusions consisting of a granular homogeneous or reticulated electrondense matrix, enclosed within dilated rough endoplasmic reticulum (RER) or remnants of its membranes (Fig. 1C,D), consistent with condensed proteinaceous material.

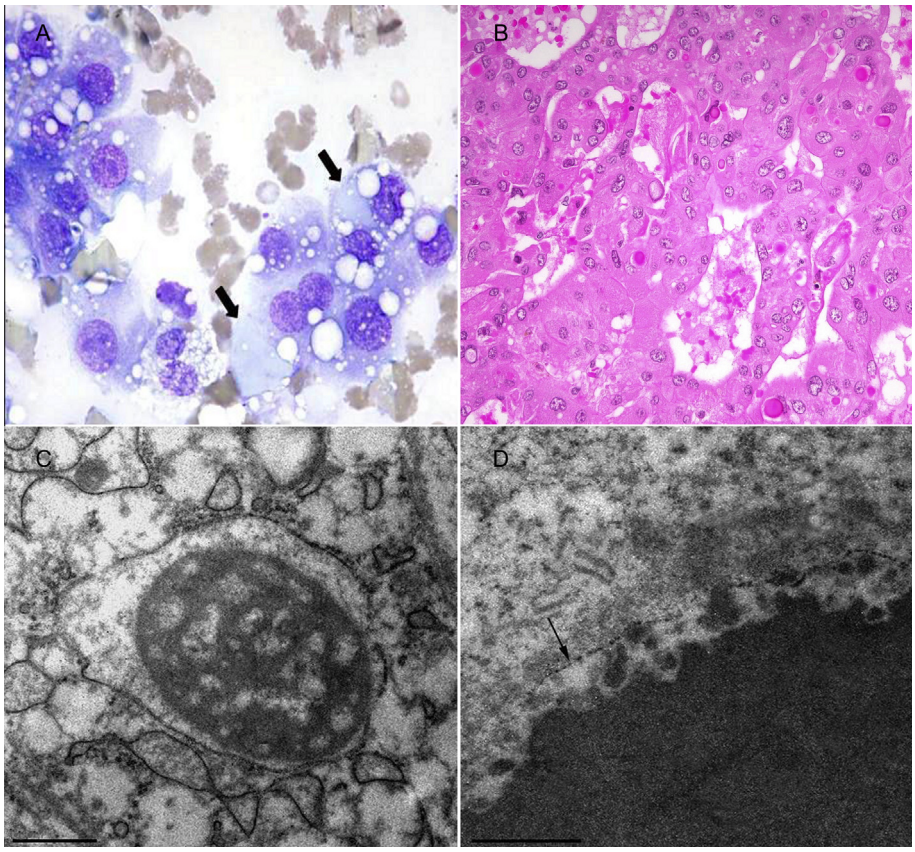


**Fig. 1.** (A) Ultrasound-guided fine-needle aspirate from a dog with a hepatocellular carcinoma: clusters of neoplastic cells containing high number of heterogeneous clear round vacuoles (lipids) and occasional variably-sized basophilic inclusions (arrows), MGG stain, 40X. (B) Liver of a dog with a trabecular-type hepatocellular carcinoma characterized by neoplastic cells arranged in irregular trabeculae admixed with vascular lacunae. Note the high number of intracytoplasmic, well-demarcated, eosinophilic inclusions, H&E stain, 40X. (C, D) Ultrastructural features of inclusion bodies in a canine hepatocellular carcinoma: prominent reticulated pattern within dilated stacks of rough endoplasmic reticulum (C) or homogeneous granular electrondense matrix surrounded by remnants of RER membranes (D, arrow). Uranyl acetate and lead citrate; Bar = 1 μm (C), 0.5 μm (D).