# Acinar cell carcinoma of exocrine pancreas in two horses. A histologic, immunohistochemical and ultrastructural study

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**Abstract**

Tumors of the pancreas are uncommon in animals. Two horses presented separately with nonspecific clinical signs of few weeks duration, and were euthanized due to their poor prognosis. At necropsy, both horses had multiple abundant, small, whitish nodules that replaced pancreatic tissue and multifocally involved the serosal surface of the abdominal cavity, and liver, and lung parenchyma. Histologically, neoplastic cells were organized in acini and contained abundant (horse No. 1) and rare (horse No. 2) intracytoplasmatic zymogen granules. Both tumors were positive by immunohistochemistry for amylase and pan-cytokeratin, and negative for insulin and neuron-specific enolase. In horse No. 2, a low percentage of neoplastic cells were also positive for glucagon and synaptophysin. The presence of zymogen granules was confirmed in both cases by electron microscopy. Moreover, occasional fibrillary and glucagon granules were observed in horse No. 1 and 2, respectively. A diagnosis of pancreatic acinar cell carcinoma was established in both horses. To the authors' knowledge, this is the first description of immunohistological and ultrastructural findings in equine patients with exocrine pancreatic tumors.

**Keywords:**

Pancreas; immunohistochemistry; electron microscopy; fibrillary granules

Neoplastic lesions of the pancreas are uncommon in domestic animals and originate more frequently from exocrine than from endocrine cells (Charles, 2007; Head, 2002; Priester, 1974). Most cases are reported in dogs (Dennis *et al.*, 2008; Rabanal *et al.*, 1992; Rowlatt, 1967), less commonly in cats (Banner *et al.*, 1979; Seaman, 2004), and rarely in horses (Carrick *et al.*, 1992; Church *et al.*, 1987; Rendle *et al.*, 2006), cattle (Kelley *et al.*, 1996; Lucena *et al.*, 2011) and pigs (Rowlatt, 1967). This differs from people, where pancreatic neoplasia is among the five most common causes of cancer-related mortality with an increasing rate of prevalence (Greenlee *et al.*, 2001; Malvezzi *et al.*, 2013).

The diagnosis of pancreatic exocrine carcinoma on routine light microscopy may be difficult since it can be confused with other tumors, especially islet cell carcinoma (Ordóñez, 2001). In people, it has been demonstrated that both immunohistochemistry (IHC) and electron microscopy (EM) are useful in establishing the diagnosis of pancreatic cell carcinomas (La Rosa *et al.*, 2012; Ordóñez, 2001). However, the few reports of equine pancreatic tumors are based on gross and light microscopic findings, and do not include immunohistochemical or ultrastructural studies. The present study describes the pathological details of two horses with pancreatic acinar cell carcinoma, including histologic, immunohistochemical and ultrastructural studies.

Horse No. 1 was a 22-year-old Polish warmblood mare that was presented to the Clinic for Equine Internal Medicine (Vetsuisse Faculty, University of Zurich) with a 3-week history of apathy, anorexia, weight loss, polyuria and polydipsia. Horse No. 2 was a 17-year-old Swiss warmblood gelding that was presented to the same institution with a several week history of weight loss and anorexia. Laboratory investigations showed significant elevation of hepatic enzymes in both cases. Transcutaneous ultrasound scan revealed the presence of multiple intrapulmonary nodules and a thickened colon wall in horse 1, and an irregular serosal surface of the small intestine in horse No. 2. Exploratory

laparotomy was performed in horse No. 2, which revealed a thickened colonic wall, enlarged abdominal lymph nodes and a firm liver. Neoplasia was suspected in both cases and the animals were euthanized due to poor prognosis and full necropsies were performed less than 6 hours later.

Samples of various organs were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 3 µm, and stained with hematoxylin and eosin (HE). Selected sections were stained with Periodic Acid-Schiff (PAS), PAS after enzymatic digestion with diastase and Masson trichrome stain. Immunohistochemical evaluation was performed using commercially available antibodies against amylase (anti-rabbit, 1:1000, Sigma Chemical, A8273) pan-cytokeratin (anti-mouse, 1:50, DAKO M082101), Glucagon (anti-human, 1:100, DAKO A0565), insulin (anti-guinea pig, 1:200, DAKO A0564), neuron-specific enolase (NSE) (anti-mouse, 1:150, DAKO M087301) and synaptophysin (anti-mouse, 1:10, DAKO M077601). The standard avidin-biotin-peroxidase technique was used to demonstrate the antigen. Normal horse pancreatic tissue was used as a positive control in all IHC. Results were semi-quantified by assessing the percentage of labeled neoplastic cells as follows: (-)positive neoplastic cells were not seen, (+) less than 10% of neoplastic cells labeled, (++) between 10 and 50% of neoplastic cells labeled, and (+++) more than 50% of neoplastic cells labeled.

For electron microscopy (EM), selected tissues were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide ($OsO_4$), buffered with 0.1M sodium phosphate (pH 7.4). After fixation, the tissues were dehydrated in an ethanol series, embedded in Epon (Fluka) and ultrathin (90nm) sections were stained with uranyl acetate and lead citrate.

Case No. 1: On post mortem examination, 4 and 30 l of sero-sanguinous fluids were present within pleural and peritoneal cavity, respectively. The pancreas was markedly irregular and diffusely disrupted by innumerable small whitish to tan, firm

nodules. Similar nodules of 2 to 15 mm in diameter, some of them umbilicated, were scattered over the mesentery and on the serosal surface of the small intestine, colon, spleen and diaphragm. Approximately 50% of hepatic parenchyma contained multifocal to coalescing miliary nodules of the same characteristics. Mesenteric lymph nodes were markedly enlarged. In the thorax, approximately 10% of the pulmonary parenchyma was infiltrated by multiple tan to reddish firm nodules of 5 to 15 mm in diameter, and mediastinal lymph nodes were also markedly enlarged.

Case No. 2: On post mortem examination, approximately 20 l of sero-sanguinous fluids were present in the abdominal cavity. Similarly to the previous case, the pancreas was extensively replaced by a firm, whitish, multinodular mass with a smooth surface that adhered to the serosa covering liver and diaphragm. The liver was firm and infiltrated with miliary multifocal to coalescing pale foci of 1 to 10 mm in diameter, which grossly occupied approximately 50% of the parenchyma. Hepatic and mesenteric lymph nodes were markedly enlarged, firm and creamy in color. Examination of the gastrointestinal tract showed a severely edematous ascending colon.

In both cases, histological examination revealed that the grossly observed nodules corresponded to a densely cellular, infiltrative and non-encapsulated neoplastic proliferation. No normal pancreatic tissue was observed in any of the studied sections. Neoplastic cells were organized mainly in acinar, and less frequently tubular or solid growth patterns. The stroma was fibrous and moderately thick, showing multifocal scirrhous reaction. Neoplastic cells were polyhedral, medium-sized (15-25 µm in diameter), and showed mainly indistinct cell borders. In horse No. 1, tumoral cells showed moderate amount of eosinophilic homogeneous cytoplasm, often containing numerous intracytoplasmic deeply eosinophilic granules (Fig. 1). However, in horse No. 2, only scant similar granules were occasionally seen within a low number of neoplastic cells.

The nuclei of neoplastic cells were round to oval, often basally located, and contained finely stippled chromatin with 1 to 3 prominent eosinophilic nucleoli. Abundant mitotic figures were seen, being more numerous in the areas with a solid growth pattern (up to 10 per high power field). The degree of anisokaryosis and anisocytosis was marked. Multifocally, there were extensive and abundant areas of necrosis, sometimes accompanied by hemorrhages. Often, lumen of lypmphatic and blood vessels showed numerous aggregates of neoplastic cells. Multifocally, areas of moderate lymphoplasmacytic infiltration could be seen. Overall, the observed features were compatible with pancreatic acinar carcinoma with metastasis to liver, lung, lymph nodes and peritoneal surfaces.

Marked submocosal edema of ascending colon was observed in case No. 2, but no other significant abnormalities were seen in any other organ examined histologically in either case.

In both cases, the intracytoplasmatic granules observed within neoplastic cells were diastase-resistant, PAS-positive and clearly visible with trichrome Masson staining. Immunohistochemical results are summarized in table 1. In both cases, diffuse, strong, intracytoplasmatic, finely granular labelling for pan-cytokeratin was observed. Staining for Amylase demonstrated intense, granular, intracytoplasmatic labelling, often forming clumps, in approximately 30-40% neoplastic cells in case No. 2, whilst only occasional neoplastic cells reacted positively in horse No. 1. Horse No. 2 had scattered, rare, intracytoplasmatic labelling for glucagon and synaptophysin, whilst sections from horse No. 1 were negative. Neoplastic cells from both cases were negative for insulin and NSE.

Ultrastructural examination of metastatic nodules showed neoplastic cells with obvious exocrine secretory characteristics that were more prominent in case No. 1. Neoplastic cells were polygonal, often organized in acini, with basally located nuclei and

an apical small lumen. Frequently, neoplastic cells contained abundant (horse No. 1) or moderate amount (horse No. 2) of intracytoplasmic round to oval and electron dense granules, measuring between 100 and 2000nm in diameter and surrounded by a limiting membrane, compatible with zymogen granules (Fig. 2). In addition, case No. 1 revealed some tumor cells that displayed another type of cytoplasmic granules, with oval to rhomboid shape, measuring up to 600 nm. These inclusions showed a limiting membrane and contained densely packed fibrillary material (Fig. 3). No evidence of these granules was seen in case No. 2. Besides, tumoral cells of case No. 2 revealed, occasionally, round to oval cytoplasmic granules of 100 to 400nm in diameter with a round to oval, eccentric, dense homogeneous core surrounded by a membraneous envelope, compatible with neuroendocrine granules, more specifically, alpha granules. Similar granules were not seen in case No. 1.

Based on gross, histological, IHC and EM findings, a diagnosis of pancreatic acinar cell carcinoma was made in both horses.

Grossly, differential diagnoses in both horses included pancreatic islet cell carcinoma, exocrine pancreatic carcinoma, cholangiocellular carcinoma and mesothelioma. During necropsy, gastrointestinal carcinoma was also considered but was soon ruled out, since no neoplastic nodules affecting mucosa or intestinal wall were observed in any portion of the gastrointestinal tract. On histological examination, the observation of granules compatible with zymogen granules within neoplastic cells that were organized in acini (mainly in horse No. 1) pointed towards a diagnosis of pancreatic exocrine carcinoma (Head, 2002; Ordóñez, 2001).

According to the International Classification of Tumours of Domestic Animals (WHO), there are three histological types of epithelial neoplasms originating from exocrine pancreas: adenomas, adenocarcinomas and non-differentiated carcinomas

(Kircher and Nielsen, 1976). Adenocarcinomas are further divided into small tubular, large tubular and acinar cell carcinomas (ACC). In both our cases, the acinar pattern was predominant, but tubular and solid patterns of undifferentiated cells were also multifocally observed. Similarly, pancreatic ACC in people frequently display variable histologic features, with multiple appearances within a single neoplasm as well as among tumors (Ordóñez, 2001) .

Zymogen granules are characteristic of exocrine pancreatic carcinomas and, more specifically, of acinar cell carcinoma subtype, since tubular cells do not contain them (La Rosa *et al.*, 2012; Ordóñez, 2001). However, it is known that zymogen granules may be unapparent, or present only in a low number of cells within the tumor (Head, 2002; Ordóñez, 2001), as it was the case in horse No. 2. In these situations, the use of IHC against pancreatic hormones, neuroendocrine granules and pancreatic enzymes is especially useful to discriminate between endocrine and exocrine pancreatic carcinomas (Head, 2002). In our cases, endocrine pancreatic neoplasia was ruled out in both horses, since negative results were obtained by IHC for insulin and NSE, and only a low percentage of tumoral cells were positive for synaptophysin and glucagon in horse No. 2 By contrast, positive results for amylase were obtained in both tumors. Surprisingly, amylase labelling was more intense in the horse No. 2, in which the zymogen granules were less frequently seen in HE, while the labelling was observed in only a low percentage of cells in horse No. 1. Interestingly, in people pancreatic ACCs often had a low percentage of positive cells (La Rosa *et al.*, 2012). In addition, among the few IHC data available in veterinary species, Rabanal et al. (1992) suggested that IHC for both amylase and carboxypeptidase are of value in the diagnosis of pancreatic exocrine neoplasms in dog, showing frequently abundant labelling within these tumors (Rabanal *et al.*, 1992). These variations in amylase IHC results might be attributed to different

200 factors such as the use of different antibodies, laboratory procedures as well as inter-
201 species differences. Finally, the presented tumors were strongly and diffusely positive for
202 pan-cytokeratin IHC, confirming their epithelial origin.

203 Ultrastructurally, zymogen granules are generally larger than neuroendocrine
204 granules. In people, zymogen granules in ACC are reported to measure between 125 to
205 more than 1000 nm in diameter, while neuroendocrine granules are usually less than 500
206 nm and commonly show a halo and granular contents (Ordóñez, 2001; Ulich *et al.*, 1982).
207 In both presented cases, most predominant granules showed ultrastuctural features
208 characteristic of zymogen granules. In addition, in horse No. 1, some neoplastic cells
209 contained large irregular granules with a fine filamentous ultrastructure. Interestingly,
210 these granules are similar to the so-called fibrillary granules, which are reported to be a
211 frequent feature in human pancreatic ACC (Ordóñez, 2001) and have only occasionally
212 been described dogs (Banner *et al.*, 1978) and a cat (Banner *et al.*, 1979). Fibrillary
213 granules are considered to be part of the spectrum of zymogen granules in neoplastic
214 acinar cells and are considered to be an important diagnostic feature of pancreatic ACC
215 in human beings (Ordóñez, 2001).

216 Finally, in horse No. 2, few neoplastic cells showed smaller round granules with
217 a highly electron opaque core surrounded by a clear halo, which were compatible with
218 glucagon granules. Accordingly, a low percentage of neoplastic cells in this horse showed
219 reactivity for both glucagon and synaptophysin IHC. Taking into account that the EM
220 technique was performed on tissue collected from metastatic nodules on intestinal serosa,
221 far away from the pancreatic anatomic area, it is suggested that pancreatic ACC in horse
222 No. 2 showed scattered neuroendocrine differentiation associated with the neoplastic
223 acinar glands. Likewise, nearly 50% of primary and metastatic human pancreatic
224 carcinomas show pluripotent capacity and express two or more cell lineage markers (Kim

*et al.*, 1990). Therefore, this finding suggests that in horses, as hypothesised in human beings, the original transformed cell type in some pancreatic exocrine carcinomas may retain the capability for differentiation along more than one cell lineage pathway (Kim *et al.*, 1990). In human beings, diagnosis of a mixed acinar-neuroendocrine carcinoma is established when more than 30% of neoplastic cells show endocrine differentiation (La Rosa *et al.*, 2012). Since this was not the case in here presented horses, pancreatic acinar cell carcinoma was the final diagnosis in both cases.

In summary, this is the first description of IHC and EM features of pancreatic carcinoma in horses. Electron microscopy identified fibrillary granules for the first time in equine cases. Findings support the utility of EM and IHC techniques in establishing ACCs, especially in cases where zymogen granules are not visible in conventional HE staining.

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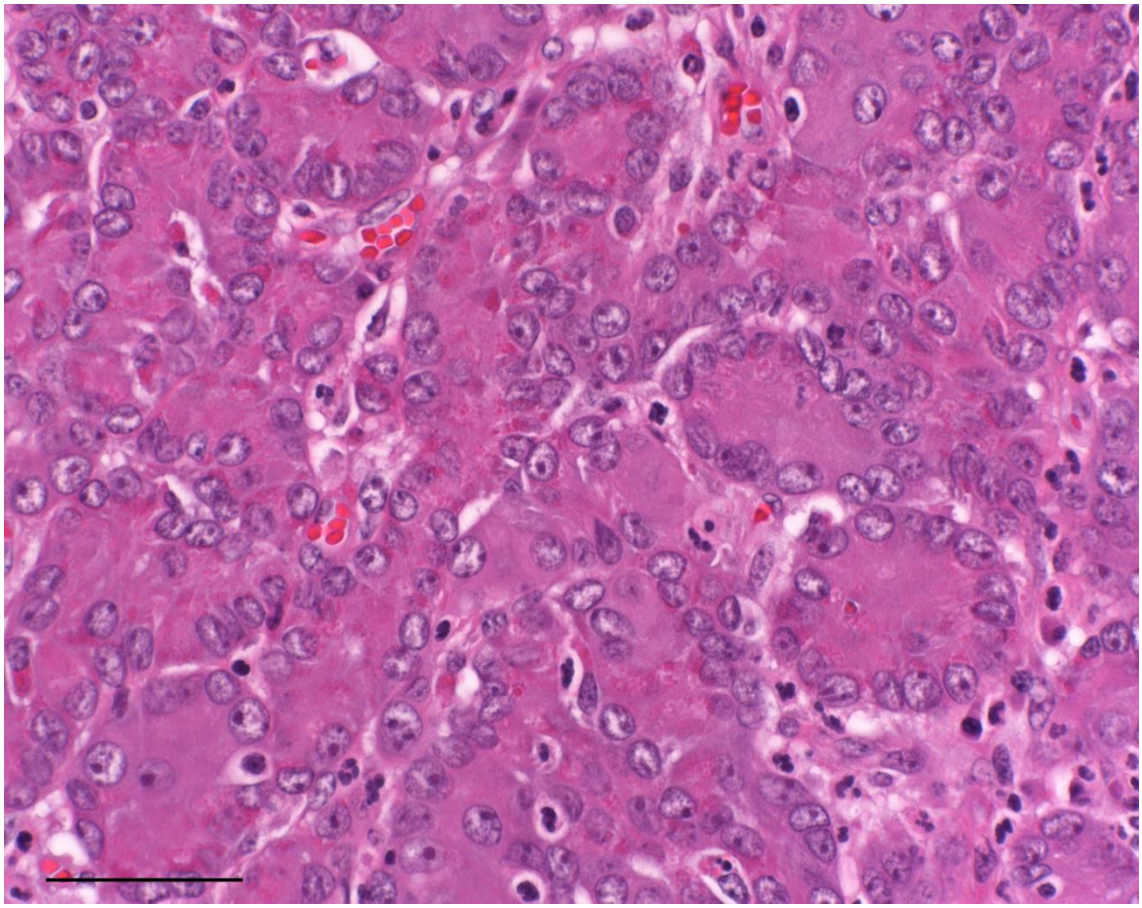
353 **Figures**



354

355 **Fig. 1.** Pancreatic acinar cell carcinoma; horse No.1. Acini of neoplastic cylindrical cells
356 with basally located nuclei and intracytoplasmic deeply eosinophilic granules (zymogen
357 granules). HE. Bar, 50 µm.

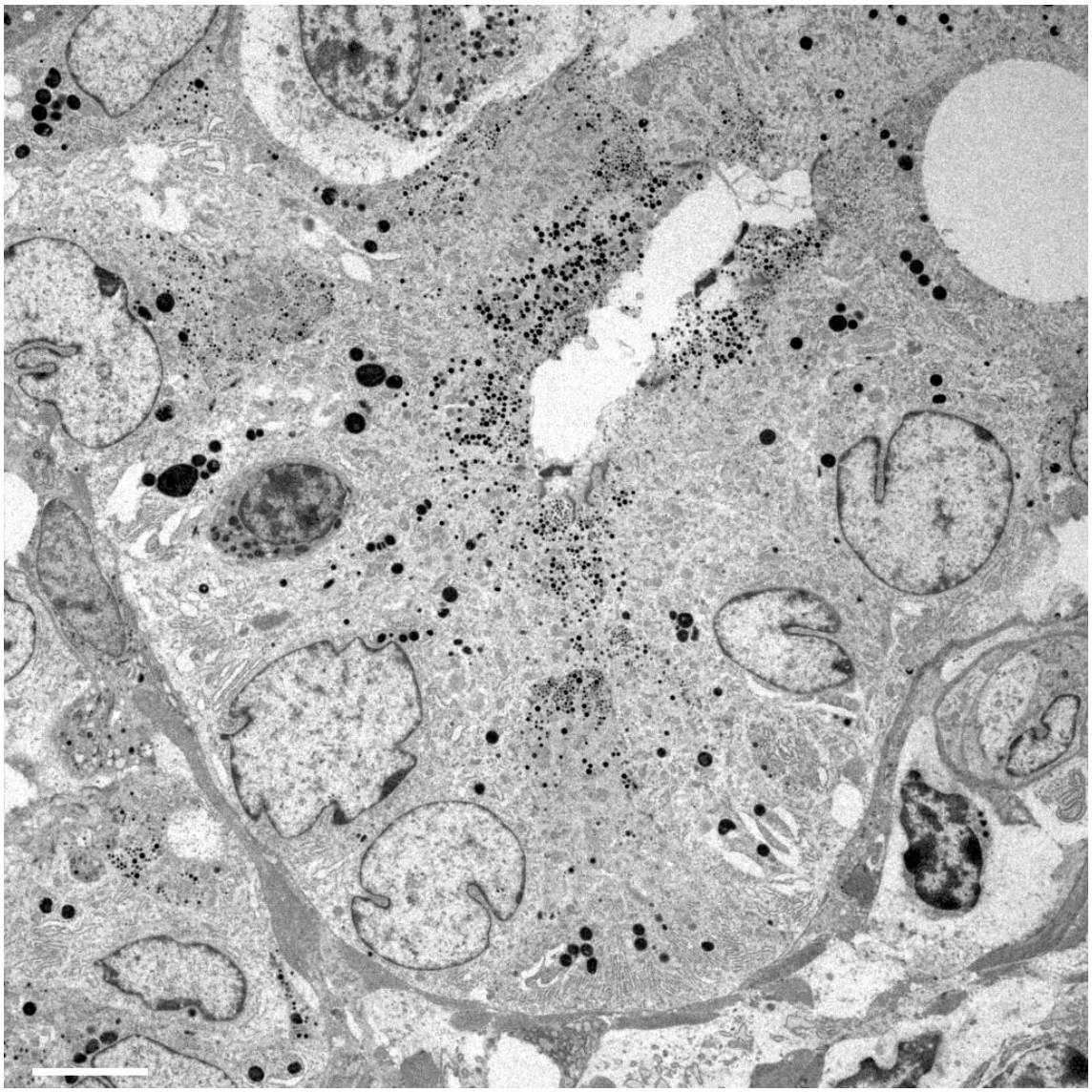


**Fig. 2**. Pancreatic acinar cell carcinoma. Horse No 1. Several neoplastic cells are organized in acini. They show basally located nuclei, contain abundant intracytoplasmic round electron dense granules (zymogen granules) of up to 2000 nm in diameter and form a small lumen bordered by small microvilli in their apical pole. Electron microscopy. Bar, 5 µm.

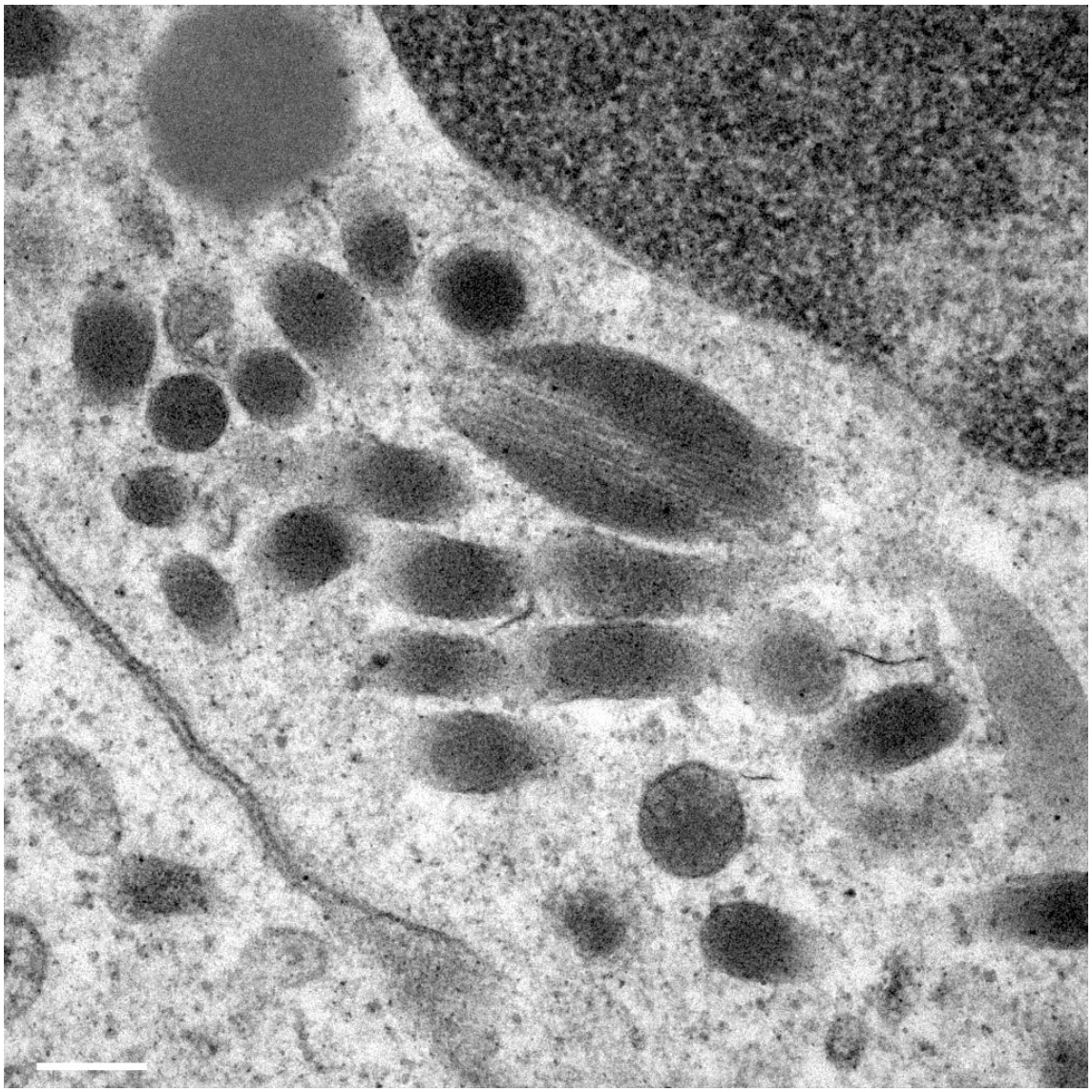


**Fig. 3.** Horse No. 1. Neoplastic cell with intracytoplasmic oval to rhomboid shaped fibrillary granules. Electron microscopy. Bar, 0.2 µm

374 **Table 1.** Immunohistochemical results in neoplastic tissues of both horses.

| Antibody | Horse No. 1 | Horse No. 2 |
|---|---|---|
| **Amylase** | + | ++ |
| **Cytokeratin** | +++ | +++ |
| **Glucagon** | - | + |
| **Insulin** | - | - |
| **NSE** | - | - |
| **Synaptophysin** | - | + |

375 (-): no positive neoplastic cells; (+) less than 10% of neoplastic cells labeled, (++)
376 between 10 and 50% of neoplastic cells labeled; (+++) more than 50% of neoplastic cells
377 labeled.
378