

Case Report

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Insulinoma in a 5-Year-Old Dexter Cow

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Key words: Cattle; Hypoglycemia; Pancreas; Tumor.

A 5-year-old Dexter cow in the fifth month of pregnancy was referred to the Clinic for Ruminants and Swine, Department of Veterinary Medicine, Freie Universität Berlin, Germany, by a local practitioner due to general weakness and ataxia that did not respond to treatment. Approximately 3 months before hospitalization the cow had suffered from two bouts of acute catarrhal mastitis, which were successfully treated by frequent milking and parenteral administration of tylosin^a at a dosage of 10 mg/kg bodyweight once daily on ten consecutive days. Approximately 6 weeks after the second bout of mastitis (2 weeks before hospitalization), the cow had weakness and ataxia. There was severe hypoglycemia^b (glucose concentration 21.6 mg/dL) while complete blood cell counts and differentials, bilirubin, blood urea, creatinine, magnesium, calcium, phosphate and iron concentrations remained within the reference range.^c

At examination, the cow's posture was characterized by a wide-based stance and reluctance to walk. The cow had symmetrical ataxia and hypermetria of all limbs, intention tremors of the head and a deficit in its menace responses. The ataxia was graded as 3–4 out of 5.¹ The cow demonstrated bilateral mydriasis and delayed pupillary reflexes (both direct and indirect). Other cranial nerve function and spinal reflexes were normal and there were no indications of head tilt, tail weakness, bladder atony, and perineal hypalgesia. The cow showed no signs of circling. Given the symmetrical ataxia and hypermetria of all limbs, the intention tremors and the deficit in its menace responses the tentative neurologic diagnosis was cerebellar ataxia. The

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Abbreviations:

RBC	red blood cell count
AST	aspartate aminotransferase
CK	creatinine kinase
NEF	non-esterified fatty acids
BHB	β-hydroxybutyrate
DAB	diaminobenzidine

body condition score was assessed 2 of 5.² Respiratory rate (36/min), heart rate (72/min), and body temperature (38.7°C) were within the reference range. Upon examination of the digestive tract, dysphagia was observed while the animal was ruminating. Rumen fluid was dripping from the oral cavity and a discharge containing rumen fluid was draining from the nostrils intermittently. Urinalysis yielded normal results. The color of urine was yellow to light amber and the specific gravity was normal (1.030; reference range 1.025–1.045). Neither glucose nor ketone bodies were detected in the urine.

CBC and serum biochemistry revealed a low reticulocyte count (5.63×10^6 μ L; reference range $6-8 \times 10^6$ μ L) and a slight left shift (band neutrophils 0.31×10^3 μ L; reference range $0-0.3 \times 10^3$ μ L, segmented neutrophils 1.26×10^3 μ L; reference range $1.3-4.5 \times 10^3$ μ L), increased AST (86 U/L; reference range 0–50 U/L) and CK activities (860 U/L; reference range 0–150 U/L). Venous blood gas analysis identified a slightly increased Base Excess (6 mmol/L; reference range -3 – $+3$ mmol/L), while the blood pH was within the reference range (7.39; reference range 7.35–7.45). NEFA^d (0.6 mmol/L, reference range ≤ 0.4 mmol/L) were slightly increased, but beta-hydroxybutyrate^e (0.2 mmol/L, reference range ≤ 1.0 mmol/L) was normal. The plasma glucose concentration revealed insulinoma as a tentative diagnosis based on the normal BHB concentration a negative energy balance was considered unlikely. The increase in the activity of AST and CK was probably because of transportation. To exclude cerebrocortical necrosis, serum total thiamine concentration was determined by HPLC technique.³ Total thiamine was 41 μ g/L (reference range of >50 μ g/L).

No abnormalities were detected on endoscopy of the upper gastrointestinal and respiratory tracts or ultrasonographic examination of the liver, gallbladder, spleen, intestine, rumen, reticulum and kidney. On transabdominal ultrasonographic examination, pregnancy in an advanced stage was ascertained and fetal viability confirmed by measuring fetal heart rate (110 beats/min; normal fetal heart rate 90–125 beats/min).

Persistent hypoglycemia was demonstrated on four consecutive days starting from the day after admission. The Dexter cow was initially treated with glucogenic precursors (40 g propylene glycol and 40 g glycerol twice daily), which were administered orally to control hypoglycemia. The treatment of hypoglycemia with corticosteroids was not performed to avoid the risks of abortion. Serum samples for determination of insulin, estrogen, and cortisol were sent to the Endocrinology Laboratory, Clinic for Cattle, Tierärztliche Hochschule Hannover. A radioimmunoassay was applied for the quantitation of serum insulin concentrations in cattle.^f The details for the analysis of above-mentioned variables have been described by Meyerholz (2014). Estrogen (246 pg/mL; reference range >20 pg/mL for pregnant cows) and progesterone (6.3 ng/mL; reference range >5 ng/mL for pregnant cows) were detectable and confirmed pregnancy. Cortisol was also detectable (19.8 ng/mL; reference range >2 ng/mL) ruling out adrenal insufficiency as a cause of persistent hypoglycemia. Plasma glucose concentrations were extremely low (33.3, 25.2, 30.6, 27.0 mg/dL; reference range 40–59 mg/dL) and serum insulin concentrations exceeded 300 pmol/L in three of the four samples (316, 333, 109, and 501 pmol/L, respectively; reference range Holstein Friesian heifers 54–194 pmol/L) on 4 days during hospitalization. The latter levels were higher compared to those reported in literature when the same assay had been applied in pregnant primiparous cows⁴ and also when a similar method had been applied in pregnant multiparous cows.⁵ No reference values, however, are available for serum insulin concentration in Dexter cows.

Due to persistent hypoglycemia and hyperinsulinemia in the absence of ketonemia, an insulin-secreting tumor was suspected. An intravenous glucose tolerance test (IVGTT) was performed to support this differential diagnosis. A long-term catheter^g was placed in the left jugular vein under aseptic conditions and connected with a three-way stopcock fitted with an extension^h tube. The tube was fixed to the skin with two simple interrupted sutures. The system was filled with heparinized normal saline solution (50 IU/mL) and blood samples for determination of glucose and insulin concentrations were drawn from the catheter into appropriate 9 mL tubesⁱ after disposal of the 5 mL of aspirated fluid. Following collection of the initial blood sample, a volume of 500 mL of 5% glucose solution^j was administered over a period of 5 minutes through the long-term catheter (170 mg glucose/kg BW) and serial blood samples were obtained at 30 minutes and 1-hour intervals following glucose administration. From an initial level of 27.7 mg/dL glucose levels crossed the lower limit of the reference range (40 mg/dL) at 30 minutes following the start of the infusion, and decreased to below baseline at 90 minutes (Fig 1). Insulin concentrations crossed a high peak concentration (2180 pmol/L) from an initial level of 54 pmol/L and decreased to a level of 322 pmol/L at 30 minutes postinfusion and remained elevated over the baseline concentration 5 hours long postinfusion (Fig 1). Determination of the glucose/

insulin ratio, which is the standard approach for diagnosis of insulinoma in dogs⁶ and humans,⁷ was not considered an appropriate diagnostic tool in cattle as the physiological range of insulin secretion and concentration in Dexter cows is unknown. The results of IVGTT reflecting persisting hyperinsulinemia after plasma glucose levels had already returned to subnormal levels further supported suspicion of an insulin-secreting tumor. From the results of the IVGTT, it was concluded that persisting hyperinsulinemia associated with hypoglycemia as observed at IVGTT in this Dexter cow was demonstrative of an insulinoma. Hypoglycemia in cattle is a common finding related to malnutrition in young stock⁸ or to negative energy balance in the transition period.⁹ In contrast to the case presented here, hypoglycemia originating from deficient energy supply or increased demands of energy due to lactation is associated with increased levels of NEFA and BHB due to lipomobilization and ketone body production. The Dexter cow did not respond with ketone body production or excessive lipomobilization in the face of hypoglycemia. This finding indicated a blockade of lipolysis and ketone body formation, most likely due to persistent hyperinsulinemia which inhibits lipolysis and subsequent ketone body production.¹⁰ The mildly increased NEFA levels of the Dexter cow might be due to catecholamine-induced lipolysis, which cannot be inhibited by insulin due to reduced antilipolytic effect of insulin in pregnancy.¹⁰ The absence of excessive lipomobilization and ketosis as well as persistence of hyperinsulinemia observed following glucose administration, however, do justify the assumption of insulinoma but are not conclusive as the insulin response to intravenous glucose infusion is physiologically high (Peak values range between 137–1728 pmol/L) in dairy cows, as demonstrated in two previous studies.^{11,12} In humans, the term insulinoma is used to describe small tumors composed of islet cell tissue that develop in the pancreas and ectopic sites and cause hypoglycemia by their ability to secrete insulin.¹³ The clinical diagnosis of insulinoma in humans is based on the presence of Whipple's triad, consisting of the presence of clinical signs such as tremor, sweating, tachycardia, loss of consciousness, giddiness, and blurring of vision that occur intermittently,¹⁴ persistent hypoglycemia during fasting and, improvement of clinical signs after infusion of glucose.¹⁵ Signs of neurologic dysfunction were reported in 30 patients with insulinoma.¹⁶ Confusion, coma, convulsions, and weakness were predominant findings in these patients.¹⁶ Magnetic resonance imaging and computed tomography are alternative diagnostic tools applied in humans and small animals. Recently, intraabdominal ultrasonography was added to the diagnostic spectrum in humans to increase the diagnostic sensitivity of insulinomas.¹⁵ Biochemical diagnosis of insulinoma in humans relies on unequivocally measurable insulin concentrations in the fasting state, the concurrent measurement of C-peptide together with quantitation of ketone bodies.⁷ Furthermore, evaluation of the insulin–glucose ratio is an important diagnostic parameter with a sensitivity of 93% and specificity of 94%.⁷ In the present

case, the two monoclonal spikes in the alpha 1-fraction, which were detected by serum electrophoresis, were probably due to pregnancy or increased levels of C-peptide. However, a validated C-peptide assay for use in cattle is not available yet.

All treatment attempts including providing glucogenic nutrients and precursors (oral administration of 40 g propylene glycol and 40 g glycerol twice daily throughout hospitalization) failed and the condition of the cow deteriorated during hospitalization. Furthermore, she had an abortion approximately in the sixth month of pregnancy. Abnormalities were not detected on post-mortem examination of the fetus. Abortion might have been caused by undersupply of the fetus with glucose due to hypoglycemia of the mother. In pregnant cows, glucose crosses the uterus and placenta insulin-independently by the primary glucose transporters (GLUT1 and GLUT3). A maternal hypoglycemia affects the fetal glucose uptake directly because the fetus and placenta cannot sequester glucose against its concentration gradient and the capacity of the fetus and placenta is limited to compensate hypoglycemia by gluconeogenesis.¹⁷ Due to the clinical condition and the unfavorable prognosis, euthanasia was elected and necropsy was performed at the Institute of Veterinary Pathology, Faculty of Veterinary Medicine (Freie Universität Berlin).

At necropsy, multifocal, partially encapsulated, highly infiltrative, white-grey nodules with a maximal diameter of 2.8 cm were present in the right lobe of the pancreas (Fig 2). White nodes in pancreaticoduodenal and mesenteric lymph nodes were seen in addition. Additional findings were chronic enteritis and a moderate chronic ulcerative abomasitis. The proposed cause of abomasitis was chronic stress during hospitalization. For histological evaluation, pancreatic tissue and lymph nodes were fixed by immersion in 10% neutral-buffered formalin for 96 hour and paraffin-embedded. Sections of 4 μ m in thickness were routinely stained with hematoxylin and eosin (HE). These showed a multiple infiltrative coalescing neoplastic masses of polygonal tumor cells arranged in nests and packets, overall showing a neuroendocrine pattern (Fig 3). Multifocal necrosis and hemorrhage were present. Tumor cells had high amounts of intensely eosinophilic cytoplasm (Fig 3) and

mitotic rate was low. Moderate pancreatic atrophy was present in the adjacent exocrine pancreas. The aforementioned lymph nodes were almost completely infiltrated by the tumor cells, resulting in a replacement of original lymphoid tissue. Intravascular tumor cells were frequently observed. For a definitive diagnosis of the neoplasm, immunohistochemistry using anti-human insulin antibodies,^k anti-chromogranin A, anti-synaptophysin and anti-melan A was performed following routine protocols with a 15 minutes microwave heating step in citrate buffer pH 6.0 as retrieval method for anti-chromogranin A, anti-synaptophysin and anti-melan A. A biotinylated goat anti-mouse antibody diluted 1 : 200 was used as secondary antibody for all antibodies except anti-insulin (goat anti-guinea pig). Color development was performed using DAB and hemalaun as counterstain. The expected staining pattern for islet cell tumors is similar in different domestic animals. Cells stain positive for chromogranin A or B depending on the neoplastic cell type, protein gene product 9.5 (PGP9.5, synonym Ubiquitin carboxy-terminal hydrolase L1), synaptophysin or neuron specific enolase (NSE). In insulinomas, tumor cells are additionally positive for insulin.¹⁸ Immunohistochemistry confirmed the clinical diagnosis of insulin-producing islet cell tumor in both the pancreatic neoplasm and the lymph nodes, with the anti-insulin antibody yielding strong intracytoplasmic signals with occasional membrane staining (Fig 4). In addition, the neoplastic cells were melan A-negative, chromogranin A-negative, and synaptophysin-positive (not shown). Although tumor cells stained unexpectedly negative for chromogranin A, the positive staining for both synaptophysin and especially insulin support the diagnosis of an insulinoma. Incubation of the slides with an irrelevant antibody as negative control did not result in specific staining (not shown).

Insulinomas are endocrinologically active tumors of the pancreas derived from pancreatic beta cells and have been reported in humans¹⁴ and a number of animal species.^{6,19,20} Insulinoma in cattle has previously been identified by immunohistochemistry and light microscopy of suspected neoplasms encountered in the slaughterhouse at routine meat inspection.²⁰ Most of pancreatic tumors have been described as islet cell tumors and most of them were classified as malignant.²⁰ In humans, insulinomas are described as usually

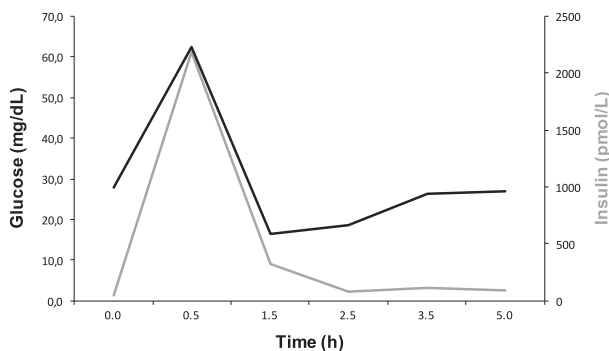


Fig 1. Plasma glucose and serum insulin concentrations during intravenous glucose tolerance test using 170 mg glucose/kg BW in the Dexter cow.

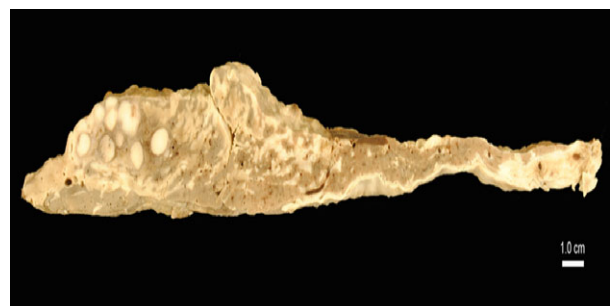


Fig 2. Pancreas with multifocal whitish nodules, formalin-fixed.

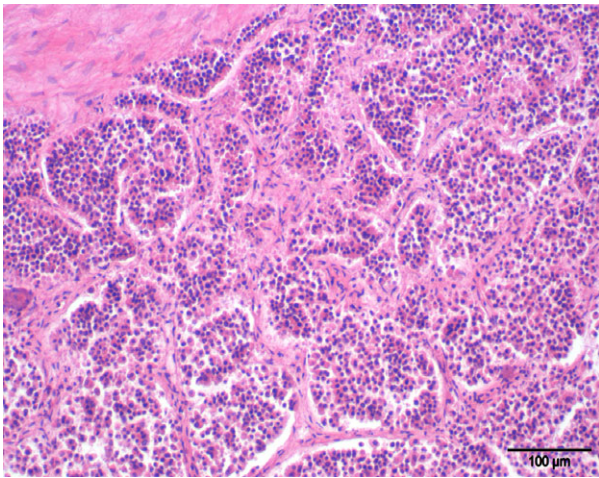


Fig 3. Polygonal neoplastic cells with high amounts of eosinophilic cytoplasm arranged in a neuroendocrine pattern of nests and packets, separated by moderate amounts of fibrovascular stroma. HE stain.

solitary, benign, and encapsulated small lesions with a diameter <2 cm.¹³ Other pancreatic tumors that can cause hyperinsulinemia include pancreatic polypeptide-secreting tumors (PPomas), as described in dogs.⁶ Clinical signs, however, are typically absent or go unnoticed in case of tumors that primarily produce pancreatic polypeptide in dogs.²¹ Pancreatic tumors, that produce both pancreatic polypeptide and insulin, can cause persistent hypoglycemia due to hyperinsulinemia.^{19,21} Therefore, histopathological examination of pancreas tissue is necessary in order to achieve an exact diagnosis of insulinoma.¹⁹ A paraneoplastic hypoglycemia is an important differential diagnosis of insulinoma and occurs due to a nonislet cell tumor that secretes incompletely processed IGF-II, which causes glucose consumption by interacting with IGF and insulin receptors directly.^{6,22} The determination of IGF-I and IGF-II levels in serum and IGF-II:IGF-I ratio can help to confirm or rule out the diagnosis of paraneoplastic hypoglycemia.^{6,22} Further differential diagnoses of insulinoma, which might cause a persistent hypoglycemia, include disorders of counter regulatory hormone release (glucagon, epinephrine, growth hormone, and cortisol), especially adrenal insufficiency.²³ Adrenal insufficiency is either congenital or acquired and characterized by an insufficient production of steroid hormones (glucocorticoids and often mineralocorticoids). ACTH stimulation test can establish the diagnosis and distinguish whether it is primary or secondary adrenal insufficiency.²³ Addison's disease (chronic adrenal insufficiency) can occur as a result of a primary disorder of the adrenal gland or secondary to a deficiency of hypothalamic and pituitary hormones such as adrenocorticotropic hormone (ACTH) or corticotropin-releasing hormone (CRH).²³ Adrenal insufficiency has never been described in cattle except one case²⁴ and was ruled out in the present case by evaluation of cortisol and potassium concentrations. Neither disorders of counter

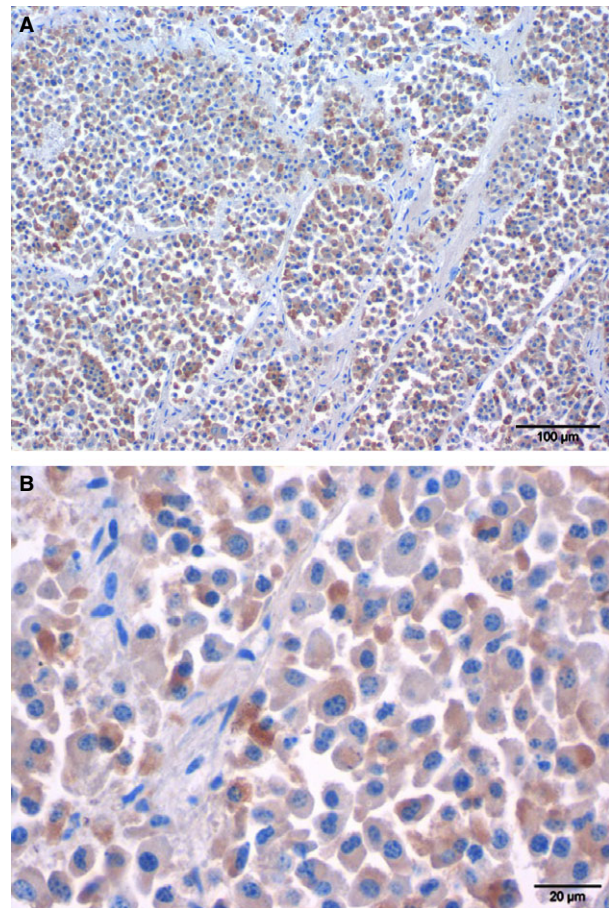


Fig 4. (A) Anti-insulin immunohistochemistry showing variably intense cytoplasmic staining of neoplastic cells. (B) Higher magnification. Note that not all neoplastic cells are positive for insulin. DAB staining (brown) with hematoxylin counterstaining (blue).

regulatory hormone release nor nonislet cell tumors are associated with hyperinsulinemia, contrary to insulinoma. Animals with insulinoma do not exhibit a compensatory drop of insulin secretion in the presence of hypoglycemia.²⁵ Therefore, IVGTT seems to be the diagnostic method of choice for insulinoma in cattle, to characterize the regulation of insulin release and the pancreatic insulin response to changes in glucose levels.

Surgical excision of neoplastic tissue is treatment of choice in humans¹⁴ and ferrets.¹⁹ In dogs, the long-term medical treatment is applied by oral administration of diazoxide possibly in combination with prednisolone.⁶ A partial pancreatectomy contributed to a longer survival time in dogs with insulinoma.⁶ Persistent hypoglycemia in the absence of ketosis in adult dairy cattle presenting with signs of neurologic dysfunction could indicate the presence of an insulinoma. The present case of a cow with signs of neurologic dysfunction associated with persistent hypoglycemia and hyperinsulinemia illustrates a case of an insulinoma with inhibition of lipolysis and ketogenesis. When diagnosis is reached in vivo, glucocorticoid medication in combination with partial pancreatectomy might be attempted, but possible metastases have to be considered.

Footnotes

- ^a Tylosel-200, Selectavet, am Kögelberg 5, 83629 Weyarn-Holzolling
- ^b Vet ABC, Fa. Scil, Viernheim
- ^c Cobas Mira Plus (Fa, Roche, Mannheim)
- ^d FA 115 kit, Randox Laboratories Ltd., Crumlin, UK
- ^e Ranbut kit RB1007, Randox Laboratories Ltd., Crumlin, UK
- ^f IM3210, insulin IRMA KIT, Immunotech, Beckman Coulter, CA
- ^g VasoVet® G14, B. Braun, Melsungen AG, Melsungen, Germany
- ^h Discifix®-3, B. Braun, Melsungen AG, Melsungen, Germany
- ⁱ Tube 9 mL Sarstedt Monovette AG & Co. Sarstedt Nürnberg, Deutschland
- ^j Glucose 5%, B. Braun Melsungen, Germany
- ^k Dako, Hamburg, Germany
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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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