Case Report
J Vet Intern Med 2014;28:1331–1335

Hyperinsulinemic Hypoglycemia Syndrome in 2 Dogs with Bartonellosis

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Key words: Dog; Hyperinsulinemia; Hypoglycemia; Monocytosis; Seizures.

Case 1

A 6-month-old male castrated, 21-kg Weimaraner developed seizures after surgical castration and umbilical hernia repair. Two weeks earlier, presurgical laboratory abnormalities included lymphocytosis (7,800/μL), eosinophilia (1,700/μL), monocytosis (1,200/μL), basophilia (170/μL), thrombocytosis (459,000/μL), and an elevated ALP activity (135 IU/L). Blood glucose was 111 mg/dL. Surgery and recovery were uncomplicated, until 2 hours postoperatively when generalized tonic clonic seizures developed. Seizures continued despite 2 doses of diazepam (1.5 mL IV), so propofol (10 mg) was administered IV. Hypoglycemia was not suspected and blood glucose was not measured. The dog was transported to an emergency clinic, where laboratory abnormalities included neutrophilia (16,210/μL) and hyperphosphatemia (7.7 mg/dL), with a normal blood glucose concentration (106 mg/dL).

When transferred to a specialty hospital the following morning, the dog was dysphoric, nonambulatory, and had absent menace reflexes bilaterally. No other cranial nerve deficits or anisocoria were noted. Serum chemistry and bile acid values before (1.3 μmol/L) and after feeding (1.8 μmol/L) were within reference intervals. After intravenous administration of mannitol and phenobarbital, no additional seizures were observed, but the dog remained stuporous, with absent menace reflexes until discharged 2 days later. Phenobarbital was continued (2 mg/kg PO q12).

The following morning, the dog was found salivating, minimally responsive, and recumbent. Laboratory abnormalities included hypoglycemia (glucose 25 mg/dL), neutrophilia (12,030/μL), and monocytosis (3,290/μL). A blood ammonia concentration (30 μmol/L) was within reference intervals. Enrofloxacin (5 mg/kg IV) and a 5% dextrose infusion were administered. The dog was fed every 2 hours and periodically determined glucose values ranged from 40 to 75 mg/dL. After transfer to a specialty hospital, hypoglycemia (59, 48, 22, 41, 23, and 40 mg/dL at 10:00 PM, 1:00, 3:00, 4:00, 5:00, and 6:00 AM, respectively) persisted, despite frequent small feedings and continuous administration of 10% dextrose solution with periodic boluses. Dexamethasone sodium phosphate (0.15 mg/kg IV) was administered, after which the dog was referred to NCSU-CVM-VTH for further evaluation.

Historically purchased from a breeder in Virginia, the dog had been healthy before surgery 6 days earlier. Littermates were reportedly healthy, vaccinations current, heartworm, flea, and tick preventive medications were used routinely. The owner denied exposure of the dog to toxins such as xylitol or oral hypoglycemic drugs. At NCSU-CVM-VTH, the dog was laterally recumbent, pupils fixed, menace reflexes absent bilaterally, and blood glucose was 20 mg/dL. Hematologic abnormalities included mild microcytic, normochromic, nonregenerative anemia (PCV 32%, reticulocytes 0.44%), and monocytosis (2,129/μL). Normoglycemia (129 mg/dL) was documented after an intravenous bolus of dextrose. Serum biochemical abnormalities included hypoalbuminemia (2.4 g/dL), low SUN (4 mg/dL) and creatinine (0.3 mg/dL), hypomagnesemia (1.7 mg/dL) and hypokalemia (3.7 mmol/L), all potentially related to fluid administration, and hemodilution. Blood ammonia (11 μmol/L) and coagulation measurements were normal, except for a mildly

Abbreviations:

APTT	activated partial thromboplastin time
BAPGM	*Bartonella* alpha proteobacteria growth medium
CBC	complete blood count
CRI	constant rate infusion
HHS	hyperinsulinemic hypoglycemia syndrome
HIF-1	hypoxia inducible factor
IGR	insulin/glucose ratio
NCSU-CVM-VTH	North Carolina State University College of Veterinary Medicine Veterinary Teaching Hospital
PCR	polymerase chain reaction
UFVMC	University of Florida Veterinary Medical Center
VEGF	vascular endothelial growth factor

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Submitted September 9, 2013; Revised January 23, 2014; Accepted April 22, 2014.

DOI: 10.1111/jvim.12381

prolonged APTT (14 seconds). Urinalysis findings included isosthenuria (specific gravity 1.008) and glucosuria (2+ urine dipstick). An ACTH stimulation test was normal (precortisol 3.5 μg/dL, post 11.9 μg/dL). Abnormalities were not detected by abdominal ultrasound examination. Hypoglycemia (0.3 mmol/L) accompanied an elevated plasma insulin concentration (397 pmol/L), and increased IGR (1,323).[a] Throughout the day, the dog became hypoglycemic 20–40 minutes after repeated IV boluses of 50% dextrose solution. An interstitial glucose monitor was placed and because of the refractory nature of the hypoglycemia, 50% dextrose solution (5 mL/h) was administered by CRI overnight. By the following afternoon, blood glucose concentration normalized, dextrose CRI was discontinued, and the dog was discharged. Oral phenobarbital (2.2 mg/kg) and amoxicillin/clavulanic acid (11.9 mg/kg) were dispensed. When rechecked 2 weeks after discharge, the owner reported no seizures, improved vision, and diminished ataxia. There were no neurologic deficits except absent bilateral menace responses. Serum insulin concentration (292 pmol/L) was increased, whereas glucose concentration (6.0 mmol/L) and IGR (32)[a] were within reference intervals.

Four days later, the dog was readmitted in lateral recumbency and minimally responsive after seizures. Blood glucose concentration was 21 mg/dL (0.7 mmol/L), serum insulin concentration 668 pmol/L, and IGR 954.[a] Because of the preoperative hematologic abnormalities[1] and unexplained, refractory hypoglycemia, blood was cultured in *Bartonella* alpha Proteobacteria growth medium (BAPGM)[2] in an effort to rule in or rule out infection with fastidious bacteria, such as a *Bartonella* sp. *Bartonella henselae*, DNA (SA2 strain type, sequence similarity 455/455 bp to Gen Bank accession AJ441256) was PCR amplified and sequenced from the canine's blood. The owner elected euthanasia and a cosmetic necropsy. Necropsy abnormalities included multifocal, neutrophilic arteritis accompanied by occasional eosinophils, lymphocytes, plasma cells, and spindle cells; coronary artery intimal proliferation; minimal, neutrophilic interstitial pneumonia; small multifocal hepatic aggregates of neutrophils; and mild multifocal, neutrophilic, and eosinophilic colitis. There was no evidence of aberrant parasitic migration. The cerebrum contained mild perivascular lymphocytic and histiocytic inflammation. There were no histologic pancreatic changes and necropsy did not identify a cause for hyperinsulinemic hypoglycemia.

Case 2

One month before referral to UFVMC, a 9-month-old male 12.0-kg Pembroke Welsh Corgi was examined by the primary veterinarian after experiencing a 1-minute duration generalized seizure. On physical examination, the dog was dull and poorly responsive to stimuli. CBC, serum biochemical profile, and blood gas analysis abnormalities included hypokalemia (3.53 mmol/L) and hypoglobulinemia (2.0 mg/dL). The blood glucose concentration was 92 mg/dL. After an 18-hour uneventful observation period, the dog was discharged without medications. Shortly after returning home, the owner witnessed another 1-minute generalized seizure. The following morning, a board-certified neurologist localized the lesion to the prosencephalon and epilepsy was suspected.

Three days later, the dog was castrated without complications, but was again hospitalized that evening in status epilepticus, with hyperthermia ($T = 104.1$). Diazepam (0.6 mg/kg IV) followed by phenobarbital (5 mg/kg IV) did not achieve seizure control, until propofol (1.7 mg/kg IV, repeated twice) was administered. Laboratory abnormalities included metabolic acidosis (pH 7.26, pCO_2 32.2 mmHg, HCO_3^- 14.7 mmHg, BE −12 mEq/L), moderate hypokalemia (2.9 mmol/L), and hypoglycemia (35 mg/dL). A commercial ELISA assay[b] that detects *Anaplasma phagocytophilum, Borrelia burgdorferi,* and *Ehrlihichia canis* antibodies, and *Dirofilaria immitis* antigen was negative. The following day, oral phenobarbital treatment (2.7 mg/kg PO q12h) was started. On hospitalization day 3, repeat examination by the neurologist identified dull mentation, diminished bilateral menace responses, diminished right fore and hindlimb proprioception, and diminished hind limb extensor postural thrust. With repeated prosencephalic neurologic localization, an MRI and CSF tap were recommended. Pre- (65.8 μmol/L) and postprandial (115 μmol/L) bile acid values were consistent with hepatobiliary disease. A portosystemic shunt was suspected and therefore phenobarbital treatment was weaned during the next 2 days and leviteracetam (0.1 mg/kg PO q8h), lactulose (0.25 mL/kg PO q8h), neomycin (15 mg/kg PO q8h), and low protein diet (Hill's L/D) were dispensed. Eight days later, the dog had a generalized seizure and was hospitalized. Neomycin was discontinued and metronidazole (20.8 mg/kg PO q12h) was started, after which the dog had seizures twice in 2 days. When an abdominal ultrasound did not identify a portosystemic shunt, the dog was referred to UFVMC.

On admission, the dog was poorly responsive, bradycardic (64 beats/min), and had decreased proprioception in the left fore and both hind limbs. A CBC, blood ammonia concentration, and urinalysis results were normal. Serum biochemical abnormalities included elevations in ALP (127 IU/L) and ALT (165 IU/L) activities, hyperphosphatemia (5.7 mg/dL), and hypoglycemia (44 mg/dL). An electrocardiogram confirmed sinus bradycardia. There were no abdominal radiographic or ultrasonographic abnormalities detected. Fine needle aspiration cytology of the liver identified normal hepatocytes. Treatment consisted of a bolus of 25% dextrose IV (2 mL/kg) followed by a CRI of 0.45% NaCl with 5% dextrose (2.9 mL/kg/h). Pre- (12.6 μmol/L) and postprandial (41.0 μmol/L) bile acids were mildly elevated. Prothrombin time, APTT, and ACTH stimulation results were normal. Blood glucose concentration was 1.9 mmol/L, insulin concentration was 102 pmol/L, and IGR 54.[a] Blood lead concentration was normal. Blood and urine metabolic

screening panels[c] (chromatographic amino acid, organic acid, and carbohydrate screenings and spot tests for ketones, methylmalonic acid, and mucopolysaccharides) for hepatic diseases, portosystemic shunts, and glycogen storage diseases were normal or negative.

On day 3 of hospitalization, a brain MRI revealed focal T2-weighted hyperintensities within the ventral right olfactory lobe and ventral left olfactory bulb, with no evidence of contrast enhancement, most consistent with seizure-induced pathology; however, inflammatory disease, metabolic disease, and toxin could not be excluded. CSF protein and cytology were normal. An abdominal CT documented normal liver size, shape, portal branching, and portal vein diameter, no abnormal contrast enhancement pattern, and no evidence of a portosystemic shunt.

Despite a CRI of 5% dextrose, blood glucose values ranged from 45 to 55 mg/dL. After a laparoscopic liver biopsy, the dog recovered uneventfully from anesthesia. Glucagon (0.03 mg/kg IM) was administered, after which blood glucose minimally increased from 47 to 57 mg/dL. Three hours later, the dog had a generalized seizure, blood glucose concentration was 39 mg/dL, with a minimal response to 55 mg/dL after an IV bolus of 25% dextrose (2 mL/kg). Seizures continued until diazepam (0.2 mg/kg IV) was administered. On day 5 of hospitalization, the dog developed cluster seizures. Despite intravenous boluses of 25% dextrose (2 mL/kg), blood glucose values ranged from 29 to 138 mg/dL and cluster seizures continued with minimal response to 3–0.4 mg/kg IV diazepam boluses, but stopped after a diazepam CRI (0.25 mg/kg/h). Phenobarbital loading (8 mg/kg over 12 hours) was begun. Seizures remained refractory to medical treatment, requiring intermittent diazepam boluses. On day 6, liver histopathology results revealed diffuse, moderate hydropic degeneration and no evidence of glycogen storage disease or microvascular dysplasia. Because of finances and the guarded prognosis, the owner elected euthanasia and necropsy.

Major findings at necropsy included severe chronic lymphocytic and suppurative cholangitis with marked peribiliary fibrosis, mild portal vein thrombosis, marked midzonal hepatocellular hydropic change associated with glycogen storage, and mild to moderate pancreatic islet cell hyperplasia. There were no brain lesions detected in 13 sections. Retrospectively, 5 small blocks of pancreas were taken from 1 larger block of formalin-fixed pancreas taken at necropsy from 2 4-month-old Beagle and 3 adult Welsh Corgi dogs. Hematoxylin and eosin-stained sections initially were prepared from paraffin-embedded blocks for routine histopathology. Recut sections for immunohistochemistry were stained with antibody to insulin detected by secondary antibody conjugated with peroxidase. Islets positive for insulin were counted per section with known area from ImageJ[d] software measurements. Individual islet cross-sectional area and number of insulin-positive cells per islet were counted. Analysis compared 5 sections of pancreas from the dog with data derived from the 5 reference cases. Based upon morphometric and immunohistochemical testing, the dog's pancreas had at least twice as many insulin-positive pancreatic islet cells per sampled pancreatic section, whereas pancreatic cell and nuclear size did not visually differ. Because *B. henselae* DNA was amplified from dog 1, a paraffin-embedded liver and spleen tissue block was submitted to the IPRL for *Bartonella* sp. PCR targeting the 16S-23S intergenic spacer region. *B. koehlerae* DNA (sequence similarity 221/224 bp (98.7% similarity with *B. koehlerae* AF3124 90)) was amplified and successfully sequenced.

Hyperinsulinemic hypoglycemia syndrome (HHS) is considered the most common cause of persistent hypoglycemia in infants and children.[3,4] To the authors' knowledge, HHS has not been previously reported as a distinct clinical entity in dogs. Ultimately, a review of the human literature suggested the possibility that HHS might be the cause of medically refractory hypoglycemia in these 2 young, 6- and 9-month-old dogs. Physiologically, hypoglycemia occurs because of excess secretion of insulin or insulin-like substances, excess glucose consumption (sepsis), decreased hepatic gluconeogenesis or glycogenolysis, ingestion of oral hypoglycemic drugs (insulin secretogogues such as sulfonylureas or xylitol), and in association with factitious hypoglycemia. Documentation of hyperinsulinemia in association with hypoglycemia supported insulin excess as the cause of the low blood glucose values (repeatedly documented in both of these dogs), which occurred despite aggressive intravenous administration of glucose. When blood glucose concentrations fall below 4.4 mmol/L, insulin production should decrease and insulin concentrations fall below 58 mmol/L. The I/G ratio (normal ratios for the endocrinology laboratory[a] used in this study are between 14 and 43) directly compares the amount of glucose to the amount of insulin in the blood at a point in time. Increased I/G ratios of 954 and 54 were documented in dogs 1 and 2, respectively; however, despite documentation of hyperinsulinemia, an earlier I/G ratio was normal, when dog 1 was minimally symptomatic. In the context of other differential diagnoses for hypoglycemia, neither dog had historical access to oral hypoglycemic agents or insulin; hypoadrenocorticism and portosystemic shunts were ruled out; metabolic screening failed to support an inborn error of metabolism in dog 2; and neonatal, hunting dog (exercise-induced) and growth hormone deficiency-induced hypoglycemia were not historically applicable. As known causes of hypoglycemia were not applicable or were ruled out, and as increased serum insulin values were documented in conjunction with low blood glucose measurements, a diagnosis of HHS in both of these young dogs seemed justified. HHS could be used as a descriptive term for any medical condition in which hyperinsulinemia accompanies hypoglycemia, such as an insulinoma, sepsis, or iatrogenic insulin administration. However, in the context of human medical usage and the findings obtained in these 2 cases, the designation HHS should be confined to excessive production of insulin by hyperplasic

pancreatic beta cells, most often occurring in a young child or dog. During the referral evaluations of these 2 dogs, blood insulin values were determined only after other differential diagnoses were ruled out, hypoglycemia was proven refractory to glucose administration, and neither a diagnosis or prognosis could be provided to the owners, ultimately resulting in the decision for euthanasia. Administration of large quantities of glucose, in conjunction with variations in rate and frequency of glucose administration, influence measured insulin and glucose values. Although other more common causes of hypoglycemia in young dogs should be concurrently ruled out, specimens should be collected for glucose and insulin measurements earlier in the course of the diagnostic evaluation of young to middle age dogs with suspected of HHS.

In dogs, hyperinsulinemia and hypoglycemia occurs in association with insulinomas and sepsis.[5,6] Septicemic dogs often have clinical, hematologic, and coagulation indictors of sepsis,[7] which were absent in our cases. Whether surgery, anesthesia, or castrations were contributing factors to the development of HHS in these dogs remains undetermined. At necropsy, an insulinoma was not found and both dogs had nonspecific histologic changes involving multiple organs, supporting the possibility that a pre-existing, ongoing systemic illness predisposed to HHS.

Because of preoperative hematologic abnormalities, the persistent monocytosis,[1] and the lack of a unifying pathogenesis for the refractory hypoglycemia, dog 1 was tested on a research basis using BAPGM enrichment blood culture/PCR in an effort to identify a fastidious bacteria, as best illustrated in the manuscript by Davenport and colleagues.[8] *B. henselae* DNA was amplified and sequenced from the canine's blood, whereas enrichment culture PCR was negative, potentially because of prior antibiotic administration. Although hematologic and biochemical findings in dogs with *Bartonella* sp. bacteremia are often normal, eosinophilia, lymphocytosis, monocytosis, thrombocytopenia, thrombocytosis, and hypogammglobulinemia occurs in a subset of dogs with serological or BAPGM enrichment blood culture/PCR evidence of *Bartonella* sp. infections.[9–11] Because *B. henselae* was amplified and sequenced from postantibiotic blood of dog 1, paraffin-embedded tissues from dog 2 were tested retrospectively, resulting in successful PCR amplification and sequencing of *B. koehlerae* DNA. In dog 2, the possibility of DNA carryover during the necropsy could not be ruled out.[12] Whether infection with *B. henselae* and *B. koehlerae* was fortuitously detected, was related to seizures secondary to vasculitis or other pathologic mechanisms, or contributed to the pathogenesis of HHS was not determined; however, future HHS cases should be examined serologically and by BAPGM enrichment blood culture/PCR for the antemortem presence of *Bartonella* sp. and potentially other fastidious infectious agents that could potentially induce local pancreatic tissue hypoxia,[13,14] providing an alternative and more directed treatment modality for dogs with HHS.

Footnotes

[a] Tests performed by the Clinical Endocrinology Laboratory, Diagnostic Center for Population and Animal Health, Michigan State University

[b] SNAP 4DX; IDEXX Laboratories, Westbrook, ME

[c] PennGen Laboratory, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA

[d] Image J Software, Public domain Java image processing program, Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, MD

Acknowledgment

This study was supported by the State of North Carolina. The authors thank Mrs. Tonya Lee for editorial assistance.

Conflict of Interest Declaration: In conjunction with Dr Sushama Sontakke and North Carolina State University, Dr Breitschwerdt holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, a newly formed company that provides advanced diagnostic testing for the detection of *Bartonella* species infection in animals. All other authors have no conflicts of interest.

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