Pancreatic islet cell tumors represent a diverse group of benign and malignant endocrine neoplasms differentiating toward one or multiple cell types of the islets of Langerhans [11]. The most frequent pancreatic islet cell tumors are adenomas and carcinomas derived from insulin-secreting beta cells (insulinoma) [2, 9, 10]. Most such neoplasms are endocrinologically active, secreting hormones, and are associated with functional disturbances relating to hypoglycemia [2, 3, 11].

In dogs, insulinomas are uncommon or rare [1, 10, 13]. Irrespective of medical rarity, scientific interest in the clinicopathological features is high and many reports have already described insulinomas in dogs [3, 4, 15]. In Japan, the only retrospective study of insulinomas focused on relationships between treatment and prognosis [4].

As a general rule, the diagnosis of insulinoma can be reached clinically or pathologically. The diagnosis of insulinoma is made following laboratory demonstration of an inappropriately high serum insulin concentration during a time of hypoglycemia (clinical or tentative diagnosis) and is confirmed by histopathology (pathological or definitive diagnosis) [1, 3, 9].

In clinical diagnosis, recent advances in image analysis for the veterinary clinical field now allow abdominal ultrasoundography or computed tomography (CT) to provide an additional clinical evidence supporting diagnosis of insulinoma [1]. However, the accuracy in diagnosis and cases examined still remain limited. Regardless of imaging results, an exploratory laparotomy is recommended as the first choice when insulinoma is suspected based on clinical signs, hypoglycemia, and inappropriately elevated concentration of serum insulin [1, 13].

The present retrospective study examined 8 cases with pathologically diagnosed insulinoma. All 8 cases showed clinical signs and hypoglycemia, but insulin concentrations varied and some were within the reference interval. To confirm the reliability of measured values, we submitted serum samples from 4 cases to two different veterinary commercial laboratories. Results differed considerably between laboratories, with no apparent correlations between the two. In Laboratory A, 3 of 4 cases were above the reference interval, and 1 case was below the reference interval. Conversely, in Laboratory B, 3 of 4 cases were above the reference interval, and 1 case was below the reference interval. Split decisions regarding the diagnosis of insulinoma were seen for 2 of the 4 cases.

KEY WORDS: canine, insulinoma, pancreas, retrospective study.
hepatic metastases (clinical stage 3) and 2 cases with metastasis to the associated lymph nodes identified intraoperatively (clinical stage 2). The remaining 3 cases were clinical stage 1. Death or euthanasia occurred in 5 cases, while the 3 remaining cases were alive at the last follow-up in November 2008. Three cases remained alive >2 years postoperatively.

Clinical signs were shown in Table 2. Clinical signs leading to suspicion of insulinoma at the referring veterinarians included muscle tremors, muscle weakness or ataxia (5 of 8 dogs), and collapse or convulsions due to hypoglycemia (5 of 8 dogs) (Table 2). All cases showed at least one of these clinical symptoms. Serum glucose levels (initial data at the referring veterinarian and preoperative data) and preoperative insulin concentrations are provided in Table 3.

Partial pancreatectomy was performed for all 8 cases and the pancreas with tumors was submitted for histopathology. In addition, 3 dogs (Cases 1, 6 and 7) underwent partial hepatectomy and 2 dogs (Cases 3 and 5) underwent excision of an enlarged associated lymph node. These samples were also submitted for histopathology. Tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Classification of either islet cell adenoma or carcinoma was made based on histopathological appearance and evidence of metastasis [2, 10]. Mitotic index was measured by calculating the total number of mitotic figures in 10 high-power fields (400 × magnification) selected at random within the tumor. In addition, to confirm the endocrine nature of tumors, chromogranin A and synaptophysin immunoreactivity were examined in all tumors. The functionality of islet cell neoplasm was demonstrated by insulin, somatostatin, glucagon, and gastrin immunocytochemistry. Classification of islet cell tumors (e.g., insulinoma, glucagonoma, gastrinoma and somatostatinoma) depended on immunostaining for these four antibodies.

For immunohistochemical staining, the following primary antibodies were used: rabbit polyclonal anti-human chromogranin A antibody (prediluted; Dako Japan, Tokyo, Japan), rabbit polyclonal anti-human synaptophysin antibody (prediluted; Dako Japan), guinea pig polyclonal anti-porcine insulin antibody (prediluted; Dako Japan), rabbit polyclonal anti-synthesized somatostatin antibody (prediluted; Nichirei, Tokyo, Japan); rabbit polyclonal anti-human glucagon antibody (prediluted; Nichirei), and rabbit polyclonal anti-human gastrin polyclonal antibody (×500; Dako Japan). Labeled antigens were detected with using a Histofine Simple Stain MAX-PO (MULTI) kit (Nichirei), with the exception of the guinea pig anti-porcine insulin polyclonal antibody, followed by DAB reaction. For the guinea pig anti-porcine insulin polyclonal antibody, an EnVision kit (Dako Japan) was applied for the detection of the labeled antigen. For all sections, high-temperature antigen retrieval was performed using commercially available retrieval solution (Target Retrieval Solution; Dako Japan) or distilled water for 5 min in a microwave at 95°C.
Veterinary Teaching Hospital. The reagent used was glucose hexokinase reagent (Roche Diagnostics, Basel, Switzerland).

Measurement of serum insulin levels for the 8 cases was outsourced to a commercial veterinary laboratory, Laboratory A. In addition, to confirm the reliability of measured values, the same samples for 4 cases (Cases 1, 4, 5 and 7 mentioned above) were also submitted to another commercial veterinary laboratory, Laboratory B. Laboratory A used a chemiluminescent immunoassay method with mouse anti-ovine insulin monoclonal antibody, while Laboratory B used competitive enzyme-linked immunosorbent assay with mouse anti-canine insulin monoclonal antibody.

RESULTS

**Abdominal ultrasonography:** Abdominal ultrasonography was performed in 6 cases (Cases 3–8), detecting tumor in 2 cases (Cases 4 and 7) (Fig. 1).

**Tumor location within the pancreas and tumor size:** Seven dogs with primary pancreatic tumor displayed a solitary neoplasm located in the pancreatic body, left lobe or right lobe of the pancreas (Table 1).

**Laboratory diagnosis:** Serum glucose levels (initial data at the referring veterinarian and preoperative data) and preoperative insulin concentrations were provided in Table 3. Mean preoperative glucose concentration for the 8 dogs was 40.4 mg/dL (range, 22–66 mg/dL). One dog (Case 4) showed a preoperative glucose level within the reference interval (>60 mg/dL). Mean preoperative insulin concentration for the 8 dogs was 37.68 μU/mL (range, 12.58–161.3 μU/mL). The reference interval for insulin concentrations in Laboratory A was 7–17 μU/mL. Five dogs were above the reference interval and 3 dogs were in the middle to high end of the reference interval. No dog displayed an insulin concentration <7 μU/mL (Table 3).

For the 4 dogs used for reliability comparisons, mean preoperative insulin concentration from Laboratory A was 53.26 μU/mL (range, 14.55–161.3 μU/mL). Three dogs were over the reference interval and 1 dog was in the middle of the reference interval. No dog displayed insulin concentration <7 μU/mL. For Laboratory B, minimum concentration was 8 mIU/L, and the maximum concentration was >300 mIU/L. The reference interval of insulin concentrations at Laboratory B was 10–25 mIU/L. Three dogs were over the reference interval and 1 dog was showing insulin concentrations <10 mIU/L (Table 4).

**Histopathology:** Eight cases with islet tumors of the pancreas were diagnosed by routine light microscopy as islet cell carcinoma. Metastasis to regional lymph nodes was identified in 2 cases (Cases 3 and 5) (Fig. 2) and hepatic metastases were observed in 3 cases (Cases 1, 6 and 7). In Case 1, no primary tumor of the pancreas was found and only hepatic involvement was demonstrated (Fig. 3).

Islet cell carcinomas displayed multilobular and trabecular neoplastic cell proliferation, partially encapsulated by various amounts of connective tissue capsule. Fine to abundant connective tissue septa were sometimes identified radiating from the surrounding capsule. In each lobule or trabecula, neoplastic cells were arranged into tubules, columns or solid sheets. Neoplastic cells had infiltrated into

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Table 4. Comparison of glucose and insulin concentrations between Laboratories A and B for 4 dogs

<table>
<thead>
<tr>
<th>Case</th>
<th>Serum* glucose (mg/dL)</th>
<th>Serum** insulin (μU/mL)</th>
<th>Serum*** insulin (mIU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>14.55</td>
<td>&gt;300</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>17.58</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>161.3</td>
<td>119.0</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>19.59</td>
<td>124.7</td>
</tr>
</tbody>
</table>

* Paired glucose concentrations were less than 60 mg/dL.
** Insulin concentrations at Laboratory A. Reference interval: 7–17 μU/mL.
*** Insulin concentrations at Laboratory B. Reference interval: 10–25 mIU/L.

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Fig. 1. Insulinoma. Case 7. Ultrasound image of a dog with insulinoma. Hypoechoic nodule with smooth contour. The right lobe of the pancreas.
surrounding capsules in adjacent exocrine pancreas as individual cells or small cell clusters (Fig. 4). Vascular or lymphatic invasion was identified in 2 cases (Cases 2 and 3). Most neoplastic cells were polygonal with mildly to moderately pleomorphic, anisokaryotic round to oval nuclei with scattered chromatin and a single small nucleolus, and moderate to abundant eosinophilic granular or vacuolar cytoplasm.

Metastatic tumors in lymph nodes were partly encapsulated, but metastatic foci in the liver were non-encapsulated. All metastatic foci were comprised of neoplastic cells identical to those in the primary pancreatic neoplasm (Table 5).

**Immunohistochemistry:** Immunohistochemical examination revealed that all carcinomas originating from islet cells expressed chromogranin A and synaptophysin. In all islet cell carcinomas, the dominant cells displayed positive immunoreactivity for insulin, but some tumors also contained a variable number of cells that stained for somatostatin (Fig. 5), glucagon or gastrin. No tumors were pure insulinomas, but no pure glucagonomas (alpha cell carcinomas), somatostatinomas (delta cell carcinomas) or gastrinomas were identified. Immunoreactivity for insulin,
**DISCUSSION**

In the present study, 7 pancreatic tumors and 1 metastatic tumor of the liver were diagnosed as islet cell carcinoma based on histological type [2, 11]. Histological evidence of invasion through the capsule and into adjacent pancreatic parenchyma, blood vessels and lymphatics, and metastasis represent the primary criteria for malignancy [2, 11]. Furthermore, islet cell neoplasms are classified into certain cell types based on hormone production [11].

Immunohistochemically, endocrine pancreatic neoplasms in animals and humans have been shown to be multihormonal and comprise multiple cell types [11, 17]. In the present cases, the predominant cells in all examined tumors showed insulin-positive immunoreactivity, but no tumors are pure insulinomas, and no pure glucagonomas, soma-
tostatinomas or gastrinomas were identified.

In general, the frequency of malignant beta cell tumors in dogs is high. Clinically, virtually all insulinomas (> 95%) are malignant [3, 13] and pathologically, carcinomas account for about 60% of pancreatic beta cell tumors [2]. Our present series of carcinomas included 3 cases without metastases (clinical stage 1), 2 cases of carcinoma with metastasis to associated lymph nodes at surgery (clinical stage 2), and 3 cases with hepatic metastases (clinical stage 3). All cases were within the 95% confidence interval of median survival times for insulinoma in each clinical stage as reported in another recent retrospective study [15].

For the diagnosis of islet tumors in dogs, hormone production responsible for the associated functional syndrome is necessary to properly classify the neoplasm and to avoid diagnostic errors that may lead to inappropriate treatment [11]. For the treatment of canine insulinoma, the efficacy of medical treatments after surgery has been suggested [4, 15], but surgical treatment after clinical diagnosis remains the treatment of choice [4, 13, 15].

In dogs, investigators agree on the significance of serum insulin concentrations for the clinical diagnosis of insulinoma, but disagree on the significance of serum insulin concentrations in confirming suspected insulinoma at one time point [10, 13].

Insulin concentrations usually vary in dogs with suspected insulinoma. Even if the patient shows an insulin concentration in the middle to high end of the reference interval, insulinoma remains possible [10, 12, 13]. About 25% of these cases have insulinoma [12]. A serum insulin concentration in the low end of the reference interval is considered equivocal or non-diagnostic. In such cases, repeating simultaneous insulin and glucose determinations may be necessary and give more convincing information whether insulinoma is present [10, 12, 13]. Furthermore, according to recent findings in veterinary clinical pathology, calculated ratios such as amended insulin:glucose ratio, insulin:glucose ratio, and glucose:insulin ratio result in a high number of false-positive results, and the use of such ratios is not recommended for domestic animals [12, 18]. The diagnosis of insulinoma therefore remains difficult.

In the retrospective study of 8 pathologically confirmed cases of insulinoma, insulin concentrations for 8 dogs showed 6 dogs over the reference interval and 2 dogs in the middle to high end of the reference interval (although insulinoma remains possible). Conversely, comparative measurements showed that in Laboratory A, 3 dogs were over the reference interval and 1 dog was in the middle of the reference interval, and in Laboratory B, 3 of 4 dogs were over the reference interval and 1 dog was below the reference interval. Split decisions were seen for the diagnosis of insulinoma in 2 of the 4 dogs. Furthermore, we submitted additional three serum samples which suspected insulinoma due to clinical signs and hypoglycemia to Laboratory A and Laboratory B and got split decisions for the diagnosis of insulinoma in all three dogs (unpublished data).

We cannot yet make any definitive conclusions regarding the significance of a single measurement of serum insulin concentration in confirming suspected insulinoma. What we can say, however, is that our study isolates the major issues of disagreement over significance. First, multiple measurements of the same serum at different laboratories cannot be recommended for the diagnosis of insulinoma. Second, discrepancies in data existed between these two laboratories and repeated simultaneous insulin and glucose determinations will not help to lessen such gaps. Third, the validity of data from each laboratory is essential and without meeting this prerequisite, repeated measurements may also not be beneficial for the clinical diagnosis of insulinoma.

Insulinomas without abnormal plasma insulin levels have been reported in human medicine [5, 7, 8]. The mechanisms remain unclear, although several possible options must be considered: 1) insulinoma may secrete insulin in short bursts, causing wide fluctuations in plasma insulin levels; 2)

<table>
<thead>
<tr>
<th>Case</th>
<th>Infiltration</th>
<th>Vascular or lymphatic invasion</th>
<th>Metastasis</th>
<th>Liver</th>
<th>Mitotic index*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td>–</td>
<td>5</td>
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<tr>
<td>3</td>
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<tr>
<td>8</td>
<td>+</td>
<td>–</td>
<td></td>
<td>–</td>
<td>5</td>
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</tbody>
</table>

* Mitotic index: total number of mitotic figures in 10 high-power fields.

Table 6. Immunoreactivity in 8 cases of canine insulinoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Somatostatin</th>
<th>Gastrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
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<tr>
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<td>0</td>
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<td>0</td>
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<tr>
<td>6</td>
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<td>1</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Immunoreactivity graded on scale from 0 to 5+: 0, negative; 1+, scattered; 2+, <25%; 3+, 25–49%; 4+, 50–74%; 5+, 75–100%.
insulinoma may secrete abnormal insulin, which is easily broken down; 3) insulinoma may release excessive amounts of proinsulin, which displays less biological potency than the corresponding insulin molecules; and 4) circulating insulin-like growth factor (IGF)-II may contribute to hypoglycemia [5]. In addition, a subgroup of patients with insulinoma demonstrates elevated insulin levels as well as insulin secretion totally uncoupled from the blood glucose concentration [16]. A similar phenomenon in which human insulin assay systems cannot yield correct detection may occur, as in our canine cases. As serum insulin assays are not species-specific [12], sufficient cross-immunoreactivity exists that commercial human assays have been validated for canine insulin [18].

In human medicine, circulating insulin levels usually vary widely. However, biochemical proof of inappropriately elevated insulin secretion during hypoglycemia is required prior to surgery in patients with insulinoma [16]. Conversely, to date, in canine surgery for insulinoma, proof of biochemical hypoglycemia with simultaneous high insulin level may not always be required for tentative diagnosis as the basis of surgical intervention [13]. In contrast to human cases, which comprise >90% benign tumors [6], the frequency of malignant tumors [3, 13] and metastatic rates at celiotomy are high for canine insulinomas [1, 3]. Earlier clinical diagnosis and decision making for treatments may thus be beneficial. In patients with clinical syndrome of hypoglycemia, collecting multiple samples for paired glucose and insulin measurements may be ideal for clinical diagnosis of insulinoma, but is impractical, particularly for decision making regarding surgical treatment. At the very least, explanation of possible causes for clinicopathological canine “equivocal” or “still possible” insulinomas is needed to recommend owners repeated insulin measurements for clinical diagnosis.

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