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Reina Fujiwara

*See next page for additional authors*

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# Retrospective Study of Computed Tomographic Characterization of Canine Oral Malignant Melanoma in 24 Dogs

Nan Choisunirachon<sup>1,2</sup> Lee-Shuan Lin<sup>1,3</sup> Yuiko Tanaka<sup>1</sup> Kohei Saeki<sup>1</sup> Reina Fujiwara<sup>1</sup>  
Ryohei Nishimura<sup>1</sup> Takayuki Nakagawa<sup>1\*</sup>

## *Abstract*

Clinical and computed tomographic (CT) information from 24 histopathological diagnosed canine oral malignant melanomas (MMs) was retrospectively investigated. The CT appearance on canine oral MM from each patient was evaluated, including both bone and soft tissue windows and pre- and post-contrast enhancement. Among the 24 dogs, canine oral MM was highly presented in small breeds, especially in miniature dachshunds older than 10 years. The gingiva was the most commonly affected site, followed by palatine, mucosal, and lingual areas. The location of the primary tumor in the oral arcade was statistically significant associated with bone invasion such as osteolysis. Canine oral MMs in the caudal arcades showed significantly greater association with osteolysis than MMs of the rostral arcades ( $p = 0.0325$ ). Among tumor locations, the upper oral arcades had a significantly higher contrast enhancement value than the lower arcades ( $p = 0.0228$ ). When metastatic and non-metastatic regional lymph nodes were compared, there was no significant difference in node size or contrast enhancement values, although metastatic mandibular lymph nodes were slightly larger than the non-metastatic nodes. Therefore, CT-based information could facilitate clinical diagnosis and treatment planning for canine oral MM.

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**Keywords:** canine, computed tomography, malignant melanoma, oral

<sup>1</sup> Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

<sup>2</sup> Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.

<sup>3</sup> Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and technology, No.1 Neipu, Pingtung 912, Taiwan.

\*Correspondence: anakaga@mail.ecc.u-tokyo.ac.jp

## Introduction

Oral tumors are common in dogs and account for 5.3% of all malignancies in this species (Niemic, 2008) and 20% of all tumors found in the head and neck regions (Bronden, et al., 2009). Malignant melanoma (MM) reportedly has a higher incidence in small breeds of dog aged over 10 years (Ramos-Vara et al., 2000). In general, canine oral MM is extremely variable with respect to anatomical site, tumor size, clinical stage, and histological parameters. Primary oral MM can be found in any part of the oral cavity, but gingiva is the most common site, with less frequent incidences on the tongue, buccal mucosa, and palate (Smith et al., 2002). One study showed that gingival MM was more likely to occur in the mandible (Ramos-Vara et al., 2000), but another study noted that the occurrence site of MM was not significantly different between the upper and lower arcades (Todoroff and Brodey, 1979). Biologically, MM tends to focally infiltrate adjacent tissues, and so invasion of the oral arcades of either the mandibles or the maxilla may be common.

Presently, there are various treatments for canine oral MM, including surgical excision, radiation, chemotherapy, or immunotherapy (Bergman, 2007). Given the poor prognosis associated with local invasiveness and the high incidence of metastasis (Bergman, 2007), accurate diagnosis based on clinical staging is required for a precise prognosis and for planning a proper treatment regimen for oral MM patients. Radiographic examination of the canine head area is less helpful for diagnosis due to superimposition of the complex structure (Park et al., 1992). In veterinary medicine, computed tomography (CT) is a superior diagnostic tool compared to other imaging modalities in terms of clinical diagnosis (Ohlerth and Scharf, 2007) and it has been shown to have better sensitivity and specificity for detecting oral cancer (Handschel et al., 2012). CT-based images can reveal details of local invasion and extension of a tumor mass, which is important for planning treatments such as surgical excision and radiotherapy. However, until present, there is no report of the CT-based imaging information on canine oral MM. The purpose of this study was to demonstrate the CT characterization of canine oral MMs based on specific clinical features of patients.

## Materials and Methods

This study was performed by a retrospective review of medical records of 24 dogs that had undergone CT and had been histologically diagnosed with oral MM at the Veterinary Medical Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, between May 2009 and September 2012. A histopathological diagnosis of the primary mass was done from tissue specimens obtained by core biopsy or surgical resection after the CT imaging. Macroscopic findings and clinical signs associated with oral tumors were noted, including mucosal rupture that was designated by laceration of mucosal epithelial, and oral discharge. Clinical staging was categorized by size of the primary mass measured by caliper, lymph node involvement investigated through histopathological or cytological samples, and

distant metastasis determined by whole body CT imaging. The patients were staged using the TNM-based staging scheme for dogs with oral melanoma in accordance with World Health Organization criteria (Bergman, 2007).

For CT investigation, the patients were immobilized according to clinician preferences which were sedation using medetomidine hydrochloride (Domitor®, Pfizer)-midazolam (Dormicum®, Roche) or general anesthesia using propofol and isoflurane. All dogs underwent an axial CT examination in a ventral recumbency, and were scanned using a 4-slice helical CT unit with a slice thickness of 2 mm, a pitch of 1.0-1.5 at 120 kV, and 100 mA (Asteion; Toshiba, Japan). A field of view was set to cover the entire head region, which was dependent on the size of the dog. After survey images were acquired, contrast studies were performed by manual intravenous injection of a non-ionic, water-soluble contrast agent at a bolus dose of 600 mgI/kg of iohexol (Omnipaque300; Daiichi-Sankyo, Tokyo, Japan). Intervals between the injection of contrast agent and scanning of post-contrast phase were less than 1 min.

The CT images were analyzed in a Digital Imaging and Communications in Medicine (DICOM) format using an open source PACS workstation DICOM viewer (Osirix) by two experienced veterinarian radiologist (NC and LL). Both bone window (window level [WL]: 500 Hounsfield units [HU] and window width [WW]: 2500 HU) and soft tissue window (WL: 70 HU and WW: 450 HU) were reviewed. The location of oral MM was designated as follows; arcades or buccal, lingual, or palatine areas. In the arcades of the mandible or maxilla, primary tumor locations were described as rostral or caudal based on the position of the 4<sup>th</sup> premolar tooth. A mass located rostral to the 4<sup>th</sup> premolar was defined as a rostral arcade mass, whereas a mass located at or caudal to the 4<sup>th</sup> premolar was defined as a caudal arcade mass. After the contrast enhancement, all information from the CT scan images, such as tumor dimension, texture, invasion of adjacent tissues, contrast enhancement pattern and the information of size and contrast enhancement of the regional lymph node (mandibular and retropharyngeal lymph nodes) was recorded. To evaluate the invasion of adjacent tissue, information on the affected bone was also reviewed, such as osteolysis, osteosclerosis, and periosteal reaction. Contrast enhancement patterns were characterized as homogeneous, rim enhancement, or heterogeneous. Contrast enhancement of the mass from the selected soft tissue area as the region of interest (ROI) in the biggest diameter of tumor mass was quantified by calculating the mean attenuation (HU) of the pre- and post-contrast images. Regional lymph nodes were also screened and classified as metastatic or non-metastatic based on cytological or histological examination. Enlarged regional lymph nodes on the pathological hemilateral side were measured as the ROI and expressed as a percentage difference related to the lymph node on the non-lesion, opposite side. Post-contrast tissue attenuation of regional lymph nodes was also compared between the pre- and post-contrast phase and then compared with the opposite normal side, as with the ROI.

**Statistical analysis:** Statistical analyses were performed using Prism 5.0 (Graphpad Software, CA, USA). All data were expressed as means and standard deviations (SD), and included the median and range (minimum-maximum) in non-Gaussian populations. Different effects of the anesthetic methods on tissue enhancement, and differences in the ROI and post-contrast attenuation values of metastatic and non-metastatic regional lymph nodes based on cytological examinations were analyzed using the Mann-Whitney test. Relationships between clinicopathological characteristics including the location of primary tumor mass and CT-based imaging information, such as bone lesions and soft tissue enhancement, were tested using Fisher's test. Differences were defined as statistically significant at  $p < 0.05$ .

### Results

**Clinical features:** The clinical features of the 24 canine oral MMs are shown in Table 1. There were 13 males and 11 females. The mean  $\pm$  SD age was  $11.9 \pm 2.8$  years. The mean  $\pm$  SD body weight was  $13.1 \pm 10.3$  kg. The body weight of 12 of the 24 dogs was  $< 10$  kg. The most common breeds were small breeds including miniature dachshunds and mixed-breed dogs ( $n = 7$  each), toy poodles ( $n = 2$ ), golden retrievers ( $n = 2$ ), and other breeds ( $n = 1$ , each) including Papillon, Labrador retriever, Scottish terrier, rough collie, standard poodle, and pug.

The oral examination showed that 17 of the 24 dogs were found to have mucosal rupture, and 10 of these 17 dogs presented either bloody or serosanguineous oral discharge. Eleven of the 24 dogs had lymph node involvement confirmed by cytological examination or histopathology. However, only 7 of the 24 dogs had distant metastasis. Based on tumor dimension and the above findings, 3 dogs were classified as stage 1, and 7 dogs each as stages II, III, and IV.

**CT-based findings:** Primary oral MM were mostly located in gingiva of the arcade ( $n = 20$ ), only 2 and 6 of the 24 dogs had MMs located in the rostral and caudal maxilla, and 8 and 4 dogs had MMs located in the rostral and caudal mandible, respectively (Table 2). Primary oral MMs were also found in palatine ( $n = 2$ ), mucosal ( $n = 1$ ), and lingual areas ( $n = 1$ ). The clinical stage of primary tumors located in rostral areas was low, whereas tumors in the caudal arcades were of a higher clinical stage (Table 3). The largest dimension of the primary tumors measured on the CT-based images was mostly the Z-dimension (rostrocaudal axis;  $n = 21$ ); however, 2 dogs had a large Y-dimension (dorsoventral axis) and 1 dog had a larger X-dimension (left-right axis).

Invasion of the tumor mass into adjacent areas was detected in 16 of the 24 dogs, and more than one structure was found to be invaded in some dogs. The adjacent organs invaded by the oral MMs were mainly bony structures, including the maxilla ( $n = 7$ ), mandible ( $n = 8$ ), or skull ( $n = 1$ ), followed by the retrobulbar area ( $n = 3$ ), oro-nasopharynx ( $n = 1$ ), and nasal passage ( $n = 1$ ). In the 15 dogs with bone invasion, the CT-based images revealed bone changes

such as osteolysis ( $n = 15$ ), osteosclerosis ( $n = 3$ ), and periosteal reaction ( $n = 2$ ). The location of the primary oral MM both in the upper and lower arcades correlated with bone invasion. Oral MMs located in the caudal arcades (10 of 10 dogs) significantly correlated with bone invasion compared with those located in the rostral arcades (5 of 10 dogs) ( $p = 0.0325$ ; Figs 1A and B; Table 2).

**Table 1** Clinical demographic data of 24 canine oral malignant melanoma patients

Clinical features	Value
No. of patients	24
Age (years)	
Median	12.5
Mean $\pm$ SD	$11.9 \pm 2.8$
Range	(7.9-17.11)
Sex	
Female	11
Intact	4
Spayed	7
Male	13
Intact	7
Castrated	6
Weight (kg)	
Median	9.9
Mean $\pm$ SD	$13.1 \pm 10.3$
Range	(2.0-43.7)
$\leq 10$ kg	12
10-20 kg	7
$> 20$ kg	5
Stage	
I	3
II	7
III	7
IV	7
Mucosal rupture	
Negative	7
Positive	17
Oral discharge	
Negative	14
Positive	10
Lymph node metastasis	
Negative	13
Positive	11
Distant metastasis	
Negative	17
Positive	7

**Table 2** Primary oral malignant melanoma (MM) location and CT-based imaging data

Primary oral MM location	No. of patients	No. of patients with osteolysis	No. of patients with high contrast enhancement*
Rostral mandible	8	4	3
Caudal mandible	4	4	-
Rostral maxilla	2	1	2
Caudal maxilla	6	6	5
Palatine	2	-	2
Buccal	1	-	1
Lingual	1	-	-

\* high contrast enhancement: more than 2-fold difference in the survey CT

**Table 3** Clinical stage and location of primary oral malignant melanoma (MM) (The representative data refer to the number of the patient)

Clinical stage	Primary oral MM location						
	Rostral maxilla	Caudal maxilla	Rostral mandible	Caudal mandible	Buccal	Palatine	Lingual
I	1	-	2	-	-	-	-
II	-	2	3	1	-	1	-
III	1	2	2	1	1	-	-
IV	-	2	1	2	-	1	1

Contrast studies showed comparable numbers of dog with homogenous (n = 11) and heterogenous (n = 13) enhancement patterns. Five dogs also showed a rim enhancement pattern accompanied by a homogenous or heterogenous pattern. The mean post-contrast attenuation (HU) compared to pre-contrast attenuation value was  $196.3 \pm 132.0\%$ . Immobilization methods did not significantly influence the post-contrast attenuation value ( $p = 0.4433$ ), although the medetomidine-midazolam method was associated with a higher attenuation mean  $\pm$  SD ( $331.2 \pm 214.0\%$ ; n = 6) than the propofol-isoflurane method ( $211.5 \pm 79.4\%$ ; n = 18). The clinical stage did not affect the attenuation values ( $P = 0.6802$ ). Ten dogs had less than 2-fold post-contrast attenuation, and 8 and 5 dogs had 2- to 3-fold and more than 3-fold post-contrast attenuation, respectively. Only 1 dog with oral MM in the rostral mandible had a post-contrast attenuation (94.5%) value lower than the pre-contrast attenuation value. Among the many dogs with primary oral MMs in the oral arcades, both the rostral and caudal maxilla had significantly higher post-contrast attenuation values (more than 2 folds) than the mandible arcades ( $p = 0.0228$ ; Figs 1A and B). Furthermore, all dogs that had oral MMs in the palatine (n = 2) and buccal (n = 1) areas had high post-contrast attenuation values (more than 2 folds; Table 2).

From this study, 9 of the 24 dogs were confirmed to have regional lymph node involvement by cytological or histological examination. Although the percentage of ROI difference between the affected sides and normal sides of the medial and lateral mandibular lymph nodes was higher in these patients compared to those without metastasis ( $143.6 \pm 49.3\%$  and  $94.2 \pm 23.6\%$  for metastatic and non-metastatic medial mandibular lymph nodes, and  $276.7 \pm 168.2\%$  and  $169.9 \pm 58.1\%$  for metastatic and non-metastatic

lateral mandibular lymph nodes, respectively), no statistically significant differences were detected ( $P = 0.1905$  and  $0.4127$ , respectively; Figs 2A and B). In addition, the percentage of post-contrast attenuation of all regional lymph nodes was comparable between the metastatic and non-metastatic lymph nodes ( $p > 0.05$ ).

## Discussion

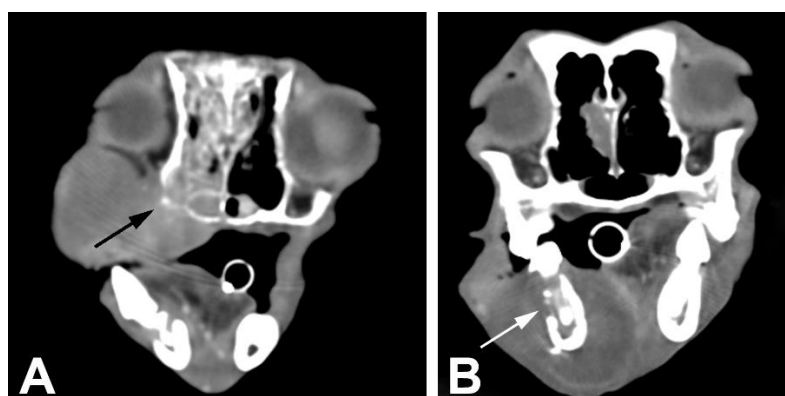
In veterinary medicine, CT-based imaging is highly valued as a diagnostic imaging tool for canine and feline head- and neck-diseases (Kafka et al., 2004; Tromblee et al., 2006; Gendler et al., 2010). CT is superior to conventional diagnostic radiography as it displays an image that includes the highly complex structure in a tomographic form and is more beneficial than magnetic resonance (MR) imaging owing to its shorter immobilization time (Kafka et al., 2004). In addition, CT-based images are more sensitive than MR images to detect bone lesion. Hence, CT-based images may be more suitable for clinical diagnosis of frail patients that could not tolerate a long period of general anesthesia, and they also provide precisely detailed information to facilitate treatment planning and clinical prognosis, especially for malignant cancers with short survival duration such as canine oral MM.

In this retrospective study, we determined the clinical features associated with CT-based imaging data of canine oral MMs, which to date are limited. The clinicopathological results from this study showed the same tendency as those in previous studies of clinical canine oral MM (Ramos-Vara et al., 2000). In our study, miniature dachshunds were highly presented with oral MM. Previous studies reported that miniature dachshunds were overrepresented in oral MM populations with a relative risk of 1.81, which is higher than that for most breeds (Ramos-Vara et al., 2000). This might suggest that breed predisposition of the

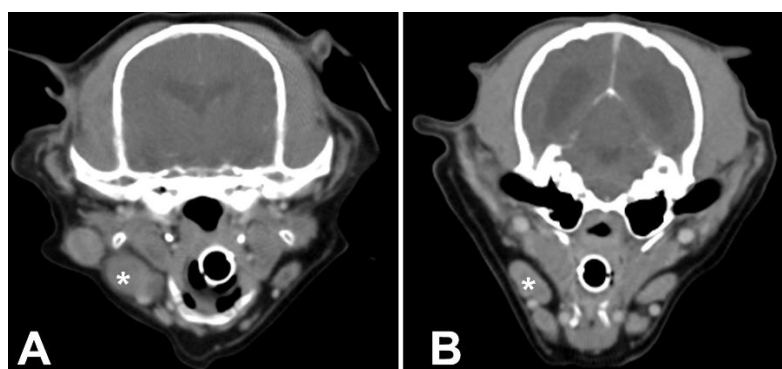
dachshund family might be at risk in developing oral MM.

The clinical stages of most of the patients in this study were stages II, III and IV at presentation. Lower stages (stage I) included MMs in rostral areas, which may easily be detected by the owners. In

contrast, clinical stage IV oral MM showed a high tendency to be located in the caudal arcades. Masses in this area are usually hidden and detection by owner may be delayed. In addition, our study confirmed a previous report that oral MMs were often found in the gingiva (Smith et al., 2002).



**Figure 1** Post contrast, CT-based images of canine oral MMs in the caudal arcades. Canine oral MMs in the caudal maxilla (A) and caudal mandible (B) with osteolysis (arrows). Almost all canine oral MMs in the caudal maxilla, but none of those in the caudal mandible, presented high attenuation values in the contrast study.



**Figure 2** CT-based images of regional mandibular lymph nodes of canine oral MM in metastatic (A) and non-metastatic (B) patients based on cytological examination. The size, measured by the region of interest (ROI) of the metastatic mandibular lymph nodes was not significantly different from that of the non-metastatic nodes; (B), the ROI of the metastatic mandibular lymph nodes was slightly larger than that of the non-metastatic nodes (asterisk: affected hemilateral side).

It is commonly known that canine oral MM is highly invasive and affects nearby soft tissue and bone structures. Several studies have reported that oral MMs caused osteogenic changes (Chenier and Dore, 1999; Oyamada et al., 2007; Ellis et al., 2010), in which cartilage and osteoid matrices might be produced by dedifferentiated melanocytes (Oyamada et al., 2007). In our study, 15 dogs that showed local invasion were also presented with osteolysis, whereas only 3 dogs showed a combination of osteolysis and osteogenic bone changes on the CT-based images. Several reports have shown that oral malignancy-related bone destruction is provoked by several factors, including parathyroid hormone-related peptide, interleukins, or the receptor activator of the NF- $\kappa$ B ligand (Kayamori et al., 2010; Nakashima et al., 2012). However, no data related to bone destruction in canine oral MM have been reported. Further investigation into molecular changes in nearby skeletons after the invasion of tumor cells may clarify the mechanism of bone changes in canine oral MMs.

In the contrast enhancement studies, the immobilization methods were found to have no significant effect on the attenuation values. This finding allowed us to proceed with the subsequent comparisons without considering the differences in immobilization methods. The location of the primary tumor correlated with the contrast enhancement, especially in the rostral and caudal areas of the upper

arcade. High contrast enhancement may indicate high neovascularization in the tumor tissue, which is well known as an important factor in tumor growth and metastasis (Hicklin and Ellis, 2005). In this study, all oral MMs in the caudal maxilla (6/6) invaded adjacent tissues and most of these (5/6) showed high contrast enhancement. These findings suggest that the caudal maxilla may be supplied by a large number of tumor vessels and that it should be considered as high risk for malignancy owing to the poor prognostic features. Unfortunately, we could not evaluate the correlation between the location of the primary tumor and the survival time of the patient because of the variety of treatment methods used in these patients. A case-control prospective study focusing on this issue is needed to investigate the clinical significance of vascularization in canine oral MMs.

When the metastatic and non-metastatic mandibular lymph nodes in the CT images were compared, the attenuation values after tissue enhancement were not significantly different. However, the size of the metastatic nodes was slightly larger than that of the non-metastatic nodes in the mandibular area. The increase in the size of the regional lymph node might be due to infiltration of the metastatic tumor or local inflammation. A previous, meta-analysis noted that using CT-based images to detect regional lymph node metastasis in the head and neck area had a lower diagnostic odds ratio than that

from using ultrasonography (de Bondt et al., 2007), however, routine follow-up by CT was superior to ultrasonography owing to the detection of both lymph node metastasis and local recurrence (Rivelli et al., 2011). Besides, in this study, we did not compare the CT-image of retropharyngeal lymph node because of the lack of cytological result. According to the retrospective study design, the small population number, and the insufficient prognostic data of this study, further large-scale prospective studies are warranted to reveal the clinical importance of CT-based images in canine patients with oral MM.

In conclusion, CT-based images are a valuable tool for obtaining clinical information for canine oral MM to facilitate diagnosis and treatment planning.

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## บทคัดย่อ

### การศึกษาย้อนหลังครั้งนี้มีวัตถุประสงค์เพื่อศึกษาข้อมูลทางคลินิกและลักษณะภาพถ่ายเอกซเรย์ คอมพิวเตอรืของสุนัขที่ป่วยด้วยโรคมะเร็งเมลาโนมาในช่องปากจำนวน 24 ตัว

แนน ซ้อยสุนิรช<sup>1,2</sup> ลี ชวน ลิน<sup>1,3</sup> ยูโยเก ทานากะ<sup>1</sup> โคเฮ ซาเอกิ<sup>1</sup> เรอน่า พูจิวาร่า<sup>1</sup> เรียวเฮ นิชิมูระ<sup>1</sup> ทากะยุกิ นาคากาวะ<sup>1\*</sup>

โดยลักษณะภาพถ่ายเอกซเรย์คอมพิวเตอรืของสุนัขป่วยแต่ละรายได้รับการประเมินลักษณะผิดปกติทั้งก่อนและหลังฉีดสารเพิ่มความชัดภาพ รวมทั้งที่ความกว้างและระดับความหนาแน่นรังสีสำหรับกระดูก และเนื้อเยื่ออ่อน จากการศึกษาพบว่าสุนัขส่วนใหญ่ที่ป่วยด้วยโรคมะเร็งเมลาโนมาเป็นสุนัขสายพันธุ์เล็ก โดยเฉพาะอย่างยิ่งสุนัขสายพันธุ์ดัชชุนที่มีอายุมากกว่า 10 ปี ตำแหน่งที่พบความผิดปกติมากที่สุดได้แก่ เหงือก ร่องลมคาคือ เพดานปาก เยื่อช่องปาก และลิ้น ตำแหน่งของมะเร็งที่گرامมีความสัมพันธ์กับการเปลี่ยนแปลงของเนื้อเยื่อกระดูกรอบข้าง โดยเฉพาะอย่างยิ่ง การสลายของเนื้อเยื่อกระดูก โดยพบการสลายของเนื้อเยื่อกระดูกเนื่องจากก้อนมะเร็งเมลาโนมาในช่องปากบริเวณกรามบนและกรามล่างทางด้านหลังมากกว่ากรามบนและกรามล่างทางด้านหน้าของสัตว์ป่วยอย่างมีนัยสำคัญทางสถิติ ( $p = 0.0325$ ) เมื่อเปรียบเทียบการเพิ่มขึ้นของสารเพิ่มความชัดภาพ มะเร็งเมลาโนมาในช่องปากบริเวณกรามบนมีการเพิ่มขึ้นของค่าที่รังสีมากกว่ามะเร็งเมลาโนมาในช่องปากบริเวณกรามล่างอย่างมีนัยสำคัญทางสถิติ ( $p = 0.0228$ ) เมื่อเปรียบเทียบลักษณะปุ่มน้ำเหลืองบริเวณใกล้เคียง พบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของขนาดปุ่มน้ำเหลืองและลักษณะการที่รังสีระหว่างปุ่มน้ำเหลืองที่มีการแพร่กระจายและไม่มีการแพร่กระจายของมะเร็งเมลาโนมา อย่างไรก็ตามพบว่า ปุ่มน้ำเหลืองใกล้เคียงที่มีการแพร่กระจายของเซลล์มะเร็งมีขนาดใหญ่กว่าปุ่มน้ำเหลืองที่ไม่พบการแพร่กระจายของเซลล์ จากผลการศึกษานี้สรุปได้ว่าข้อมูลจากภาพถ่ายเอกซเรย์คอมพิวเตอรืสามารถให้ข้อมูลที่สำคัญและเหมาะสมสำหรับการตรวจวินิจฉัยและวางแผนการรักษาสำหรับสุนัขป่วยด้วยโรคมะเร็งเมลาโนมาในช่องปาก

**คำสำคัญ:** สุนัข ภาพถ่ายเอกซเรย์คอมพิวเตอรื มะเร็งเมลาโนมา ช่องปาก

<sup>1</sup> หน่วยปฏิบัติการสัตวศาสตร์ทางสัตวแพทย์ ภาควิชาวิทยาศาสตร์ทางสัตวแพทย์ บัณฑิตวิทยาลัยทางเกษตรศาสตร์และวิทยาศาสตร์สิ่งมีชีวิต มหาวิทยาลัยโตเกียว โตเกียว ประเทศญี่ปุ่น

<sup>2</sup> ภาควิชาสัตวศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

<sup>3</sup> ภาควิชาสัตวศาสตร์ วิทยาลัยสัตวแพทยศาสตร์ มหาวิทยาลัยวิทยาศาสตร์และเทคโนโลยีฟินทง เนพู ฟินทง ประเทศไต้หวัน

\*ผู้รับผิดชอบบทความ E-mail: anakaga@mail.ecc.u-tokyo.ac.jp