Diagnostic value of cytological analysis of tumours and tumour-like lesions of the oral cavity in dogs and cats: A prospective study on 114 cases

Bonfanti, U; Bertazzolo, W; Gracis, M; Roccabianca, P; Romanelli, G; Palermo, G; Zini, E

Abstract: Neoplastic or non-neoplastic masses are common findings in the oral cavity of cats and dogs. The aim of this prospective study was to compare the results of cytological examinations of lesions of the oral cavity following fine-needle aspiration (FNA), fine-needle insertion (FNI), and impression smear (IS) with histopathological results being considered as the diagnostic gold standard. In total, 85 dogs and 29 cats were included in the study. Cases were included when histology and cytology (FNA, FNI, and/or IS) were available from the same lesion. Agreement and accuracy between cytological and histopathological results were calculated. Eighteen cytological specimens were excluded, with a retrieval rate of 84.2%. Of the 96 samples analysed, FNA, FNI, and IS were available from 80, 76, and 73 animals, respectively. Overall, 60/67 (89.6%) and 21/29 (72.4%) lesions were neoplastic in dogs and cats, respectively, with the remaining being non-neoplastic. For all lesions, \( \kappa \)-values obtained by FNA, FNI, and IS were in dogs 0.83 (95% confidence interval [CI]: 0.77-0.90), 0.87 (95% CI: 0.81-0.93) and 0.75 (95% CI: 0.67-0.84), respectively, and in cats 0.92 (95% CI: 0.87-0.96), 0.92 (95% CI: 0.88-0.97) and 0.86 (95% CI: 0.79-0.92), respectively. The diagnostic accuracies of FNA, FNI, and IS in dogs with neoplasia were 98.2%, 98.1%, and 91.8%, respectively, and in cats with neoplasia were 95.6%, 95.6% and 93.8%, respectively. In conclusion, the high agreement with histopathology suggests that cytological examinations by FNI, FNA, and IS are all appropriate methods to correctly diagnose lesions of the oral cavity in dogs and cats.

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Diagnostic value of cytological analysis of tumors and tumor-like lesions of the oral cavity in dogs and cats: A prospective case study on 114 cases

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Abstract

Oral cavity masses are common findings in canine and feline clinical practice, either neoplastic or non-neoplastic. The aim of this prospective study was to compare results of cytologic examinations by fine-needle aspiration (FNA), fine-needle insertion (FNI) and impression smear (IS) obtained from lesions of the oral cavity with histology set as the diagnostic gold standard.

Eighty-five dogs and 29 cats were included in the study. Specimens were included when histology and cytology (FNA, FNI and/or IS) were available from the same lesion; $k$-agreement and accuracy between cytological and histological results were calculated. Eighteen cytological specimens were excluded with a retrieval rate of 85.7%. Out of the 96 samples used for the analysis, FNA, FNI and IS were available from 80, 76 and 73 animals, respectively. Sixty of 67 (89.6%) dog and 21 of 29 (72.4%) cat lesions were neoplastic and the remaining were non-neoplastic. For all lesions $k$-values obtained by FNA, FNI and IS in dogs were 0.83 (confidence interval [CI] 95%: 0.77-0.90), 0.87 (CI 95%: 0.81-0.93) and 0.75 (CI 95%: 0.67-0.84), respectively, and in cats 0.92 (CI 95%: 0.87-0.96), 0.92 (CI 95%: 0.88-0.97) and 0.86 (CI 95%: 0.79-0.92), respectively. Diagnostic accuracy of FNA, FNI and IS in dogs with neoplasia was 98.2%, 98.1% and 91.8%, respectively, and in cats was 95.6%, 95.6% and 95.8%, respectively. In both species the elevated agreement and accuracy suggest that cytological examination by FNI, FNA and IS are effective methods to correctly diagnose mass lesions of the oral cavity when compared to histopathology, which represents the gold standard in particular for unsatisfactory cytological samples.

Keywords: Oral cavity; Neoplasia; Cytology; Canine; Feline.
Introduction

Oral cavity masses represent common findings in dogs and cats in clinical practice, with a large variety of diagnoses spanning from benign and malignant tumors to tumor-like conditions (Spodnick and Page, 1995; Goldschmidt and Hendrick, 2002). The most frequent oropharyngeal cancer in dogs is melanoma (Smith et al., 2002), and the majority of these are malignant (Bradley et al., 1984; Spodnick and Page, 1995). whereas in cats is squamous cell carcinoma (SCC) (Bradley et al., 1984; Spodnick and Page, 1995; Liptak and Withrow, 2006). SCCs account for 70% of feline and 25% of canine oral neoplasms and may arise from virtually any surface of the oral cavity (Bradley et al., 1984).

Cytological examination represents a minimally invasive and easily available diagnostic tool that is routinely used in companion animal medicine. The results of this technique correlate well with histopathological findings for numerous mass lesions (Bonfanti et al., 2006; Ghisleni et al., 2006; Simon et al., 2009) including angiosarcoma, mammary tumors and osteosarcoma in dogs (Allen et al., 1986; Bertazzolo et al., 2005; Reinhardt et al., 2005; Simeonov and Stoikov, 2006; Simon et al., 2009; Sontas et al., 2012), thymoma, lymph nodal and splenic lesions, and abdominal, cutaneous or subcutaneous masses in both species (Rae et al., 1989; Menard et al., 1996; Chalita et al., 2001; Bonfanti et al., 2004; Ghisleni et al., 2006; Ovejero Braun and Hauser, 2007). However, the diagnostic reliability of cytology in the evaluation of oral masses has not yet been previously investigated in dogs and cats. In human medicine, few reports have explored the diagnostic potential of fine-needle aspiration (FNA) for intraoral lesions and for lesions of the maxillofacial region. These studies support the clinical usefulness of cytological analysis, with a sensitivity ranging from about 75% to 96% and a high specificity and positive predictive value, reaching almost 100% (Cramer et al., 1995; Singh et al., 2011).
The aim of this prospective study was to determine the diagnostic reliability of cytology obtained by fine-needle aspiration (FNA), fine-needle insertion (FNI) – ie, aspiration and non-aspiration technique, respectively, and impression smear (IS) from mass lesions of the oral cavity of dogs and cats, as compared to histopathology.

**Materials and methods**

*Criteria for selection of cases*

Dogs and cats with mass lesions of the oral cavity that were examined at the authors’ institutions (MG, GR, and WB) between 2007 and 2010. Most of patients examined came from the northern part of Italy, and were referred to large clinics of this region. Cases were included when cytological and histological specimens were available from the same lesion.

*Procedures*

From oral cavity lesions, cytological specimens were obtained by FNA and FNI using different Gauge needles (21-25 G) using 2.5-5 ml syringes for aspiration. All samples were obtained by inserting the needle through the oral mucosa. The insertion path was placed in the anatomic region included in the planned excisional procedure of the mass. IS were obtained from lesions surgically excised and prepared after accurate blotting of the specimen with a clean absorbent paper to remove blood and tissue fluid in excess. When possible, FNA, FNI and IS were performed on the same lesion. All cytological smears were stained with May-Grünwald-Giemsa. For each case, 1 to 5 slides from every available sampling technique were reviewed by two board-certified clinical pathologists (UB, WB) unaware of the histological diagnosis. Histological specimens were fixed in 10% neutral buffered formalin and bisected along their longer axis with a scalpel blade. Tissues were embedded in paraffin and stained
with hematoxylin and eosin. Then, all samples were reviewed by a single board-certified pathologist (PR), not aware of the previous cytological diagnosis. “Histological Classification of Tumors of the Alimentary System of Domestic Animals” (Head et al., 2003a,b) was used to categorize the neoplastic conditions. When necessary, immunohistochemical labeling was additionally requested to allow a definitive histological diagnosis.

**Data analysis**

For all cases, every cytological diagnosis made by FNA, FNI or IS was compared with its paired histological diagnosis, with the latter set as gold standard. Agreements between cytological methods and histopathology were assessed using Cohen's kappa coefficient ($k$) and were calculated for all lesions and for all tumors, either in dogs or cats.

The extent of concordance between cytological and histological diagnosis was classified as complete agreement and no agreement, or undetermined. Complete agreement was defined as concordance for both cell lineage (i.e. epithelial, mesenchymal, hematopoietic or melanocytic) and cell type (e.g. squamous epithelium, odontogenic epithelium, fibroblastic cells). No agreement was defined as the lack of concordance for cell lineage (e.g. mesenchymal instead of epithelial) or cell type in case of neoplasia (e.g. acanthomatous ameloblastoma instead of squamous cell carcinoma), or if a cytological diagnosis of any non-neoplastic lesion (e.g., inflammation) instead of neoplastic was obtained and vice versa. Agreement was classified as undetermined if the cytological specimen was unsatisfactory because of hypocellularity, hemodilution, or necrosis. Values of $k < 0$ indicated no agreement, values between 0–0.20 indicated a slight agreement, values between 0.21–0.40 indicated a fair agreement, values between 0.41–0.60 indicated a moderate agreement, values between 0.61–
0.80 indicated a substantial agreement, values between 0.81–0.99 indicated an almost perfect agreement, and values of 1 indicated a perfect agreement (Landis and Koch, 1977).

In addition, diagnostic reliability of each of the 3 cytological methods to identify neoplastic and non-neoplastic lesions was further tested in dogs and in cats separately by calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Sensitivity, specificity, PPV, NPV and accuracy were arbitrarily considered low if <70%, moderate if ≥70% and <80%, high if ≥80 and <90 high, and very high if ≥90%.

Clinical usefulness of the 3 cytological methods was also calculated for the most represented tumors collected in this series of dogs and cats. For this purpose, k-agreement as well as sensitivity, specificity, PPV, NPV and accuracy were calculated if more than 10 cases were available for every cytological method in each species. For every analysis histopathology was considered the gold standard. Statistical analysis was conducted with a software package.¹

Results

Animals and samples

A total of 114 animals were initially retrieved for the study, including 85 dogs and 29 cats. The dogs consisted of 39 males, 25 females, 12 spayed females, and 9 neutered males. Median age of dogs was 9 years (range, 1 to 17 years). There were 30 mongrels, 8 Labrador Retriever dogs, 6 Boxer dogs, 4 Yorkshire Terrier dogs, 3 Rottweilers, 2 each American

¹ Microsoft Office Excel 2007 for Windows 7
Cocker Spaniel, Bernese Mountain dogs, Dachshund, English Setter, Fox Terrier dogs, German Shepherd dogs, Pinscher, Poodle and Shih-Tzu, and one each Alaskan Malamute, American Staffordshire Terrier dog, Andes Shepherd dog, Bobtail, Chihuahua, Dobermann, Dogue de Bordeaux, English Cocker Spaniel, Golden Retriever dog, Italian Bloodhound, Maltese, Maremma Sheepdog, Newfoundland, Rhodesian Ridgeback, Schipperke and Schnauzer. The cats included were 13 spayed females, 10 neutered males, 3 males, and 3 females. Median age of cats was 11 years (range, 1 to 17 years). There were 19 domestic shorthaired cats, 4 Persians, 2 Exotic shorthaired, and 1 each for the following breeds: Siamese, Scottish Fold, Norwegian Forest and Sphynx.

Oral lesions

Of the 114 animals, 110 (96.5%) had single oral lesions and 4 (3.5%) multiple lesions (Table 1). Sixteen (14.0%) of the 114 cases were excluded from the study because cytological results were unsatisfactory with every method (i.e., FNA, FNI and IS); therefore, no cytological specimen was available for review.

The 16 cases that were excluded belonged to dogs, and histological diagnoses included peripheral odontogenic fibroma (former fibromatous epulis) \((n=7)\), gingival fibroepithelial hyperplasia \((n=3)\), chronic mixed inflammation \((n=2)\), acanthomatous ameloblastoma \((n=1)\), adenocarcinoma \((n=1)\), fibroma \((n=1)\) and myopericitoma \((n=1)\). Two other cases were excluded because histopathology showed two different concurrent neoplasms in the same lesion, including a dog with melanoma and osteosarcoma, and a dog with ossifying fibroma and acanthomatous ameloblastoma.
Overall, 96 oral lesions were considered for further analysis, yielding a retrieval rate of 84.2%. Of the 96 cases, 67 were obtained from dogs and 29 from cats; tumors were diagnosed in 81 (84.4%) cases and non-neoplastic lesions in the remaining 15 (15.6%) cases. Sixty-eight (83.9%) of the 81 animals with tumors were affected by a malignant neoplasm and 13 (16.1%) by a benign neoplasm. Considering dogs and cats separately, 50 dogs (74.6%) and 18 cats (62.1%) had malignant oral tumors and 10 dogs (14.9%) and 3 cats (10.3%) had a benign oral neoplasia. Among the 81 animals with oral cavity tumors, including 60 (89.6%) of the 67 dogs and 21 (72.4%) of the 29 cats, histological diagnoses were squamous cell carcinoma (n=23, 14 cats and 9 dogs), melanoma (n=22, 21 dogs and 1 cat), undifferentiated malignant spindle cell tumor (n=8, 7 dogs and 1 cat), acanthomatous ameloblastoma (n=5, all dogs), lymphoma (n=4, 2 dogs and 2 cats), ameloblastoma (n=3, all dogs), adenocarcinoma (n=2, 1 dog and 1 cat), peripheral odontogenic fibroma (n=3, all dogs), fibrosarcoma (n=2, all 2 dogs), plasma cell tumor (n=2, all dogs), undifferentiated neoplasia (n=2, all dogs), ameloblastic keratinizing carcinoma (n=1, a cat), anaplastic carcinoma (n=1, a dog), chondrosarcoma (n=1, a dog), mast cell tumor (n=1, a dog) and osteoma (n=1, a cat).

Of the 15 animals with non-neoplastic lesions, 7 (10.4%) of the 67 dogs and 8 (27.6%) of the 29 cats were identified. Histological diagnoses for these 15 cases consisted of chronic mixed inflammation (n=8, 4 dogs and 4 cats), eosinophilic inflammation (n=4, 3 cats and 1 dog), gingival fibroepithelial hyperplasia (n=1, a dog), reactive histocytosis (n=1, a dog) and reactive fibroplasia (n=1, a cat).

From the 96 oral lesions included, FNA, FNI and IS was not performed in 2, 7 and 22 cases, respectively. Additionally, 14 (14.6%), 13 (13.5%) and 1 (1.0%) had FNA, FNI and IS, respectively, that were excluded because classified as unsatisfactory due to hypocellularity,
hemodilution, or necrosis. The final number of cytological cases included in the FNA, FNI and IS analyses were 80 (57 dogs and 23 cats), 76 (53 dogs and 23 cats) and 73 (49 dogs and 24 cats), respectively.

Diagnostic reliability of FNA, FNI and IS

For all oral lesions grouped together and for all oral neoplastic lesions, FNI yielded the highest agreement with the histopathological diagnosis in dogs (87.0%) while FNA or FNI provided the same agreement in cats (100.0%). Similarly, for specific oral tumors FNI gave the highest agreement in canine melanoma (87.0%) and FNA or FNI in feline SCC (92.0%) (Table 2). In particular, for all oral lesions, FNA and FNI yielded almost perfect agreement in dogs and cats (83.0-92.0%), while IS showed substantial agreement only in dogs (75.0%). For all oral tumors, FNA and FNI yielded almost perfect to perfect agreement in both species (82.0-100.0%), while IS showed an almost perfect agreement in dogs (82.0%), and a substantial agreement in cats (77.0%). For canine oral melanoma, FNA and FNI yielded almost perfect agreement (86.0% and 87.0%, respectively), and IS substantial agreement (77.0%). For feline oral SCC, all methods showed almost perfect agreement (86.0-92.0%) (Table 2).

In dogs, the highest sensitivity and specificity for the diagnosis of oral tumors was recorded using FNI (98.0% and 100.0%, respectively). For diagnosing non-neoplastic lesions both FNA and FNI showed very high specificity (100.0%) with a moderate sensitivity (75.0%) (Table 3). The PPV was very high for neoplastic and non-neoplastic lesions with all methods (98.1-100.0%), and the NPV was very high with neoplastic and non-neoplastic lesions for all methods, except for tumors using IS and FNI which was low or moderate.
(50.0% and 75.0%, respectively). Accuracy was very high for both neoplastic and non-neoplastic lesions with all methods (91.8-98.2%).

In cats all cytological methods yielded very high sensitivity and specificity for the diagnosis of oral tumors and non-neoplastic lesions (94.1-100.0%) (Table 4). PPV for tumors and NPV for non-neoplastic lesions were very high with all methods (100.0%), and PPV for non-neoplastic lesions and NPV for tumors were high (80.0-87.5%). Accuracy was very high for both neoplastic and non-neoplastic lesions with all methods (95.6-95.8%).

The most represented oral tumors, with more than 10 cases, were melanoma (n=22) in dogs and SCC (n=14) in cats. IS was the most reliable cytological method in the diagnosis of canine melanoma, with high or very high sensitivity, specificity, PPV, NPV and accuracy (88.9-100.0%) (Table 3). FNI was the most reliable technique for the diagnosis of feline SCC, with high or very high sensitivity, specificity, PPV, NPV and accuracy (83.3-100.0%) (Table 4).

Discussion

The reliability of a diagnosis obtained from a biopsy of an oral lesion is critical in veterinary oncology. Biopsies provide information that is necessary to select the most appropriate treatment protocol, whether it is surgery, radiation therapy, or chemotherapy, and to select the extent of the treatment, either conservative or aggressive. The results of this study demonstrate an elevated agreement and accuracy of FNA, FNI and IS to identify canine and feline oral cavity lesions when compared with the definitive histological diagnosis, thereby suggesting that the three cytological methods are effective procedures in both species.
In particular, FNA technique consists in inserting the tip of the needle in the tissue of interest, retracting slightly the plunger (½ to 1 cc of vacuum) of the syringe, advancing the needle and retracting it in several different directions, releasing the plunger and withdrawing the needle. Later the specimen is placed on a glass slide. FNI (i.e. fine-needle capillary technique, “stab” technique) consists in the above described procedure, avoiding the use of the syringe and the plunger. Cells are displaced into the cylinder of the needle by capillary action as the needle is incompletely retracted and redirected into the tissue. Its major advantages are to reduce blood contamination and to preserve cellular integrity.

For oral cavity neoplasm FNA showed an almost perfect agreement in dogs ($k=0.82$) and a perfect agreement in cats ($k=1.00$), whereas with IS the agreement remained almost perfect in dogs ($k=0.82$) and decreased to substantial in cats ($k=0.77$).

In a previous study from our group on tumors located in the gastrointestinal tract, IS had a better diagnostic agreement compared to FNA, with histopathological diagnosis as the gold standard, in both species (Bonfanti et al., 2006). The difference is likely due to the type of tumors investigated, with those pertaining to the gastrointestinal tract often being of different origin from those of the oral cavity. In particular, in the gastrointestinal tract, many of the neoplastic lesions evaluated were round cell tumors (i.e., lymphoma) (Bonfanti et al., 2006). Lymphoma can be readily diagnosed by IS, and more easily than using either FNA or FNI methods which often yield a high proportion of naked nuclei. A second explanation could be that oral neoplastic lesions are easily reached using needle biopsy techniques, therefore allowing a higher percentage of retrieval success, as compared to gastrointestinal lesions that, owing to their localization, can be better investigated after collection of biopsy samples followed by IS. In the present study, FNI for oral tumors showed the highest agreement
compared with histological examination in dogs ($k=0.85$) and was equal to FNA in cats ($k=1.00$).

In particular, FNI showed a slightly higher agreement for all lesions in dogs and for oral canine melanoma. Even if values for FNA and FNI can be considered rather comparable, the explanation for the slightly higher agreement documented with the latter might be due to the intrinsic nature of the technique. By inserting the needle without aspiration, cells may be collected limiting their damage and better preserving cytological features that are necessary for the diagnosis. However, this would not explain the similar agreements obtained with FNA and FNI in cats. Whether aspiration of lesions in cats was less aggressively performed than in dogs, or if feline oral lesions are more resistant to aspiration than those of dogs, obtaining therefore a higher percentage of intact cells, cannot be answered. Alternatively, although speculative, the use of different Gauge needles might have played a role on cell retrieval. Further studies are therefore required to explain and confirm this finding.

According to the literature, there are yet no studies that have evaluated sensitivity, specificity, PPV and NPV of cytological examination of oral cavity lesions compared with histopathology in dogs and cats.

In humans, the sensitivity of FNA cytology in the identification of oral and maxillofacial lesions ranges from 75% to 96%, and specificity and PPV approximate 100% (Cramer et al., 1995; Singh et al., 2011). The results of our investigation demonstrated similar sensitivity, specificity and PPV for FNA in dogs and cats (from 75% to 100%). However, among the three methods used for diagnosing oral tumors in dogs, FNI showed the highest sensitivity and specificity (98% and 100%, respectively), and for non-neoplastic lesions FNI
performed equal to FNA, with very high specificity (100%) and moderate sensitivity (75%). The IS method performed less than FNI and FNA in dog oral tumors, yielding a low NPV (50%) which was also reflected in a low sensitivity in the diagnosis of non-neoplastic lesions. Accuracy of the three methods was very high for the identification of neoplastic and non-neoplastic lesions, with slightly lower levels recorded for IS (91.8%).

Therefore, in dogs FNI and FNA may be superior to IS but the results, overall, suggest that all the three methods are useful to achieve a diagnosis of neoplastic and non-neoplastic oral lesions in this species. In cats, for both neoplastic and non-neoplastic lesions, sensitivity, specificity and accuracy were all very high, and PPV and NPV were high, suggesting optimal performance of each of the three methods.

Of note, despite the elevated performance of cytological examinations, those methods did not replace histology. Indeed 14 FNA, 13 FNI and 1 IS samples, were excluded since were classified as unsatisfactory due to hypocellularity, hemodilution, or necrosis. Furthermore, another 16 of the 114 cases were excluded from the analysis because cytological results were unsatisfactory with every method. Regarding these latter cases it is worth mentioning that 7 (43.7%) were diagnosed as peripheral odontogenic fibroma, suggesting this particular tumor may not be suited for cytological examination. The stromal and firm tissue that characterizes the peripheral odontogenic fibroma - as well as fibroma and gingival fibroepithelial hyperplasia, another two causes of unsatisfactory cytological results - may prevent adequate sampling and make histopathology the only reliable tool for achieving a correct diagnosis.
Additionally, although less common, in the present series a few cases showed two associated tumor types in the same lesion. In particular two dogs were excluded from the analysis because of the presence of two concomitant neoplastic processes in the same mass. Similar observations in the oral cavity are rare but described in humans (Dallera et al., 1982; Ryu et al., 2000; Lim et al., 2008) and also in dogs (Watrach et al., 1970; Pérez-Martinez et al., 2000; Sitzman, 2000). The above results highlight the primary importance of histopathology to achieve a correct diagnosis in some oral cavity lesions.

Conclusions

In conclusion, to the best of our knowledge, this is the first report evaluating the diagnostic usefulness of FNA, FNI and IS to diagnose oral cavity lesions in dogs and cats. The elevated agreement and accuracy suggested that cytological examination of oral cavity lesions is an effective procedure in both species when compared with histopathology. Because cytological examination performed either with FNA or FNI allow immediate evaluation, may not need anesthesia and is cost effective, in a clinical setting may represent the first diagnostic approach of mass lesions of the oral cavity in dogs or cats. Our results, however, also highlight the primary importance of histopathology to achieve a correct diagnosis in oral cavity lesions, emphasizing its role as gold standard in particular for unsatisfactory cytological samples.

Conflict of interest statement

None of the authors of this paper has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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### Table 1

Localization of oral masses in 114 dogs and cats.

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>Dogs</th>
<th>Cats</th>
<th>TOTAL</th>
</tr>
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<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Jaw</td>
<td>30 (35.3)</td>
<td>8 (27.6)</td>
<td>38 (33.3)</td>
</tr>
<tr>
<td>Mandible</td>
<td>25 (29.4)</td>
<td>12 (41.4)</td>
<td>37 (32.5)</td>
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<tr>
<td>Hard palate</td>
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<td>0 (0.0)</td>
<td>3 (2.6)</td>
</tr>
<tr>
<td>Buccal mucosa</td>
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<td>0 (0.0)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>Sublingual mucosa</td>
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<td>2 (6.9)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>Tongue</td>
<td>1 (1.2)</td>
<td>1 (3.5)</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Others</td>
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<td>3 (10.3)</td>
<td>22 (19.3)</td>
</tr>
<tr>
<td>Multiple</td>
<td>1 (1.2)</td>
<td>3 (10.3)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>85 (100.0)</strong></td>
<td><strong>29 (100.0)</strong></td>
<td><strong>114 (100.0)</strong></td>
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