This is the final peer-reviewed accepted manuscript of:

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.
Vet J. 2015 Aug;205(2):322-7
Bonfanti U, Bertazzolo W, Gracis M, Roccabianca P, Romanelli G, Palermo G, Zini E.

The final published version is available at . doi: 10.1016/j.tvjl.2014.10.022.

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

U. Bonfanti [a,*], W. Bertazzolo [b], M. Gracis [c], P. Roccabianca [d], G. Romanelli [e], G. Palermo [f], E. Zini [g]

[a] *Biessea Veterinary Diagnostic Laboratory, Via A. D'Aosta 7, 20129 Milano, Italy*
[b] *Ospedale Veterinario Città di Pavia, Viale Cremona 179, 27100 Pavia, Italy*
[c] *Clinica Veterinaria San Siro Via Lampugnano 99, 20151 Milano*
[d] *DIVET-Sezione anatomia patologica veterinaria e patologia aviare, University of Milan, Via Celoria 10, 20133 Milano, Italy*
[e] *Clinica Veterinaria Nerviano, Via Lampugnani 3, 20014 Nerviano (MI), Italy*
[f] *Istituto Veterinario di Novara, S.P. 9, 28060 Granozzo con Monticello (NO), Italy*
[g] *Istituto Veterinario di Novara, S.P. 9, 28060 Granozzo con Monticello (NO), Italy; Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland; Department of Animal Medicine, Production and Health, University of Padua, viale dell'Università 16, 35020 Legnaro, (PD), Italy*

* Corresponding author. Tel.: +39 335 5435425.
E-mail address: ugo.bonfanti@gmail.com (U. Bonfanti).

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.

172 Overall, 96 oral lesions were considered for further analysis, yielding a retrieval rate
173 of 84.2%. Of the 96 cases, 67 were obtained from dogs and 29 from cats; tumors were
174 diagnosed in 81 (84.4%) cases and non-neoplastic lesions in the remaining 15 (15.6%) cases.
175 Sixty-eight (83.9%) of the 81 animals with tumors were affected by a malignant neoplasm and
176 13 (16.1%) by a benign neoplasm. Considering dogs and cats separately, 50 dogs (74.6%) and
177 18 cats (62.1%) had malignant oral tumors and 10 dogs (14.9%) and 3 cats (10.3%) had a
178 benign oral neoplasia. Among the 81 animals with oral cavity tumors, including 60 (89.6%) of
179 the 67 dogs and 21 (72.4%) of the 29 cats, histological diagnoses were squamous cell
180 carcinoma (*n*=23, 14 cats and 9 dogs) (Fig. 1 and Fig. 2), melanoma (*n*=22, 21 dogs and 1 cat)
181 (Fig. 3 and Fig. 4), undifferentiated malignant spindle cell tumor (*n*= 8, 7 dogs and 1 cat),
182 acanthomatous ameloblastoma (*n*=5, all dogs), lymphoma (*n*=4, 2 dogs and 2 cats) (Fig. 5
183 and Fig. 6), ameloblastoma (*n*=3, all dogs), adenocarcinoma (*n*=2, 1 dog and 1 cat),
184 peripheral odontogenic fibroma (*n*=3, all dogs), fibrosarcoma (*n*=2, all 2 dogs), plasma cell
185 tumor (*n*=2, all dogs), undifferentiated neoplasia (*n*=2, all dogs), ameloblastic keratinizing
186 carcinoma (*n*=1, a cat), anaplastic carcinoma (*n*=1, a dog), chondrosarcoma (*n*=1, a dog), mast
187 cell tumor (*n*=1, a dog) and osteoma (*n*=1, a cat).
188
189 Of the 15 animals with non-neoplastic lesions, 7 (10.4%) of the 67 dogs and 8 (27.6%)
190 of the 29 cats were identified. Histological diagnoses for these 15 cases consisted of chronic
191 mixed inflammation (*n*=8, 4 dogs and 4 cats) (Fig. 7 and Fig. 8), eosinophilic inflammation
192 (*n*=4, 3 cats and 1 dog), gingival fibroepithelial hyperplasia (*n*=1, a dog), reactive histocytosis
193 (*n*=1, a dog) and reactive fibroplasia (*n*=1, a cat).
194
195 From the 96 oral lesions included, FNA, FNI and IS was not performed in 2, 7 and 22
196 cases, respectively. Additionally, 14 (14.6%), 13 (13.5%) and 1 (1.0%) had FNA, FNI and IS,

197 respectively, that were excluded because classified as unsatisfactory due to hypocellularity,
198 hemodilution, or necrosis. The final number of cytological cases included in the FNA, FNI
199 and IS analyses were 80 (57 dogs and 23 cats), 76 (53 dogs and 23 cats) and 73 (49 dogs and
200 24 cats), respectively.
201
202 *Diagnostic reliability of FNA, FNI and IS*
203 For all oral lesions grouped together and for all oral neoplastic lesions, FNI yielded the
204 highest agreement with the histopathological diagnosis in dogs (87.0%) while FNA or FNI
205 provided the same agreement in cats (100.0%). Similarly, for specific oral tumors FNI gave
206 the highest agreement in canine melanoma (87.0%) and FNA or FNI in feline SCC (92.0%)
207 (Table 2). In particular, for all oral lesions, FNA and FNI yielded almost perfect agreement in
208 dogs and cats (83.0-92.0%), while IS showed substantial agreement only in dogs (75.0%). For
209 all oral tumors, FNA and FNI yielded almost perfect to perfect agreement in both species
210 (82.0-100.0%), while IS showed an almost perfect agreement in dogs (82.0%), and a
211 substantial agreement in cats (77.0%). For canine oral melanoma, FNA and FNI yielded
212 almost perfect agreement (86.0% and 87.0%, respectively), and IS substantial agreement
213 (77.0%). For feline oral SCC, all methods showed almost perfect agreement (86.0-92.0%)
214 (Table 2).
215
216 In dogs, the highest sensitivity and specificity for the diagnosis of oral tumors was
217 recorded using FNI (98.0% and 100.0%, respectively). For diagnosing non-neoplastic lesions
218 both FNA and FNI showed very high specificity (100.0%) with a moderate sensitivity
219 (75.0%) (Table 3). The PPV was very high for neoplastic and non-neoplastic lesions with all
220 methods (98.1-100.0%), and the NPV was very high with neoplastic and non-neoplastic
221 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.

247 In particular, FNA technique consists in inserting the tip of the needle in the tissue of
248 interest, retracting slightly the plunger (½ to 1 cc of vacuum) of the syringe, advancing the
249 needle and retracting it in several different directions, releasing the plunger and withdrawing
250 the needle. Later the specimen is placed on a glass slide. FNI (i.e fine-needle capillary
251 technique, "stab" technique) consists in the above described procedure, avoiding the use of
252 the syringe and the plunger. Cells are displaced into the cylinder of the needle by capillary
253 action as the needle is incompletely retracted and redirected into the tissue. Its major
254 advantages are to reduce blood contamination and to preserve cellular integrity.
255
256 For oral cavity neoplasm FNA showed an almost perfect agreement in dogs (k=0.82)
257 and a perfect agreement in cats (k=1.00), whereas with IS the agreement remained almost
258 perfect in dogs (k=0.82) and decreased to substantial in cats (k=0.77).
259
260 In a previous study from our group on tumors located in the gastrointestinal tract, IS
261 had a better diagnostic agreement compared to FNA, with histopathological diagnosis as the
262 gold standard, in both species (Bonfanti et al., 2006). The difference is likely due to the type
263 of tumors investigated, with those pertaining to the gastrointestinal tract often being of
264 different origin from those of the oral cavity. In particular, in the gastrointestinal tract, many
265 of the neoplastic lesions evaluated were round cell tumors (i.e., lymphoma) (Bonfanti et al.,
266 2006). Lymphoma can be readily diagnosed by IS, and more easily than using either FNA or
267 FNI methods which often yield a high proportion of naked nuclei. A second explanation could
268 be that oral neoplastic lesions are easily reached using needle biopsy techniques, therefore
269 allowing a higher percentage of retrieval success, as compared to gastrointestinal lesions that,
270 owing to their localization, can be better investigated after collection of biopsy samples
271 followed by IS. In the present study, FNI for oral tumors showed the highest agreement

272 compared with histological examination in dogs (k=0.85) and was equal to FNA in cats
273 (k=1.00).
274
275 In particular, FNI showed a slightly higher agreement for all lesions in dogs and for
276 oral canine melanoma. Even if values for FNA and FNI can be considered rather comparable,
277 the explanation for the slightly higher agreement documented with the latter might be due to
278 the intrinsic nature of the technique. By inserting the needle without aspiration, cells may be
279 collected limiting their damage and better preserving cytological features that are necessary
280 for the diagnosis. However, this would not explain the similar agreements obtained with FNA
281 and FNI in cats. Whether aspiration of lesions in cats was less aggressively performed than in
282 dogs, or if feline oral lesions are more resistant to aspiration than those of dogs, obtaining
283 therefore a higher percentage of intact cells, cannot be answered. Alternatively, although
284 speculative, the use of different Gauge needles might have played a role on cell retrieval.
285 Further studies are therefore required to explain and confirm this finding.
286
287 According to the literature, there are yet no studies that have evaluated sensitivity,
288 specificity, PPV and NPV of cytological examination of oral cavity lesions compared with
289 histopathology in dogs and cats.
290
291 In humans, the sensitivity of FNA cytology in the identification of oral and
292 maxillofacial lesions ranges from 75% to 96%, and specificity and PPV approximate 100%
293 (Cramer et al., 1995; Singh et al., 2011). The results of our investigation demonstrated similar
294 sensitivity, specificity and PPV for FNA in dogs and cats (from 75% to 100%). However,
295 among the three methods used for diagnosing oral tumors in dogs, FNI showed the highest
296 sensitivity and specificity (98% and 100%, respectively), and for non-neoplastic lesions FNI

297 performed equal to FNA, with very high specificity (100%) and moderate sensitivity (75%).
298 The IS method performed less than FNI and FNA in dog oral tumors, yielding a low NPV
299 (50%) which was also reflected in a low sensitivity in the diagnosis of non-neoplastic lesions.
300 Accuracy of the three methods was very high for the identification of neoplastic and non-
301 neoplastic lesions, with slightly lower levels recorded for IS (91.8%).
302
303 Therefore, in dogs FNI and FNA may be superior to IS but the results, overall, suggest
304 that all the three methods are useful to achieve a diagnosis of neoplastic and non-neoplastic
305 oral lesions in this species. In cats, for both neoplastic and non-neoplastic lesions, sensitivity,
306 specificity and accuracy were all very high, and PPV and NPV were high, suggesting optimal
307 performance of each of the three methods.
308
309 Of note, despite the elevated performance of cytological examinations, those methods
310 did not replace histology. Indeed 14 FNA, 13 FNI and 1 IS samples, were excluded since were
311 classified as unsatisfactory due to hypocellularity, hemodilution, or necrosis. Furthermore,
312 another 16 of the 114 cases were excluded from the analysis because cytological results were
313 unsatisfactory with every method. Regarding these latter cases it is worth mentioning that 7
314 (43.7%) were diagnosed as peripheral odontogenic fibroma, suggesting this particular tumor
315 may not be suited for cytological examination. The stromal and firm tissue that characterizes
316 the peripheral odontogenic fibroma - as well as fibroma and gingival fibroepithelial
317 hyperplasia, another two causes of unsatisfactory cytological results - may prevent adequate
318 sampling and make histopathology the only reliable tool for achieving a correct diagnosis.
319
320 Additionally, although less common, in the present series a few cases showed two
321 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.

Acknowledgements

Preliminary results were presented as an Abstract form at the 13th Annual Meeting of the European Society of Veterinary Clinical Pathology and the European College of

Veterinary Clinical Pathology, Trinity College Dublin, Dublin, Ireland, 31st August – 3rd September 2011.

References

Allen, S.W., Prasse, K.W., Mahaffey, E.A., 1986. Cytologic differentiation of benign from malignant canine mammary tumors. Veterinary Pathology 23, 649-655.

Bertazzolo, W., Dell'Orco, M., Bonfanti, U., Ghisleni, G.., Caniatti, M., Masserdotti, C., Antoniazzi, E., Crippa, L., Roccabianca, P., 2005. Canine angiosarcoma: cytologic, histologic, and immunohistochemical correlations. Veterinary Clinical Pathology 34, 28-34.

Bonfanti, U., Bussadori, C., Zatelli, A., De Lorenzi, D., Masserdotti, C., Bertazzolo W., Faverzani, S., Ghisleni, G. Capobianco, R., Caniatti, M., 2004. Percutaneous fine-needle biopsy of deep thoracic and abdominal masses in dogs and cats. Journal of Small Animal Practice 45, 191-198.

Bonfanti, U., Bertazzolo, W., Bottero, E., De Lorenzi, D., Marconato L., Masserdotti, C., Zatelli, A., Zini, E., 2006. Diagnostic value of cytologic examination of gastrointestinal tract tumors in dogs and cats: 83 cases (2001-2004). Journal of the American Veterinary Medical Association 229, 1130-1133.

Bradley, R.L., MacEwen, E.G., Loar, A.S., 1984. Mandibular resection for removal of oral tumors in 30 dogs and 6 cats. Journal of the American Veterinary Medical Association 184, 460-463.

Chalita, M.C., Matera, J.M., Alves, M.T., Longatto-Filho, A., 2001. Nonaspiration fine needle cytology and its histologic correlation in canine skin and soft tissue tumors. Analytical and Quantitative Cytology and Histology 23:395-399.

Cramer, H., Lampe, H., Downing, P., 1995. Intraoral and transoral fine needle aspiration. A review of 25 cases. Acta Cytol 39, 683-688.

Dallera, P., Bertoni, F., Marchetti, C., Bacchini, P., 1982. Atypical melanocytic hyperplasia concomitant with malignant desmoplastic melanoma of the oral cavity. Minerva Stomatologica 31,153-164.

Ghisleni, G., Roccabianca, P., Ceruti, R., Stefanello, D., Bertazzolo W., Bonfanti, U., Caniatti, M., 2006. Correlation between fine-needle aspiration cytology and histopathology in the evaluation of cutaneous and subcutaneous masses from dogs and cats. Veterinary Clinical Pathology 35, 24-30.

Goldschmidt, M.H., Hendrick, M.J., 2002. Tumors of the skin and soft tissues. In: Tumors in Domestic Animals, Fourth Ed. Blackwell Publishing, Ames, IA, USA, pp. 45-83.

Head, K.W., Cullen, J.M., Dubielzig, R.R., Else, R.W., Misdorp, W., Patnaik, A.K., Tateyama, S., van der Gaag, I., 2003a. Histological Classification of Tumors of the Upper Alimentary Tract of Domestic Animals. In: Histological Classification of Tumors of the Alimentary System of Domestic Animals, Second Series, Volume X, Armed Forces of Pathology, Washington, DC, USA, pp.27-45.

Head, K.W., Cullen, J.M., Dubielzig, R.R., Else, R.W., Misdorp, W., Patnaik, A.K., Tateyama, S., van der Gaag, I., 2003b. Histological Classification of Tumors of Odontogenic Origin of Domestic Animals. In: Histological Classification of Tumors of the Alimentary System of Domestic Animals, Second Series, Volume X, Armed Forces of Pathology, Washington, DC, USA, pp.46-57.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159–174.

Lim, Y.C., Kim, W.S., Choi, E.C., 2008. Collision metastasis of squamous carcinoma of the oral tongue and incidental thyroid papillary carcinoma to a single cervical lymph node. International Journal of Oral Maxillofacial Surgery 37, 494-496.

Liptak, J.M., Withrow, S.J., 2006. Oral Tumors. In: Small Animal Clinical Oncology, Fourth Ed. WB Saunders, Philadelphia, PA, USA, pp. 455-475.

Ménard, M., Fontaine, M., Morin, M. 1986. Fine Needle Aspiration Biopsy of Malignant Tumors in Dogs and Cats: A Report of 102 Cases. Canadian Veterinary Journal 27, 504-510.

Ovejero Braun, A., Hauser, B., 2007. Correlation between cytopathology and histopathology of the skin, lymph node and spleen in 500 dogs and cats. Schweizer Archiv für Tierheilkunde 149, 249-257.

Pérez-Martínez, C., García Fernández, R.A., Reyes Avila, L.E., Pérez-Pérez, V., Gonzalez, N., Garcìa-Iglesias, M.J., 2000. Malignant fibrous histiocytoma (giant cell type) associated with a malignant mixed tumor in the salivary gland of a dog. Veterinary Pathology 37, 350-353.

Rae, C.A., Jacobs, R.M., Couto, C.G., 1989. A comparison between the cytological and histological characteristics in thirteen canine and feline thymomas. Canadian Veterinary Journal 30, 497-500.

Reinhardt, S., Stockhaus, C., Teske, E., Rudolph, R., Brunnberg, L., 2005. Assessment of cytological criteria for diagnosing osteosarcoma in dogs. Journal of Small Animal Practice 46, 65–70.

Ryu, D.M., Kwon, Y.D., Lee, B.S., Kim, Y.G., 2000. Concomitant occurrence of squamous cell carcinoma and myxoma of the mandible: a case report. International Journal of Oral Maxillofacial Surgery 58, 425-430.

Simeonov, R., Stoikov, D., 2006. Study on the correlation between the cytological and histological tests in the diagnostics of canine spontaneous mammary neoplasms. Bulgarian Journal of Veterinary Internal Medicine 9, 211−219.

443 Simon, D., Schoenrock. D., Nolte, I., Baumgärtner, W., Barron, R., Mischke, R., 2009. Cytologic
444 examination of fine-needle aspirates from mammary gland tumors in the dog:
445 diagnostic accuracy with comparison to histopathology and association with
446 postoperative outcome. Veterinary Clinical Pathology 38, 521-528.
447
448 Singh, S., Garg, N., Gupta, S., Marvah, N., Kalra, R., Singh, V., Sen, R., 2011. Fine needle
449 aspiration cytology in lesions of oral and maxillofacial region: Diagnostic pitfalls.
450 Journal of Cytology 28, 93-97.
451
452 Sitzman, C., 2000. Simultaneous hyperplasia, metaplasia, and neoplasia in an 8 year-old boxer
453 dog: a case report. Journal of Veterinary Dentistry 17, 27-30.
454
455 Smith, S.H., Goldschmidt, M.H., McManus, P.M., 2002. A comparative review of melanocytic
456 neoplasms. Veterinary Pathology 39, 665-678.
457
458 Sontas, B.H., Yüzbaşıoğlu Öztürk, G., Toydemir, T.F., Arun, S.S., Ekici, H., 2012. Fine-needle
459 aspiration biopsy of canine mammary gland tumours: a comparison between cytology
460 and histopathology. Reproduction in Domestic Animals 47, 125-130.
461
462 Spodnick, G.J., Page, R.L., 1995. Canine and feline oropharyngeal neoplasms. In: Current
463 Veterinary Therapy, Eighth Ed. WB Saunders, Philadelphia, PA, USA, pp. 691-695.
464
465 Watrach, A.M., Small, E., Case, M.T., 1970. Canine papilloma: progression of oral papilloma to
466 carcinoma. Journal of the National Cancer Institute 45, 915-920.
467

468 **Table 1**

469 Localization of oral masses in 114 dogs and cats.

470

LOCATION	Dogs n (%)	Cats n (%)	TOTAL n (%)
Jaw	39 (45.9)	8 (27.6)	47 (41.2)
Mandible	29 (34.1)	12 (41.4)	41 (36.0)
Hard palate	5 (5.9)	0 (0.0)	5 (4.4)
Buccal mucosa	4 (4.7)	3 (0.0)	7 (6.1)
Sublingual mucosa	2 (2.4)	2 (6.9)	4 (3.5)
Tongue	1 (1.2)	1 (3.4)	2 (1.8)
Tongue-Pharynx	1 (1.2)	1 (3.4)	2 (1.8)
Tongue-Tonsil	0 (0.0)	2 (6.9)	2 (1.8)
Others[2]	4 (4.7)	0 (0.0)	4 (19.3)
Multiple	1 (1.2)	3 (10.3)	4 (3.5)
TOTAL	85 (100.0)	29 (100.0)	114 (100.0)

471

[2] Others: Gingival fornix, Multiple mucosal masses, Soft palate, Lip-Hard palate

Table 2

Value of *k*-agreement of cytological methods for all oral lesions and tumors in each species, for canine melanoma and for feline SCC.

	Dogs			**Cats**		
	FNA	FNI	IS	FNA	FNI	IS
	k (CI 95%)	*k* (CI 95%)	*k* (CI 95%)	*k* (CI 95%)	*k* (CI 95%)	*k* (CI 95%)
All lesions	0.83 (0.77-0.90)	0.87 (0.81-0.93)	0.75 (0.67-0.84)	0.92 (0.87-0.96)	0.92 (0.88-0.97)	0.86 (0.79-0.92)
Tumors	0.82 (0.75-0.88)	0.85 (0.79-0.91)	0.82 (0.75-0.89)	1.00 (0.87-0.96)	1.00 (0.88-0.97)	0.77 (0.74-0.80)
Melanoma	0.86 (0.81-0.91)	0.87 (0.83-0.92)	0.77 (0.70-0.83)			
SCC				0.92 (0.87-0.96)	0.92 (0.88-0.97)	0.86 (0.79-0.92)

SCC, squamous cell carcinoma; CI, confidence interval.

Table 3

Sensitivity, specificity, PPV, NPV and accuracy of cytology for diagnosing neoplasia, non-neoplastic lesions and melanoma in dogs using FNA, FNI, IS.

Parameter	**Dogs**								
	Neoplasia			Non-neoplastic lesions			Melanoma		
	FNA	**FNI**	**IS**	**FNA**	**FNI**	**IS**	**FNA**	**FNI**	**IS**
Sensitivity	100.0%	98.0%	91.1%	75.0%	75.0%	50.0%	85.0%	80.0%	88.9%
Specificity	75.0%	100.0%	100.0%	100.0%	100.0%	100.0%	97.0%	100.0%	100.0%
PPV	98.1%	100.0%	100.0%	100.0%	100.0%	100.0%	94.4%	100.0%	100.0%
NPV	100.0%	75.0%	50.0%	98.1%	98.0%	91.1%	91.4%	89.2%	94.6%
Accuracy	98.2%	98.1%	91.8%	98.2%	98.1%	91.8%	92.4%	92.4%	96.2%

PPV, positive predictive value; NPV, negative predictive value; FNA, fine-needle aspiration; FNI, fine-needle insertion; IS, impression smear.

Table 4

Sensitivity, specificity, PPV, NPV and accuracy of cytology for diagnosing neoplasia, non-neoplastic lesions and SCC in cats using FNA, FNI, IS.

Parameter	Cats								
	Neoplasia			Non-neoplastic lesions			SCC		
	FNA	**FNI**	**IS**	**FNA**	**FNI**	**IS**	**FNA**	**FNI**	**IS**
Sensitivity	94.7%	94.4%	94.1%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Specificity	100.0%	100.0%	100.0%	94.7%	94.4%	94.1%	88.9%	90.0%	83.3%
PPV	100.0%	100.0%	100.0%	80.0%	83.3%	87.5%	93.3%	92.9%	85.7%
NPV	80.0%	83.3%	87.5%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Accuracy	95.6%	95.6%	95.8%	95.6%	95.6%	95.8%	95.6%	95.6%	91.7%

PPV, positive predictive value; NPV, negative predictive value; SCC, squamous cell carcinoma; FNA, fine-needle aspiration; FNI, fine-needle insertion; IS, impression smear.

Figure 1

Cytology - Squamous cell carcinoma. Cat.

Epithelial cells in small cluster. The cells display moderate to marked anisocytosis and mild anisokaryosis, with a variable amount of keratinized cytoplasm and large atypical nuclei (nuclear-cytoplasmic asynchrony). Non degenerated neutrophils and red blood cells are present among the neoplastic elements (FNA; May-Grünwald-Giemsa; 400X).

Figure 2

Histology – Squamous cell carcinoma. Cat.

Cohesive groups of polygonal, large (10-30 microns in largest diameter) neoplastic squamous epithelial cells with indistinct cell borders and intensely eosinophilic cytoplasm and round to oval to irregular often vesicular 10-20 micron nuclei with one to two blue small nucleoli. Anisocytosis and anisokaryosis are prominent. Presence of keratinized groups of cells (horn pearls) and single intensely eosinophilic keratinized/dyskeratotic cells. (Haematoxylin and Eosin; 400X).

Figure 3

Cytology - Melanoma. Dog.

Pleomorphic oval to spindle shaped cells, single or clustered in an aggregate. The cells contain dark green to black melanin granules, and variably sized atypical nuclei. Free melanin granules are present in the background of the smear. (FNI; May-Grünwald-Giemsa; 600X).

Figure 4

Histology - Epithelioid melanoma. Dog.

Densely cellular neoplasm characterized by sheets of round to polygonal cells with indistinct cell borders, intermediate nuclear/cytoplasmic ratio, abundant lightly basophilic granular cytoplasm containing in approximately 50% of cells rare brown black irregularly sized granular pigment

(melanin). Nuclei are round to oval, 8-14 micron in diameter, vesicular with one to three small round basophilic nucleoli. Occasional mitotic figures and melanomachrophages are present. (Haematoxylin and Eosin; 400X).

Figure 5

Cytology - Lymphoma. Cat.

Prevalence of large immature round lymphoid cells. Blastic cells have a high nuclear-to-cytoplasmic ratio with clear cytoplasm and large nuclei with prominent nucleoli. Few small mature lymphoid cells are detected. Naked nuclei and basophilic fragments of cytoplasm (lymphoglandular bodies) are scattered among the cells. (FNI; May-Grünwald-Giemsa; 1000X).

Figure 6

Histology - Lymphoma. Cat.

Neoplasm composed of sheets of round cells with variably distinct call margins, intermediate to high nuclear to cytoplasmic ratio and variable size ranging from 10 to 25 micron in diameter. Cells have moderate amount of granular cytoplasm and a round to oval paracentral nucleus with finely clumped chromatin and one central round blue nucleolus. Occasional mitoses are present. Anisocytosis and anisokaryosis are prominent. (Haematoxylin and Eosin; 400X).

Figure 7

Cytology - Mixed inflammation with lymphoplasmacellular component. Cat.

Mixed cell population represented mainly by small lymphocytes and plasma cells, as well as non degenerated neutrophils and few macrophages / histiocytes. (IS; May-Grünwald-Giemsa; 600X).

Figure 8

Histology – Moderate, diffuse chronic mixed lymphoplasmacellular inflammation with neutrophils.

Cat.

The superficial mucosa is hyperplastic and characterized by spongiosis and infiltration by non degenerated neutrophils. The lamina propria is diffusely infiltrated by a prevalence of mature plasma cells, lesser numbers of non degenerated neutrophils and occasional small mature lymphocytes. Hyperemia is also present. (Haematoxylin and Eosin; 400X).