EPIDEMIOLOGIC STUDY AND MORPHOLOGIC DIAGNOSIS ON LESIONS IDENTIFIED IN PSITTACINES

Iulia Paraschiv, Manuella Militaru, Laurenţiu Tudor

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, iuliaaparaschiv@yahoo.ro

Abstract

Tumoral lesions in psittacines are, nowadays, clinically diagnosed with increasing frequency. This study is aimed to evaluate clinic and epidemiologic characteristics together with the efficiency of the cytologic, histopathologic and necropsic diagnosis on lesions in parrots. A total of 23 cases were examined at the Department of Pathological Anatomy of Faculty of Veterinary Medicine, Bucharest during September 2011 - October 2012.
19 standard budgerigars (Melopsittacus undulatus), two cockatiels (Nymphicus hollandicus), one lovebird (Peach-Faced Agapornis) and one cockatoo (Cacatua sulphurea) were clinically examined. Sex of the birds was not significant in the tumor incidence. Regarding age, 17 cases were 1-5 years old and only 6 over 5. Regarding topography, 7 cases presented lesions in the pectoral area, 6 cases in the abdominal area and, same number, in the wing region and one case each for the uropygial region, legs, eye, cere and beak.
Microscopically, most of the cases were diagnosed as tumors and only one as inflamatory process. The majority were classified as malignant proliferations, from which five had mesenchymal origin (four fibrosarcomas and one hystiocitic cell sarcoma) and one, epithelial origin (a basal-cell carcinoma). The benign lesions had a mesenchymal origin (one hemangyoma and two lypomas). Malignant cases had a poor survival rate, under three weeks for mesenchymal neoplasms and one week for the epithelial one.
All in all, this study revealed that most cases of lesions in parrots were 1-4 year old, located either on trunk or wing and the majority confirmed a malignant proliferation.

Key words: *parrot, tumor, epidemiology, morphologic diagnosis*

INTRODUCTION

Psittacines, like all species, suffer from a range of cutaneous, subcutaneous and internal neoplasms. Approach to these should be similar to that used in other species. Fine-needle aspiration or biopsy is indicated prior to removal.

Species, site and age may be predictive in relation to tumor type (Samour, 2008).
Classification of tumors include pseudoneoplasies, benign lesions and malignant tumors, either solid or systemic, with epithelial or mesenchymal origin. Xanthomas are non-neoplastic, proliferative lesions, seen most commonly in budgerigars and cockatiels as a variable-sized, yellow mass. Benign lesions are most comonly diagnosed as lipomas, usually located on the sterno-pubic area, or hemagiomas, situated internal or external (Harrison 2007, Lightfoot, 2007 and 2009). Fibrosarcomas are neoplasies, most comonly seen in the oral cavity, associated with long bones, or in the abdominal cavity (Harrison and Lightfoot, 2007, Palmieri C et al., 2011). Squamous cell carcinoma is another neoplastic lesion, located anywhere on the body, ocasionally cited at the uropigyal gland (Paterson, 2006). Internal carcinomas are generally diagnosed at necropsy and include ovarian, renal, hepato-biliary and pancreatic neoplasies. Numerous reports of exophthalmos in psittacines, particularly young African greys, have been diagnosed as retrobulbar lymphoma.(Harrison and Lightfoot, 2007 and Samour, 2008)

MATERIALS AND METHODS

The present study involved a number of 23 psittacine cases, examined at the Department of Pathological Anatomy from Faculty of Veterinary Medicine, Bucharest, during september 2011-october 2012. They were submitted to a macroscopic examination, together with a general state evaluation. Clinical anamnesis was carefully carried to offer information about the age and sex of the bird, the time the owner observed the lesion, the growth rhythm and the eventual, previous feather or soft tissue trauma on the site of the lesion.
Citologic examination for the live cases was carried through fine-niddle aspiration technique, using 22G and 23G gauge needles. Of considerable importance is the physical contention of the parrot. In the case of the budgerigar (*Mellopsitacus undulatus),* this procedure means catching the bird and restraining the neck between index and middle finger and the feet supported by the thumb and the ring finger. This method ensures movement restriction, calming of the bird, and most important, proper breathing. As for the middle and large psittacine species (*Agapornis spp., Nymphicus hollandicus, Cacatua sulphurea*), a towel is used for restraint. For surgical excisions, the techniques used in citologic examination included scrape and imprint technique. The coloration method used was May-Grümwald-Giemsa and only for a limited number of cases, MGG-Quick.

Necropsies were performed in the Department's Necropsy Laboratory.
Histopathologic examinations were performed on lesions and organ samples obtained during necropsies. They were fixed in 10% neutral buffered formaldehid and tricloracetic acid, if they included bone structures. In the end, the samples were embedded in paraffin, sectioned at 4-6 microns and stained with Hematoxilin-Eosin (H&E).
The microscopic examination of citologic and histopathologic samples was carried at a Carl Zeiss Axio Imager A1 microscope with photographic system integrated.

RESULTS AND DISCUSIONS

A total of 23 parrots were examined, out of which 19 cases of budgerigars (*Melopsittacus undulatus*), two cockatiels (*Nymphicus hollandicus*) and one lovebird (*Agapornis roseicollis*) and one cacadu (*Cacatua sulphurea*).
Sex prevalence was not revealed, both were almost equally affected, 56,5% females and 43,5% males. As for the cases belonging to species without sexual dimorphism, as *Agapornis roseicollis* and *Cacatua sulphurea*, it was taken into consideration the existance of a mate, egg laying and behaviour.
As for the age of the birds, some important aspects were highlighted. A percentage of 73.9 % were aged between 1 and 5 years old, only 26.1% over 5 and none after the age of 8. (Table.1)

Table.1 Association between age and number of cases

Age of the parrot (years)	Number of cases
<1 year	1
1-2	2
2-3	3
3-4	7
4-5	4
5-6	1
6-7	4
7-8	1

Similar results were observed in 1983 by Neumann and Kummerfeld in a study regarding 74 cases of internal neoplasms in budgerigars. The highest incidence was registered between age 3 and 5 years old.
Regarding age of the parrots, lifespan is of great importance. *Melopsittacus undulatus* is considered to have a lifespan of 8-10 years (Jepson, 2009), *Nymphicus hollandicus* and *Genus Agapornis*, generally, live up to 15 years,

sometimes longer (Jepson, 2009). *Cacatua sulphurea* is the species with the highest longevity from the cases examined, up to 40 -50 years, with sexual maturity reached between 3 and 4 years of age. (Jepson, 2009) The case included in the study was two and half years old, which means the tumoral lesion developed at a young age, similar to ages before one year of small parrot species.

Regarding topography of the lesions, Table 2 reveals the anatomical regions of tumoral lesions identified in this study.

Table 2. Anatomic location frequency of lesions identified in psittacines

Topography of the lesion	Number of cases	Percentage
Pectoral region	7	30.6%
Coelomic region	6	26.1%
Wing region	6	26.1%
Uropigyal gland region	1	4.3%
Foot region	1	4.3%
Ceroma and beak region	1	4.3%
Eye region	1	4.3%

Survival rate proved, in most cases, to be correlated with the location of the lesion, meaning that lesions located at anatomical junctions, such as the carpal joint, tarso-metatarsal joint or maxilo-mandibular joint, are predisposed to rapid growth. These regions are intensely used, accesible to self mutilation, prone to septic or haemorhagic complications and, usually they were the site of repeatedly trauma, preliminary to the macroscopic lesion. On the other hand, pectoral or coelomic locations affect less the general state of the animal, due to the fact they infiltrate the pectoral muscle or the cavity with lateral deviation of the digestive organs, without self mutilation or septic complications. 7 cases with lesions located at jonctional site presented a survival rate under 30 days from the moment of diagnosis and only 2 cases overcame one year of survival. The cases with lesions on the torso showed a survival rate between 6 months and 3 years.

Another important epidemiologic aspect revealed in the study was the seasonal dynamics of the lesions. As a result, 10 cases (43.5%) were identified in spring, followed by 6 cases (26.1%) in automn, 5 cases (21.7%) in summer and in winter only 2 cases (8.7%). A high incidence in spring and automn is favored by thermal changes, photoperiod, atmospheric pressure changes, all of them reflected in metabolic and hormonal stress, to which parrots are vulnerable.

The cases submitted to anatomopathologic examination were represented by seven cases of *Melopsittacus undulatus* and one case, each, for *Nymphicus hollandicus, Agapornis roseicollis* and *Cacatua sulphurea*.
Microscopic examination revealed only one case of nontumoral lesion and the rest, tumoral lesions of different degrees and origins.
The nontumoral case was a *Nymphicus hollandicus* female with a lesion located at the uropigyal gland region. The area revealed a significant inflamation, with a diameter of aproximately 2 cm, coloured dark red, firm at palpation. The scientific data reveal cases of adenocarcinoma or squamous cell carcinoma, that evolve with swelling also and, as a result may be difficult cu differentiate from solely gland inflamation (Reavill D. R., 2004 and Samour J., 2008). In order to differentiate the two entities in the case studied, citologic examination was performed and, afterwards, coloration by May-Grümwald-Giemsa technique. Microscopic examination revealed the presence of a significant number of inflamatory cells, blood cells and cellular detritus. None atipic cells were identified, no atipic mitosis and nucleus-citoplasm ratio in normal limits. As a result, the citologic diagnosis was of cronic inflamation together with secretion impactation of the uropigyal gland. To support the diagnosis, at 10 days after the mechanical removal of the secretion, the inflamatory area retracted and the gland resumed normal function.
Regardind the tumoral lesions, 3 cases were identified as benign, all with mesenchymal origin. Pectoral lipomas were identified in two cases of Melopsittacus undulatus. Anamnesis recorded favoring factors as inappropriate feeding and limited or no daily physical training. Fine needle aspiration was performed at the edge of the pectoral deformation and a greasy material, hard to dry was noticed on the microscope slide. May-Grümwald-Giemsa was the stain used and citologic interpretation revealed a rare, uniform cellular population of adypocites, without inflamatory reaction. The cells appear as contour coloured vacuoles with a peripheric, small nucleus, pushed by the lipidic drop. Clinically, the size of these lesions remained constant or in little regression during 6 months of study, although the dietary and exercise deficiencies were corrected.
The third case of benign tumor was diagnosed at a specimen of *Cacatua sulphurea*. Anamnesis revealed two, parallel, round formations , with a diameter of 1 cm , located prepectorally, depicted by the owner in march 2011. In may, surgical excision of the left formation was performed with a rapid post-sugery recovery. During summer season, the right formation

presented daily size variations of up to 1-1.5 cm, enlarging at midday and regressing during night, sign of important vascular envolvement at this level. In september, a second surgery was performed for the right formation. Macroscopically, the tumor had a 2.5 cm diameter, well delimited from adjacent tissues due to a fibrous capsule and intense red colour. At sectioning, the formation expressed a quantity of 2 ml of intensely red liquid. Citology of this sample revealed a large cellular population composed of erythrocytes and groups of blood platelets which are of great significance in blood clotting. Among normal cellular forms, few bizzare erythrocytes were identified, such as schystocytes and acantocytes. These appear in local hemodynamic changes due to endothelial distruction. Another category, diagnosed as endothelial cells, were identified in the microscopic evaluation. These cells were characterized by poligonal shape, weak contour, ovalar nucleus, in groups of 2 or 3 cells, well differentiated without abnormal mitotic activity. After surgery, healing was conducted per primam, none relapse observed. The final diagnosis was of hemangyoma, based on anamnetic data, citologic examination and post-surgery recovery.

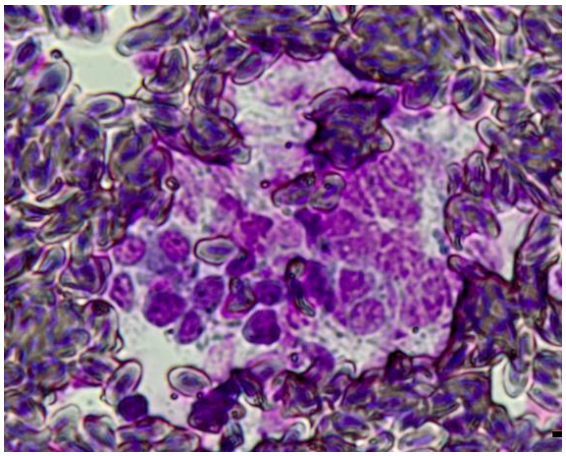
Of the 6 malignant lesions, 5 had mesenchymal origin and only one epithelial origin.

The epithelial neoplasm was diagnosed at a female of *Melopsittacus undulatus*, located on the left wing at the carpal joint. Initially, the lesion started as a plumage follicle inflamation and local irritation, evolved into a nodule with aproximately 5 mm diameter and, afterwards tripled in size in aproximately 2 weeks time. The ulceration area, presented a round shape, without any cicatrization tendancy. A citologic sample was obtained by fine-needle aspiration technique and stained M.G.G-Quick. The microscopic evaluation revealed cellular groups, scattered all around the sample, characterized by a very pale citoplasm, a high nucleus-citoplasm ratio, relative anisocariosis and nuclear hiperchromasia. The citologic aspects conducted to a suspicion diagnosis of basal cell carcinoma. Self mutilation lead to intense haemorrhage and death of the bird only a week after diagnosis.

Of mesenchymal origin neoplasms, one case presented unusual location of the lesion, the muscular region of the right leg of a budgerigar. The formation deformed roundly the whole region, was firm at palpation, dark red in colour with self mutilation crusts. Citologic examination of cellular aspirate revealed an important population of erythrocytes together with groups of large, ovoido-spherical cells, with large nucleus, multiple mitosis and cellular anisocytosis, as seen in Figure 1. The diagnosis for the case was

of histiocytic sarcoma.

Figure 1. *Melopsittacus undulatus*. Histiocytic sarcoma. Group of modified histyocites surrounded by a large population of erythrocytes (M.G.G. stain, 40x)

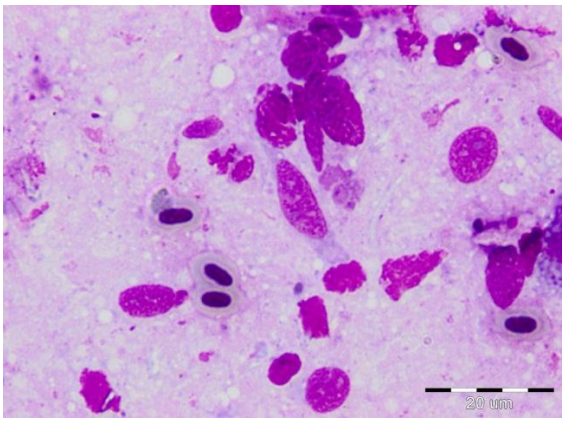


The survival rate, in this case, was of 1 month after examination.
The other 4 mesenchymal neoplasms examined in this study were represented by fibrosarcomas. Three cases were reported in *Melopsittacus undulatus,* out of which two in the wing region and one case in the caelomic cavity. One case, was diagnosed at a specimen of *Agapornis roseicollis,* located at the carpal joint. The lesions identified at the budgerigars were submitted to citologic, necropsic and histopathologic examination and for the Agapornis parrot a citologic examination was performed. The citologic examination revealed, in all four cases, a population of large, basophilic, spindle cells with large, round nuclei and obvious nucleoli. The mitotic activity was present in different degrees, same as the presence of bizzare cellular forms. The necropsies for the two cases of wing lesions revealed an obvious deformation of both muscular and bone structures at the site of the neoplasm as seen in Figure 2.

Figure 2. *Melopsittacus undulatus*. Macroscopic aspects of a fibrosarcoma located on the left wing

The emaciation of the pectoral muscles observed in these cases, together with the presence of seed content in the digestive tract was attributed to paraneoplasic syndrome of protein loss. Histopathologic examination of the tumor samples confirmed the diagnosis of fibrosarcoma, bizzare fibroblastic cells displayed in the internal derm layer, with a typical herringbone pattern. The examination of organ samples did not reveal any metastasis in those cases. The case of internal fibrosarcoma was submitted to necropsy after sudden death, in order to perform a differential diagnosis between egg binding, colibacilary granuloma and a neoplastic formation. After caelomic cavity opening, a spheroidal formation was depicted between the intestinal loops, logged to the mesentery, compressing regional organs. The tumor had a 2 cm diameter, external yellow colour and, after sectioning the internal structure, presented a compact aspect and whitish-grey colour. The citologic examination was performed as impression smears and the microscopic characteristics revealed atypical forms of fibroblastic cells, multiple mitosis, anysocytosis and anysocariosis.(Figure 3)

Figure 3. *Melopsittacus undulatus*. Abdominal fibrosarcoma. Pleomorphic spindle cells, rare erythrocytes (M.G.G. stain, 100x)



The cases of fibrosarcoma diagnosed at budgerigars had a survival rate of up to 2 months, while the lesion identified at the Agapornis presented a survival rate of over 6 months.

CONCLUSIONS

The most frequent location for tumoral lesions in common caged parrots are the ventral torso and the wing region.
Young age, up to 4 years old, involved most of the cases with tumors.
Seasonal change is envolved in tumoral development and clinical signs of disconfort.
Superficial neoplasies, located at anatomical junctions, are prone to bleeding or septic complications and a low survival rate.
Most cases diagnosed had mesenchymal origin out of which the most frequent type was the fibrosarcoma.

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