Original Research

Cytological and Histopathological Studies of Canine Round Cell Tumors

Kotrappa Yankappa Mathur [1], Suguna Rao[2], Girish Bekkare Chandrashekaraiah[3*] and Byregowda Sonnahallipura Munivenkatappa[4]

[1]Syngene International Ltd, Biocon Park, Jigani Link Road, Bommasandra, Bengaluru-560099, Karnataka, INDIA
[2]Department of Pathology, Veterinary College, Hebbal, Bengaluru-560024, Karnataka, INDIA
[3]Department of Pathology, Veterinary College, Gokula, Vidyanagara-Post, Hassan-573202, Karnataka, INDIA
[4]Institute of Animal Health and Veterinary Biologicals, Bengaluru-560024, Karnataka, INDIA

***Corresponding author:** girishbekkare@gmail.com

Rec. Date:	Sep 11, 2017 11:35
Accept Date:	Jan 18, 2018 02:57
DOI	10.5455/ijlr.20170911113547

Abstract

Cancer is the most important cause of death in canines. Round cell tumors are the most common type of tumors that develop in dogs and it involves cutaneous regions the most. Round cell tumors include mast cell tumors, histiocytomas, plasmacytomas, transmissable venereal tumors (TVTs) and cutaneous Lymphomas in dogs. In the current investigation involving 215 tumor and tumor like growths, a total of 35 (16.3%) round cell tumors were diagnosed. This included transmissible venereal tumor (# 23), mast cell tumor (# 10), and plasmacytoma (# 2). The TVTs cytologically showed round to oval shaped cells with anisokaryosis and punctate cytoplasmic vacuoles. The metachromatically stained granules in the cells of mast cell tumors were characteristic. Plasmacytomas showed eccentrically placed round to oval shaped nucleus with coarse chromatin and prominent nucleoli. The current paper describes the cytopathological features of these round cell neoplasms in canines.

Key words: Cancer, Cytology, Mast Cell Tumor, Round Cell Tumors, Transmissible Venereal Tumor

How to cite: Mathur, K., Rao, S., Chandrashekaraiah, G., & Munivenkatappa, B. (2018). Cytological and Histopathological Studies of Canine Round Cell Tumors. International Journal of Livestock Research, 8(6),88-95. doi: 10.5455/ijlr.20170911113547

Introduction

Cancer is the most recurrent and the leading cause of death in canines (Girish *et al.*, 2011; Gardener *et al.*, 2016; Pang and Argyle, 2016). Dogs being companion animals live to their maximum life expectancy similar to humans and develop variety of spontaneous neoplasms. In one of the studies, it is estimated that one in every three dogs develops cancer proving the enormity of the problem in small animal veterinary medicine. Dogs therefore, have been considered as an informative system for the pathobiological and

genetic investigation of human cancers (Girish *et al.,* 2011; Pang and Argyle, 2016). Data from canines as animal models in preclinical safety assessment for carcinogenic risk assessment has been found to be more superior to other conventional rodents such as rats or mice. Round cell tumors are considered to among the most common tumors that develop in dogs especially involving the skin. They are usually subcutaneous in location however can be found in any part of the body. Early diagnosis of these tumors is very essential as to increase the chances of better clinical response and favorable prognosis. Round cell tumors seen in dogs generally include mast cell tumors, histiocytomas, plasmacytomas, transmissible venereal tumors and cutaneous Lymphomas (Ramos-Vara *et al.,* 2011; Meuten, 2017). As per the observation made by Welle *et al.,* 2008, mast cell tumor alone accounts for approximately one fourth of all skin tumors in dogs. Histiocytomas are generally benign and are seen in head, neck and appendages. Plasmacsytomas/ Muliple myelomas seen in older dogs are generally malignant and TVTs (Transmissible Venereal Tumors) are sexually transmitted. Cutaneous lymphoma which appears as multiple growths is said to be common in middle to older aged dogs.

Cytologically, round cell tumors is characterized by cells that are round to oval in shape compared to other tumor types involving epithelial or mesenchymal origin. Cytological examination for the material collected from excision biopsy/fine needle aspirations or punch biopsy is a faster yet reliable method in diagnosis of these round cell neoplasms. The observations recorded systematically from cytological smear can present many architectural characteristics can be very useful and handy in addition to histopathology or immunohistochemical investigations.

Material and Methods

Tumor Sample Collection

The current study was carried out in the Department of Veterinary Pathology, Veterinary College, Bengaluru and Veterinary College, Hassan. 215 dogs presented to college hospitals and other nearby private clinics with the history of tumor growths and post mortems during the period 2005-2015 formed the source for the present investigation.

Details of Growth and Animals

Information of animals including breed, age, sex, color, clinical findings shown by the animals such as shape, size, color and location of the growths were recorded.

Cytological Smear Preparation and Examination

Different methods were used to collect tissue samples for cytological examination based on the nature of the growths. Fine needle aspiration cytology (FNAC) and fine needle aspiration biopsy (FNAB)

 DOI 10.5455/ijlr.20170911113547

were employed for superficial nodular growths. Impression smears and scraping smears were used for neoplasms with ulcers. For surgically excised growths, scraping method was used. The smears were stained using standard Giemsa, New Methylene blue, Toluidine blue and Papanicolaou standard staining techniques as outlined by Luna, 1968. Criteria for malignancy were adopted by the previous works (Villiers and Dunn, 1998; Alleman and Bain, 2000; Kotrappa *et al.,* 2014) objectively describing cellular, cytoplasmic and nuclear details.

Histopathological Examination

Tissue samples collected were immediately fixed in 10% neutral buffered formalin. The samples were processed by routine paraffin embedding technique and sectioned at five micron thickness. The sections were stained with routine hematoxylene and eosin (H & E) method. Special stains such as Van Gieson's (Connective tissue and Keratin), Masson's trichrome (Connective tissue and Keratin) and Toluidine blue (mast cell granules) were used on need basis.

Results and Discussion

In the current investigation involving 215 tumor and tumor like growths, a total of 35 (16.3%) round cell tumors were diagnosed. Among the various methods of smear preparations used, scraping smear yielded consistent results and a large number of cells for examination. Mast cell granules were well appreciated in New methylene blue, Toluidine blue and Giemsa stain. The round cell tumors observed included transmissible venereal tumor (23), mast cell tumor (10) and plasmacytoma (2).

Transmissible Venereal Tumor (TVT)

The age of the tumor bearing dogs ranged from 5 to 14 years with an average age of 9.42 years. Out of 23 cases, Seventeen cases were from females which included eleven nondescript and three Pomeranian dogs and one each in Labrador, German Shepherd and Doberman. In case of female dogs the tumors were found originating from vulva, vestibule and vagina. The remaining six cases were recorded in nondescript males. In four cases, the tumorous mass was located at the base of the penis and prepuce and in two instances, the diffuse and ulcerated growth was found in the inguinal region.

Grossly, the size of the tumor masses varied from one to six cm at their greatest diameters and was lobulated, friable and pedunculated growths. They were friable, cauliflower like, pinkish red, ulcerated growths with serosanguinous or haemorrhagic discharge and tendency to bleed on manipulation as has been observed previously (Jain *et al.,* 2002; Ganguly *et al.,* 2013; Milo and Snead, 2014). Cytologically, the smears revealed high cellularity. The cells were round to oval shaped and showed anisokaryosis(Fig.1). The nuclei of the cells contained coarsely aggregated chromatin with a prominent nucleolus which was well appreciated in Toluidine blue stained smears. Mitotic figures were numerous.

DOI 10.5455/ijlr.20170911113547

The cytoplasm was lightly bluish in Giemsa and Toluidine blue stained smears and contained distinct typical punctuate vacuoles (Fig. 2).Similar findings were described previously (Duncan and Prasse, 1979; Alleman and Bain, 2000; Milo and Snead, 2014). Further, it has been clearly indicated that the punctate cytoplasmic vacuoles were characteristics of TVT and lack of vacuoles may confuse TVT with other round cell tumors.

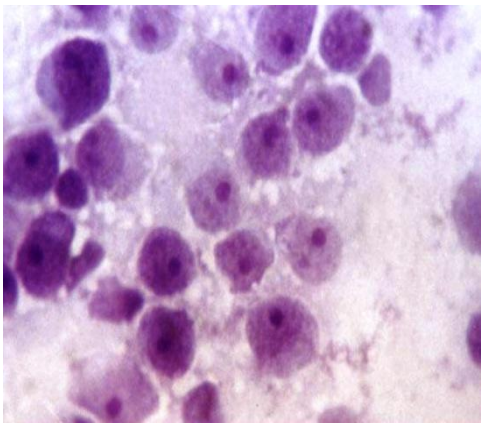

Fig.1: Cytological smear of TVT showing round to oval shaped cells with a prominent nucleolus. (Toluidine blue X125)

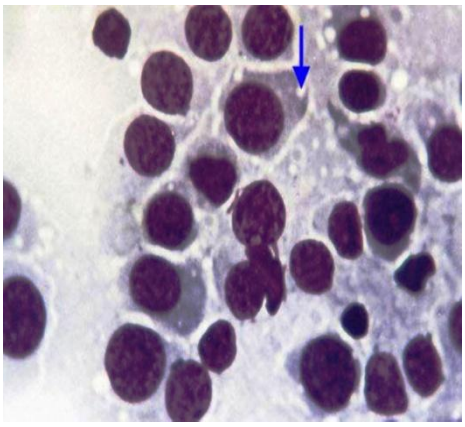

Fig. 2: Cytological smear of TVT showing high cellularity, anisokaryosis, nuclei with coarsely aggregated chromatin and basophilic cytoplasm containing punctate vacuoles shown by arrow. (Giemsa X125)

Histopathologically, the section from TVT revealed solid sheets of compactly arranged cells with minimal amount of fibrous stroma. The cells were round to oval shaped with vesicular nuclei and distinct nucleolus along with dispersed chromatin aggregates around. Numerous mitotic figures were observed. A few cases revealed focal areas of mucosal necrosis and infiltration of plasma cells, eosinophils and occasional histiocytes amidst the proliferating neoplastic cells (Fig. 3).Similar findings have been appreciated and documented (Jain *et al.*, 2002; Milo and Snead, 2014).

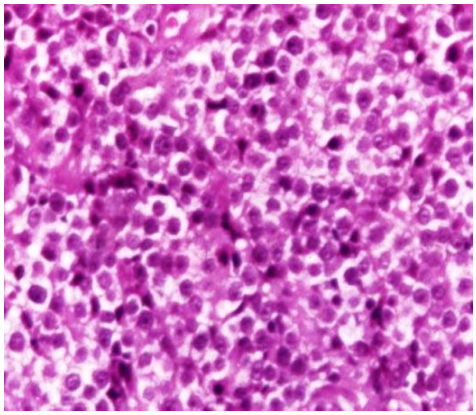

Fig. 3: Section of TVT showing compactly arranged round to oval shaped cells with vesicular nucleus and distinct nucleolus. (H&E X50)

DOI 10.5455/ijlr.20170911113547

Mast Cell Tumor

The age of the affected dogs varied from 6 to 10 years with an average of 7.8 years. Six cases occurred in males (three in Nondescript, two in Labrador and one in Golden retriever) and four in female (three in Mongrel and one in Pomeranian). Two affected dogs were nondescript The location of the mast cell tumors varied from ventral aspect of neck, lateral aspect of thigh, thoracic and abdominal regions. The mast cell tumors, which grossly appeared as firm raised, circumscribed, blackish brown coloured and ulcerated masses in the present study were similar to previous findings (Dean, 1988; Simoes *et al.*, 1994; Girish *et al.*, 2010).

The cytological findings such as high cellularity, pleomorphic cells with oval or round shaped nucleus consisting of chromatin aggregates, indistinct nucleolus and intracytoplasmic and extracellular pinkish colored granules (Fig. 4 and 5) observed in this study have also been described earlier (Duncan and Prasse, 1979; Tvedten, 1994; Alleman and Bain, 2000; Girish *et al.*, 2010).

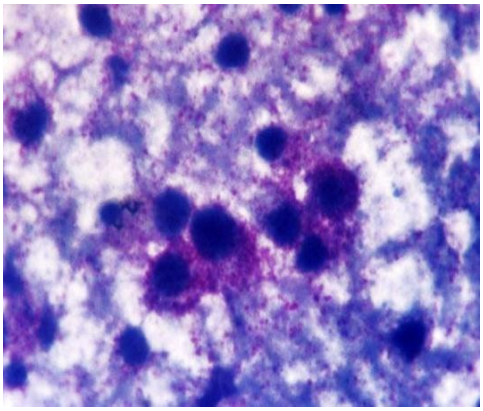

Fig. 4: Cytological smear of mast cell tumour showing pleomorphic cells with pinkish coloured distinct cytoplasmic granules in some of the cells. Note also the eosinophilic granules scattered extracellularly. (New methylene blue X125).

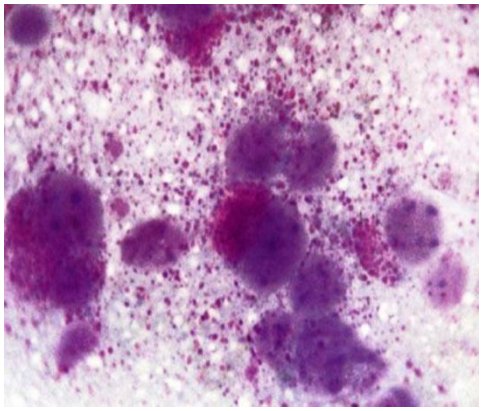

Fig. 5: Cytological smear showing mast cells with prominent multiple clumps of chromatin and pinkish coloured intra and extracellular granules. (Toluidine blue X125)

Further, it is also indicated that mast cell tumors could be diagnosed easily based on cytology alone, because of metachromatically stained granules in the cells (Duncan and Prasse, 1979; Dean, 1988). However, presence of eosinophils as observed earlier (Dean, 1988; Tvedten, 1994) in the cytological preparations and finding that the eosinophils in cytological preparations might be a clue for diagnosis of mast cell tumors was not observed in the present study.

Histopathologically, the presence of spherical to oval shaped cells, eosinophilic granular cytoplasm (Fig. 6), oval to spherical nuclei, dust like purplish cytoplasmic granules in Toluidine blue stained

DOI 10.5455/ijlr.20170911113547

sections and infiltration of eosinophils confirmed the tumor as mast cell tumor and the descriptions were in accordance with previous findings (Simoes *et al.,* 1994; Girish *et al.,* 2010).

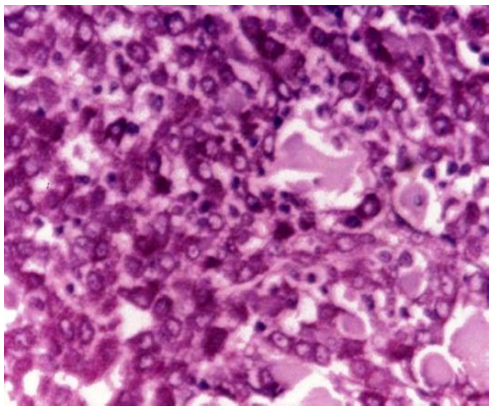

Fig. 6: Section of mast cell tumour showing spherical to oval shaped cells with eosinophilic granular cytoplasm. (H&E X50)

Plasmacytoma

Of the two plasmacytomas recorded, one was encountered in a five year old male nondescript (left thoracic region) and another in a four year old female Pomeranian (located over the dorsal wall of the lower abdominal region). The mass was yellowish white colored and ulcerated a finding also reported by Lucke, 1987. Smears revealed presence of individually arranged round to oval shaped cells. They showed eccentrically placed round to oval shaped nucleus with coarse chromatin and prominent nucleoli (Fig.7).

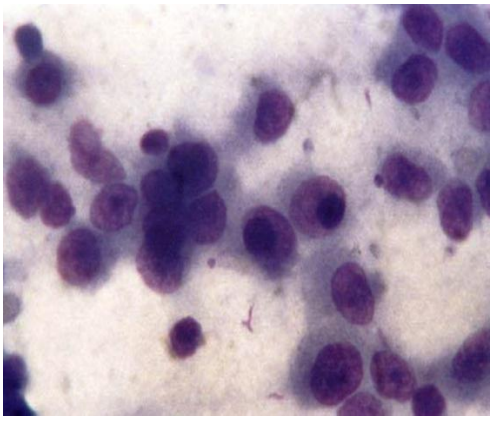

Fig. 7: Cytological smear showing plasma cells with eccentrically placed nucleus, prominent nucleolus in some cells and basophilic cytoplasm in plasmacytoma.(Giemsa X125)

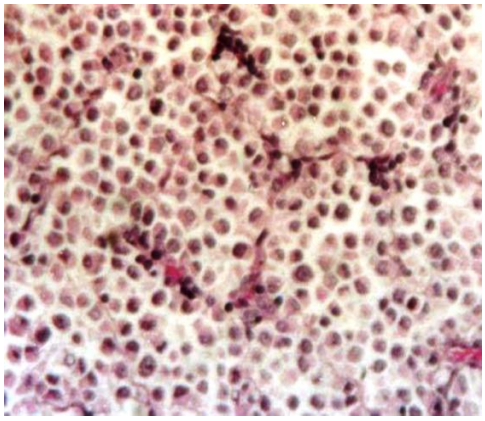

Fig.8: Section of plasmacytoma showing plasma cells consisting of eccentrically placed nucleus with abundant eosinophilic cytoplasm. (H&E X50).

DOI 10.5455/ijlr.20170911113547

The cells had variable cytoplasmic basophilia and these findings were in accordance with the earlier descriptions (Lucke, 1987; Alleman and Bain, 2000; Boostrom *et al.*, 2017). The eccentrically placed nucleus and abundant basophilic cytoplasm helped in the precise identification of cells as plasma cells. However, amyloid material, which was observed earlier (Raskin, 2001) could not be demonstrated in cytological smears in the present study. Histopathologically, the plasmacytoma was characterized by solid sheets of plasma cells, eccentrically placed round to oval shaped nucleus with coarse chromatin, abundant eosinophilic to lightly basophilic cytoplasm and thin fibrovascular stroma (Fig. 8). In addition, amorphous eosinophilic amyloid material, multiple areas of necrosis and infiltration of lymphocytes were also observed. These findings were in total agreement with earlier findings (Lucke, 1987; Boostrom *et al.*, 2017).

Conclusion

Cancer is the most important and one of the leading causes of death in dogs. Like humans dogs also develop variety of spontaneous neoplasms as they up to their life term. Recent trends shows use of canine neoplasms as animal models to study their human counterparts. Round cell tumors include mast cell tumors, histiocytomas, plasmacytomas, transmissable venereal tumors (TVTs) and cutaneous Lymphomas in dogs. In the current investigation involving 215 tumor and tumor like growths, a total of 35 (16.3%) round cell tumors were diagnosed based on clinical history, gross findings, cytology and histopathology. The round cell tumors diagnosed included transmissible venereal tumors (23), mast cell tumors (10), and plasmacytomas (2). The various pathological features shown by these cancerous growths were documented and described in detail in the current investigation.

References

1. Girish, BC., Suguna, R, Byregowda, SM and Kotrappa, YM. 2011. Canine Squamous Cell Carcinoma: a Review of 17 Cases, *Brazilian Journal of Veterinary Pathology*, 4(2):79-86.
2. Gardner, HL, Fenger, JM and London, CA. 2016. Dogs as a Model for Cancer, *Annual Review of Animal Biosciences*, 4 (1): 199-222.
3. Pang, LY and Argyle, DJ. 2016. Veterinary oncology: Biology, big data and precision medicine, *The Veterinary Journal*, 213: 38-45.
4. Ramos-Vara, JA and Miller, MA. 2011. Immunohistochemical expression of E-cadherin does not distinguish canine cutaneous histiocytoma from other canine round cell tumors, *Veterinary Pathology*, 48: 758-763.
5. Meuten, DJ. 2017. In, Tumors in domestic animals. 5th Edn, John Wiley & Sons Inc, Iowa, USA. pp. 142-202.
6. Welle, MM, Bley, CR, Howard, J and Rüfenacht, S. 2008. Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment, *Veterinary Dermatology*, 19(6):321-329.
7. Luna, AG. 1968. In, Manual of histology and special staining technique. 3rd Edn, Armed Force Institute of Pathology, Magraw Hill Book Co, London.

DOI 10.5455/ijlr.20170911113547

8. Kotrappa, YM, Suguna, R, Girish, BC, Byregowda, SM and Manjunatha, K. 2014. Cytological and histopathological studies of mesenchymal and melonotic neoplasms in dogs, *Journal of Cell and Tissue Research*, 14(3):4477-4483.
9. Villiers, EJ and Dunn, JK. 1998. Collection and preparation of smears for cytological examination, *In Practice*, 20: 370-377.
10. Alleman, AR and Bain, PJ. 2000. Diagnosing neoplasia: The cytologic criteria for malignancy, *Veterinary Medicine*, 3: 204-223.
11. Jain, A, Tiwari, RP, Tiwari, SK, Neelu, G and Awasthi, MK. 2002. Histopathological studies in canine transmissible venereal tumour, *Indian Journal of Animal Reproduction*, 23: 60-62.
12. Ganguly, B, Das, U and Das, AK. 2013. Canine transmissible venereal tumor, *Veterinary and Comparative Oncology*, 14 (1): 1-12.
13. Milo, J and Snead, E. 2014. A case of ocular canine transmissible venereal tumor, *Canadian Veterinary Journal*, 55:1245-1249
14. Duncan, JR and Prasse, KW. 1979. Cytology of canine cutaneous round cell tumours: Mast cell tumour, histiocytoma, lymphosarcoma and transmissible venereal tumour, *Veterinary Pathology*, 16: 673-679.
15. Dean, PW. 1988. Mast cell tumours in dogs: Diagnosis, treatment and prognosis, *Veterinary Medicine*, 2:185-192.
16. Simoes, JPC, Schoning, P and Butine, M. 1994. Prognosis of canine mast cell tumours: A comparison of three methods, *Veterinary Pathology*, 31: 637-647.
17. Girish, BC, Suguna, R, Byregowda, SM, Satyanarayana, ML and Sreekanth, P. 2010. Mast cell tumour in a Labrador dog, *Indian Veterinary Journal*, 87: 1276.
18. Tvedten, H. 1994. Cytology of neoplastic and inflammatory masses. In: Small Animal Clinical Diagnosis by Laboratory Methods. 2nd Edn., W.B. Saunders Co., Philadelphia, pp. 321-341.
19. Lucke, VM. 1987. Primary cutaneous plasmacytomas in the dog and cat, *Journal of Small Animal Practice*, 28: 49-55.
20. Boostrom, BO, Moore, AS, DeRegis, CJ, Robat, C, Freeman, K and Thamm, DH. 2017. Canine Cutaneous Plasmacytosis: 21 Cases (2005–2015), *Journal of Veterinary Internal Medicine*, 1:1-7.
21. Raskin, RE. 2001. Skin and subcutaneous tissues. In: Atlas of Canine and Feline Cytology. 1st Edn., W.B.Saunders Co., Philadelphia, pp. 35.

DOI 10.5455/ijlr.20170911113547