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Transmissible venereal tumours (TVT) in bitches: A haematological, biochemical and histopathological study

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Abstract

The dogs affected with transmissible venereal tumours were investigated for haematological, biochemical, cytological and histopathological study. The prevalence of canine venereal tumour was found to be 25.46 per cent with respect to all reproductive diseases and 34.15 per cent of all types of tumours in dogs in and around Bhubaneswar, Odisha. The incidence was more in young female dogs of age group 2 to 11 years (46.34%) with a mean age of 5.17 years. Non-descript/ indigenous breeds (73.17%) were more affected as compared to pure breeds of dogs. Haematological examination of suspected cases revealed normocytic –hypochromic anaemia, neutrophilic leucocytosis with thrombocytopenia and lymphopenia. Serum biochemical examination of affected cases revealed hypoproteinemia, hypoalbuminemia, hypoglobulinemia, hypoglycemia, decreased triglyceride and higher levels of LDH, blood urea nitrogen, creatinine, calcium, increased levels of alanine aminotransferase and alkaline phosphatase. Gross examination revealed presence of tumour masses around the genital organs with pinkish or hyperemic cauliflower like appearance with ulcerations and bleeding. Cytological and histopathological examinations of suspected cases revealed typical pleomorphic neoplastic round cells with marked anisocytosis, anisokaryosis, vacuolated cytoplasm, large central to eccentric nuclei, increased mitoses, hyperchromasia which are suggestive of Transmissible venereal tumours. Cytological, histopathological along with haematological and biochemical studies can be profitably utilized for early diagnosis and prognosis of canine transmissible venereal tumours.

Keywords: Haematology, biochemistry, histopathology, TVT, Tumour

Introduction

Canine breeding has gained a tremendous popularity within this decade and the dog is considered as a best companion to human beings. In general, the breeders and most pet owners are very much concerned about the reproductive health of their pet to prevent reproductive diseases and for future breeding. Still pathologies of variable etiologies like congenital, endocrine, autoimmune, infectious, traumatic and neoplastic disorders exist in canine reproductive tract. The most common hormonal disorder reported in female dogs include cystic endometrial hyperplasia-pyometra complex which has been studied extensively since years and several therapeutic agents have been recommended for successful intervention (Jena *et al.*, 2013, Rosa *et al.*, 2020 and Singh *et al.*, 2020) [10, 19, 20]. The next commonly reported contagious disorder is canine transmissible venereal tumour which is a naturally occurring allograft, an undifferentiated neoplasm of round cells of reticuloendothelial origin that is sexually transmitted and primarily affects the external genitalia of both sexes. Despite its ancient origin, it is still a commonly observed cutaneous canine tumour with a greater incidence in females throughout the world. Close apposition with stray and wild dogs makes the disease more complicated and difficult to control (Kumar *et al.*, 2007, Honparkhe *et al.*, 2010 and Ramsingh *et al.*, 2013) [13, 9, 17]. Canine tumours are also considered as an excellent tumor models which mimic human tumors to be used for diagnostic, prognostic and therapeutic studies on tumors of other animals including human beings also. Among them canine venereal tumor has got immense importance because of its peculiar proliferative behavior. It is so because, it shows growth, stagnant and regressive phases due to which it always remained an interesting area of tumor research. There is always a need for a simple, inexpensive and rapid diagnostic method for detecting TVT in dogs under field conditions.

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The current study is hypothesized with the following objectives: Taking into consideration all these the present study was conducted to study the pathological aspects of this life threatening tumours for early and rapid diagnosis.

Materials and Methods

The Research work was carried out at Department of Veterinary Pathology, College of Veterinary Science & A.H., OUAT, Bhubaneswar. The dogs, presented with clinical symptoms like bleeding from genital region or having tumorous growths in the genital organs suspected for transmissible venereal tumours were taken for the study. Their haematological and biochemical parameters were compared with six healthy animals presented for vaccination, routine checkup or breeding advice (control group). Cytological and histopathological investigation was done for the TVT affected dogs.

Results & Discussion

During the present study period 41 dogs were diagnosed to be transmissible venereal tumours (TVT) out of 161 dogs presented with the complaints of various reproductive disorders. The prevalence of TVT was found to be 25.46 per cent. TVT is most commonly found in tropical and subtropical regions where large populations of free roaming dogs exist (Rogers, 1997) [18] and prevalence of TVT was up to 37% when the density of stray dogs is high (Batamuzi *et al.*, 1992) [3]. A great variation among years may be expected, due to factors like uncontrolled breeding and increase of the population at risk. This type of tumours constituted 34.15 per cent of all types of tumours presented in dogs for treatment. Goswami *et al.* (2008) [8] reported that out of 44 cutaneous growths removed from dogs of various age, breed and sex, 12 were diagnosed as transmissible venereal tumour. In the present study, the mean age of occurrence of TVT was 5.17 years with an age range of 2 to 11 years and a peak at two to four years (46.34%) followed by 31.71 per cent in 4-6 years age group. Thus this study revealed that young aged dogs of 2 - 6 years were more prone for TVT which is similar to the earlier reports of Honparkhe *et al.*, (2010) [9]. The lowest age at which TVT recorded was 2.0 years. However Rogers (1997) [18] reported that TVT usually occurs in young sexually mature canines. Out of 41 dogs that had TVT, non-descript/indigenous breeds constituted 73.17% of the total cases. High incidence of venereal tumor in stray dogs could be due to frequent contact between infected animals particularly during breeding season. Among the pure breeds, Labrador breed showed a higher incidence (9.75%) followed by Pomeranian, Spitz and German shepherd breeds. This variation among the pure breeds may be due to breed predisposition in the locality. Out of the 41 dogs, 07 were males and 34 were female dogs. Female dogs are more susceptible to TVT than males (Honparkhe *et al.*, 2010) [9] which might be due to large scale of castration in male dogs thereby eliminating the sexually activity.

The cases were presented with the complaints of inappetance, loss of body weight, body condition, weakness, anorexia and depression etc. The tumour was found on scrotal region, penis, prepuce, cervix, vaginal opening and vulval opening. The growths were of variable size, ulcerating, infected etc. In some cases, there was history of spontaneous regression with recurrence of new growths at different sites. There was also bleeding from the genital parts and animals refused to copulate in some cases. No involvement of adjacent tissues

was noticed. Similar reports were also given by Behera *et al.* (2012) [4]. No extragenital lesion of the disorder had been recorded during the study period. There was generalized lymphadenopathy characterized by marked nodular enlargement of the lymph nodes.

In the present study the mean haemoglobin level was found to be 12.65 ± 0.387 and 11.59 ± 0.201 g/dl in control group and TVT affected dogs respectively. The mean packed cell volume was found to be 37.92 ± 0.823 and 36.19 ± 0.149 per cent in control group and TVT affected dogs respectively. The mean total erythrocyte count level ($\times 10^6/\mu\text{l}$) was found to be 6.29 ± 0.419 and 5.86 ± 0.316 in control group and TVT affected dogs respectively. There was a significant decrease ($p < 0.01$) in haemoglobin, packed cell volume and total erythrocyte count level in the dogs affected with TVT when compared with healthy control group of animals indicating normocytic –hypochromic anaemia which is in agreement with the findings of Cizmeci *et al.*, (2012) [21] who reported that normocytic-hypochromic non-regenerative anemia developed in due to tumoral bleeding and lower urinary system infection in TVT cases. However Kose *et al.*, (2013) [11] reported that microcytic hypochromic regenerative anemia was linked to hemorrhagic anemia due to tumoral bleeding.

The mean TLC level was found to be 10609.31 ± 291.76 and 34517.82 ± 1012.23 in control group and TVT affected dogs respectively. There was significant increase ($p < 0.05$) in mean TLC level in the dogs affected with TVT when compared with healthy control group of animals. There was significant increase ($p < 0.05$) in mean neutrophil percentage and significant decrease ($p < 0.05$) in lymphocyte percentage in the dogs affected with TVT when compared with healthy control group of animals. The mean thrombocyte count ($\times 10^3/\mu\text{l}$) was found to be 350.57 ± 21.78 and 25.76 ± 1.248 in control group and TVT affected dogs respectively. There was significant decrease ($p < 0.05$) in mean thrombocyte count in the dogs affected with TVT when compared with healthy control group of animals. Neutrophilia, thrombocytopenia and lymphopenia in the dogs affected with TVT reported in our study is in accordance of the result of Cizmeci *et al.* (2012) [21], Behera *et al.* (2012) [4] and Kose *et al.* (2013) [11] who reported leucocytosis and lymphopenia as a common finding in TVT due to infection in tumoral tissue and its periphery as a result of immunosuppression, leading to secondary bacterial invasion. Haematological abnormalities are the common paraneoplastic syndromes resulting due to neoplastic infiltration of bone marrow, splenic malfunctioning or immune mediated abnormalities (Alley and Schecter, 2002) [2]. No significant difference was observed regarding the other haematological parameters when compared with healthy control group of animals.

The values of serum biochemical parameters like blood urea nitrogen, serum creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, albumin, globulin, glucose, lactate dehydrogenase (LDH), triglyceride and calcium in TVT affected animals have been described in Table 1. There was significant increase ($p < 0.05$) in mean BUN, creatinin, LDH, ALT, ALP and calcium level in the dogs affected with TVT when compared with healthy control group of animals that might be due to metastasis to other organs affecting the organ function (Behera *et al.*, 2012) [4]. In all the cases, significant increase of LDH value was observed which might be due to cellular damage and necrosis of the affected tissues. Necrosis is generally observed in malignant tissue growths. Similar report was also given by earlier

workers (Goswami *et al.*, 2008) [8]. Affected cases also showed significant increase in calcium value which may be associated with paraneoplastic syndrome (PNS) as being reported by (Goswami *et al.*, 2008) [8]. There was significant decrease ($p < 0.05$) in mean total protein, albumin, glucose and triglyceride level in the dogs affected with TVT when compared with healthy control group of animals. There was also decrease of mean globulin value in the dogs affected with TVT, but not up to the significant level. TVT affected canines revealed hypoproteinemia, hypoalbuminemia, hypoglobulinemia which might due to malnutrition and wasting condition in conjunction with the disease (Behera *et al.*, 2012) [4]. Hypoglycemia might have resulted due to cell and tissue damage affecting glucose metabolism. In our study though some individual animals showed abnormal serum biochemistry, the mean values remained within the normal range which might be due to early presentation of the disease and absence of metastatic lesions.

Cytological smears prepared from fine needle aspiration biopsy (FNAB) and impression of the tumour masses revealed very high cellularity of discrete cells with round to oval shape with moderate to abundant pale cytoplasm containing varying sized multiple punctate clear vacuoles known as histiocytes. Central to eccentrically placed nuclei; anisokaryosis and coarse chromatin were observed. Nucleolus was prominent. Numerous mitotic figures were found. Occasional binucleated cells were seen (Fig 2). Occasional presence of fibroblasts was also observed. Similar findings were reported by Duncan and Prasse (1979) [6] and Goswami *et al.* (2008) [8]. Alleman and Bain (2000) [1] also observed the neoplastic cells with numerous distinct vacuoles.

Meinkoth and Cowell (2002) [15] also recorded large number of mitotic figures. Similarly, Krithiga (2005) [12] stated that the neoplastic cells were arranged individually with round nuclei and cytoplasmic vacuoles. Grossly, the mass was bright pinkish or hyperemic with cauliflower like appearance with ulcerations and bleeding. Tumour size varied from 3 cm to 11 cm. In bitches, the growths hanging from vagina and were well vascularised (Fig 1). Marchal *et al.* (1997) [14], Goswami *et al.* (2008) [8] and Behera *et al.* (2012) [4] reported that TVT grossly appeared as a solitary or multiple reddish mass with cauliflower like appearance and hyperaemic, as observed in the present study. Duncan and Prasse (1979) [6], Moulton (1990) [16] and recorded transmissible veneral tumour as cauliflower like pedunculated nodular, papillary or multilobulated, firm to friable mass involving external genitalia and oral, nasal and conjunctival muscoa.

Histopathology revealed sheets or cords of round, ovoid or polyhedral cells with large round nuclei and large central nucleoli, which concurred with the observations of Marchal *et al.* (1997) [14]. The cytoplasmic boundaries are indistinct. There was vacuolation of cytoplasm of histiocytes depicting foam cells. Thin delicate strands of fibrous tissue stroma were observed as reported by Rogers (1997) [18]. Nuclei were large, round, hyperchromatic with large central nucleoli. Cytoplasm was faintly eosinophilic with indistinct cell outline. There was perivascular infiltration, congestion and necrosis. Mitotic figures were common and the cells were not arranged in obvious architectural pattern (Fig 3). These findings in the present study are quite similar to the earlier studies reported by Gobello and Corrada (2002) [7], Krithiga *et al.* (2005) [12] and Goswami *et al.* (2008) [8].

Table 1: Comparative biochemical findings (Mean \pm SE) in canine TVT

Parameters	Control Group (N=6)	TVT (N=41)
BUN (mg/dL)	17.64 ^a \pm 0.391	37.91 ^b \pm 0.981
Creatinine (mg/dL)	2.18 ^a \pm 0.345	5.31 ^b \pm 0.841
LDH (U/L)	148.74 ^a \pm 12.371	359.71 ^b \pm 21.163
ALT (U/L)	28.39 ^a \pm 2.483	57.42 ^b \pm 5.236
ALP (U/L)	126.73 ^a \pm 4.627	210.38 ^b \pm 11.314
TP (g/dL)	7.42 ^a \pm 0.187	4.82 ^b \pm 0.798
Albumin (g/dL)	3.77 ^a \pm 0.312	2.49 ^b \pm 0.051
Globulin (g/dL)	3.63 ^a \pm 0.207	3.23 ^a \pm 0.105
Glucose (mg/dL)	105.46 ^a \pm 6.71	67.98 ^b \pm 4.69
Calcium (mg/dL)	9.71 ^a \pm 0.517	13.52 ^b \pm 0.613
Triglyceride (mg/dL)	178.48 ^a \pm 6.214	110.34 ^b \pm 8.973

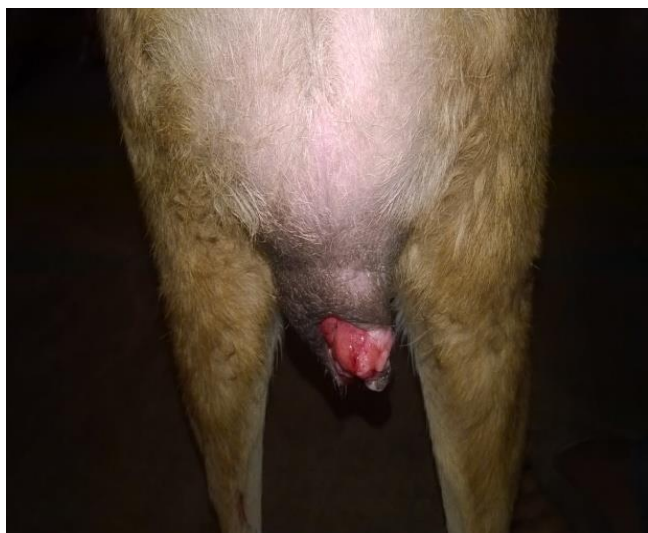


Fig 1: Tumour mass-vagina

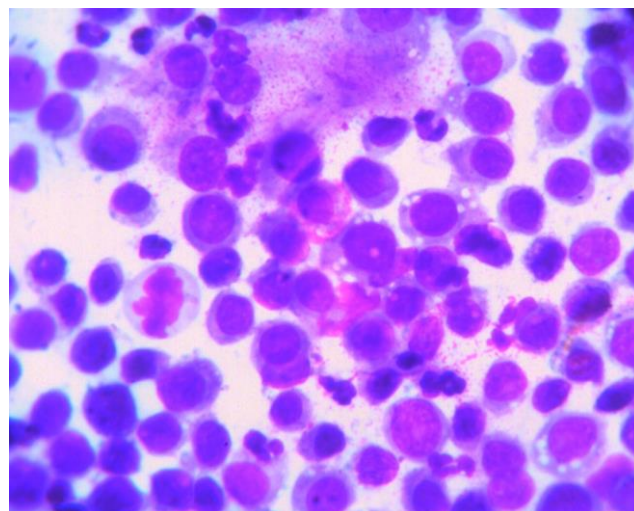


Fig 2: Pleomormphic round cells-vacuolated cytoplasm, anisokaryosis-Geimsa (X1000)

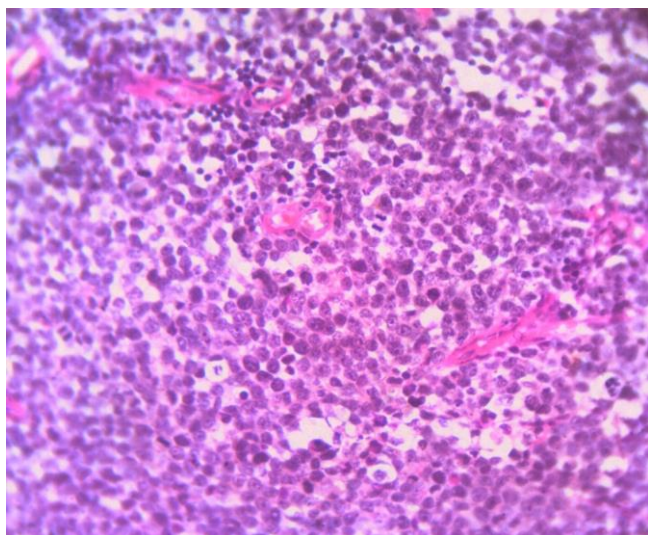


Fig 3: Pleomorphic round cells-vacuolated cytoplasm, anisocytosis, congestion (X400)

In spite of advances made in molecular biology, diagnosis and development of various therapeutic protocols, canine venereal tumour is still prevalent affecting a large canine population. Cytological, histopathological along with haematological and biochemical studies can be profitably utilized for early diagnosis and prognosis of canine transmissible venereal tumours.

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