REVIEW ARTICLE

TRANSMISSIBLE VENEREAL TUMOUR (TVT) IN BITCHES AND THERAPY: A REVİEW

SAKINE ÜLKÜM ÇIZMECI* AND MEHMET GULER

Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Selcuk, Selçuklu, 42003 Konya-Turkey
* ulkum@selcuk.edu.tr

ABSTRACT. TVT, also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or sticker tumour is a benign reticuloendothelial tumour that affects particularly mucosa of external genital organs and rarely internal genital organs in dogs of both genders. TVT is usually transmitted by coitus but also can be transmitted by licking, sniffing, biting, and scrabbling of the tumour affected area or through damaged skin of mucosa. Transmissible venereal tumour (TVT) is usually observed in stray animals live in tropical and subtropical lands. The affected animals are usually within 9-13 months of age and with high sexual activity. Tumour is frequently located in posterior vagina and vestibulovaginal junction. The average chromosome count of TVT cells is 59 (57-64). TVT specific antibodies were found in blood samples of affected animals which suggest that they may have a role in natural regression mechanism. The primary objective of tumour treatment is total elimination by surgery, radiotherapy, immunotherapy and/or chemotherapy. Controlling of the disease is very difficult because stray dogs are carriers.

Keywords: dog, transmissible venereal tumour, diagnosis, treatment, review.

INTRODUCTION

Dogs are most tumour observed domestic animals. Tumours observed in dogs vary depending on breed, gender, age, system, organ and tissue. Besides, although tumoural formations may occur in any age, the frequency of benign ones is higher in younger ages while that of malign ones is higher in older ages (Gulcubuk and Gurel, 2003). The great majority (63%) of vaginal tumours in dogs is reported to be TVT (Misirlioglu *et al.*, 1994).

TVT, which is also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or sticker tumour (Oruc *et al.,* 2011; Erer and Kiran, 2000) is a benign reticuloendothelial tumour that affects particularly mucosa of external genital organs and rarely internal genital organs in dogs of both gender (Sankar ve ark, 2016; VonHoldt and Ostrander, 2006; Katzir *et al.*, 1985).

INCIDENCE

TVT is usually observed in stray animals which have high sexual activity live in tropical and subtropical regions (Oruc *et al.,* 2011; Nak *et al.*, 2004; Das and Das,

2000). TVT is reported to have about 2,000-2,500 years of history and originated from dogs or wolves of East Asia according to the results of DNA and microsatellite tests performed in 40 dogs from five different continents (Murgia *et al.*, 2001). It is reported that 44.1% of tumours observed in dogs are in genital system and 14.7% of them are venereal tumours (Erer and Kiran 1993). In a study, reported that in 1,522 dogs medical examination was made in a five year period and, Of these, 123 (8,08%) cases were diagnosed of canine TVT (Araujo *et al.*, 2016).

TVT is usually observed in adult dogs with average age of 9 to 13 months (Salt *et al.,* 2005; Morris and Dobson, 2001). Those dogs usually have body weight more than 18 kg to 20 kg (Nak, 2001). Animals under 1 year of age are within risk group in endemic regions. The incidence of the disease is greater in female animals than in male animals. This is due to spread of disease by carrier male animals via copulation with many female animals (Das and Das, 2000). Although it is known that there is no breed predisposition for that disease, it is reported this terrier dogs are more susceptible for mammary tumours, mastocytoma, melanoma and TVT (Gonzalez *et al.*, 2000).

PATHOGENESIS

TVT is located in external genital organs of dogs and usually transmitted by transplantation of viable tumour cells during the coitus (Stockmann *et al.*, 2011). That tumour is unique because of being first experimentally induced tumour by veterinary surgeon Nowinsky in 1876 in Russia (Purohit, 2009; Nak, 2001). TVT is usually transmitted by coitus but also can be transmitted by licking, sniffing, biting, scrabbling of the tumour affected area or through damaged skin or mucosa (Ozyurtlu *et al.*, 2008; Gulcubuk and Gurel, 2003; Liao *et al.,* 2003; Jonston *et al.*, 2001; Konuk *et al.*, 2001). Cellular origin of TVT is unknown and incidence of physical transmission is higher than that of infectious transmission (Goldschmidt and Hendrick, 2002; Erer and Kiran, 2000).

Transmission is realized by direct transplantation of live tumour cells (Ozyurtlu *et al.,* 2008; Murgia *et al.*, 2006; Erer and Kiran, 2000). However, frozen, heat or glycerin treated tumoural cells or filtrates without cell do not form tumour (Purohit, 2009; Murgia *et al.*, 2006; Erer and Kiran, 2000). Also, some researchers link this disease to a viral agent due to cytoplasmic inclusions in tumour cells (Martins *et al.*, 2010). Besides, some virus particles were observed during electron microscopic examinations but viral etiology could not proved accurately (Erer and Kiran, 2000).

Experimental transmission of TVT can be obtained by subcutaneous inoculation of live tumour cells. Tumoural tissue usually regresses spontaneously after experimental infections. Spontaneous regression occurs less frequently in natural disease. It is believed that regression is achieved by tumour antibodies (Harmelin *et al.*, 2002; Katzir *et al.,* 1985). It is reported that tumour regression can be achieved through passive immunity provided by blood or serum transfusion from the animals recovered from the disease (Konuk *et al.,* 2001).

Tumoural growth happens between 15^{th} and 60^{th} days following transmission.

TVT either grows slowly through years or invades and finally becomes malign and metastatic (Martins *et al*., 2010; Purohit, 2009). Initially tumour develops in external genital organs of females and males (Goldschmidt and Hendrick, 2002). Besides external genital organs, it is reported that tumour can also be found in bucca, nasal and anal mucosa, conjunctiva and skin as well (Martins *et al.,* 2010; Nak *et al*., 2005; Perez *et al*., 1998).

CYTOGENETIC ORIGIN

There are obvious differences in chromosomal morphologies and counts of TVT cells (Katzir *et al*., 1985) and their average chromosomal count is reported as 59 (57-64) as reported (Ekici, 2015; Martins *et al.,* 2010; Mukaratirwa and Gruys, 2003; Erer and Kiran, 2000). 17 of those chromosomes are metacentric and 42 of them are acrocentric (Ekici, 2015; Nak, 2001). The normal diploid number of chromosomes in the cell of dods is 78, and 76 of these are acrosentric and while 2 chromosomes are metacentris (Mukaratirwa and Gruys, 2003). Similarity between cytogenetical appearance of primary tumour in genital area and that of metastatic one fortifies the evidences about consistency of cytogenetical abnormalities of TVT cells. The same chromosomal sequence is also preserved in cell cultures (Das and Das, 2000).

DIAGNOSIS

Diagnosis depends on anamnesis, clinical and laboratory results. Histological examination is a highly reliable method for diagnosis. Besides, chromosomal analysis and experimental transmission may also be used (Das and Das, 2000). Usually serosanguinous and haemorrhagic genital discharge may be observed in clinical inspection. Other signs may be licking external genital organs and a prolapsed mass in this region (Ekici, 2015). Tumour is usually located in posterior vagina and frequently in vestibulovaginal junction in bitches. In male dogs, it is usually found in caudal penis, glans penis or crura bulbis glandis and preputium (Purohit, 2009; Nak *et al*., 2005; Nak *et al*., 2004). TVT in external genital organs are 0.5 mm to 10 cm in diameter, cauliflower like, rubescent, fresh-looking and easily bleeds when touched (Purohit, 2009; Jonston *et al*., 2001). In most cases, they are proliferative, verrucous, papillar or nodular bunch of masses prolapsed out of vulva of bitches (Konuk *et al*., 2001; Erer and Kirar, 2000). Sometimes necrosis and petechies, superficial ulcerations and secondary infections may be observed in tumoural tissue (Purohit, 2009). There is a serosanguinous or haemorrhagic fluid discharge from the tumoural tissue which quickly ulcerates and has a necrotic appearance subsequently (Das and Das, 2000). Haemorrhagic discharge may be confused with estrus, uretritis and cystitis in bitches and prostatitis in male dogs (Konuk *et al*., 2001). Animals may have anemia in case of continuous bleeding (Purohit, 2009).

Tumoural cells are observed as big, round, polygonal or oval in histopthological inspection. There are vesicles and big nucleuses in those cells and each nucleus has a big, dark purple tainted nucleolus in its center. Mitotic activities of cells are

increased (Ozyurtlu *et al.*, 2008; Salt *et al.,* 2005; Misirlioglu *et al.*, 1994). Cytoplasm is slightly eosinophilic (Erer and Kirar, 2000). Localisation of tumour has an important role in diagnosis. Round cell tumours of genital organs should be considered as TVT until diagnosed by special techniques such as electron microscopic examination or immunohistochemistry (Ekici, 2015)

METASTATIC LESIONS

It is reported that metastasis is observed in 5-17% of TVT cases (Purohit, 2009). The tumour may metastasize to regional lymph nodes, tonsils, eye, brain, adenohypophysis, nose, maxillary bones, pancreas, liver, spleen, lung, bone, kidney, subcutis and other regions (Kose *et al.,* 2013; Purohit, 2009; Stettner *et al.,* 2005; Ozyurtlu *et al.,* 2008; Gulcubuk and Gurel, 2003; Jonston *et al.,* 2001; Konuk *et al.*, 2001). Tumours located in lips are similar to those in genital organs while others in mouth and tonsils are more diffuse and bright. Eye tumours may lead to blindness (Purohit, 2009). Tumour grows rapidly and metastasizes in immunosuppressed adult dogs and puppies (Perez et al., 1998). It was reported that metastasis occurs more in bitches (16%) than in male dogs (2%) (Salt et al., 2005).

PROGNOSIS

Immunologic studies show obviously that TVT in dogs is antigenic and immune response developed against tumour has a great importance in prognosis. Tumour regresses spontaneously after a logarithmic growth period in adult dogs and immune response developed against tumour may prevent future proliferations. However, tumour ulcerates and metastasizes in cases in immunodeficient individuals. Biological behavior of TVT in dogs may be predicted by determination of Nuclear Organizer Regions (AgNORs). Bad prognosis in TVT depends on increased AgNORs in nucleuses of cells (Purohit, 2009).

TREATMENT

The primary objective of tumour treatment is total elimination by surgery, radiotherapy, immunotherapy and/or chemotherapy (Eze *et al.*, 2007).

Surgical Practices

Surgical practices are very important in diagnosis and treatment of tumours and it is a very efficient way of treatment for most of the solid tumours. Surgery cannot be applicable in diffuse TVT cases (Norris and Withrow, 1984). Tumour may be removed by electrosurgical excision or cryosurgery if it is in suitable size. Traditional surgical methods are not preferred because they hasten metastasis and increase recurrence rate. Recurrence is reported approximately in 30% of the cases. Recurrence rates are decreased if castration or ovariohysterectomy applied together with chemotherapy. An autogenous vaccination supported by Levamisol may be used to prevent recurrence after surgical operation (Das and Das, 2000).

Radiotherapy

Radiation which is a kind of energy formed by stimulation of ionization of atoms or molecule components present in the line of beam and absorbed by live tissues. It requires professional staff and special devices for application and also chemical immobilization of the dog throughout the radiotherapy (Morris and Dobson, 2001). The applied dose is 10 Gy (1000 rads) per session. Radiotherapy is reported to be effective against TVT (Purohit, 2009; Jonston *et al.*, 2001).

Immunotherapy

TVT can be treated by whole blood or serum transfusion from a fully recovered animal or by administration of an autocutaneous vaccine obtained from a tumoural tissue homogenate (Cizmeci *et al.*, 2012a; Jonston *et al.*, 2001). Bacterial toxins may also be used in TVT treatment. It is reported that administration of dead suspension of *Chromobacterium prodigiosum* alone or combined with other organisms may provide satisfactory results (Das and Das, 2000).

Chemotherapy

Cytotoxic drugs are classified as alkaline agents, antimetabolites, antitumour (cytotoxic) antibiotics, vinca alcoloids, hormones, enzymes and other agents depending on their mode of action, target tissues and antitumoural activities (Chun *et al.*, 2001; Morris and Dobson, 2001; Norris and Withrow, 1984). Doses of the cytotoxic drugs are usually calculated by functional measurement of body surface area instead on body weight. Because, blood quantity in detoxifying and excreting organs is more related to body surface than it is related to body weight. Body weight is used in body surface area calculation shown in Eq. (1) (Dobson and Gorman, 1993; Chun *et al.*, 2001).

$$\text{Formula for surface area } (m^2) = \frac{10.1 \times (\text{body weight gr.})^{2/3}}{10000} \quad (1)$$

Alkaline agents, antitumour antibiotics and some of the other agents block DNA replication at single point. Antimetabolites prevent DNA or RNA synthesis via synthesis of enzyme blockers or non-functional molecules. Vinca alcoloids are antimitotics and their effect is through blocking metaphase in mitotic line (Dobson and Gorman, 1993).

Although surgery and radiotherapy is effective only for local neoplastic diseases, chemotherapy is very efficient against systemic diseases (Dobson and Gorman, 1993). One of the most challenging problems in chemotherapy against cancer is rapid tumoural growth before it was diagnosed (Misirlioglu *et al.*, 1994). Optimum drug dose in chemotherapy is the quantity to make a significant effect on tumour cells without any evident toxic effect on normal tissues (Dobson and Gorman, 1993). The drugs must be administered at maximum tolerance rates and minimum dose range. 20% decrease in dose causes greater than 50% decrease in drug efficacy (Chun *et al.,* 2001)

Some cytotoxic agents used in TVT treatment are as follows:

Cyclophosphamide

It is used combined with other chemotherapeutics in treatment of lymphoma, some carcinomas and sarcomas (Chun *et al.,* 2001).

Methotrexate

It is used combined with other chemotherapeutics in treatment of lymphomas of cats and dogs (Norris and Withrow, 1984). Besides, it can be used in treatment of myoproliferative disorders and transmissible venereal tumour (Oglivie, 1998).

Doxorubicin

That drug is used against lymphoma, osteosarcoma, hemangiosarcoma, thyroid carcinoma and some other carcinomas (Cizmeci *et al.,* 2012b; Chun *et al.*, 2001; Oglivie, 1998). Doxorubicin has a high antitumoural activity in treatment of malign tumours (Oglivie, 1998).

Mitomycin

Mitomycin is used in adenocarcinoma and carcinomas of mammary gland, cervix, colon, rectum, bladder, neck and pancreas (Dobson and Gorman, 1993).

Vinblastine

It is used in mast cell tumours and lymphomas of dogs. Besides, it is very effective in malign testis tumours (Chun *et al.*, 2001).

Vincristine Sulfate

Vincristine sulfate is used in treatment of lymhoproliferative cancers, transmissible venereal tumours, thrombocytopenia and some sarcomas (Sankar *et al.,* 2016; Chun *et al.,* 2001).

Involution is rapid in the beginning of the treatment but slows down in time and the involution of the lesions is gradual. Full recovery is achieved after 2-8 cures generally and the success in treatment is 90%. There are some side-effects expected. Cytotoxic agents such as vincristine may cause side-effects like myelosuppression and gastrointestinal disorders which may result in leucopenia and vomit in 5-7% of the patients (Das and Das, 2000).

IMMUNITY

Specific antibodies against TVT antigens are found in blood of animals having tumour and they are believed to have role in natural regression mechanism. Newborn puppies of bitches having tumour need a longer latent period for tumoural growth. Dimensions of tumoural tissue in those puppies are smaller and they have a faster spontaneous regression (Das and Das, 2000).

CONTROL

Controlling of the disease is very difficult because stray dogs are of carrier role. Dog owners and breeders should check all bitches and male dogs prior to coitus and prevent the copulation between stray and valuable dogs. Checking the dogs carefully in kennels before coitus, prevention

of breeding animals, dog licence laws, control of potentially infected reserve and preventing roaming of stray animals would help in controlling rate of the disease (Gurel *et al.,* 2002).

REFERENCES

1. Araujo D.C.C., Antonioli T., Costa T.S., Gomez de Carvalho J.R., Laguna A.G.V., Ramadinha R.H.R. and Fernandes J. F. (2016). Occurrence and location of transmissible venereal tumours in dogs seen at the Universidade Federal Rural do Rio de Janeiro Veterinary Hospital: Oncology Sector between 2010 and 2014. *Rev Bras Med Vet*, **38(3):**277-280.
2. Chun R., Garrett L. and MacEwen E.G. (2001). Cancer chemotherapy. In: *Small Animal Clinical Oncology*. Withrow SJ, MacEwen E.G. (Eds). W.B. Saunders Company, USA.
3. Cizmeci S.U., Kose A.M., Aydin I., Dinc D.A., Maden M. and Kose S.I. (2012). Transmissible venereal tümörlü dişi köpeklerde otojen aşı uygulaması. *Eurasian J Vet Sci*, **28:** 220-223.
4. Cizmeci S.U., Kose A.M., Aydin I., Dinc D.A., Maden M., Kose S.I. (2012). Clinical efficiency of doxorubicin and cisplatin in treatment of transmissible venereal tumour of bitches. *Revue Méd Vét*, **163(11)**: 516-521
5. Das U. and Das K. (2000). Review of canine transmissible venereal sarcoma. *Veterinary Research Communications*, **24:** 545-556.
6. Dobson J.M. and Gorman N.T. (1993). Basic principles of anticancer chemotherapy. In: *Cancer chemoteraphy in small animal practice*. Price C.J., Bedford P.G., Sutton J.B. (Eds). Blackwell Scientific Publications, Great Britain.
7. Ekici H. (2015). Tumours of reproductive organs in carnivores. In: *Birth and infertility in domestic animals*. Alaçam E. (ed). Medisan publications. Ankar. pp 353-356.
8. Erer H. and Kiran M.N. (1993). Tumours of dogs between the years 1985-1992 in Konya. *SU Vet Fak Derg,* **9:** 87-89.
9. Erer H. and Kiran M.N. (2000). Other Tumours and Neoplastic Diseases*, Veterinary Oncology*. Damla Press, Konya.
10. Eze C.A., Anyanwu H.C. and Kene R.O.C. (2007). Review of canine transmissible venereal tumour (TVT) in dogs. *Nigerian Veterinary Journal*, **28:** 54-70.
11. Goldschmidt M.H. and Hendrick M.J. (2002). Tumours of the skin and soft tissues. In: *Tumours in Domestic Animals*. Donald J. M. (ed). Iowa State Press.
12. Gonzalez C.M., Griffey S.M., Naydan D.K., Flores E., Cepeda R., Cattaneo G. and Madewell B.R. (2000). Canine transmissible venereal tumour: a morphological and immunohistochemical study of 11 tumours in growth phase and during regression after chemoteraphy. *Journal of Comparative Pathology*, **122:** 241-248.
13. Gulcubuk A. and Gurel A. (2003). dog tumours observed in Istanbul between the years of 1995 to 2000. *İstanbul Univ Vet Fak Derg,* **19:** 83-91.
14. Gurel A., Kuscu B., Gulanber E.G. and Arun S.S. (2002). Transmissible venereal tumours detected in the extragenital organs of dogs. *Isr. J Vet Med,* **57:** 23-27.
15. Harmelin A., Pinthus J.H., Friedman-Morvinski D., Kaufman K. and Brenner O. (2002). Lack of MHC expression and retention of ultrastructural characteristic by xenograft transmissible venereal tumour cells in SCID mice. *Vet Immunol Immunop*, **86:** 245-249.
16. Jonston S.D., Kustritz M.V.R. and Olson P.N.S. (2001). Disorders of the canine vagina, vestibule and vulva. In: *Canine and Feline Theriogenology*. Kersy R. (ed). W.B. Saunders, Philadelphia.
17. Katzir N., Rechavi G., Cohen J.B., Unger T., Simoni F., Segal S., Cohen D. and Givol D. (1985). 'Retroposon' insertion into cellular oncogene c-myc in canine transmissible venereal tumour. *Proc Natl Acad Sci,* **82:**1054-1058.
18. Konuk C.S., Gurbulak K., Kilicarslan M.R., Gurel A. and Senunver A. (2001) Treatment of transmissible venereal tumours in the bitch using the combination of triple chemotherapeutic agents. *İstanbul Univ Vet Fak Derg,* **27:** 139-149.
19. Kose A.M., Cizmeci S.U., Aydin I., Dinc D.A., Maden M. and Kanat O. (2013). Disseminated Metastasized Transmissible Venereal Tumour Case in a Bitch. *Eurasian J Vet Sci*, **29:1**, 53-57.
20. Liao K.W., Hung S.W., Hsiao Y.W., Bennett M. and Chu R.M. (2003). Canine transmissible venereal tumour cell depletion of B lymphocytes: molecule(s) specifically toxic for B cells. *Vet Immunol Immunop*, **92:** 149-162.
21. Martins M.I.M., Ferreira de Souza F. and Gobello C. (2005). The canine transmissible venereal tumour: etiology, pathology, diagnosis and treatment. *Recent Advances in Small Animal Reproduction*, A1233.0405. www.ivis.org, accessed 17 Dec 2010.
22. Misirlioglu D., Unal E.F., Nak D., Nak Y. and Ozmen O. (1994). General evaluation of tumours which was observed frequently in department of obstetrics and gynecology in the last years I. genital system tumours. *UU Vet Fak Der*g, **13:** 49-56.
23. Morris J. and Dobson J. (2001). Pathogenesis and Tumour Biology. In: *Small Animal Oncology*. Morris J., Dobson J. (eds). Blackwell science Ltd, United Kingdom.

24. Mukaratirwa S. and Gruys E. (2003). Canine transmissible venereal tumour: cytogenetic origin, immunophenotype and immunobiology. A review. *Veterinary Quarterly*, **25:** 102-111.
25. Murgia C., Pritchard J.K., Kim S.Y., Fassati A. and Weiss R.A. (2006). Clonal origin and evolution of a transmissible cancer. *NIH Public Access*, **126:** 477-487.
26. Nak D. (2001). Transmissible venereal tumour in bitches. *YYU Vet Fak Derg,* **7:** 152-155.
27. Nak D., Misirlioglu D., Nak Y., Seyrek-Intas K. and Tek H.B. (2004). Transmissible venereal tumour with mammary gland metastases in a bitch. *Vet Bil Derg,* **20:** 99-102.
28. Nak D., Nak Y., Cangül I.T. and Tuna B. (2005). A clinico-pathological study on the effect of vincristine on transmissible venereal tumour in dogs. *J Vet Med,* **52:** 366-370.
29. Norris A.M. and Withrow S.J. (1984). A review of cancer chemotherapy for pet animals. *Can Vet J,* **25:**153-157.
30. Oglivie G.K. (1998). Chemoteraphy and the surgery patient: Principles and recent advances. *Clin Tech Small Anim Pract*, **13(1):**22-32.
31. Oruc E., Saglam Y.S., Cengiz M. and Polat B. (2011). Cytological diagnosis of breast metastasis of transmissible venereal tumour by fine needle aspiration and the treatment with vincristine sulphate in a dog. *Ataturk University Journal of Veterinary Sciences*, **6:** 63-69.
32. Ozyurtlu N., Bademkiran S., Unver O., Yildiz F. and İcen H. (2008). Abdominal and subcutan inguinal metastasis of Transmissible Venereal Tumour in a bitch. *Dicle Univ Vet Fak Derg*, **1:** 48-51.
33. Perez J., Day M.J. and Mozos E. (1998). Immunohistohistochemical study of the local inflamatory infiltrate in spontaneous canine transmissible venereal tumour at different stages of growth. *Vet Immunol Immunop,* **64:** 133-147.
34. Purohit G.N. (2009). Canine transmissible venereal tumour: a review. *The Internet Journal of Veterinary Medicine*, **6(1)**, http://ispub.com/IJVM/6/1/5692 accessed 17 Dec 2011.
35. Salt S., Yanik K,. Seyrek-Intas D., Alper E., Misirlioglu D., Ozmen O. and Seyrek-Intas K. (2005). Clinic, radiographic, ultrasonographic, scintigraphic and histopathologic findings of canine transmissible venereal tumour (tvt) in two dogs. *Journal of Turkish Veterinary Surgery*, **11:** 48-55.
36. Sankar P., Ramya R. and Mohamed Ali M.G. (2016). Therapeutic management of intranasal transmissible venereal tumour in a dog, *Intas Polivet,* **17(2):** 543-545.
37. Stettner N., Brenner O., Eilam R. and Harmelin A. (2005). Pegylated liposomal doxorubicin as a chemoterapeutic agents for treatment of canine transmissible venereal tumour in murine models. *J Vet Med,* **67:** 1133-1139.
38. Stockmann D., Ferrar H.F., Andrade A.L., Lopes R.A., Cardose T.C. and Luvizotto M.C.R. (2011). Canine transmissible venereal tumours: aspects related to programmed cell death. *J Vet Pathol,* **4(1)**: 67-75.
39. VonHoldt B.M. and Ostrander E.A. (2006). The singular history of a canine transmissible tumour. *Elsevier Cell,* **126:** 445-447.