

# Factors Affecting Treatment Outcome of Canine Transmissible Venereal Tumor: A Retrospective Study

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## Abstract

Canine transmissible venereal tumor (TVT) is a contagious neoplastic disease commonly seen in tropical and subtropical areas in the countries where the control of stray dog population is poor. This study aimed to summarize the current disease status and evaluate factors affecting treatment outcome to vincristine sulfate, which is the chemotherapeutic drug of choice. Studied factors were divided into three categories; general background (i.e. age, gender, breed), specific descriptions (i.e. tumor diameter, tumor site, season of the year), and treatment details (number of vincristine shots, meteorological parameters). One hundred and fifty-seven medical records were included with 100 achieved complete treatments and enrolled in the investigation of treatment outcome. Treatment outcome was classified as complete remission (CR) and partial remission (PR) at the eighth week of treatment. Moreover, the corresponding factors affecting number of vincristine shot to commit CR were evaluated. The age, gender and breed had no effect on treatment outcome or number of vincristine shots. The tumors at the genital area were prone to be recovered (CR) than the others ( $P = 0.08$ ). The tumors with diameter  $>6$  cm required higher number of vincristine shots than that of diameter  $<2$  cm ( $P = 0.05$ ). Moreover, there was a tendency that CR was observed with lower mean temperature at 60 days after enrolled (Temp60) and lower maximal temperature (MaxTemp60) than the PR cases ( $P = 0.08$  and  $P = 0.07$ , respectively). These results may contribute to the prognosis of the treatment with vincristine sulfate particularly when the treatment is influenced by tumor site, tumor size, and climate.

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**Keywords:** canine, disease status, transmissible venereal tumor, treatment outcome

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## Introduction

Canine transmissible venereal tumor (TVT) is an immune-related contagious neoplasm that commonly occurs in the reproductive tract (Das and Das, 2000). The tumor spreads widely in tropical and subtropical urban areas where controls of free-roaming dog population are limited (Ganguly et al., 2013). The transmission occurs through mucosal abrasion during natural coitus or behavioral social expression between dogs, such as sniffing, licking or biting (Otomo et al., 1981). TVT is seen in genital and extra-genital sites (Das and Das, 2000). The prevalence has been reported in different geographical areas: 11% of the total number of tumor in canine patients was observed in Kenya, 32% in Sri Lanka, 23.5 – 28.6% in India and 10% in Maryland (USA) (for review: Das and Das, 2000). Chemotherapy with vincristine sulfate, given intravenously once a week, is the treatment of choice yielding 90% of complete response after treatment (Ganguly et al., 2013). However, partial responses is possible with variable remission rate (Rogers et al., 1998). Treatment outcome is generally individual depending on disease severity, environment and self-immunity (Das and Das, 2000). Experimental inoculation suggests that spontaneous regression of TVT is related to interleukin-6 (IL-6) released by tumor-infiltrating lymphocytes, which restore host defense mechanism by suppressing TGF- $\beta$ 1 and promoting CD8+ T-lymphocyte to initiate tumor cell destruction (Hsiao et al., 2004). This scheme is also presented in natural infected cases treated with vincristine. Thus, host immunity may play an important role in the regression of clinical TVT (Gonzalez et al., 2000).

Scarpelli et al. (2010) revealed that TVT cases required more numbers of vincristine shots to suppress tumor growth during hot and wet season, compared to cool and dry season. This might be due to the heat stress resulting in impairment of peripheral natural killer cell function as shown in the pigs (Hicks et al., 1998). The increase in somatic cell count and incidence of clinical mastitis was also presented in dairy cows raised in high ambient temperature (Lacetera et al., 2005). Thus, the host defense immunity is likely compromised by thermal or heat stress resulting in impaired pathogen clearance.

The aim of this study was to retrospectively find the associations between host details, tumor characteristics, seasons and host responses to vincristine treatments in Bangkok Metropolitan, a city in the tropical area where stray dog population remains a social problem.

## Materials and Methods

**Data Source:** Medical records of 157 TVT cases of total 48,173 cases that visited the Division of Obstetrics, Gynaecology and Reproduction at Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, between March 2007 to March 2012 (60 months) were included. TVT was diagnosed by cytological method: impression smears or fine needle aspiration and stained with commercial modified Giemsa (Diff-Quick, SE Supply, Bangkok, Thailand). TVT cells are characterized by round to oval

cells, high nucleus-cytoplasmic ration, large round eccentric nucleus with coarse chromatin and vacuolated cytoplasm. Tissue biopsy and histopathological method were used to diagnose extra-genital TVT.

**Data category:** Data collected from medical records were: 1) General background including the animal age at the day of diagnosis, gender and breed; 2) Specific details including tumor diameter (cm), tumor site and season; and 3) treatment details (number of vincristine shots and temperature parameter 60 days during treatment). All cases were treated with vincristine intravenously once a week. Treatment outcome were classified into complete remission (CR) and partial remission (PR) after eight vincristine continuous administrations (complete treatment) (Rogers et al., 1998). Complete remission was defined as total regression of TVT mass while partial remission was classified as tumor regressed less than 50% compared with tumor size before treatment.

Of 152 dogs, 100 had regular visits throughout eight treatments, therefore, were enrolled to test effects of gender, breed, tumor site, season, number of vincristine shots and climate parameters during 60 days of treatment on treatment outcome, whereas only 69 and 51 had records for age and tumor diameter, respectively. To evaluate the influence of age, gender, breed, season and tumor diameter on treatment outcome, the data was classified using the following criteria; age was classified into two groups as less than 5 and more than 5 years old, gender was classified as male and female, breed was classified as pure and mixed breed, tumor diameter was categorized into 4 groups as less than 2 cm, 2 to 4 cm, 4 to 6 cm and more than 6 cm, which divided from frequency distribution, tumor sites was classified as genital TVT, extra-genital TVT and mixed type, seasons were divided into hot, rainy and cool seasons.

**Meteorological data:** Meteorological data were collected from Bangkok Metropolitan meteorological station, Thai Meteorological Department, Ministry of Information and Communication Technology, Thailand from January, 2007 to June, 2012. Seasons in Thailand were classified as hot (February 15<sup>th</sup> to May 14<sup>th</sup>), rainy (May 15<sup>th</sup> to October 14<sup>th</sup>) and cool (October 15<sup>th</sup> to February 14<sup>th</sup>). Climates in each season were described as mean temperature, mean relative humidity (RH) and mean temperature heat index (THI). The THI was calculated for each day from the formula:  $THI = DB - [0.55 - (0.55 \times RH)] \times (DB - 58)$ . DB represents the average daily temperature and RH represents the average daily humidity (Kelly and Bond, 1971). Mean temperatures in hot, rainy and cool season were  $29.8 \pm 1.71^\circ C$  (range: 18.8 – 33.7),  $29.1 \pm 1.16^\circ C$  (range: 25 – 33.6) and  $27.8 \pm 1.56^\circ C$  (range: 21.4 – 31), respectively. Mean relative humidity was  $71.8 \pm 6.9\%$  (range: 46 – 92) in hot season,  $77.1 \pm 6.0\%$  (range: 61 – 94) in rainy season and  $67.2 \pm 9.24\%$  (range: 46 – 95) in cool season. Mean THI in hot, rainy and cool seasons were 81.2, 81.0 and 77.8.

**Temperature classification:** The first day dogs enrolled in the treatment program was used to define cases to

each season. Comparisons of means temperature (Temp60), 24-h maximum daily temperature (MaxTemp60), and 24-h minimum daily temperature (MinTemp60) during 60 days period after the onset of treatment between CR and PR cases were calculated. Similarly, means daily RH and THI during 60 days periods after the onset of treatment (RH60 and THI60, respectively) of CR and PR cases were compared.

**Statistical analysis:** Statistical test was carried out by Statistical Analysis System software (SAS version 9.0, Cary, NC, USA). The continuous data were presented as mean  $\pm$  SD and discrete data were presented as percentage. Chi's square was used to compare percentages of CR within ages, genders, breeds, tumor diameters, tumor sites, seasons and numbers of vincristine shots. General linear model (GLM) was used to analyze effects of climate factors (i.e., Temp, Temp60, Max Temp60, Min Temp60, RH60 and THI60) with treatment outcome. The statistical model included the effects of age, gender, breed, tumor diameter, tumor site, season, and number of vincristine shots. Dependent variable was treatment outcome (%CR). Least-square means were calculated from each class of factors and were compared by using least significant difference test.  $P < 0.05$  was regarded to be statistically significant.

**Table 1** Factors affecting treatment outcome classified as complete remission (CR) and partial remission (PR) of 100 dogs developing transmissible venereal tumor.

	CR	PR	p-value
<b>General details</b>			
Age (years), N (%)			
≤5 years	29 (76.32)	9 (23.68)	0.49
>5 years	29 (82.86)	6 (17.14)	
Gender, N (%)			
male	39 (82.98)	8 (17.02)	0.81
female	43 (81.13)	10 (18.87)	
Breed, N (%)			
pure breed	16 (84.21)	3 (15.79)	0.78
mixed breed	66 (81.48)	15 (18.52)	
<b>Specific details</b>			
Diameter, N (%)			
<2 cm	18 (85.71)	3 (14.29)	0.20
2 - 4 cm	16 (100)	0	
4 - 6 cm	6 (75)	2 (25)	
>6 cm	7 (87.5)	1 (12.5)	
Tumor site, N (%)			
genital	70 (83.33)	14 (16.67)	0.08
extra-genital	11 (84.62)	2 (15.38)	
mixed	1 (33.33)	2 (66.67)	
Season, N (%)			
hot	27 (77.14)	8 (22.86)	0.13
rainy	26 (76.47)	8 (23.53)	
cool	29 (93.55)	2 (6.45)	
<b>Treatment details</b>			
Shots, N (%)			
≤5 shots	26 (92.86)	2 (7.14)	0.08
>5 shots	56 (77.78)	16 (22.22)	

Different superscript indicates statistically difference of the data in the same column ( $p < 0.05$ )

## Results

**General background:** The mean body weight of 157 dogs affected with TTV was  $19.6 \pm 7.11$  kg (ranged 4 - 46.5 kg) with the mean age at  $6.4 \pm 3.61$  years (ranged 1 - 21 years). The proportion of male (75/157) and female (82/157) was similar (47.7% and 52.3%, respectively). The dogs were pure breed (24/157) (i.e. 6 bangkaew, 3 golden retriever, 5 labrador retriever and others) and mixed breed (133/157). All cases were petted outside the house and got infection from free-roaming dogs.

**Specific details:** TTV was presented mostly in the genital area (135/157, 86.0%) with minority in the extra-genital area (17/157, 10.8%) and five cases in both areas (5/157, 3.2%). Specific sites of TTV mass recorded in 153 of 157 were penile surface (30/153, 19.7%), bulbous glandis (21/153, 13.7%), prepuce (10/153, 6.5%), vagina (52/153, 34.0%) vulva (23/153, 15.0%) and other areas (17/153, 11.1%). Bleeding was found in most cases (143/153, 93.2%). Nine extra-genital TTV cases and one case with 0.5-cm diameter located in the vagina without bleeding were presented. Of 157 cases, 81 cases were reported of tumor diameter of  $3.8 \pm 3.9$  centimeters (range: 0.2 - 30 centimeters).

**Table 2** Characteristics and clinical findings with transmissible venereal tumor and factors affecting on number of vincristine shots to commit complete remission (76 cases)

	Mean shots	p-value
<b>General details</b>		
Age		
≤5 years	7.2±0.32	0.12
>5 years	6.6±0.28	
Gender		
male	6.9±0.32	0.87
female	7.0±0.28	
Breed		
pure breed	6.5±0.55	0.20
mixed breed	7.1±0.40	
<b>Specific details</b>		
Diameter		
<2 cm	6.6±0.43 <sup>a</sup>	0.05
2 - 4 cm	7.2±0.45 <sup>a,b</sup>	
4 - 6 cm	6.8±0.67 <sup>a,b</sup>	
>6 cm	8.2±0.62 <sup>b</sup>	
Tumor site		
genital	6.8±0.26	0.34
extra-genital	7.6±0.52	
mixed	6.5±0.98	
Season		
hot	7.2±0.36	0.37
rainy	7.3±0.37	
cool	6.6±0.36	

Different superscript indicates statistically difference of the data in the same column ( $p<0.05$ )

**Table 3** Response to vincristine treatment according to temperature parameter 60 days during treatment (N=100)

	CR (N=76)	PR (N=24)	p-value
Temp60 (°C)	28.7±0.11	29.1±0.24	0.08
MaxTemp60 (°C)	33.6±0.12	34.1±0.26	0.07
MinTemp60 (°C)	25.3±0.15	25.7±0.31	0.21
RH60 (%)	72.2±0.58	72.9±1.24	0.60
THI60	79.7±0.21	80.5±0.45	0.13

CR = complete remission, PR = partial remission, Temp60 = mean temperature, MaxTemp60 = mean maximum temperature, MinTemp60 = mean minimal temperature, RH60 = mean percentage of relative humidity, THI60 = mean temperature heat index

**Treatment outcome:** Analysis of treatment outcome was performed in 100 dogs. The rest were excluded due to discontinuation of treatment by some reasons: the dogs had clinical signs of side effects or owner visits were aborted with unknown reasons. Of 100 dogs, 76 cases (76%) were treated with CR and 24 cases (24%) showed PR. Mean numbers of vincristine shot to CR was 5.0±2.6 (median was 5 injections). From this finding, median of this treatment leaded to classify the number of vincristine shot as less than 5 and more than 5 for the analyses. There were no significant differences of treatment outcome (%CR) between/among groups of age, gender, breed, tumor diameter, tumor site, season and number of vincristine shots (Table 1).

However, there was a tendency that the dogs with tumor mass at the genital site had higher percentage of CR than those at both sites ( $p=0.08$ ). Moreover, the dogs that received >5 vincristine shots were prone to commit CR than those received ≤5 shots ( $p=0.08$ ). There were no effects of age, gender, breed, tumor site and season on the number of vincristine shot required to complete remission (Table 2). The dogs with tumor diameter <2 cm required less number of vincristine shots than those with >6 cm tumor masses ( $p=0.05$ ) (Table 2). Also, the PR cases were likely observed at higher Temp60 ( $p=0.08$ ) and MaxTemp60 temperature ( $p=0.07$ ) than the CR cases (Table 3).

## Discussion

**Factors affecting treatment outcome:** The mean age of the dogs in this study was  $6.4 \pm 3.6$  years old, which was higher than the previous studies; 3.9 - 4.5 years (Das and Das, 2000). This finding suggested that older dogs could be infected with TTV similar to younger dogs, although, the infected cases usually were in the age of maximum sexual activity (Das and Das, 2000). TTV in this study presented an equal ration between the male and female, which was similar to the previous study (Scarpelli et al., 2010). While the female dogs have been reported as more susceptible to the infection than the males (Das and Das, 2000), some studies reported more cases in males than females (Osipov and Golubeva, 1976; Brown et al., 1980; Boscos, 1988). Breed susceptibility for TTV infection has not been reported (Ganguly et al., 2013). In this study, most of the infected cases were mixed breed (84.2%) similar to the study by Kunakornsawat et al. (2010). Because mixed breed dogs are mostly free-roaming pets, these dogs have increase chances to get infected with TTV. In this study, most of the tumor masses in the extra-genital organs (9/10) had no bleeding, indicating that bleeding is not a common sign in the extra-genital TTV cases. Tumor characteristics likely influenced treatment outcome. Moreover, in this study, the extra-genital TTV was prone to PR than the genital TTV which was similar to the study of Gaspar et al. (2010) stating that extra-genital TTV responded to vincristine treatment less than genital TTV.

**Factors affecting number of vincristine shots:** In this study, PR was 24%, which was nearly one-fourth of all cases. Said et al. (2009) showed one case (1/30) refractory to vincristine treatment after six-week administration. Scarpelli et al. (2010) suggested that the older cases used more injections than younger cases. However, in the current study, there was no significant difference between age groups. The other factor affecting number of vincristine injections required to CR was the tumor diameter demonstrated by the more vincristine injections needed to reduce the tumor size of over 6 cm than those less than 2 cm, which was similar to the study of Scarpelli et al. (2010) stated that larger tumor required more time to regress completely than a smaller one. Scarpelli et al. (2010) mentioned that larger tumors contain more tumor cells that produce more immunosuppressive factors could retard tumor clearance and require more injections of vincristine treatment.

In this study, there was a tendency that less number of vincristine shots was required in the cool season than the hot season. Moreover, the Temp60 and MaxTemp60 were higher in the PR than CR cases suggesting that high temperature likely decreases tumor reduction via suppression of host immunity system. Similar findings have been reported by Scarpelli et al. (2010) suggesting that TTV treatment by vincristine administration during hot and rainy months was longer and less effective than therapy in cold and dry months. High temperature condition might attribute stress to tumor-bearing dogs, which interferes the cellular immunity and delay tumor clearance resulting in the more vincristine shots for

treatment (Medary et al., 1996; Scarpelli et al., 2010). During high temperature condition, cellular mechanism suppression has been reported in the cows (Lacetera et al., 2005). Hekman et al. (2014) suggested that chronic stress, e.g. due to environmental temperature, might induce the release of glucocorticoids which is associated with leukopenia, lymphopenia and reduction of leucocyte phagocytic activity. From this finding, high temperature could limit tumor clearance in TTV cases.

Understanding factors affecting vincristine therapy might help practitioners to predict the success of treatment outcome. TTVs at extra-genital areas and large tumor sizes seem to compromise the treatment outcome by vincristine sulfate. Moreover, the treatment response in TTV is likely affected by the host's immune response. Immunosuppression during high environmental temperatures may delay tumor clearance.

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## บทคัดย่อ

### ปัจจัยที่มีผลต่อผลการรักษาเนื้องอกติดต่อระบบสืบพันธุ์ในสุนัข: การศึกษาข้อเสนอ

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เนื้องอกติดต่อระบบสืบพันธุ์ในสุนัขเป็นเนื้องอกติดต่อที่พบได้บ่อยในประเทศไทยร้อนและใกล้เขตร้อนที่มีปัญหาการควบคุมประชากรสุนัข การศึกานี้มีจุดประสงค์เพื่อสรุปสถานการณ์ของโรคในปัจจุบันและประเมินปัจจัยที่มีผลต่อผลการรักษาด้วยยาในคริสทีนชั้ลเพตซึ่งเป็นยาหลักที่ใช้ในการรักษา การศึกษาแบ่งปัจจัยที่เกี่ยวข้องออกเป็น 3 กลุ่มคือ ข้อมูลทั่วไปจากตัวสัตว์ (เช่น อายุ เพศ พันธุ์ เป็นต้น) ข้อมูลจำเพาะ (เช่น เส้นผ่านศูนย์กลางของเนื้องอก ตำแหน่งที่พบ ถูกที่ได้รับการรักษา) และ รายละเอียดที่เกี่ยวกับการรักษา (เช่น จำนวนเข็มที่ใช้ในการรักษา ข้อมูลทางอุตุนิยมวิทยา) ข้อมูลดังกล่าวได้รับจากประวัติสัตว์ป่วยจำนวน 157 ฉบับในขณะที่ ประวัติจำนวน 100 ฉบับได้รับการรักษาจนจากระบวนการภายนอก 8 สัปดาห์ของการรักษา จึงนำมาประเมินผลต่อการรักษาซึ่งผลของการรักษาแบ่งเป็น 2 ส่วนคือ การหายแบบสมบูรณ์ (CR) และการหายแบบบางส่วน (PR) และปัจจัยอื่นที่เกี่ยวข้องกับจำนวนครั้งของการฉีดยาในคริสทีนได้นำมาใช้ในการประเมินผลของการรักษาด้วย จากการศึกษาพบว่า อายุ เพศหรือพันธุ์ ไม่ส่งผลต่อการรักษาหรือจำนวนครั้งของการรักษา เนื้องอกที่พบในตำแหน่งอวัยวะเพศมีแนวโน้มที่จะหายมากกว่าเนื้องอกที่พบในตำแหน่งอื่น ( $p=0.08$ ) เส้นผ่านศูนย์กลางของเนื้องอกที่มีขนาดใหญ่กว่า 6 เซนติเมตร ต้องการจำนวนครั้งในการรักษามากกว่าเนื้องอกที่มีขนาดเส้นผ่านศูนย์กลางที่เล็กกว่า 2 เซนติเมตร ( $p=0.05$ ) ยิ่งกว่านั้น มีแนวโน้มที่การหายแบบสมบูรณ์จะเกิดขึ้นในสภาวะอุณหภูมิเฉลี่ยตลอดการรักษา 60 วันและในสภาวะอุณหภูมิเฉลี่ยสูงมากกว่าการหายแบบบางส่วน ( $p=0.08$  และ  $p=0.07$  ตามลำดับ) จากผลดังกล่าวอาจให้ข้อมูลเพื่อทำนายผลต่อการรักษาด้วยยาในคริสทีนชัลเพต หากมีผลกระทบจากตำแหน่งและขนาดของเนื้องอก รวมทั้งสภาพอากาศในสภาพแวดล้อมนั้นๆ

**คำสำคัญ:** สุนัข สถานการณ์ของโรค เนื้องอกติดต่อระบบสืบพันธุ์ ผลของการรักษา

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