

Diagnose Enforcement of Case Canine Transmissible Venereal Tumor (CTVT) on Domestic Dog

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ABSTRACT

Canine Transmissible Venereal Tumor (CTVT) also known as venereal sarcoma is an inflammation mediated by reticuloendothelial tumors and infects the canine genitalia. This study aimed to diagnose CTVT cases in domestic dogs by looking at clinical symptoms, macroscopic, hematologic, blood chemistry and cytology, and also by histopathological features. Based on clinical symptoms or anatomical pathology (macroscopic) on CTVT appears in the form of lumps and blood mucus on the vestibule. On the vulva were visible hemorrhagic fluid secretions. The nodules that form on the genital organs resemble cauliflower and they were fragile, hyperemic, hemorrhagic and multilobular masses. Hematological and cytological examinations showed a decrease in the value of red blood cells, hemoglobin, and hematocrit which as a sign of anemia and showed increase in aspartate transaminase (AST), alanine transaminase (ALT), glucose, and ureum values. Meanwhile, the decrease occurred in total protein, albumin, globulin, and A/ G ratio. The results of the CTVT histopathology examination by biopsy of vulva vagina indicate perivascular inflammatory cell infiltration, neovascularization, and proliferation of fibroblast cells accompanied by necrosis and the formation of cytoplasmic vacuole. This paper is expected to increase knowledge and information about confirmation of case diagnosis canine transmissible venereal tumor on domestic dog.

Keywords: CTVT, Canine Transmissible Venereal Tumor, Venereal sarcoma, Domestic dog tumor

1. INTRODUCTION

Canine transmissible venereal tumor (CTVT) is an inflammation mediated by reticuloendothelial tumors and infects parts of the dog's genitalia [1]. CTVT tumors are transmitted during coitus in sexually mature young dogs and transmitted through the physical transfer of viable tumor cells through direct contact with injured skin or mucous tissue [2]. This is due to the unrestricted sexual behavior of the dog resulting in overpopulated. At the time of coitus there is often bleeding due to friction of the mucosa from the penis with the vagina so that transmission from the tumor will be easy. Canine transmissible venereal tumors usually infect both male and female genital devices. However, female dogs are more often exposed to these tumors [3].

One of the tumor diseases that often infect domestic dogs is the canine CTVT or venereal sarcoma. CTVT cases are often a serious problem around the world that occurs at the same frequency in both males and females. Canine transmissible venereal tumors mostly occur in dog populations with free roaming without marital control in warm-temperature tropical environments [4]. According to Jacob et al [5], the CTVT incidence occurred in sexually active adult dogs with age range from 2 to 5. The CTVTs are common in domestic dogs released due to uncontrolled dog sexual habits and actively transmitted through the need for genitalia mucosa during coitus [6].

Exfoliation and transplantation of tumor cells during coitus are the main causes of metastasis into the genital mucosa, as well as to the nose or mucosa of the mouth,

when mating or licking the genitals of dogs affected by CTVT. Tumor growth can generally be seen within 2-6 months after first mating, but can grow slowly for years or more then become invasive and eventually become malignant and mutate. Metastasis reported less than 5-17% of total cases [3].

Diagnosis of CTVT cases in domestic dogs can be done clinically by looking at the visible symptoms. According to Das and Das [7], clinical symptoms of CTVT in dogs are characterized by swelling of the genitalia organs in the form of papillae and small reddish nodules. The method of examination that can be done to enforce the diagnosis of CTVT is to conduct laboratory examinations in the form of hematological examinations, blood chemistry, macroscopic examinations, and microscopic examinations to see the morphology of cells suspected of experiencing CTVT and several other tests cellularly and molecularly needed to determine the character of CTVT [8]. According to Loremier and Fan [9], these examinations are indispensable for the determination of CTVT diagnosis.

In the case of CTVT, clinical marks vary depending on the location of the tumor. Macroscopically, tumors in female dogs look the same as in male dogs and their occurrence can be localized in the vestibulum area, or vaginal caterine, spreading to the vulva so that it often causes deformities in the perineal region. Dogs with tumor sites in their genital area will see fluid mixed with blood from their genitals. The liquid will often be confused with the incidence of erethritis, cystitis, or prostatitis [10]. Cytology examination of CTVT generally shows cell mass growth.

Based on the description above the diagnosis of domestic dogs suspected of suffering from Canine Transmissible Venereal Tumor (CTVT) can be properly enforced so it is necessary to conduct further examination and analysis macroscopic, hematological, blood chemical and microscopic images.

2. MATERIALS AND METHODS

2.1 Signalment and Anamneses

Examination in the patient begins with signaling and anamneses with the owner of the animal. Signals obtained from clients include the name of the animal, breed of the animal, gender, age, name of the owner, address, and telephone number. Furthermore, anamneses with animal owners is what the client's complaint to the patient, how the status of eating, drinking, urination, and defecation of the patient a few days before being taken to the KIHA-ZIMA veterinary clinic.

2.2 Clinical Symptom Screening

Clinical examination is a series of activities in establishing a diagnosis. Inspections are inspection, auscultation, palpation, and [11]. Examination of clinical symptoms includes the state of the skin/hair, natural holes, the presence of ectoparasites, mucous color. General examination of the outer state includes the type of animal, sex, age, dental condition, skin condition, mucous membranes of the eyes, oral cavity, ears, neck, abdomen, the inside of the thighs the possibility of vesicles, or other lesions. Joints, soles of the feet, base of the tail, around the anus, and genitals and udder.

Diagnose enforcement in cases of domestic dogs experiencing CTVT is carried out through macroscopic, hematological, blood chemistry and histopathological examinations. Macroscopic examination is done by initiating the vulva-vagina that has excess swelling that forms the inflammatory mass of irregular tumors (cauliflower). Hematology and blood chemistry examination CTVT using hematology analyzer, blood examination done is Complete Blood Count. Blood collection is carried out in the veins of cephalic antibrachia as much as 1.5 ml. The blood as much as 0.5 ml is inserted into a vacuum tube with anticoagulant EDTA for complete blood count examination with hematology analyzer.

CTVT cytology examination is done by swab on the mucosa vulvovaginal using sterile cotton bud. Next the tip of the cotton swab is applied along the object glass/slide preparation. Then the preparation is colored using giemsa consisting of a fixative solution, eosinophilic solution, and basophilic solution. Coloring begins from the dyeing of the preparation slide on a fixative solution for 1-2 minutes. After that on the eosinophilic solution for 30 seconds and lastly on the basophilic solution for 15 seconds, then rinsed with running water and dried. After that the preparation slide is examined under a microscope with a magnification of 100x and 400x.

Histopathological examination of CTVT is done by means of tumor mass first fixated into NBF 10% for 24 hours. After that stopping point in alcohol 70% for 12 hours, then dehydrated into storied alcohol 80%, 90%, 95%, and absolute alcohol I and II for 2 hours each. Then sample in clearing in xylol liquid I and II 45 minutes each. Furthermore, samples are infiltrated in paraffin I, II, III each 45 minutes, then done embedding process until it becomes a paraffin block. The sample is slicing using a microtome with a thickness of 5 microns and then placed on top of the object glass and incubated into a warmer slide to be continued in the coloring process.

Coloring begins with the process of deparaffinization using xylol I for 5 minutes, xylol II for 2 minutes. Then followed by the rehydration process with alcohol decreased from absolute alcohol I and II, alcohol 96% I and II, alcohol 90% respectively for 2 minutes, then put in water. Then the slide is put in a hematoxylin solution for 5 minutes, then put in water. After that put in eosin solution for 5 minutes, then done dehydration process again with alcohol 96% I and II, absolute I and II two dip each. Furthermore, the clearing process is carried out with xylol I, II, and III for 3 minutes each. Then done mounting with entellan. Observations were made with light microscopes and photographed with photomicrographs [14].

Data obtained are presented descriptively based on changes in clinical, macroscopic, hematological, blood chemistry, cytology and histopathology CTVT in domestic dog.

3. RESULTS

3.1 Signalment and Anamneses

A female domestic breed named Finni aged 1.3 years, 7.8 kg body weight with reddish brown hair color is examined at the KIHA-ZIMA Pet Care Veterinary Clinic, Payakumbuh, West Sumatra. Anamneses results from dog owner Finni said that there was a drastic decrease in appetite since 5 weeks after returning from hunting activity, easily tired and slow in responding to calls. There is a lump accompanied by a reddish discharge that comes out of the vagina since a week ago, and the right leg of the back is lifted during walking.

3.2 Clinical Symptom Screening

The result of clinical symptom examination showed that mucosa in mouth looks anemic, the heart rate was 78x/minute, the respiratory rate was 18x/min, body temperature 39.5 °C and Body Condition Score II. During examination by inspection and palpation, there was a bleeding mucus (discharge) (Figure 1) and the presence of a tender swelling of vulvavaginal organ (Figure 2).

3.3 Laboratory Examination

3.3.1 Macroscopic Examination

The results of macroscopic examination showed the presence of necrotic tumor mass with the white color (Figure 3) and a multinodular that grows irregularly in shape look like a cauliflower with a fragile consistency and hemorrhagic (Figure 4).

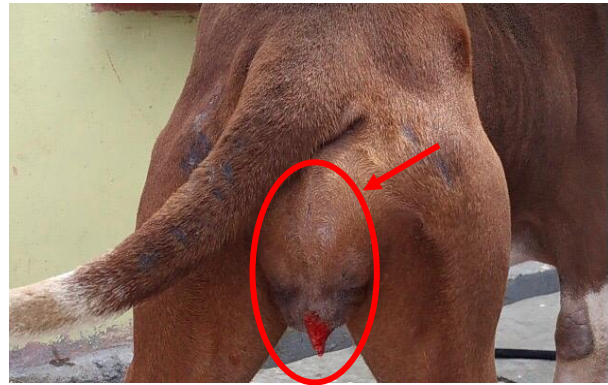


Figure 1 Mucus (discharge) hemorrhagic in the vulvavagina.



Figure 2 Presence of a tender swelling of vulvavaginal organ



Figure 3 Necrotic tumor mass with the white color



Figure 4 Multinodular like cauliflower

3.3.2 Hematology and Blood Chemistry Examination

The results of the CTVT blood hematology and chemical examination in domestic dogs are shown in Table 1.

Table 1. Hematology examination

Examination	Result	Normal range
Hematology		
White blood cell ($10^3/\mu\text{L}$)	↑ 47.1	16.0 – 17.0
Red blood cell ($10^6/\mu\text{L}$)	↓ 1.12	5.5 – 8.5
Hemoglobin (g/dL)	↓ 3.3	12.0 – 18.0
Hematocrit (%)	↓ 8.1	37.0 – 55.0
MCV (fL)	72.3	60.0 – 77.0
MCH (Pg)	↑ 29.4	19.5 – 24.5
MCHC (g/dL)	↑ 40.7	32.0 – 36.0
Platelets ($10^3/\mu\text{L}$)	337	200 – 500
Lymphocytes (%)	16.1	12.0 – 30.0
Granulocytes (%)	↑ 83.9	60.0 – 80.0
Lymphocytes ($10^3/\mu\text{L}$)	↑ 7.6	1.0 – 4.8
Blood chemistry		
AST (U/L)	↑ 173	8.9 – 48.5
ALT (U/L)	↑ 154	8.2 – 57.3
Ureum (mg/dL)	↑ 56.9	10 – 20
Creatinine (mg/dL)	↓ 0.72	1 – 2
Total protein (g/dL)	↓ 4.8	5.4 – 7.5
Albumin (g/dL)	↓ 1.6	2.6 – 4.0
Globulin (g/dL)	3.2	2.7 – 4.4
A/ G ratio	↓ 0.50	0.6 – 1.1

NOTE:

- MCV : Mean Corpuscular Volume
- ALT : Alanine Transaminase
- MCH : Mean Corpuscular Hemoglobin
- AST : Aspartate Transaminase

3.3.3 Cytology Examination

The results of cytological examination showed that the cells had a polyhedral round shape with a high level of cellularity Figure 5 that filled the viewpoint. In addition, the cytoplasm of CTVT cells has basophilic vacuoles and several mitotic cells that showed in Figure 6.



Figure 5 High cellularity is seen to form a pattern (in Circle), Giemsa staining: 100X

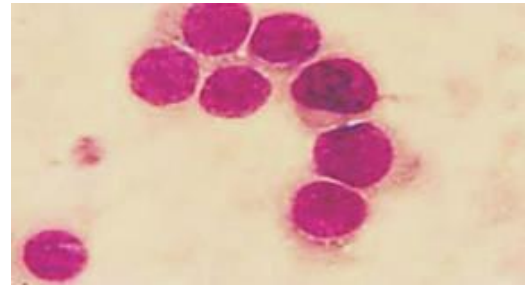


Figure 6 Polyhedral round cells, mitosis (Blue arrow), and cytoplasm with vacuoles (Black arrow). Giemsa staining: 400X.

3.3.4 Histopathology

The histopathology showed the infiltration of perivascular inflammatory cells, neurovascularization, and proliferation of fibroblast cells accompanied by necrosis and formation of cytoplasmic vacuoles (Figure 7).

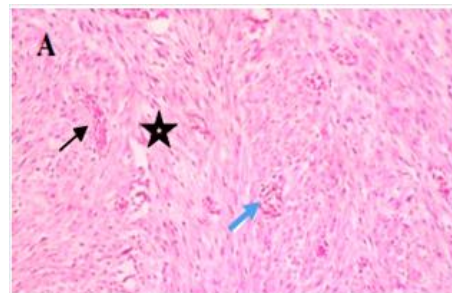


Figure 7 The presence of perivascular inflammatory cell infiltration (arrow blue), neurovascularization (black arrow) and proliferation of Fibroblast cells (asterisk), Hematoxylin Eosin staining :100 x

4. DISCUSSION

Based on clinical symptoms or anatomical pathology of Canine Transmissible Venereal Tumor (CTVT) that appears in the form of lumps and blood mucus in vestibula, and incision, it will appear irregular and multinodular tumor mass growth [12]. Nodules that form on the genitalia organs resemble cauliflower and the lesions are brittle, hyperemic, hemorrhagic, and multilobular. This is due to the allogenic implantation of tumor cells that aggressively damage local skin and muscle tissue, excesses, and metastasis potential [3,13]. CTVT tumor cells consist of forms ranging from small to large (5 μm – 10 cm), soft or hard, gray to reddish, nodular and papillary forms in the vulva-vagina (Aiello, 2002). The picture of a tumor in a female dog is usually present in the vestibule or vaginal canal, transverse to the vulva [14]. In the vulva part of the dog infected with CTVT is seen a secretion of hemorrhagic fluid that can cause permanent anemia. This fluid can trigger males around and such circumstances in female dogs are often

confused with estrus and reproductive disorder diseases [15].

CTVT hematology examination in domestic dogs showed a decrease in red blood cells, hemoglobin, and hematocrit which are a picture of anemia. Anemia that occurs in this case is hypochromic normocytic anemia because the mean corpuscular volume (MCV) value is normal and the mean corpuscular hemoglobin concentration (MCHC) is in an abnormal state. Anemia that occurs in the case of Finni dogs occurs due to the presence of inflammatory tumors or neoplasia in the vagina supported by clinical examination of pale mucous membranes, anorexia, cachexia, dyspnea, and lumps in the vulvavagina.

In addition, hematological examination results also showed leukocytosis and granulocytosis that can occur in inflammatory conditions, response to epinephrine, response to corticosteroids, pain and stress [16]. Leukocytosis is a granulocytosis that is thought to be dominated by neutrophilia especially in the vagina and perianal glands and also occurs due to an increase in endogenous corticosteroids in response to pain and stress [17].

Results of blood chemical examination in CTVT domestic dogs showed increased values of aspartate transaminase (AST), alanine transaminase (ALT), glucose, and ureum. While decreases were found in the total protein, albumin, globulin, and A/G ratio of dogs. Increased ALT and AST are possible due to hepatocellular disease. The damage to acute liver cells that occur is characterized by an increase in the value of ALT higher than the value of AST.

Hypoproteinemia, hypoalbuminemia, and hypoglobulinemia that occur are thought to be due to impaired liver function that accompanies the event. Hyperglycemia can occur due to impaired secretion of the insulin, decreased hepatic function as a storage place of glycogen [18]. In addition, cases of CTVT in domestic dogs also cause an increase in blood glucose due to lowering of liver function as a storage place for glycogen and hyperglycemia occurs naturally during the period of infection and inflammation. When the body is stressed, endogenous catecholamines released among others serve to increase blood glucose levels. The amount of increase varies with each animal and the inflammatory response.

The results of CTVT cytology examination in Domestic Dogs showed that the cellularity level is high and appears to form a diverse pattern (non-uniform). This is in accordance with the Pashkevych *et al.* research report [19], Stockmann *et al.* [2], which states CTVT tumor cells are cytologically visible patterns vary with the shape of rounded cells to polyhedral homogeneous hypercormatic. Furthermore, the results of CTVT cytology showed the presence of cytoplasm

that is vaccinated and some cells undergo mitosis that signify the regression phase of the tumor. This is very helpful in diagnosing morphology and CTVT tumor cell location [5, 20, 21].

Results of CTVT histopathological examination in Domestic Dogs from vulvavagina biopsy showed the presence of infiltration of perivascular inflammatory cells, neovascularization, and proliferation of fibroblast cells accompanied by necrosis and the formation of cytoplasmic vacuoles. The results of this examination are in accordance with the results of research by Zayas *et al* [22], which suggests that the proliferation of fibroblast cells indicates the expansion of tumor cells in tissues or organs. Fibroblasts or connective tissues have a function as a supporting framework and binders of parenchymal neoplastic cells forming tissue masses. This phenomenon is identical to the wound healing process. The infiltration of perivascular cells, in the case of CTVT indicates the body's immune response to tumors [23, 24]. According to Gonzalez *et al.* [25], inflammatory cells generally appear in the early stages of the appearance of tumors associated with the process of apoptosis in tumor cells against degeneration in the cells.

5. CONCLUSION

The study has showed some changes in hematology, cytology and biochemistry as well as and histopathological findings of vulva vagina of domestic dogs suffered from the canine transmissible venereal tumor (CTVT). The information can be used as supporting data for confirmation of the disease in the CTVT suspected dogs.

AUTHORS' CONTRIBUTIONS

All authors were equally contributed to the design, preparation, and editing of the manuscript.

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REFERENCES

- [1] M.H. Goldschmidt, M.J. Hendrick, Tumours of the skin and soft tissues. In: Tumours in Domestic Animals, Iowa State Press, Iowa, 2002.
- [2] D. Stockmann, H.F. Ferrari, A. L. Andrade, R.A. Lopes, T.C. Cardoso, M.C.R. Luvizotto, Canine transmissible venereal tumor; aspects related to programmed cell death, Brazil J. Vet. Pathol. 4 (2011) 67-75.

- [3] K. Berata, I.B.O. Winaya, M. Adi, W. Adyana, I.M. Kardena, *Patologi Veteriner Umum, Swasta Nulus, Denpasar*, 2011
- [4] S. Hantrakul, K. Narumol, K. Sunee, T. Tawewan, P. Ammart, P. Saranya, Clinical pharmacokinetics and effects of vincristine sulfate in dogs with transmissible venereal tumor (TVT). *Vet. Med. Sci.* 12 (2014) 1549-1553.
- [5] S. Amaral, S.B. Silva, I. Ferreira, L.S. Fonseca, F.H.E. Andrade, L.F.J. Gaspar, N.S. Rocha, Cytomorphological characterization of transmissible canine venereal tumor. *Revista Portuguesa Ciencias veterinarias* 3 (2007) 53-60.
- [6] S.D. Johnston, K. Kamolpatana, M.V. Root-Kustritz, G.R. Johnston, Prostatic disorders in the dog. *An. Rep Sci.* 60-61 (2000) 405- 415.
- [7] U. Das, A.K Das, Review of canine transmissible venereal sarcoma, *Vet. Res. Commun.* 24 (2000) 545-556.
- [8] Ganguly, U. Das, A.K. Das, Canine transmissible venereal tumor: a review, *Vet. Comp. Oncol.* 1 (2013) 1-12.
- [9] P. De Lorimier, T.M. Fan, *Small animal clinical oncology*, St. Louis, MO, Elsevier, 2007.
- [10] Purohit, Canine transmissible venereal tumor: a review, *J. Vet. Med.* 1 (2009) 1-6.
- [11] S. Widodo, D. Sajuthi, C. Kholiq, A. Wijaya, R. Wulansari, R.P.A. Lelana, *Diagnosa klinis hewan kecil*, IPB Press, Bogor, 2011.
- [12] Z.G. Ucmak, I. Kirsan, M. Ucmak, O.B. Erdogan, A. Gurel, Clinical approaches for genital and extragenital metastasis of transmissible venereal tumor in a bitch with ovarian remnant syndrome, *Ankara Univ. Vet Fak Derg.* 66 (2019) 417-421.
- [13] J.K. Murgia, S.Y. Pritchard, Kim, Clonal origin and evolution of a transmissible cancer cell, *J. Vet. Med.* 126 (2006) 477-487.
- [14] M.N. Erunal, M. Fidnik, S. Aslan, Use of exfoliative cytology for diagnosis of transmissible venereal tumor and controlling the recovery period in the Bitch, *Tierarzt wochenschr* 5 (2000) 107-175.
- [15] I. Martins, F.S. Ferreira, C. Gobello, *The Canine Transmissible Venereal Tumor: Etiology, Pathology, Diagnosis and Treatment*, Faculty of Veterinary Science, Argentina, 2005.
- [16] S.I.O. Salasia, H. Bambang, *Patologi Klinik Veteriner: Kasus Patologi Klinis*. Samudra Biru, Yogyakarta, 2010.
- [17] R. Duncan, K.W. Prasse, E.A. Mahaffey, *Veterinary Laboratory Medicine Clinical Pathology*, Iowa State University Press, Iowa, 1995.
- [18] S. Letimer, Duncan and Prasse's *Veterinary Laboratory Medicine, Clinical Pathology*, Wiley-Balckwall 5th Ed, United States of America, 2011.
- [19] Pashkevych, S. Volodymyr, S. Natalia, *Diagnostic Methods of Canine Transmissible Venereal Sarcoma*, Eureka: Health Sci. (3) (2018) 67-75.
- [20] Alkan, F. Satilmis, M. E Alcigir, M. B Kivrak, I. Aydin, clinicopathological evaluation of disseminated metastases of transmissible venereal tumor in spayed bitch, *Acta Sci. Vet.* 1 (2017) 2-5.
- [21] R. Thangathurai, A. B. Gurusamypalayam, D. Sarayamuthu, B. Periasamy, S. Palani, S. Soundarapandian, M.M. Bhakthavasalam, Cytological diagnosis and its histological correlation in canine transmissible venereal tumor, *Veterinarski Arhiv.* 5 (2008) 69-76.
- [22] Y.R. Zayas, M. A. F. Molina, R. T. Guerra, C. R. Padilla, Evaluation of a canine transmissible venereal tumor cell line with tumor immunity capacity but without tumorigenic property. *J. Vet. Res.* 63 (2019) 1-9.
- [23] M. Tella, V.O. Taiwo, Complete regression of transmissible venereal tumour (TVT) in Nigerian Mongrel dogs with vincristine sulphate chemotherapy. *Afr. J. Biomed. Res.* 7 (2004) 133-138.
- [24] R. Marcos, M. Santos, C. Marrinhas, E. Rocha, Cutaneous transmissible venereal tumor without genital involvement in a prepubertal female dog, *Vet. Clin. Pathol.* 35 (2006) 106-109.
- [25] M. Gonzales, S.M. Griffey, D.K. Naydan, E. Flores, R. Cepeda, G. Cattaneo, B.R. Madewell, Canine transmissible venereal tumor: a morphological and histochemical study of 11 tumor in growth phase and during regression after chemotherapy. *J. Compar. Pathol.* 122 (2000) 241-248.