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Original Article

Immunohistochemical expression of vimentin, E-cadherin, and CD45 in natural cases of canine cutaneous round tumors

Expressão imuno-histoquímica de vimentina, E-caderina e CD45 em casos naturais de tumores redondos cutâneos em cães

S. S. Oda[a], M. F. Tawfik[a], S. I. Othman[b], H. A. Rudayni[c], A. A. Allam[c,d*] and A. F. Khafaga[a*]

[a]Alexandria University, Faculty of Veterinary Medicine, Pathology Department, Edfina, Egypt
[b]Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, Riyadh, Saudi Arabia
[c]Imam Muhammad Ibn Saud Islamic University, College of Science, Department of Biology, Riyadh, Saudi Arabia
[d]Beni-suef University, Faculty of Science, Department of Zoology, Beni-suef, Egypt

Abstract

Round cell tumors are common cutaneous lesions in dogs, with increased occurrence percentages among different skin tumors. This study aimed to investigate the frequency as well as gross and pathological characteristics of round cell tumors in natural cases of tumorous dogs in relation to breed, sex, and age. Moreover, it aimed to evaluate the immunohistochemical expression of a panel of immunohistochemical stains, including vimentin, E-cadherin, and cluster of differentiation (CD45) as an adjunct technique for the differential diagnosis of cutaneous round cell neoplasm. Data were collected from 64 dogs of both sexes (36 females and 28 males), various breeds, and different ages (8 months to 7 years). The histopathological nature of neoplastic growth was reported, and neoplasm prevalence was classified using age, sex, breed, and site on the body. We observed 48 cases of transmissible venereal tumors, 12 cutaneous histiocytomas, and 4 histiocytic sarcoma. Immunohistochemical characterization revealed an intense positive immunoreactivity for vimentin in transmissible venereal tumor cells and moderate positive immunoreactivity for E-cadherin and CD45 in cutaneous histiocytoma and histiocytic sarcoma cells. In conclusion, the canine transmissible venereal tumor was the most frequent form of round cell tumor; thus, a definitive cutaneous neoplasm diagnosis should be based on histopathological morphology and immunohistochemical findings.

Keywords: dogs, histopathology, immunohistochemistry, round cell tumors.

Resumo

Os tumores de células redondas são lesões cutâneas comuns em cães, com percentuais de ocorrência aumentados entre os diferentes tumores de pele. Este estudo teve como objetivo investigar a frequência e as características macroscópicas e patológicas dos tumores de células redondas em casos naturais de cães tumorais em relação à raça, sexo e idade. Além disso, objetivou avaliar a expressão imuno-histoquímica de um painel de colorações imuno-histoquímicas, incluindo vimentina, E-caderina e cluster de diferenciação (CD45) como técnica adjuvante para o diagnóstico diferencial de neoplasia cutânea de células redondas. Foram coletados dados de 64 cães de ambos os sexos (36 fêmeas e 28 machos), de diversas raças e diferentes idades (8 meses a 7 anos). A natureza histopatológica do crescimento neoplásico foi relatada e a prevalência da neoplasia foi classificada de acordo com idade, sexo, raça e localização no corpo. Observamos 48 casos de tumores venéreos transmissíveis, 12 histiocitomas cutâneos e 4 sarcomas histiocíticos. A caracterização imuno-histoquímica revelou intensa imunorreatividade positiva para vimentina em células tumorais venéreas transmissíveis e imunorreatividade positiva moderada para E-caderina e CD45 em células de histiocitoma cutâneo e sarcoma histiocítico. Dessa forma, é possível concluir que o tumor venéreo transmissível canino foi a forma mais frequente de tumor de células redondas; assim, um diagnóstico definitivo de neoplasia cutânea deve basear-se na morfologia histopatológica e nos achados imuno-histoquímicos.

Palavras-chave: cães, histopatologia, imunohistoquímica, tumores de células redondas.

*e-mail: asmaa.khafaga@alexu.edu.eg; aallam@imamu.edu.sa
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1. Introduction

One of the most typical locations for neoplasms is the skin, which accounts for 9.5%-51% of all neoplasms in dogs (Bronden et al., 2010). Undifferentiated cutaneous and subcutaneous tissue neoplasms represent a diagnostic defiance to the surgical pathologist. Small round and spindle cell tumors often pose differential diagnosis problems when examined under light microscopy (Choi and Ro, 2018). Round cell tumors are usually cutaneous or subcutaneous masses; however, most also appear at other sites (Lafta and Alabbody, 2020). They are common skin lesions in dogs and account for many skin tumors. (Cora et al., 2017).

Round cell tumors on the canine skin are a diverse group of neoplastic conditions with a similar morphologic structure but different histological origins, along with basically various prognoses and treatments. They consist of discrete round to oval rather than fusiform cells (Daniel and Fabiano, 2022). Generally, the cancers include poorly differentiated mast cell tumors, cutaneous lymphoma, canine cutaneous histiocytoma, and plasmacytoma (Moore, 2020). However, some researchers have also considered transmissible venereal tumors, amelanotic melanoma, histiocytic sarcoma, and neuroendocrine tumors in this group (Tostes et al., 2017; Erich et al., 2018; Goldschmidt and Goldschmidt, 2020).

In veterinary medicine, immunohistochemistry is an important technique for characterizing neoplastic diseases and is an essential adjunct to light microscopy. Because of similar morphologies observed in neoplastic cells, routine histopathologic techniques are insufficient to diagnose several canine cutaneous round-cell tumor cases precisely. Moreover, veterinary oncologists need specific diagnoses to contribute to further treatment (Ramos-Vara et al., 2008). Previous investigations have reported that histopathological diagnoses of a marked percentage of canine cutaneous round cell tumors have been modified post-immunohistochemical analysis (Araujo et al., 2012; Paździor-Czapula et al., 2015).

Cutaneous histiocytomas are benign neoplasms that emerged from Langerhans cells (Baines et al., 2008). Langerhans cell-based immunohistochemical markers include E-cadherin, major histocompatibility complex (MHC)-I, MHC-II, intercellular adhesion molecule (ICAM)-1, cluster of differentiation (CD)11b, CD11c, CD1a, CD1c, CD18, CD44, CD45, CD45RA, and CD49d (Ramos-Vara and Miller, 2011; Elizabeth and Jeanine, 2016). Although most antibodies associated with these markers can be utilized in frozen or fresh tissue, the veterinarian might not have access to them. Only antibodies against E-cadherin, CD45RA, CD45, CD11d, and CD18 are allowed in formalin-fixed, paraffin-embedded tissues. (Fulmer and Mauldin, 2007; Gross et al., 2009).

A naturally occurring contagious round cell neoplasm that mostly affects both sexes' external genital mucosae is known as a canine transmissible venereal tumor (Danielle et al., 2021). The tumors are occasionally referred to as canine condyloma, venereal granuloma (Alexandre et al., 2022), canine transmissible sarcoma, contagious lymphoma, or sticker tumors and are recognized only in dogs (Park et al., 2006; Schlafer and Foster, 2016). They are distributed by living tumor cells that spread during coitus from afflicted animals or by rubbing or licking (Gross et al., 2009). Clinically, the tumor presents as a single, red, friable, often "cauliflower-like mass" (Papazoglou et al., 2001). It is usually difficult to apply a differential diagnosis with various canine round cell tumors like histiocytomas, lymphomas, melanomas, poorly differentiated mast cell tumors, poorly differentiated carcinomas, and amelanotic epithelioid (Vail and Withrow, 2020). There are still unidentified specific markers for transmissible venereal tumor cells; however, immunoexpression for vimentin (Pereira et al., 2000), lysozyme (Park et al., 2006), and CD45RA (Gross et al., 2009) is associated with histopathological analyses and supports neoplastic diagnoses.

The prevalence of neoplastic diseases, especially round-cell neoplasms, is continuously rising in veterinary medicine, necessitating ongoing research and development from the field's tumor experts. In this sense, observational studies are a practical technique, a good information source, and the evolution of neoplastic diseases. Based on clinical and histological characteristics, these data may create prognosticating recommendations. This may then be transformed into reliable empirical data that can serve as the basis for laboratory investigation. This study's goal was to ascertain the prevalence of canine cutaneous round cell tumors in naturally examined cases of dogs in relationship to breed, age, sex, and site in dogs in addition to the expression of vimentin, E-cadherin, and CD45 in immunohistochemistry as additional histopathological techniques.

2. Materials and Methods

2.1. Animals and ethical statement

One hundred and forty dog skin tumor samples were collected from the Alexandria Veterinary Medicine Directorate and the Private Clinics in Alexandria, Egypt, between February 2020 and February 2022. Dogs of various breeds and both sexes (thirty-six females and twenty-eight males) were included, with ages ranging from 8 months to 7 years. This research was approved by the Animal Experiment Local Ethics Committee of the Faculty of Veterinary Medicine, Alexandria University, Egypt.

2.2. Clinical investigation and surgical intervention

Case history and clinical symptoms of all animals was carfully reported and complete surgical removal of the tumor was conducted. For all dogs, premedication was administered with xylazine at 1 mg/kg intramuscularly (IM), whereas anesthesia was induced with ketamine at 15 mg/kg IM (Niwar and Mohammed, 2023). After complete sedation, a wide excision of the tumor was conducted.

2.3. Gross and histopathological examinations

Samples were collected from various sites, including the external genitalia mucosal surface of male and female dogs, flank, hip, pastern region, and ventral aspect of the tail.

Resected samples ranged in size from 5 to 50 mm. After careful gross examination, tissue specimens were trimmed and rapidly fixed in a neutral buffered formalin solution (10%) for histopathological and immunohistochemical examination. The paraffin embedding method was applied to the fixed specimens. Samples were quickly dehydrated using ethanol of increasing concentration, cleaned in xylene, and then embedded in paraffin wax. Five micrometer-thick sections were cut. Hematoxylin and eosin (H&E) staining was applied to the prepared slide sections (Bancroft and Gamble, 2013).

2.4. Immunohistochemical examinations

Tissue sections were treated following the established methods from the National Cancer Institute in Cairo Using an UltraVision Large Volume Detection Anti-Polyvalent horseradish peroxidase (HRP) system from Thermo Fisher Scientific. Paraffin sections of about four micrometer-thick were prepared by deparaffinizing them in xylene, rehydrating them in decreased alcohol concentrations, and then washing them in distilled water. Antigen retrieval was carried out by boiling for 10–20 minutes in 0.1 M citrate buffer (pH 6), followed by 20 minutes of cooling to room temperature. Endogenous peroxidase was blocked for 30 minutes with 3% hydrogen peroxide in methanol at 4°C after washing in distilled water. Then, sections were rinsed in phosphate-buffered saline (PBS) (pH 7.4). Non-specific reactions were blocked at room temperature for 60 minutes in 10% normal blocking serum (Ramos- Vara et al., 2008). Then, primary antibodies were included, and they were incubated at 4°C overnight. Primary monoclonal antibodies detected vimentin. (1:50–1:100, Dako, M0725), E-cadherin (1:50, Dako, M3612), and CD45 (1:200, Dako, M0701). After being cleaned in PBS, sections were incubated for 60 min with biotin-conjugated goat anti-polyvalent IgG antiserum using the Histofine kit from Nichirei Corporation in Tokyo, Japan. Following a PBS wash, the sections were incubated with streptavidin-peroxidase conjugate (Histofine kit, Nichirei Corporation, Tokyo, Japan) for 30 minutes. Streptavidin-biotin complexes were seen using a 3,3′-diaminobenzidine tetrahydrochloride (DAB)-hydrogen peroxide solution (pH 7.0) for 3 min. Sections were then counterstained with Mayer's hematoxylin and rinsed in distilled water (Fernandez et al., 2005). Leica DM500 microscope and a digital camera (Leica EC3, Leica, Germany) were used to take micrograph slices.

2.5. Scoring immunohistochemical data

Positive immunoexpression for vimentin and CD45 was noted as a brown cytoplasmic precipitate, and for E-cadherin, as a brown precipitate in epithelial cells underneath cell membranes and keratinocytes. Immunoreactive cells were counted and classified as follows: slight (positive cells form less than 10% of the observed field); moderate (10%–50% of cells in the observed field were positive); and intense (>50% of cells in the observed field were positive) (Gama et al., 2003). All sections were observed at high magnification (× 400).

2.6. Statistical analysis

Frequencies of different types of round cell tumers were subjected to statistical analysis using Chi-square test with the aid of statistical analysis system (SAS Institute, Inc., 2004) software, p value considered significant at $P < 0.05$.

3. Results

3.1. Clinical symptoms and samples site

Examined animals had a history of skin or genital abnormal growths, either hairless or ulcerated surfaces. In addition, bloody or purulent genital discharge, excessive licking of the vaginal region and genital edema were observed. The dogs showed other signs, including dysuria, ulcers in the perineum area, mating refusal, weakness, anorexia, and weight loss.

Among specimens, **64** (45.71%) were diagnosed as skin round cell tumors and were included in this study (Table 1). The samples collected from various sites, including external genitalia mucosal surface of male (25%) and female (50%) dogs, flank (6.25%), hip (6.25%), pastern (6.25%) region, and ventral aspect of the tail (6.25%).

Table 1. Histopathologic classification of round cell tumors.

Lesions	No. (Total number, 64)	%	Breeds	Age	Sex
Canine transmissible venereal tumor	48	75	German shepherd	1.2y-7y	F/M
				2-3y	F
			Pitbull	1y	M
			Boxer		
Cutaneous histiocytoma	12	18.75	German shepherd	3y	M
			Golden retriever	8m-1.6y	F/M
Histiocytic sarcoma with apocrine cyst	4	6.25	Rottweiler	4.8y-5y	M
Chi-square and *P*-value	77.25 (P<0.0001)				

M: male; F: female.

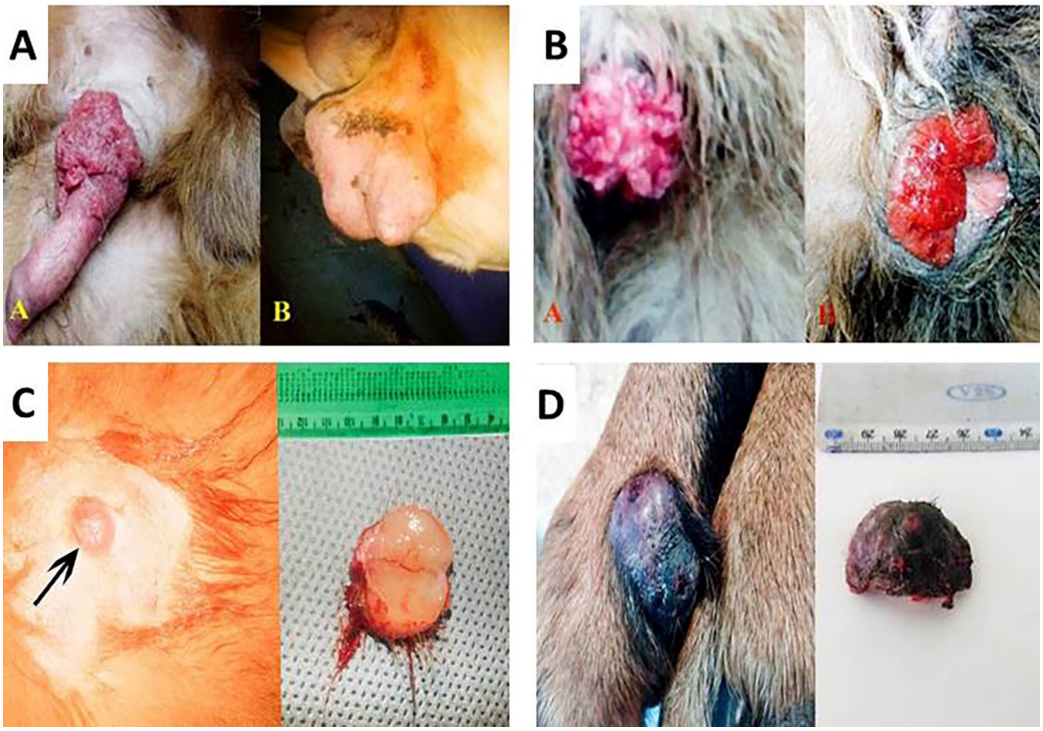

Figure 1. Growth morphology of various canine cutaneous round cell tumor types (A–D). (A–B) Canine transmissible venereal tumor ((A) 1.2-year-old German shepherd, (B) 1-year-old Boxer in A; (A) 3.2-year-old German shepherd, (B) 1.2-year-old German shepherd in B). (A) (A) Multiple, sessile nodular mass on the penis; (B) large papillary mass on the scrotum. (B) (A) Cauliflower-like mass on the vagina; (B) solitary, sessile reddish mass on the vulva. (C) Cutaneous histiocytoma, 8-month-old Golden Retriever showing button-shaped, solitary elevated mass (arrow). (D) Histiocytic sarcoma with apocrine cyst, 5-year-old Rottweiler showing firm, well-circumscribed mass with fluid-filled cysts and alopecic and blackish-red skin.

3.2. Tumor prevalence, site, and morphology

Samples were reported and classified into three types of round cell tumor: canine transmissible venereal tumors, cutaneous histiocytoma, and histiocytic sarcoma with apocrine cyst. Statistical analysis for frequencies of different types revealed highly significant variation ($P<0.0001$) between different types of round cell tumors. The most common types is canine transmissible venereal tumors followed by cutaneous histiocytoma then histiocytic sarcoma with apocrine cyst.

3.2.1. Canine transmissible venereal tumors

Forty eight samples were reported (75% of specimens). Tumors were located on the penis (Figure 1A (A)) or scrotum (Figure 1A (B)) of male dogs. In females, tumors were located in the vagina (Figure 1B (A)) or vulva (Figure 1B (B)). Masses were solitary or multiple, sessile, nodular, papillary, pedunculated, or multilobulated, forming a cauliflower-like appearance. They could be as tiny as a (5 mm) nodule or as huge as a mass (50 mm). They were pink to red in color and soft. The skin's surface was frequently ulcerated, irritated, and easily bled.

3.2.2. Cutaneous histiocytoma

Specimens from twelve dogs (18.75%) were button-shaped, solitarily elevated, and round masses with bright red coloration at the flank, hip region (Figure 1C), and ventral aspect of the tail.

3.2.3. Histiocytic sarcoma with apocrine cyst

Four specimen (6.25%) was a firm, well-circumscribed mass approximately 45 mm in diameter, with fluid-filled cysts and alopecic, blackish-red skin, located at the pastern region (Figure 1D).

3.3. Histopathological Findings

Microscopic examinations of collected samples showed three categories of description as following:

3.3.1. Canine transmissible venereal tumors

Microscopic examinations showed sheets or loosely spaced rows of uniformly sized, round, or slightly oval tumor cells with thin fibrous connective tissue stroma (Figure 2A). Tumor cell's cytoplasm contained a moderate amount of pale eosinophilic cytoplasm with conspicuous nucleoli, spherical nucleus, and chromatin that ranges from fine to coarse. There was modest to severe anisocytosis and anisokaryosis. Vacuolated cells were seen (Figure 2B), with many of mitoses. Numerous blood arteries were present in the stroma (Figure 2C). Necrotic debris was present among neoplastic cells (Figure 2D).

Additionally, hemorrhage was common. Variable counts of eosinophils, macrophages, plasma cells, neutrophils, and lymphocytes were scattered throughout the tumor (Figure 2E). The tumor's developmental stages were divided into phases of progression, initial regression, and final regression. The progression phase was characterized by a diffuse arrangement of round cells, fragile stroma, and frequent mitotic figures presence (Figure 2F). The conjunctival stroma was widely dispersed or linked with tumor-infiltrating lymphocytes during the initial regression phase (Figure 2G). Neoplastic tissue collapsed and was replaced by fibrous tissue, and apoptotic bodies were frequently seen during the final regression phase (Figure 2H).

3.3.2. Cutaneous histiocytoma

Microscopic examinations showed tightly packed sheets of cells in the dermis (Figure 2I). Neoplastic cells were round to oval, arranged in sheets and cords (Figure 3A) that variedly obliterated adnexal structures. Cells contained bean-shaped to ovoid nuclei and a pale to deep eosinophilic cytoplasm, and nuclear mitotic division were observed (Figure 3B). In one case, the epidermis was hyperplastic and marked rete formation was observed, besides the presence of dermal inflammatory cell infiltrates, particularly lymphocytes (Figure 3C).

3.3.3. Histiocytic sarcoma with apocrine cyst

Microscopic examinations showed the infiltration of large rounded to polygonal tumor cells with hyperchromatic ovoid nuclei, and plenty of eosinophilic cytoplasm (Figure 3D). Siderocytes and cellular debris, in addition to congestion, were observed (Fig. 3E). Mitotic figures were also frequently observed. Multinucleated giant tumor cells were common (Figure 3F), and other neoplastic cells showed marked atypia. Inflammatory cells were also seen, scattered among neoplastic cells, especially neutrophils (Figure 3G). Cells had one or more clear cytoplasmic vacuoles. Hemorrhage and necrosis were often detected (Figure 3H). The associated apocrine glands were cystically dilated and contained pale eosinophilic secretions. Apocrine cysts were surrounded by neoplastic and inflammatory cells, especially neutrophils (Figure 3I).

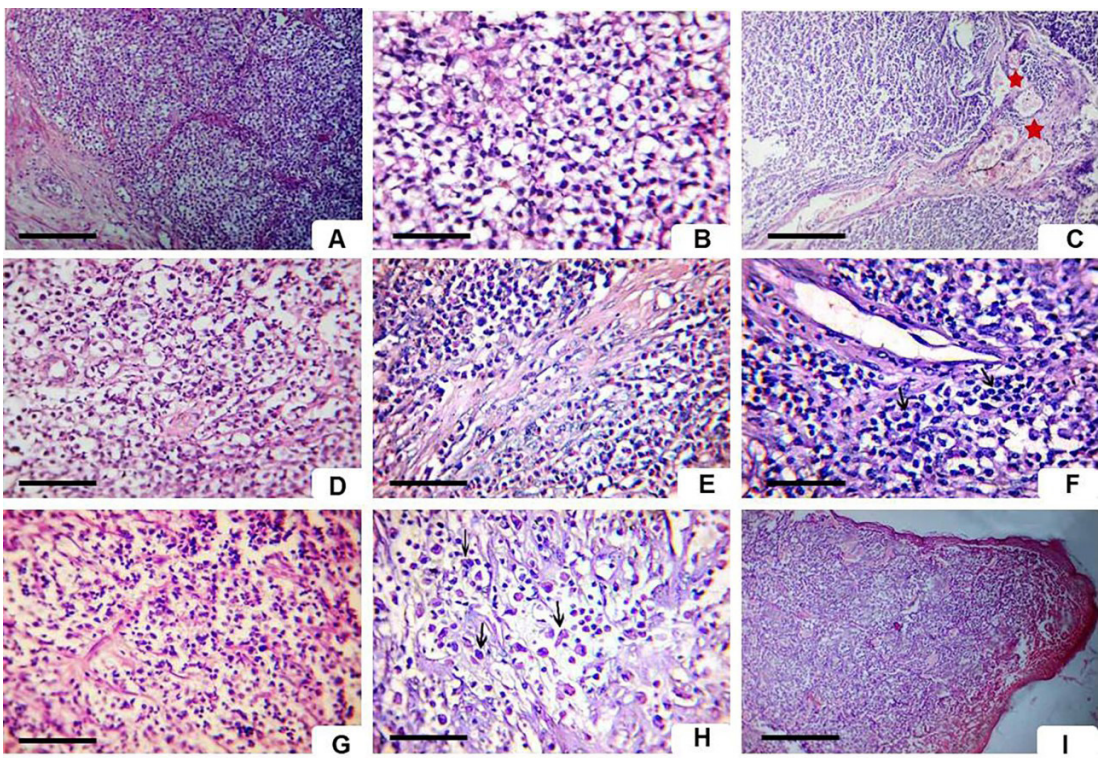

Figure 2. Histopathological findings of various canine cutaneous round cell tumor types, hematoxylin and eosin, scale bar = 50 µm (A–I). (A–H) Canine transmissible venereal tumor (vagina, 3.2-year-old German shepherd in A, D; vagina, 1.5-year-old German shepherd in B; vulva, 1.2-year-old German shepherd in C; scrotum, 1- year-old Boxer in E–F, H; vulva, 7-year-old German shepherd in G). (A) Round or slightly oval tumor cells, uniform in size, and arranged in sheets or more loosely in rows within a stroma of thin fibrous connective tissue. (B) Vacuolated cells and moderate anisokaryosis and anisocytosis. (C) Abundant blood vessels (*). (D) Necrotic debris present among neoplastic cells. (E) Variable numbers of lymphocytes, macrophages, plasma cells, eosinophils, and neutrophils. (F) The progression phase is presented as round cells arranged diffusely, interspersed by delicate stroma and the frequent presence of mitotic structures (arrows). (G) In the initial phase of regression, lymphocytes are widely distributed or associated with the conjunctival stroma. (H) The final regression phase involves the collapse of neoplastic tissue and substitution by fibrous tissue and the frequent presence of apoptotic bodies (arrows). (I) Cutaneous histiocytoma, 8-monthold Golden Retriever showing tightly packed sheets of cells in the dermis.

3.4. Immunohistochemical analysis

Specific antibodies for vimentin, CD 45, and E-cadherin were utilized for different tissue sections, verifying the origin and type of cancerous cells. Antibody immunoexpression is summarized (Table 2). The immunoreactive cells of tumor sections were evaluated as mild (less than 10% of cells were positive), moderate (between 10% and 50%), and intense (more than 50%) cell positivity.

3.4.1. Canine transmissible venereal tumors

Vimentin-positive immunoreactivity was intense (Figure 4A, B) and appeared as brown-stained granules in the cytoplasm of both vacuolated (Figure 4C) and non-vacuolated (Figure 4D) tumor cells. Additionally, antibody immunoexpression was observed in tumor stroma inside fibroblasts and the vascular endothelium (Figure 4E).

3.4.2. Cutaneous histiocytoma

E-cadherin-positive immunoreactivity was moderate in epithelial cells under the cell membrane (Figure 4F) of cutaneous histiocytoma (Figure 4G). Additionally, immunoreactivity to E-cadherin was observed in keratinocytes (Figure 4H).

3.4.3. Histiocytic sarcoma with apocrine cyst

CD45-positive immunoreactivity was moderate and appeared as golden-brown cytoplasmic staining (Figure 4I) in histiocytic sarcoma tumor cells.

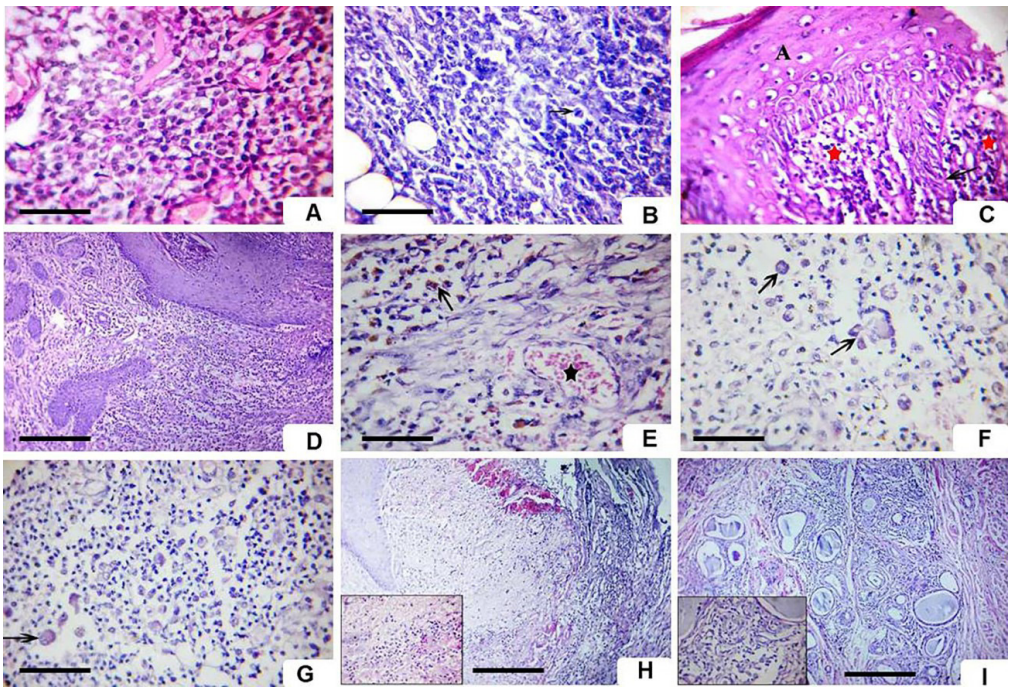

Figure 3. Histopathological findings of various canine cutaneous round cell tumor types, hematoxylin and eosin, scale bar = 200 µm (D, H); scale bar = 50 µm (A–C, E–G, I). (A–C) Cutaneous histiocytoma in 8-month-old Golden Retriever in A; 1.6-year-old Golden Retriever in B; 3-year-old German shepherd in C. (A) Round to oval neoplastic cells arranged in cords and sheets variably obliterating adnexal structures. (B) Mitotic figures (arrow). (C) Epidermal hyperplasia (A) with marked rete formation (arrow) and dermal lymphocytic infiltrates (*). (D–I) Histiocytic sarcoma with apocrine cyst, 5-year-old Rottweiler. (D) Large round to polygonal cells with ovoid hyperchromatic nuclei and abundant eosinophilic cytoplasm. (E) Siderocytes (arrow) and cellular debris plus congestion (*). (F) Multinucleated giant cells (arrows). (G) Marked atypia with multinucleated giant cells (arrow) and inflammatory cells scattered among neoplastic cells, especially neutrophils. (H) Hemorrhage and necrosis. (I) Cystically dilated apocrine glands containing pale eosinophilic secretions and surrounded by neoplastic cells and inflammatory cells, especially neutrophils.

Table 2. Immunoexpression of vimentin, CD 45, E-cadherin in different types of tumors.

specificity	Type of tumor	Immunoreactivity
Vimentin	Canine transmissible venereal tumor	Intense
E-cadherin	Cutaneous histiocytoma	Moderate
CD 45	Histiocytic sarcoma	Moderate

The immunoreactivity cells of tumor sections were evaluated as: slight (positive cells constituted less than 10%); moderate (10-50% of cells were positive); and intense (more than 50% of cells were positive).

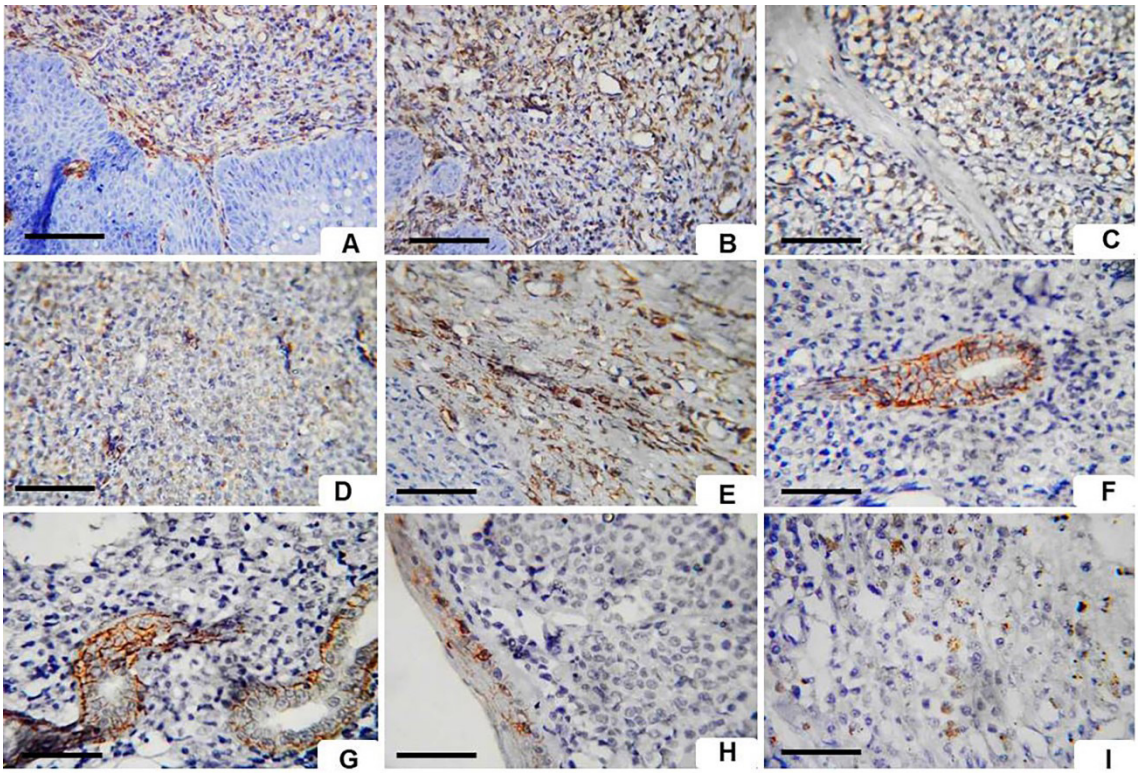

Figure 4. Immunohistochemical analysis of various canine cutaneous round cell tumor types. Immunohistochemical vimentin expression in canine transmissible venereal tumor. Vagina, 3.2-year-old German shepherd, scale bar = 200 (A), scale bar = 50 µm (B–E). (A) Positive intense immunoreactivity to vimentin. (B) High magnification of (A). (C) Intense brown cytoplasmic vimentin immunoexpression in vacuolated tumor cells. (D) Intense brown cytoplasmic vimentin immunoexpression in non-vacuolated tumor cells. (E) Intense brown vimentin immunoexpression in the tumor stroma inside fibroblasts and vascular endothelium. (F–H) Immunohistochemical E-cadherin expression in cutaneous histiocytoma, 8-month-old Golden Retriever, scale bar = 50 µm. (F) Moderate E-cadherin immunoexpression in epithelial cells beneath the cell membrane. (G) Moderate immunoreactivity to E-cadherin. (H) Positive immunoreactivity to E-cadherin in keratinocytes. (I) Immunohistochemical CD45 expression in histiocytic sarcoma, 5-year-old Rottweiler, scale Bar = 50 µm. (I) Moderate golden-brown CD45 cytoplasmic staining in tumor cells.

4. Discussion

Canine transmissible venereal neoplasm is a highly contagious, round-cell tumor of mesenchymal origin (Spugnini et al., 2008). The external genitalia of both sexes' mucosal surfaces show the disease most often. (Kabuusu et al., 2010; Ostrander et al., 2016). In this study, in contrast to female dogs, which had lesions at the vulva and vagina, male dogs' lesions were restricted to the penis and scrotum. Goldschmidt and Goldschmidt (2020) reported that the tumor was more common in females, particularly 2–5 years old. Consistent with these findings, male (n = 16) and female (n = 32) dogs in our study had an average age of 2.5 years. Fathi et al. (2018) reported that the incidence of transmissible venereal tumor infection was 51.92% for Great Danes and 36.53% for German shepherds. In our study, the incidence was 75% for German shepherds, 16.67% for Pitbulls, and 8.33% for Boxers. This statistic may reflect breed ownership preferences across Egypt.

Macroscopically, the canine transmissible venereal tumors are firm in consistency with 0.5–10 cm in diameter and are accidental surface ulceration (Schlafer and Foster, 2016). However, masses reported in our study were between 5 mm and 5 cm in diameter. We observed also that mass surfaces were often ulcerated and inflamed and bled easily. In agree with our study, Vural et al. (2018) reported that the progressive tumor growth (P-phase) was followed by spontaneous tumor regression (R-phase). Our histopathological data also agreed with those descriped before by Stthawongsin et al. (2016) and Vural et al. (2018).

Canine cutaneous histiocytomas are sometimes termed "button tumors" (Dysko et al., 2002). Oftentimes, one or rarely multiple masses exist. The frequent histiocytoma site is the head, ears, extremities, and neck, although they may be present anywhere on the body (DeLorimier and Fan, 2020). In our study, the tumor mass was located at the flank, hip region, and ventral tail aspect. This tumor is frequent in dogs, with increased incidence in animals under 4 years old and rare in felines (Amy 2017). According to Moore (2020), Rottweilers, Bernese Mountain Dogs and Golden Retrievers are the most susceptible breeds to cutaneous histiocytoma. Consistent with these findings, cutaneous histiocytomas were reported in twelve dogs: a Golden Retriever (n = 8) and a German shepherd (n = 4) with an average age of 1.7 years.

A pink, smooth, elevated lump known as a classic button tumor is typically covered with alopecic skin. (Hendrick, 2020). Skin sections show dense variably sized proliferating cells with oval to round nuclei (Sawale et al., 2014). Our gross and microscopic findings in our study were similar to those of Hendrick (2020); and Sawale et al. (2014). Also, Canine histiocytic sarcomas are locally aggressive, quickly developing tumors that only affect one place. Histiocytic sarcoma is more likely to develop in Rottweilers, Golden Retrievers, Flat-Coated Retrievers, Bernese Mountain, and Labrador Retrievers Dogs (Moore, 2020). Most of the age ranges are between 6 and 11 years old, and there is no obvious sex preference. The syndrome has been noted in dogs as young as two years old, with a preference for the extremities of the skin, subcutis, and underlying tissue, particularly close to the joints (Affolter, 2004). Our case involved a 5-year-old male Rottweiler, whose mass was in the pastern region. It was poorly restricted and consisted of bundles of plump spindle cells with a significant proportion of eosinophilic pale cytoplasm and large round to vesicular oval nuclei or a dense proliferation of huge, pleomorphic, individual round cells. We also discovered huge, stellate-shaped, multinucleated gigantic neoplastic cells with a high mitotic rate. These findings were consistent with those of Fidel et al. (2006).

Immunohistochemistry is a highly specific diagnostic method widely used for neoplastic diseases. Vimentin is important for the comparative diagnosis of canine transmissible tumors with other round-cell tumor types, e.g., lymphomas, melanomas, and amelanotic melanomas (Ramos-Vara et al., 2008). In our research, canine transmissible tumors displayed intense vimentin immunoreactivity in the cytoplasm of tumor cells, in agreement with Mozos et al. (1996), Marchal et al. (1997), Mascarenhas et al. (2014). E-cadherin is a constant feature of the canine cutaneous histiocytoma immunophenotype. Its expression is unique to histiocytomas, CD4, and negative Thy-1 expression helps differentiation between histiocytomas and reactive histiocytosis, systemic histiocytosis, and cutaneous histiocytosis (Moore, 2010). It is critical to understand that E-cadherin is expressed in keratinocytes (DeLorimier and Fan, 2020) and Langerhans cells along the cell membrane (Valli et al., 2016). The true incidence of E-cadherin expression in canine cutaneous histiocytomas is unknown; however, it is unquestionably fewer than 100%. (Moore, 2004). In our study, E-cadherin expression in canine cutaneous histiocytomas was moderate in epithelial cells underneath the tumor cell membrane and keratinocytes.

Leukocyte antigen CD45 is a widespread one (Araujo et al., 2012). This marker's expression in histiocytic sarcoma was described by Moore et al. in 1996 (Moore et al., 1996) and by Affolter and Moore in 2002 (Affolter and Moore, 2002). The absence of a common leukocyte marker allows the anaplastic fibrosarcomas (including myofibroblastic forms), hemangiopericytomas, synovial cell sarcomas, leiomyosarcomas, peripheral nerve sheath tumors, and pleomorphic liposarcomas to be distinguished from histiocytic sarcomas. (Gross et al., 2009). Additionally, Mastrorilli et al. (2012) concluded that the immunohistochemical analysis of neoplastic cells showed that most had moderate to golden-brown cytoplasmic immunoexpression for CD1a, CD11b, CD45, CD11c, and CD18. In our study, CD45 expression was moderate and agreed with that of Mastrorilli et al. (2012).

5. Conclusions

Based on the presented results, canine transmissible venereal tumors are the most significantly prevalent round-cell tumors, followed by canine cutaneous histiocytomas and histiocytic sarcoma. A confirmatory diagnostic method for canine transmissible venereal tumors could be the immunohistochemistry expression of vimentin. Additionally, cutaneous histiocytomas and histiocytic sarcoma cells might be diagnosed using the immunohistochemistry expression of E-cadherin and CD45. These results may aid veterinary oncologists in expanding their understanding of the behavior of neoplastic diseases as they progress over time and in developing recommendations for histopathologic and immunohistochemical diagnosis, which may result in establishing a reliable basis for future laboratory studies.

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