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https://dx.doi.org/10.4314/nvj.v43i3.6 **ORIGINAL ARTICLE**

CANINE CUTANEOUS TUMOURS FROM THREE SOUTHWESTERN STATES OF NIGERIA: A RETROSPECTIVE STUDY.

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SUMMARY

Over the years, studies have shown that the skin is the most commonly affected organ for both neoplastic and non-neoplastic conditions (Broden *et al.,* 2010). This occurrence has been attributed to continuous exposure of the skin to physical, chemical, and environmental factors (Guzman *et al.,* 2003; Pakhrin *et al.,* 2007). Cutaneous tumours are the second most frequently diagnosed cancer in dogs resulting in approximately 30% of all tumours reported and are usually excised due to easy surgical access than tumours in other organs (Moraes *et al.,* 2009). Several authors have reported the occurrence of tumours by utilizing data obtained from registries, animal hospital reports, and diagnostic laboratory reports. Most of these reports vary greatly in terms of data sources, sample numbers, the size of geographical regions assessed (Graf *et al.,* 2018; Kok *et al.,* 2019), prevalent environmental influences, and breed populations (Kaldrymidou *et al.,* 2002; Sanja *et al.,* 2005; Pakhrin *et al.,* 2007). Previous studies have shown that dogs are susceptible to tumours at any age and there is a marked predisposition in aged dogs than young dogs because of a progressive accumulation of genetic mutations (Reif, 2007). Environmental chemical contaminants (especially pyrethroids) also have been implicated in the occurrence of mammary adenocarcinomas (Reif, 2007). Ultraviolet light rays have also been studied as a possible aetiology of squamous cell carcinoma (SCC) in animals with poor skin pigmentation (Reif, 2007). In Nigeria, different case reports on canine tumours have been documented (Amber and Ralph, 1982; Faramade *et al*., 2017), but the risk factors and distribution of the disease in these species are very limited and restricted to the databases of Veterinary Teaching Hospitals of universities and private veterinary clinics in the major cities. The documentation of occurrences of different tumour types in a particular geographical region is necessary because it provides easy access to data for further investigation and helps in prompt diagnosis and management by clinicians (Moraes *et al.,* 2009).

INTRODUCTION

Over the years, there is apaucity of information on the prevalence of Canine Cutaneous Tumours (CCT) in Nigeria. It is noteworthy that no prevalence studies on CCTs have been reported in Nigeria to date. This information justifies and warrants this retrospective study, which aimed to determine the incidence of skin tumours in dogs diagnosed at the two Veterinary Teaching Hospitals of the Federal University of Agriculture Abeokuta and the University of Ibadan and some veterinary clinics in southwestern Nigeria over a period of 10-years.Therefore, this study was designed to determine the prevalence of CCT and associated risk factors in southwest Nigeria using cytology, histopathology, and immunohistochemistry.

MATERIALS AND METHODS

Data Source

Records of cases of CCT presented to the Departments of Veterinary Pathology, Federal University of Agriculture, Abeokuta, and the University of Ibadan (U.I) from three states (Ogun, Oyo, and Lagos) in the southwestern part of Nigeria. The three states serve as the commercial nerve centers of the southwestern part of the country with most people having one pet or more. The record cases included cytological and surgical biopsy samples submitted between January 2008 and December 2018 by different private and public veterinary clinics as well as in-patient biopsy samples from the teaching hospital were for the purpose of this study. Breed, age, and sex records as well as predilection site were obtained from forms that accompanied the samples and these were analyzed.

Breed identification was based on the information provided in the diagnostic report. Dogs with mixed breeds were grouped as "mongrel" while any unspecified breed was recorded as "unknown''. Cystic and hyperplastic epithelial lesions were not included. The details of tumour types, state of origin, and predilection site were recorded. The predilection site of the tumour on the body was categorized into six groups; head and neck, trunk, limbs, peri-anal, tail, and multiple sites.
A total of 82 CCTs were obtained from the two Veterinary teaching hospitals and from the various Veterinary clinics at Lagos. These samples were both examined histopathologically (82 samples) and cytologically (17 samples from the 82 samples) during this period. Only 27 replicate sections of the cases were examined using immunohistochemistry.

Cytological evaluations

Fine needle aspirates, scrapping, and touch imprints were used for collections of cytological samples (Duncan and Prasses, 2013). For touch imprints, impression smears were made by touching the cut surfaces of the mass on different portions of the slide and allowed to dry. Ulcerated masses were first cleaned with wet cotton wool before an impression smear. All smears were air-dried, fixed with methanol, and stained with A and B stains (Indian) for 5 minutes and some with giemsa for 10minutes.

Histopathological and Immunohistochemical procedures

All the tumour samples were fixed in 10%

neutral buffered formalin, dehydrated in the graded level of alcohol (50%, 70%, 90%, and 100%), and cleared in xylene. Tissues were processed and embedded in paraffin wax from which 5 µm were cut and stained with haematoxylin and eosin. These sections were examined under a light microscope. The diagnoses of various tumour conditions were made based on the characteristic histopathological features and classified according to the World Health Organization (WHO) International histological classification of tumours of domestic animal guidelines (Goldschmidt *et al.,* 1998; Hendrick, 2017). Immunohistochemical examinations of 27 replicate sections were carried out to determine the cellular origin of the neoplastic mass. The source, type, and dilution factor of primary antibodies used in the immunohistochemical examination are depicted in Table I.

Table I: Source, type, and dilution of primary antibodies used in the immunohistochemical examination.

Antibody	Type/Clone	Dilution	Source
Anti-pan Cytokeratin	Monoclonal mouse, PCK-26	1: 200	Abcam, USA
Anti- Vimentin	Monoclonal mouse, V9	1: 50	Dako A/S Denmark
Anti-S100	Polyclonal rabbit, Z0311	1: 300	Dako A/S Denmark
Anti-Human Desmin	Monoclonal mouse D33	1: 50	Dako A/S Denmark
Anti-Human Smooth Muscle Actin	Monoclonal mouse 1A4	1: 200	Dako A/S Denmark

The immunohistochemical procedure was performed according to the method of Feuchtinger *et al.,* (2015). Briefly, replicate sections were deparaffinized in four changes of xylene for 3 minutes each, rehydrated in graded levels of alcohol, and washed with deionized water. Antigens were unmasked with 0.01M sodium citrate buffer (pH 6.0) using a microwave oven for 30 minutes at 650W. This was allowed to cool to room temperature for 20 minutes and followed by 5 minutes of washing in 0.05M Phosphate Buffer Saline-Tween 20 (TPBS) (pH 7.6). All other steps were performed at room temperature. The sections were incubated in 3% hydrogen peroxide for 15 minutes to quench endogenous peroxide, after a brief wash TPBS, Non-specific binding was done by bathing in normal goat serum for 10 minutes.

The tissues were then incubated with specific antibodies overnight (Table I). After the last wash in TPBS, the slides were incubated with streptavidin-biotin-horseradish peroxidase (Histomark, Goat anti-Rabbit IgG (H+L) KLP, Gaithersburg U.S.A) for 15 minutes. Slides were then rinsed with distilled water and incubated with DAB (3,3′-Diaminobenzidine) for 10 minutes, rinsed with distilled water and counterstained with Mayer haematoxylin, rinsed and mounted with mountant for microscopic evaluation.

S**tatistical Analysis**

Results were presented using a descriptive statistic, the association between the prevalence of cutaneous tumours were determined using the Chi-square test while odd-ratio was used to determine the risk factors such as breed, age, sex, and predilection site (IBM® SPSS® Statistics version 23 (IBM SPSS, Armonk, NY, U.S.A.). A p-value $\leq$ 0.05 was considered significant.

RESULTS

From January 2008 to December 2018,158 neoplastic conditions were diagnosed in dogs using cytology and surgical biopsy. Of the 158 neoplastic masses, 82(51.9%) cases were of cutaneous origin. Out of the 82 cases, 79, 27, and 17 samples were diagnosed histopathologically, immunohistochemistry, and cytologically respectively, and 3 samples were unknown (Table II).

Table II: Histological classification, prevalence, breed, sex and age distribution of cutaneous tumours in 82 dogs from Southwest.

Tumour types (n=82)	No	%	95% CI	M: F ratio	Mean Age	Range Age	Breeds (No affected)	Methodologies Used
Epithelial and adnexal tumours (n=34)								
Papilloma	7	10.5	7.18-14.8	7:0	3.0	0.8-6.0	BB(1), GS(4), RR(1), MX(1)	CYT, HP, IH
Squamous Cell Carcinoma	13	10.5	7.18-14.8	6:7	7.0	4.0-11.0	RW(2), BB(5), GS(3), LA(2), BT(1)	CYT, HP, IH
Basosquamous Carcinoma	1	1.49	0.20-2.20	0:1	6.0	6.0	GS(1)	HP
Sebaceous carcinoma	1	1.49	0.20-2.20	0:1	8.0	8.0	BB(1)	CYT, HP, IH
Meibomian gland adenoma	5	1.49	0.20-2.20	1:4	7.9	1.0-14.0	MX (1)BB(4),	HP, IH
Malignant Pilomatricoma	1	1.49	0.20-2.20	1:0	7.0	7.0	MX(1)	CYT, HP, IH
Hepatoid gland adenoma	2	2.98	0.30-3.70	2:0	9.5	9.0-10.0	MX(1), CS(1)	CYT, HP
Complex Apocrine Carcinoma	1	1.49	0.20-2.20	1:0	7.0	7.0	BB(1)	HP
Apocrine anal sac adenocarcinoma	3	2.98	0.30-3.70	1:2	11.5	9.0-14.0	GS(1)LA(2)	CYT, HP
Mesenchymal tumours (n=15)								
Fibroma	1	1.49	0.20-2.20	1:0	9.0	9.0	GS(1)	HP
Fibrosarcoma	4	5.97	1.60-6.40	3:1	5.1	0.4-8.0	BB(2), GS(2)	CYT,HP, IH
Lipoma	3	1.49	0.20-2.20	3:0	7.0	5.0-7.0	GS(3)	HP
Liposarcoma	1	1.49	0.20-2.20	0:1	6.0	6.0	GS(1)	HP
Malignant Fibrous Histiocytoma	4	5.97	1.60-6.40	1:3	7.5	1.6-14	BB(1), MX (1), GS(1), RW(1)	HP,
Myxosarcoma	1	1.49	0.20-2.20	0:1	0.8	0.8	CC(1)	HP, IH
Peripheral Nerve Sheath	1	1.49	0.20-2.20	1:0	4.0	4.0	BB(1)	HP, IH
Haematopoietic tumours (n=7)								
Haemangioma	1	1.49	0.20-2.20	0:1	1.1	1.1	BB(1)	HP
Haemangiosarcoma	6	1.49	0.20-2.20	1:5	8.0	7.0-9.0	BB(4)GS(2)	HP
Round cell tumours (n=22)								
Cutaneous Histiocytoma	12	11.94	7.90-15.9	4:8	5.2	0.1-8.5	GS(4), BM(1), BB(4), MG(1), RW(1), UN(1)	HP, IH
Transmissible Venereal Tumour	10	11.94	7.90-15.9	2:8	3.7	1.5-8.0	GS(1), MX(7), BB(2)	CYT, HP,IH
Melanocytic tumours (n=4)								
Malignant melanoma	4	2.98	0.30-3.70	1:3	8.5	6.0-11	GS(2), MG(2)	HP,IH

Notes: BB=Boerboel, GS= German shepherd, RR= Rhodesian ridgeback, MX= Mixed Breed, RW= Rottweiler, LA= Lhasa apso, BT= Boston terrier, CS= Cocker Spaniel, CC=Cane corso, BM=Bull mastiff, UN= Unknown, M: F ratio= Male: female ratio, 95%CI=95 percent confident interval, CYT=Cytology, HP= Histopathology, IH=Immunohistochemistry

Twenty-one different types of cutaneous tumours (CTs) were identified. Based on the World Health Organization (WHO) (1998) criteria, 9 different types of epithelial skin tumours(34/82, 41.5%), 7mesenchymal (15/82, 18.3%), 2haemopoietic (7/82, 8.5%), 2 round cell tumours (22/82, 26.8%) and 1 melanocytic tumour (4/82, 4.9%)were diagnosed as depicted in Table III. Benign tumours were 42 (51.2%) cases while malignant were 40 (48.8%) cases (Table III). The Five most frequent individual tumour types were Squamous Cell Carcinoma (15.9%), Histiocytoma (14.6%), Transmissible venereal tumour (12.2%), Papilloma (8.5%) and Haemangiosarcoma (7.3%).

Table III: Percentage of histological classification of cutaneous tumours into benign and malignant

Tumour Type	N	Benign (%)	Malignant (%)
Epithelial and Adnexal	34	14(41.2)	20(58.8)
Mesenchymal	15	5(33.3)	10 (66.7)
Haematopoetic	7	1(14.3)	6 (85.7)
Round Cell Tumour	22	18 (81.8)	4(18.2)
Melanocytic	4	-	4 (100)
Total	82	39 (47.6)	61 (74.4)

Breed distribution

The prevalence of CT in relation to breed from 10 different breeds of dogs is depicted in Table IV. Boerboel showed the highest prevalence (n= 27, 32.9%) in which epithelial (n=12, 44.5%), round cell (n=6, 22.2%) and haematopoietic (n=5, 18.5%) tumours were the most prevalent. This is closely followed by German shepherd (n=26, 31.7%) with epithelial (n=9, 34.6%), mesenchymal (n=8, 30.8%), and round cell (n=5, 19.2%) tumours having the highest prevalence. Increased cases of the round cell (n=8, 53.3%) and epithelial (n=4,26.7%) tumours were also recorded in Mixed breeds as well as Lhasa Apso and Rottweiler, while only one epithelial tumour was documented each in unknown and other breeds of dogs.

Table IV: Prevalence of canine cutaneous tumours in relation to breed in 10 breeds from three Southwest Nigeria

Breeds	Total Prevalence n=82	N (%) EPT	N (%) MST	N (%) HPT	N (%)RCT	N (%)MLT
Boerboel	27(32.9)	12(44.5)	4 (14.8)	5 (18.5)	6 (22.2)	-
German shepherd	26(31.7)	9(34.6)	8(30.8)	2 (7.6)	5(19.2)	2(7.6)
Mixed Breed	15(18.3)	4(26.7)	1(6.7)	-	8(53.3)	2(13.3)
Lhasa Apso	4(4.9)	4 (100)	-	-	-	-
Rottweiler	4 (4.9)	2 (50)	1 (25)	-	1(25)	-
Rhodesian ridgeback	1(1.0)	1(100)	-	-	-	-
Boston terrier	1(1.0)	1(100)	-	-	-	-
Cocker Spaniel	1(1.0)	1(100)	-	-	-	-
Cane corso	1(1.0)	1(100)	-	-	-	-
Bullmastiff	1(1.0)	-	-	-	1 (100)	-
Unknown	1(1.0)	-	-	-	1(100)	-

Note: EPT-Epithelial tumour, MST-Mesenchymal tumour, HPT-Haemopoietic tumour, RCT-Round Cell Tumour, MLT-Melanocytic tumour

Sex distribution

The distribution of CCT in relation to sex is depicted in Table V. Most cutaneous tumours submitted were from female dogs (n=46, 56.1%). The epithelial and adnexal tumours (n= 34, 55.9%) and mesenchymal tumours (n=15, 60%) were more prevalent in the male dogs compared with the female dogs, although there was no significant difference, while haemopoietic, round cell tumours and melanocytic tumours were more common in the female dogs.

Table V: Sex distribution of cutaneous tumours in dogs.

Tumours	N (%) Male	N (%) Female	p-value
Epithelial and adnexal (n=34)	19 (55.9)	15 (44.1)	0.53
Mesenchymal (n=15)	9 (60.0)	6 (40.0)	0.53
Haemopoetic (n=7)	1 (14.3)	6(85.7)	4.57
Round cell tumour (n=22)	6 (27.3)	16(72.7)	7.36
Melanocytic (n=4)	1(25.0)	3(75.0)	0.50
Total	36 (43.9)	46 (56.1)	1.97

Age distribution

The mean age of dogs with CT according to the histopathological classification ranged from 0.1 to 14 years with an overall mean of 11.5 years. Tumours of the epithelial and mesenchymal origins had the highest mean age of 11.5 and 9 years respectively, compared with melanocytic (8.5years), haematopoietic (8.0years), and round cell (5.2years) tumours.

Predilection site distribution

Of the 82 cutaneous tumours, 30 (36.6%) neoplasms were localized on the trunk, 18 (22%) were found on the head and neck, 17 (20.7%) on the extremities of the limbs, 12 (14.6%) on the perianal region while 2 (2.4%) were observed on the tail. Only 3 (3.7%) were observed from multiple sites, which were mainly round cell tumours (histocytoma=12 and TVT=10). Epithelial tumours were mostly found on the trunk, head, and neck. The location of one of the tumour on the skin was not specified (Table VI).

Table VI: Predilection site distribution of cutaneous tumours in dogs.

Tumour types	N (%) He& Ne	N (%) Trunk	N (%)Limbs	N (%)Perianal	N (%) Tail	N (%) Multiple sites
Epithelial and Adnexal (n=34)	12 (35.3)	13 (38.2)	5 (14.7)	4 (11.8)	-	-
Mesenchymal (n-15)	4 (26.7)	5 (33.3)	6 (40.0)	-	-	-
Haemopoetic (n=7)	-	1 (14.3)	6 (85.7)	-	-	-
Round cell tumour (n=22)	2 (9.1)	9 (40.9)	-	8 (36.4)	-	3 (13.6)
Melanocytic (n=4)	-	2 (50.0)	-	-	2 (50.0)	-
Total (n=82)	18 (22.0)	30 (36.6)	17 (20.7)	12 (14.6)	2 (2.4)	3 (3.7)

Note: He&Ne - Head and Neck.

There was a significant association between breed, sex as well as predilection site of tumour occurrence ($p<0.05$). Generally, the risk of developing cutaneous tumours increased in dogs aged 4 and above (4-14years) but there was no significant association between age ($p>0.05$) and cutaneous tumour development.

Five most frequent cutaneous tumours

The 5 most common cutaneous tumour types and the breeds of dog with the highest frequency for the respective tumour type in relation to sex, age-range, and predilection site (Table VII).

Table VII: Five most frequent cutaneous tumour types in association with the breed, sex, age, and predilection site.

Tumour types	n Tumour	Breed of highest frequency	% Sex(M:F ratio)	% Age	% Predilection site
Squamous Cell Carcinoma	13	Boerboel	15.9 (7:6)	9.8	13.3
Cutaneous histiocytoma	12	Boerboel German shepherd	7.3 (4:8)	7.2	14.6
Transmissible Venereal Tumour	10	Mixed Breed	12.2 (2:8)	7.2	12.2
Papilloma	7	German shepherd	8.5 (0:7)	8.5	8.5
Haemagiosarcoma	6	Boerboel	7.3 (1:5)	2.4	7.3

Squamous Cell Carcinoma (15.9% of all tumours), Histiocytoma (14.6% of all tumours), Transmissible venereal tumour (12.2% of all tumours), Papilloma (8.5% of all tumours) and Haemangiosarcoma (7.3% of all tumours) were among the 5 most common tumour types. Squamous Cell Carcinoma (SCC) was the most common cutaneous tumour in this study that was mostly diagnosed in Boerboel (38.5%), German shepherd (23.1%), mixed breed (23.2%), and Lhasa Apso (15.2%). SCCs were mostly observed in dogs of age 6-7years (46.2%) with a higher frequency being recorded in female dogs (male to female ratio=7:6). This tumour occurred more on the hind limb (46.2%), followed by the head and trunk (15.4%). Histiocytoma was the second most common cutaneous tumour diagnosed mostly in Boerboel (66.7%) and German shepherd (33.3%). It developed more in dogs between ages 5 and 7 years, with a higher tumour frequency being recorded in female dogs (male to female ratio = 4:8). The trunk had the highest frequency of this tumour (83.4%). Transmissible venereal tumours were the third most common cutaneous tumour diagnosed mostly in Mongrel (70%) followed by Boerboel (30%). Dogs of< 1-3 years were mostly affected with the female dogs having the highest frequency (male to female ratio =2:8). Tumours occurred mostly on the perianal (70%) and trunk (30%) in cases where metastases were observed. Papilloma was the fourth most commonly observed CT. It was mostly diagnosed in German shepherds (57.1%) and it occurred between ages1-2 years and 6-8years. Dogs affected were mostly female (male to female ratio =0:7) with 75% of the tumour occurring on the head. Haemangiosarcoma was the fifth most commonly diagnosed CT. Boerboel was mostly affected (66.7%) followed by German shepherd (33.3%), female had the highest frequency (male to female ratio=1:5).The ages affected were between 7 and 9 years. It occurred mostly on the limbs. Among the pure breed of dogs, the Boerboel breed was

mostly affected with epithelial and haematopoietic cutaneous tumours, German shepherd with mesenchymal tumours, mixed breed with round cell tumour while melanocytic tumours had an equal occurrence in both German shepherd and mixed breeds. The cutaneous tumours diagnosed were highest in tumour samples submitted from Lagos 44 (53.7%) than those submitted from Oyo state 20 (24.4%) and Ogun state 18 (21.9%) though there was no significant association between the prevalence of cutaneous tumours and these locations. Tumours that were cytologically evaluated include; papilloma (2, 11.8%), Squamous Cell Carcinoma (2, 11.8%), sebaceous carcinoma (1, 5.9%), Hepatoid gland adenoma (2, 11.8%), apocrine adenocarcinoma (1, 5.9%), fibrosarcoma (2, 11.8%), and transmissible venereal tumour (7,41.2%).Of the 17 cases examined, only 13 cases were cytologically well correlated with histopathological findings while 4 cases were not correlated.

Immunohistochemistry

Of the 27 sections examined immunohistochemically (12 epithelial, 9mesenchymal, 4 round cell, and 2 melanocytic tumours) only 22 sections were immunoreactive to the proteins used in this study (Table VIII).

Table VIII: Immunoreactivity of CCT to the antibodies

		Immunohistochemistry				
Tumours	N	N positive	Pan-cytokeratin	Desmin	Smooth Actin	Vimentin	S-100
Epithelial and adnexal	12	10	+	-	-	-	-
Mesenchymal	9	7	-	+	+	+	+
Round cell	4	4	-	-	-	+	+
Melanocytic	2	1	-	-	-	+	+

Ten sections showed tumours of epithelial origin which include 2 papilloma, 3 squamous cell carcinoma, 1 sebaceous carcinoma, 3 meibomian gland adenoma, and 1 malignant pilomatricoma were immunohistochemically reactive to pan-cytokeratin protein. Tumours of the mesenchymal origin including fibrosarcoma (n=3,42.8%), myxosarcoma (n=1, 14.3%), malignant fibrous histiocytoma (n=2, 28.5%), and malignant peripheral nerve sheath (n=1, 14.3%) showed variations in their reactivity to desmin, vimentin, smooth muscle actin, and S-100 proteins while the round cell tumours including TVT (n=3, 75%) and histiocytoma (n=1, 25%) were reactive to s-100 and vimentin. Malignant melanoma (n=1, 50%) was also positive to s-100.

DISCUSSION

This study revealed the prevalence of CCT in the three southwest states of Nigeria, based on the analysis of 82 cases submitted to the diagnostic units of the departments of Veterinary Pathology of the two Veterinary schools over 10 years (2008–2018). Regardless of the relatively small sample size as compared

with studies carried out in Europe,the United States, Asia, and Zimbabwe (Mukaratirwa *et al.,* 2005; Pakhrin *et al.,* 2007; Villamil *et al.,* 2011; Graf *et al.,* 2018;Kok*et al.,* 2019), this study demonstrated the commonly diagnosed CCT types with the associated risk factors such as breed, age, sex and site of tumour occurrence. Despite the large number of pet owners in this part of the country, the reason for the small occurrence of skin tumours in this region is not known. Nevertheless, one would consider the limited number of cases in the study as a result of dog owners being hesitant to shoulder the financial burden associated with diagnostic services. Furthermore, the unwillingness of pet owners to submit their cases, coupled with inadequate record keeping and the unfortunate circumstance of dogs passing away before seeking diagnostic and therapeutic measures, might also contribute to the small sample size. In addition to this isthe failure of some private veterinary clinicians in referring cutaneous masses for appropriate diagnosis after surgical excision. The increased prevalence of cutaneous tumours submitted from Lagos was because it is a cosmopolitan city where most people own one dog or more. Results from this study on epithelial cutaneous tumours having the highest prevalence are in tandem with previously reported data on cutaneous tumours in dogs (Sanja *et al.,* 2005; Mukaratirwa *et al.,* 2005; Pakhrin *et al.,* 2007). This study recorded an increase in the frequency of benign tumours (51.2%) which is contrary to previous reports of Moraes *et al.,* (2009) and Kok *et al.,*(2019) but in agreement with Mukaratirwa *et al.,*(2005); Pakhrin *et al.,* (2007) and Graf *et al.,* (2018). Histiocytoma had the highest frequency as a benign tumour, indicating an increased rate of its occurrence among dogs.The highest prevalence of epithelial tumours in this study agrees with previous studies (Pakhrin *et al.,* 2005; Sanja *et al.,* 2005). SCC had the highest frequency among the epithelial tumours. This tumour has been associated with increased exposure to UV light and most dogs are kept outdoors, thereby increasing their exposure to sunlight. Round cell tumour is the second most diagnosed tumour in this study, unlike the previous studies. Mukaratirwa *et al.,*(2005) and Kok *et al.,*(2019) reported mesenchymal cutaneous tumours as the second most frequently diagnosed tumours. Comparing the prevalence of the five most common cutaneous tumour types described in this study (Squamous Cell Carcinoma, Histiocytoma, Transmissible venereal tumour, Papilloma, and Haemangiosarcoma), with previous studies, the order of prevalence varied between studies with most studies reporting Mast cell tumouras the most prevalent tumour. However, this is not in agreement with the reports of Chikweto *et al.,* (2011) and Reddy *et al.,* (2009) who reported haemangiosarcoma and lipoma respectively as the most prevalent CCT.In this study, mast cell tumour was not diagnosed unlike in other studies. These disparities between our data and the data obtained in previous studies from frequently diagnosed tumours may be attributed mainly to differences in the diagnostic criteria, the classification system used by various authors, tumour sample size and breed, the geographical locations as well as environmental influences. Although in our study, cutaneous transmissible venereal tumour (CTVT) was the third most frequently diagnosed tumour compared to other studies in which CTVT in dogs was not frequently reported except in studies from Korea, Brazil, and Grenada. This might be attributed to uncontrolled breeding due to unrestricted movement of unneutered dogs in the study areas, and the most affected breed with

higher risk for this tumour was the mixed breed dogs. Previous studies have shown that the pure breed of dogs was mostly affected or had an increased risk of cutaneous tumour development than the mixed breed (Mongrels), (Moraes *et al.*, 2009; Graf *et al.*, 2018; Kok *et al.*, 2019). The pure breeds mostly affected in this study with SCC and histiocytoma were Boerboel and German shepherd respectively, this might probably be due to the frequencies of each of these breeds in the study locations for security purposes. The occurrence of cutaneous tumours in the pure breed is well known (Moraes *et al.*, 2009; Chikweto *et al.*, 2011; Kok *et al.*, 2019). Boxers, Labrador retrievers, and Cocker spaniels are examples of breeds that are commonly affected with mast cell tumours as reported in previous studies. Murkaratirwa *et al.*, (2005) reported a higher frequency of epithelial tumours in German shepherds, which is not in tandem with this study where the highest frequency of epithelial tumours was recorded in Boerboel while German shepherd recorded the highest frequency in mesenchymal cutaneous tumours. Round cell tumours mostly affected a mixed breed of dogs. The reason for this occurrence is unknown but it is possible to suggest that this might be due to alterations in the genetic makeup of this breed of dogs during breeding. Our study revealed a predominance of female dogs (55%)over their male counterpart (45%). This might be due to the preference for female dogs by dog owners for breeding and financial gains in the study area. The risk of developing cutaneous tumours increased in dogs aged 4 to 14 years, although there was no significant association between the occurrence of tumours and age. Advancement in age has been reported as a predisposing factor for the occurrence of cutaneous tumours (Moraes *et al.*, 2009). SCC has been previously reported in older dogs and this is in tandem with this study. Some studies reported a greater predisposition towards developing histiocytoma among young dogs (Pires *et al.*, 2003), but it was frequent in dogs between ages 5 and 7 years which could be due to the relatively small sample size used for this study. It was also observed that TVT had a higher prevalence in younger dogs (< 1-3 years) in this study which could be a result ofthe unrestricted movement of young dogs, though no age predilection has been reported in previous studies. It was also observed that most dogs affected with this tumour were mostly intact female mixed breeds that were often allowed to wander at will. The risk of cutaneous tumour occurring on the head, trunk, and extremities (fore and hind limbs) was prevalent in this study. SCC occurred more on the limbs, head and trunk and this agrees with Graf *et al.*,(2018) who reported SCC occurring primarily on the head, limb extremities, and ear while Kok *et al.*, (2019) reported a high occurrence on the digit.

Cytology was carried out on 17 cases of primary cutaneous tumours. The cytological examination was helpful for recognizing the characteristic features of some tumours (Plate 4), but it was difficult to classify them in many cases. Immunohistochemical staining for actin, vimentin, desmin, pan-cytokeratin, S-100 protein, and alpha-smooth muscle actin proteins characterized the immunophenotype of CCT in previous studies (Perez *et al.*, 1999; Chijiwa*et al.*, 2004). In our study,S-100, muscle actin, desmin, vimentin, and pan-cytokeratin were used in making adifferential diagnosis of CCT (Plate 1-5).

Plate 1a: Squamous Cell Carcinoma revealed the extension of squamous epithelial cells (SQ) in the dermis (D), forming islands and a focal area of keratin "pearls" (K). H&E

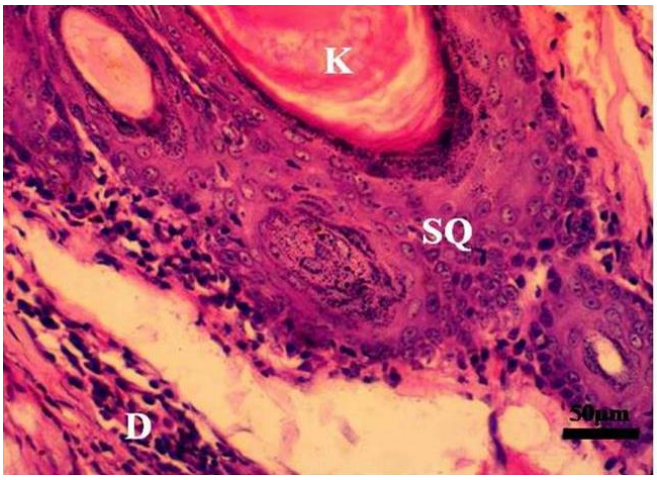


Plate 1b: Squamous Cell Carcinoma showed strong cytoplasmic expression to pan-cytokeratin in the neoplastic squamous cells (SQ). Biotin Streptavidin peroxidase, DAB.

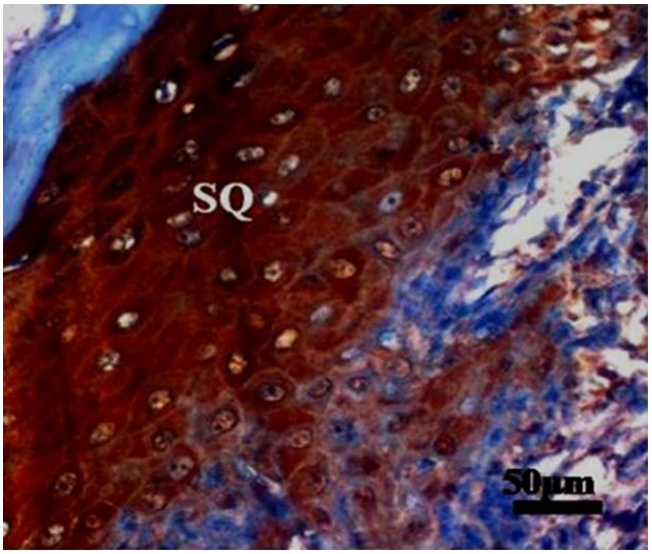


Plate 2a: Fibrosarcoma tissue scraping, showing elongated or spindle or cigar-shaped cells (CS) with high nuclear-cytoplasm ratio (arrow) with tapered cytoplasm, neutrophils (N) and red blood cells (RB) at the background. Giemsa Stain

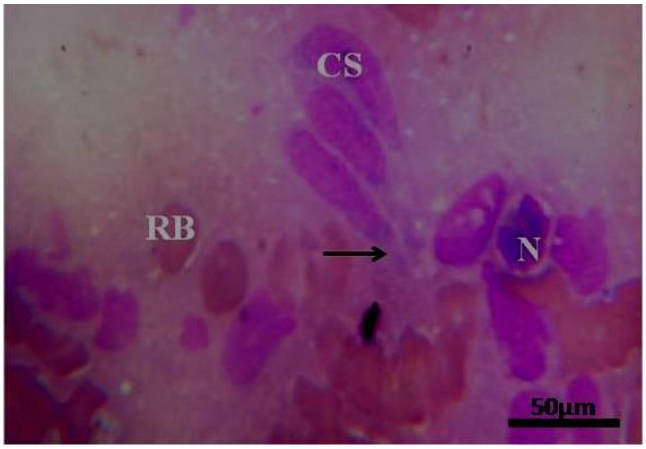


Plate 2b: Fibrosarcoma showing the presence of oval, elongated shaped cells, multinucleated giant cells (Arrow) and mitotic figures (Arrow head). H&E

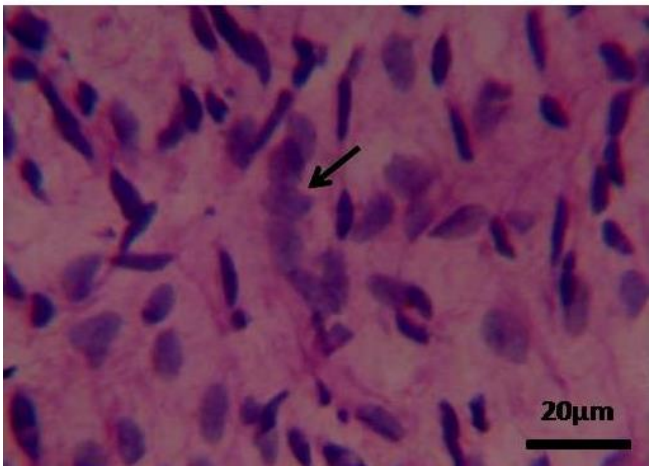


Plate 2c: Fibrosarcoma, the neoplastic cells are showing moderate cytoplasmic expression of vimentin. Biotin Streptavidin peroxidase, DAB

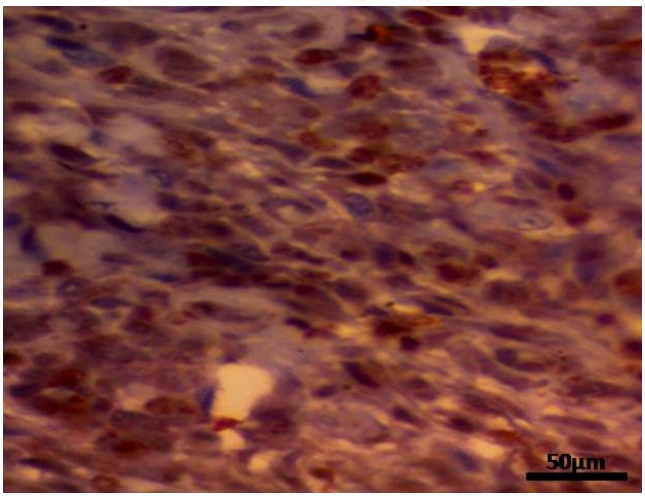


Plate 3a: Malignant melanoma showing neoplastic cells contained abundant intracytoplasmic melanin (M) and some nuclei displayed prominent 2-3 nucleoli (Arrow). H&E

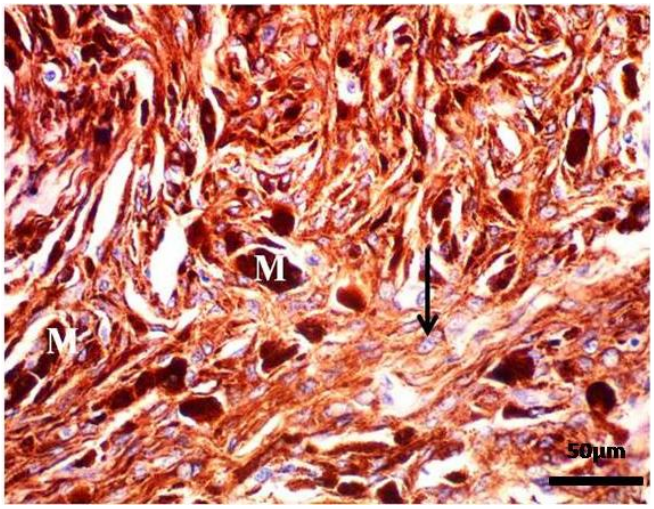


Plate 3b : Malignant melanoma showing neoplastic cells which are mainly in the dermis and expressing severe cytoplasmic immunoreactivity to S-100 protein (Arrow). Biotin Streptavidin peroxidase, DAB

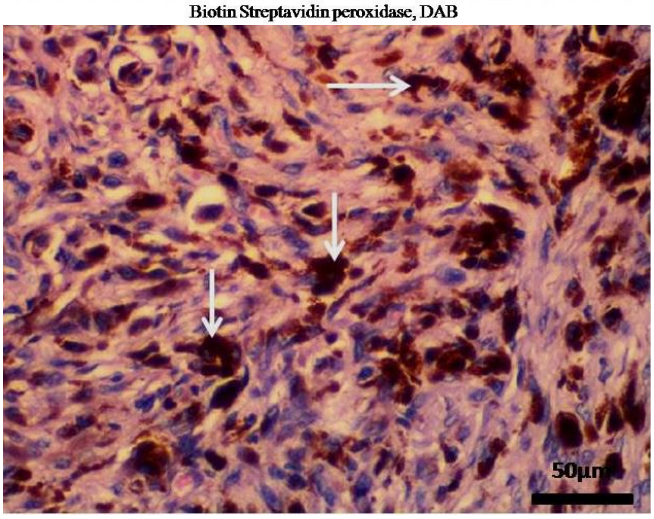


Plate 4a: Cytology of Transmissible Venereal Tumour showing numerous mitotic figures and both cytoplasmic and nuclear vacuolations. Field stain.

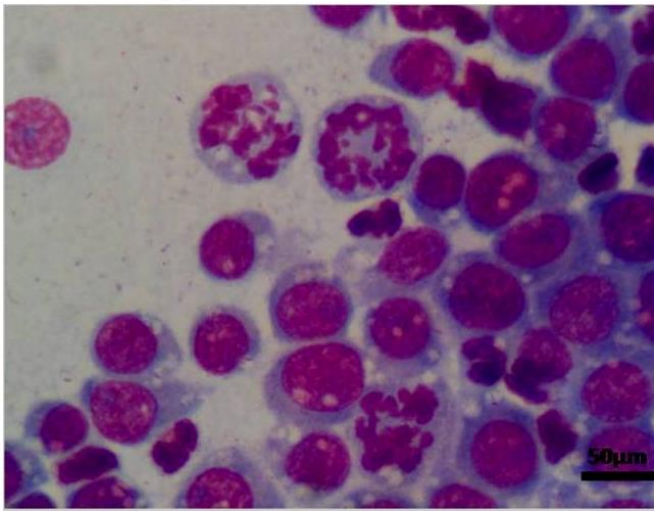


Plate 4b: Transmissible venereal tumour showing loose sheets of neoplastic cells with a few giant cells (Arrow), mitotic figure (Arrow head) and infiltration by a few neutrophils, and numerous lymphocytes. H&E.

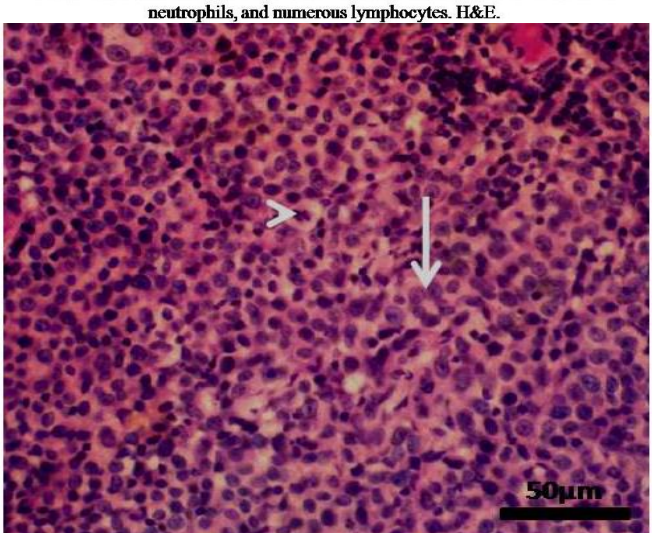


Plate 4c: Immunohistochemistry, Nuclear and cytoplasmic Immunoreactivity of Transmissible Venereal Tumour to S-100. Biotin Streptavidin peroxidase, DAB.

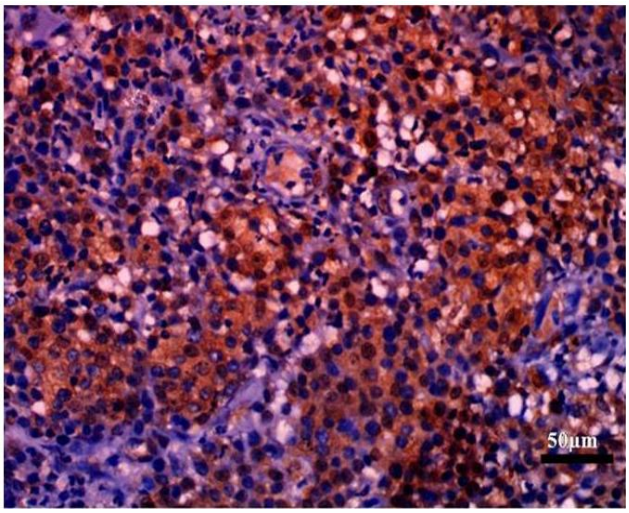


Plate 5a : Myxosarcoma showing round to spindle shaped fibroblast (arrow head) and presence of sparse lymphocytic infiltrates (arrow). H&E

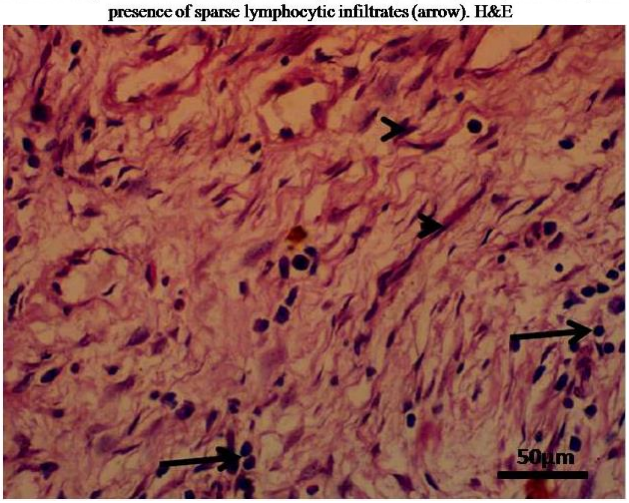
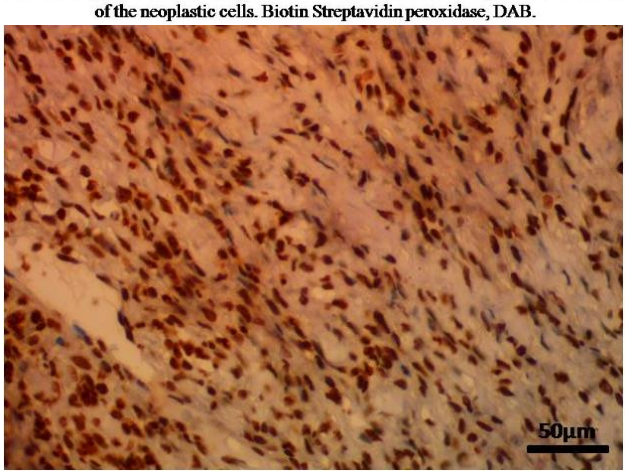


Plate 5b: Myxosarcoma showing severe nuclear immunoreactivity to desmin in most of the neoplastic cells. Biotin Streptavidin peroxidase, DAB.



Tumors of the epithelial and adnexal origin were immunohistochemically positive to pan-cytokeratin (papilloma, squamous cell carcinoma, sebaceous carcinoma, meibomian gland adenoma, and malignant pilomatricoma) while vimentin, a-SMA, desmin, and S-100 proteins differentiated tumours of the mesenchymal origin such as fibrosarcoma, malignant histiocytoma, and peripheral nerve sheath. These immunohistochemical observations are in tandem with previous reports (Perez *et al.*, 1999; Chijiwa*et al.*, 2004). However, these tumour markers identified not all these neoplasms.Admittedly, this retrospective study showed some limitations. These include a lack of standardization of sample collections; a lack of proper follow-up of some cytological samples with histopathological evaluation; incomplete medical records of few cases and alimited number of cases identified. The availability of this information would have translated directly into therapeutic interventions in these cases. However, these inadequacies might have been due to the fact that only 2 diagnostic laboratories offer histopathological and other Veterinary diagnostic services, and not all tumours were sent for histopathological diagnosis in the study area, thus it is possible to suggest that the overall cutaneous tumour incidence in southwest Nigeria dog population is underestimated. Although, histopathology has been recommended as the best diagnostic tool in the diagnosis of tumours but its inability to differentiate some CCT was another limitation encountered in this study, so the immunohistochemical method was used to determine their tissue or cellular origin. In the context of tumour follow-up after surgical removal, the time of freedom from disease, survival period, and time of recurrent tumour could not be ascertained in this study, thus hampering the potential prognostic value of the diagnostic methodologies used in this study. While this study is limited in scope, there are some benefits associated with the study, notably the identification of risk factors, cytological and histopathological features that assisted us in the classification of the CCTs, and immunohistochemical confirmation of the cellular origin of some of them. Moreover, the results of this study elucidate the prevailing types in the canine population in the southwestern part of Nigeria and would be useful for further research on canine oncology.

CONCLUSION

The study demonstrated that CCTs were observed to be influenced by breed, sex, and predilection site, except for age. These identified risk factors were considered as predisposing factor for the development of these tumours in the study population. Consequently, this research provides valuable insights into the epidemiological patterns of CCT in the three southwest states of Nigeria. Moreover, it holds significant importance in the field of small animal practice as it facilitates prompt and accurate diagnosis of suspected cutaneous tumours in dogs. Additionally, these findings can serve as a point of reference for future investigations into CCT in any geographic region within the country and throughout sub-Saharan Africa as a whole.

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