ULPGC
Universidad de Las Palmas de Gran Canaria

Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

IUSA

TESIS DOCTORAL

VETERINARY CANCER REGISTRIES. EPIDEMIOLOGY OF CANCER ON THE CANINE POPULATION OF THE CANARY ARCHIPELAGO

JOSÉ RODRÍGUEZ TORRES

DOCTORADO EN SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA

LAS PALMAS DE GRAN CANARIA

JUNIO 2023

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MAYO 2023

D ANTONIO JESÚS FERNÁNDEZ RODRÍGUEZ COORDINADOR DEL PROGRAMA DE DOCTORADO DE SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

INFORMA,

Que la Comisión Académica del Programa de Doctorado, en su sesión de fecha / / tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada **"Veterinary Cancer Registries. Epidemiology of cancer on the canine population of the Canary Archipelago"** presentada por el doctorando **D. José Rodríguez Torres** y dirigida por el **Doctor Antonio Espinosa de los Monteros y Zayas y el Doctor Ángelo Santana del Pino.**

Y para que así conste, y a efectos de lo previsto en el Arto 25 del Reglamento 1/2023, de Estudios de Doctorado (BOULPGC 27/01/2023) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 24 de mayo de 2023.

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

ESCUELA DE DOCTORADO

Programa de doctorado

DOCTORADO EN SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA

Título de la Tesis

VETERINARY CANCER REGISTRIES. EPIDEMIOLOGY OF CANCER ON THE CANINE POPULATION OF THE CANARY ARCHIPELAGO

Tesis Doctoral presentada por D. JOSÉ RODRÍGUEZ TORRES

Dirigida por el Dr. D ANTONIO ESPINOSA DE LOS MONTEROS Y ZAYAS

Codirigida por el Dr. D ÁNGELO SANTANA DEL PINO

El Director **El Codirector** **El Doctorando**

Las Palmas de Gran Canaria, a 24 de mayo de 2023.

D ANTONIO ESPINOSA DE LOS MONTEROS Y ZAYAS, CATEDRÁTICO DEL DEPARTAMENTO DE MORFOLOGÍA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

INFORMA:

Que D. José Rodríguez Torres, ha realizado bajo mi dirección y asesoramiento técnico y científico, el presente trabajo titulado: **"VETERINARY CANCER REGISTRIES. EPIDEMIOLOGY OF CANCER ON THE CANINE POPULATION OF THE CANARY ARCHIPELAGO"**, el cual considero reúne las condiciones y calidad científica para optar al grado de Doctor.

Y para que así conste, firmo el presente en Las Palmas de Gran Canaria, 24 de mayo de 2023.

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D ANGELO SANTANA DEL PINO, PROFESOR TITULAR DEL DEPARTAMENTO DE MATEMÁTICAS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

INFORMA:

Que D. José Rodríguez Torres, ha realizado bajo mi dirección y asesoramiento técnico y científico, el presente trabajo titulado: **"VETERINARY CANCER REGISTRIES. EPIDEMIOLOGY OF CANCER ON THE CANINE POPULATION OF THE CANARY ARCHIPELAGO"**, el cual considero reúne las condiciones y calidad científica para optar al grado de Doctor.

Y para que así conste, firmo el presente en Las Palmas de Gran Canaria, 24 de mayo de 2023.

…it’s about how hard you can get hit, and keep moving forward.

How much you can take, and keep moving forward.

That’s how winning is done.

Now, if you know what you’re worth,

then go out and get what you’re worth.

Rocky Balboa talking to his son.

The highway's jammed with broken heroes.

On a last chance power drive

Everybody's out on the run tonight

But there's no place left to hide

Together, Wendy, we can live with the sadness

I'll love you with all the madness in my soul

Oh, someday, girl, I don't know when

We're gonna get to that place

Where we really wanna go and we'll walk in the sun

But 'til then, tramps like us

Baby, we were born to run.

Bruce Springsteen on Bon to Run.

A mi mujer, Patricia, por todo.

A mis padres, por su eterno apoyo.

A mis perros, Bonati y Ron, mis mejores maestros y mis mayores fuentes de inspiración.

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TABLA DE CONTENIDOS.

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1.- REGULATIONS AND ADAPTATION TO DOCTORAL THESIS BY COMPENDIUM OF PUBLICATIONS.

Regulation 1/2023 on Doctoral Studies at the University of Las Palmas de Gran Canaria (ULPGC) is the regulatory framework that defines the content and format of doctoral thesis presented at the University.

Thus, in Chapter III (Doctoral Thesis), Section Two (Content and Format of the Doctoral Thesis), it is established that the doctoral thesis consists of an original piece of research carried out by the doctoral student on a subject related to the doctoral programme in which he/she is enrolled and that it must contain at least an introduction or statement of the problem, the objectives proposed, the methodology developed, the results and their discussion, the main conclusions and the bibliography used.

Doctoral thesis may be written and defended in the languages commonly used for scientific communication in their field of knowledge, although in the case of doctoral thesis written in a language other than Spanish, a summary of the contents of the thesis in Spanish, between 3 and 15 pages in length, must be provided, including the objectives and conclusions.

Article 24 describes the requirements for doctoral thesis by means of a compendium of publications, which must include at least three publications with a thematic unity, indexed in the Journal Citations Reports, Arts and Humanities Citation Index or equivalent, of which the doctoral candidate is the first or main author. Similarly, at least one of these publications must have been published in a journal whose impact index places it in the first half, in decreasing order of impact index, of the journals in the field.

In terms of content, the thesis by compendium of publications must contain an introduction that presents the objectives of the thesis, the published works and the

justification of the thematic unit of the thesis, a copy of the published works and some final conclusions.

In this sense, this thesis fulfils the above requirements in that it presents three publications that have been published in scientific journals in the field of knowledge of the doctoral programme and that have been indexed in the Journal Citations Report, in which the doctoral candidate appears as first author and whose quality indicators are indicated below:

1.- José Rodríguez, David R. Killick, Lorenzo Ressel, Antonio Espinosa de los Monteros, Ángelo Santana, Samuel Beck, Francesco Cian, Jenny S. McKay, P.J. Noble, Gina L. Pinchbeck, David A. Singleton & Alan D. Radford. "A text-mining based analysis of 100,000 tumours affecting dogs and cats in the United Kingdom". https://doi.org/10.1038/s41597-021-01039-x. Scientific Data. Impact Factor 2021: 8.501. Journal Rank in Multidisciplinary Sciences: 13/135 (Q1).

2.- José Rodríguez, Ángelo Santana, Pedro Herráez, David R. Killick & Antonio Espinosa de los Monteros. "Epidemiology of canine mammary tumours on the Canary Archipelago in Spain". https://doi.org/10.1186/s12917-022-03363-9. BMC Veterinary Research. Impact Factor 2021: 2.792. Journal Rank in Veterinary Sciences: 25/145 (Q1).

3.- José Rodríguez, Ángelo Santana, Marisa Andrada Borzollino, Pedro Herráez, David R. Killick & Antonio Espinosa de los Monteros. "Epidemiology of canine cutaneous round cell tumours on the canary archipelago in Spain". DOI: 10.1111/vco.12899. Veterinary and Comparative Oncology. Impact Factor 2021: 2.385. Journal Rank in Veterinary Sciences category: 40/145 (Q2).

2.- INTRODUCTION: THE ROLE OF EPIDEMIOLOGY TO MINIMIZE THE IMPACT OF CANCER.

Cancer is one of the most important public health concerns of our time, both in the human and pet companion animal populations, and perhaps no other diagnosis is more concerning when it comes to our own health or that of our loved ones[1,2] and pets[3,4].

Under our care, our dogs and cats spend a lifetime with us, providing loyal companionship and enriching our lives in so many ways[5–8] that their welfare and health becomes an important part of our daily concerns.

For this reason, the study of the epidemiological risk and protective factors associated with the different types of cancer has become an important branch of veterinary science[9] with an important role to play in preventing cancer and ensuring the optimal health status of various pet populations.

Furthermore, from a 'One Health' approach, cancer prevention in our dogs and cats could potentially be useful for humans[10,11], as both owners and pets share a common environment. We both breathe the same air, drink the same water and are exposed to the same food sources and environmental pollutants, hence the importance of pets as sentinels and models of human health[12,13].

From this perspective, there is a clear need to monitor the health status of our pet populations and this is where the various animal health surveillance systems in general and veterinary cancer registries in particular come into play.

3.- BACKGROUND: VETERINARY CANCER REGISTRIES AS ANIMAL HEALTH SURVEILLANCE SYSTEMS.

3.1.- Animal health surveillance systems.

Animal health surveillance systems are key to maintaining a satisfactory level of health in any defined population. However, the usefulness and capacity of these systems is heavily dependent on the quality of the data collection systems that support them. In other words, the quality of the data determines the quality of the surveillance system in achieving its objective.

In the companion animal population, there are several data collection systems such as pet insurance databases, referral practice clinical records, primary care practice clinical records, questionnaire-based data collections, canine health schemes and veterinary cancer registries[14].

Despite their importance in improving the health of animal populations, there is no such thing as a perfect surveillance system and researchers operating these systems need to be aware of their inherent strengths and weaknesses, the latter expressed in the form of various types of bias that potentially undermine the capacity of the surveillance system to play its role effectively.

As veterinary cancer registries have been the backbone of this thesis, a more detailed explanation of where they come from, how they work and the challenges they face is described below.

3.2- Tumour registries in human populations and its role in cancer control.

The general idea behind the role of tumour registries in cancer control is very simple. All tumours diagnosed in a particular population over time are recorded and compared with

the total population in that area to obtain the incidence of the different cancers in that area. In addition, by following up people with these cancers and determining the time of death, the survival time for each tumour could be determined. Over time, the information provided by the system will make it possible to analyse trends in cancer incidence in the population and, if run in the same way on different geographical areas, it will be possible to analyse differences between different cities, regions and countries.

However, although easy to understand as a concept, the practical implementation of cancer registries as a cancer control tool involves some aspects that should be considered and managed in order to assess the data quality within the cancer registry and its capacity to serve as a cancer control tool. These difficulties are classified into four recognised dimensions of data quality[15,16]: [1] Comparability, which refers to the extent to which coding and classification schemes conform to agreed international guidelines, [2] Validity/accuracy, which is defined as the proportion of cases in a dataset with a given characteristic, [3] Timeliness, which has no formal definition in this context but can be thought of as the speed with which a registry can collect, process and report sufficiently reliable and complete cancer data, and [4] Completeness, or the extent to which all incident cancers in the population are included in the registry database. In addition, depending on the sources from which the information is collected, TRs can be hospital-based (HTR), pathology-based (PTR) or population-based[17] , the latter being the gold standard in human oncology as it systematically collects information on all reportable neoplasms occurring in a geographically defined population from multiple sources - all those where cancer cases can be diagnosed or treated, such as hospital records, pathology diagnostic laboratories and even, where possible, death certificates where cancer is listed as a principal or contributory cause of death[18] .

Thus, the historical development of cancer registration is essentially the story of how researchers in the field have dealt with these four dimensions of data quality to facilitate the evolution from the first recognised population-based cancer registration in Hamburg

in 1927[18], whose main role was to provide information on cancer incidence in a defined area, to the much more powerful, networked[19,20] and multi-purpose modern registries that are active in several areas of cancer, such as epidemiological research into the causes of cancer, monitoring and evaluation of screening programmes, and follow-up of cancer patients in relation to the quality of cancer care.

3.3.- Tumour registries in pet companion populations and its role in cancer control.

The idea behind using cancer registries as a key tool to control cancer in human populations is essentially the same for using these surveillance systems in pet populations. In fact, in an ideal scenario, tumour registries in companion animals would strive for the same multipurpose use as their human counterparts. However, veterinary research faces greater challenges in this area than in human medicine.

However, from a didactic point of view, it is useful to consider human cancer registries as role models for companion animal cancer registries in order to gain a better understanding of the current limitations that these cancer registries may face and, more importantly, understanding these limitations will be the starting point to navigate through the different options to overcome these adversities and thus improve the capacity of cancer registries as health surveillance systems.

In this sense, with regard to the four dimensions of quality previously described, completeness would be the main limitation of cancer registries in the veterinary field, because, unlike the population-based tumour registries used in human oncology, most epidemiological cancer-related studies carried out in the veterinary field have been based either on data from one or more pathology laboratories[21] or on data from insurance companies[22,23] or other networks collecting data from veterinary practitioners[24,25]. Consequently, since not all cases are reported and those that are come from provided by a limited types of data provider (either pathology laboratories or insurance

companies), the animal cancer profile obtained is potentially biased (selection or referral bias) and not complete (under-reporting)[14].

Reasons for referral or selection bias could be motivated by socio-economic reasons and the fact that some low-income owners may not have the economic resources to pay for the surgery necessary to remove a lump recently detected in their dog or cat[26]. Researchers have attempted to minimise this problem of under-reporting by offering free histopathological diagnosis to veterinarians working in their respective areas[27–31], although this may have led to over-reporting of cases[14]. In addition, most studies in this area describe selection bias as a result of superficial tumours such as mammary or skin tumours which are obvious to owners and veterinarians and may therefore be over-reported compared to other tumours such as those affecting internal organs. Finally, selection bias in this area may occur when reference pathology laboratories only receive samples of certain types of tumours, the diagnosis of which requires special equipment or very specific expertise on the part of pathologists. In these cases, the most common tumours (e.g. lipomas, mast cell tumours, breast tumours) will be under-represented compared to other less commonly diagnosed tumours (e.g. histiocytic sarcomas, leiomyosarcomas, etc.).

Validity/accuracy also represents a limitation in veterinary cancer registries particularly in cases where diagnosis-related data are not obtained by histopathology but are reported as clinical finding. This limitation is mainly described in cases where data providers are insurance companies[22,23] or other medical networks[24,25] rather than pathology laboratories, although the inherent subjectivity of pathological diagnoses which may involve some degree of bias should be taken into account.

In addition to the limitations of data quality in veterinary tumour registries, another major limitation is the lack of a background population (denominator) to which the sample population affected by a tumour can be compared[32], given the general lack of mandatory census data for pet populations in many countries[21].

Over the years, this limitation has led researchers to use different approaches to obtain estimates of a population denominator such as conducting surveys to calculate the baseline data of the animal population in the study area[27,29,33] or using animal databases of defined populations such as canine associations[30,34], diagnostic laboratories[35–38], vaccinated animal databases[39], insurance databases[22,23], or specific networks of animals attending veterinary practices[24,25,40,41].

4.- OBJETIVES.

The main general objective of this thesis was to provide the basis for studying the epidemiology of cancer in the pet population of the Canary Islands.

In order to achieve this general objective, three specific objectives were proposed:

1- To develop a methodology for extracting, classifying and standardising data on tumours in companion animals (dogs and cats) in order to create a database based on unstructured documents and reports, specifically the reports of the Anatomopathological Diagnostic Service of the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria.

2- To analyse mammary neoplasms and cutaneous round cell tumours in dogs and the characteristics of the animals affected.

3- To carry out an epidemiological study of cancer in pets on the Canary Islands by comparing the distribution of breeds, sexes and islands of residence of the animals in the tumour database with those in a pet database (ZOOCAN).

5.- MATERIAL AND METHODS.

Three studies on animal tumour databases have been published as part of this thesis project. The first was published as a data descriptor using data from the Small Animal Veterinary Surveillance Network (SAVSNET) in the UK, while the second and third studies were published as research articles focusing on the epidemiology of specific tumour groups affecting the canine population of the Canary Islands.

Therefore, we will briefly describe firstly the material and methods used in the first publication and secondly the material and methods used in the second and third articles, given their similarities. It should be noted, however, that this section only provides a general description of the materials and methods used to carry out the aforementioned studies. A more detailed description of the study designs, statistical analyses and

limitations can be found in the specific sections of each paper and in the Results and Discussion section below.

For the **first publication**, we used data from SAVSNET, a national surveillance network based at the University of Liverpool Veterinary School, which collects approximately 10000 diagnostic test results daily from participating laboratories, including haematology, pathology, biochemistry and infectious disease tests, and uses them to develop research and support national surveillance of companion animals[40]. In this study, we developed a text mining methodology to extract, classify and normalise an original dataset of 180232 free text (unstructured) electronic pathology records (EPRs) for dogs and cats obtained from three diagnostic laboratories in the UK between April 2018 and June 2019. As a result, 109895 canine and feline tumours were identified, along with a description of the tumour and the animal, and all this information was coded in a properly structured and ordered database.

This database was mainly created using Microsoft Excel and RStudio software and is available on Figshare[42].

With the **second and third publications**, we started our series of articles dedicated to the analysis of the epidemiology of cancer in companion animals in the Canary Islands, with the study of the two most important groups of tumours from a population perspective: mammary tumours (second paper) and cutaneous round cell tumours (third paper) in the dog population during the period 2003-2020.

These epidemiological studies are based on data from two main sources: The Anatomopathology Diagnostic Service (APDS) and the ZOOCAN databases, which are described below.

1.- The APDS receives approximately 1500 animal tissue samples per year from private and official veterinarians throughout the Canary Islands, together with a submission form describing the animal from which the sample was taken (species, breed, sex, neuter

status, age and location of the lesion). These specimens are processed and prepared for examination by the attending pathologist, who will ultimately provide a diagnosis of the specimen in a diagnostic report, which is kept in the ADPS archives.

In our case, one of the main achievements of this thesis project was to build, from these archives, a normalised database of the more than 20000 tumours diagnosed over a period of 18 years, from which we extracted data to carry out our first studies on the two groups of tumours previously mentioned: the mammary tumours (second paper) and the cutaneous round cell tumours (third paper).

2.- Our second source of data was the ZOOCAN database, a centralised web-based registry where veterinarians throughout the Canary Islands are required to register all companion animals under their care[43]. The database is managed by the Regional College of Veterinary Surgeons, who provided us with an anonymised dataset of registered animals to be used in our studies as a baseline population.

Both research articles were structured around two main parts. Firstly, we developed a longitudinal study of the main variables, such as age at tumour onset, presence of single vs multiple tumours or proportion of malignant vs benign cases, as well as the main trends observed, where we examined how the proportions of the different types of tumours have evolved over the study period.

Secondly, we conducted a case-control study comparing data on animals and tumours from the APDS database (cases) with animals from the baseline population described in ZOOCAN (controls) to analyse the associations of the variables breed (mainly), sex and island with the occurrence of the type of tumour studied in each of the publications.

An R script was developed for each of these studies and the result databases were hosted on Figshare[44].

6.- RESULTS AND DISCUSSION: SCIENTIFIC PUBLICATIONS.

6.1.- Paper 1: A text-mining based analysis of 100,000 tumours affecting dogs and cats in the United Kingdom.

scientific data

OPEN

DATA DESCRIPTOR

A text-mining based analysis of 100,000 tumours affecting dogs and cats in the United Kingdom

José Rodríguez[1], David R. Killick[2], Lorenzo Ressel[2], Antonio Espinosa de los Monteros[1], Angelo Santana[3], Samuel Beck[4], Francesco Cian[5], Jenny S. McKay[6], P. J. Noble[2], Gina L. Pinchbeck[2], David A. Singleton[2] & Alan D. Radford[2] ✉

Cancer is a major reason for veterinary consultation, especially in companion animals. Cancer surveillance plays a key role in prevention but opportunities for such surveillance in companion animals are limited by the lack of suitable veterinary population health infrastructures. In this paper we describe a pathology-based animal tumour registry (PTR) developed within the Small Animal Veterinary Surveillance Network (SAVSNET) built from electronic pathology records (EPR) submitted to this network. From an original collection of 180232 free text (non-structured) EPRs reported between April 2018 and June 2019, we used specific text-mining methodologies to identify 109895 neoplasias. These data were normalized to describe both the tumour (type and location) and the animal (breed, neutering status and veterinary practice postcode). The resulting PTR, the largest of its kind for companion animals to date, is an important research resource being able to facilitate a wide array of research in areas including surveillance, clinical decision making and comparative cancer biology.

Background & Summary

A tumour registry (TR) systematically collects and stores data allowing analysis and interpretation of these data from subjects with cancer providing useful information that may be used in different areas such as epidemiology, health care planning and monitoring[1].

Based on the sources from which the information is collected, TRs can be hospital-based (HTR), pathology-based (PTR) or population-based[2] with the latter being the gold standard in human oncology since it provides an unbiased profile of the cancer epidemiology in a defined population.

However, in the veterinary field, most previous animal TRs have been hospital-based or pathology-based[3] neither of which are appropriate for cancer surveillance purposes by themselves given that both provide an incomplete (underreporting) and inaccurate (biased) sample based either on patient attendance at a given hospital or on laboratory-based surveillance.

Additionally, the lack of a background population to which compare the sample population affected by a tumour has remained a key limitation to developing population-based veterinary cancer registries[3].

Researchers have tried to minimize this underreporting issue with different approaches to encourage participation of veterinary surgeons when it comes to submit samples for pathology diagnosis.

One approach adopted in TRs in the US (in 1968[4] and 1978[5]), involved researchers asking all veterinarians in their respective areas to submit reports for all confirmed tumours. In an adaptation of this method in Italy, national[6] and regional[7,8] TRs have offered free histopathologic diagnosis for practitioners operating in their respective areas. A similar process was used in the "Cancer in the Dog" project (1990–1998)[9], in Norway, and further updated in the Danish Veterinary Cancer Registry (2005–2008)[10], in which veterinarians were invited to submit their tumour diagnosis (TD) through a web-based application. Veterinary insurance databases have also been used[11,12] to obtain data from insured animal populations and finally, more recently, researchers have sought

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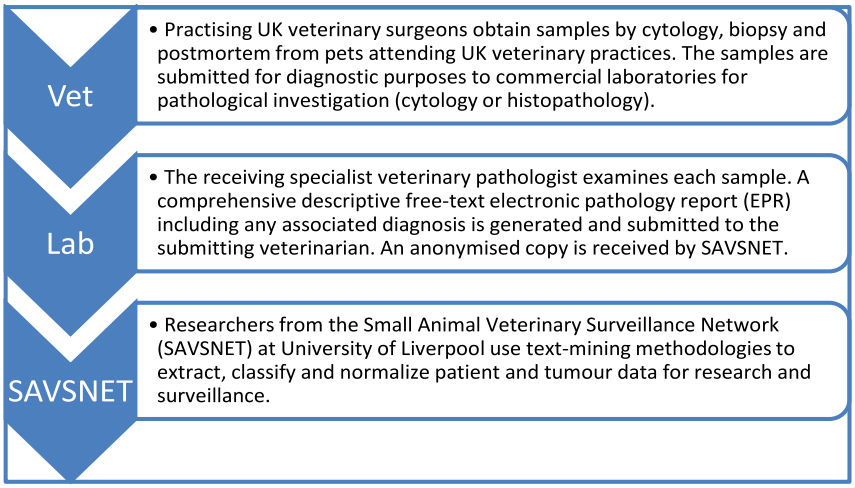

Fig. 1 Schematic overview of the methodology.

to harness data available in individual electronic pathology records (EPRs). In 2015, records from three diagnostic laboratories in Switzerland were used to create the Swiss Canine[13] and Feline[14] Cancer Registries, with more than 85000 tumour cases; the largest PTR so far.

Overall, animal TRs have been sporadic and usually been of limited duration[15] and have never provided a comprehensive and detailed tumour dataset but a selection of their general results such as the most frequent tumours, locations, breed, age, etc.

Ideally, to create a useful surveillance tool, underlying data flows should be continuous and large enough to represent the population being studied. The data should be available in databases as near to real time as possible and be easily searchable without a requirement for particular technical skills. Here we describe our approach to meet these targets, of a sustainable PTR covering a large population with national coverage and open access, using a health informatic approach to efficiently extract anonymised tumour data from large volumes of routinely collected companion animal EPRs.

Figure 1 shows our new approach that capitalises on existing data flows to an established national surveillance network (SAVSNET) which collects approximately 10000 diagnostic test results daily[16] from participating laboratories, including haematology, pathology, biochemistry and infectious disease assays and uses them to support national surveillance and research[17,18]. For this study, we employed a text-mining methodology to extract, classify and normalize animal tumour data from three diagnostic laboratories, encompassing a total of 180232 canine and feline EPRs across the UK between April 2018 and June 2019. The result is a normalized animal PTR of 109895 tumours pertaining predominantly to dogs (91.6%) and diagnosed more commonly by histology (63.4%) than cytology (36.6%). The most common tumours in dogs were lipomas (21.7%), mast cell tumours (13.1%), and histiocytomas (7.7%) and in cats, lymphomas (14%) and squamous cell carcinomas (11.1%).

To our knowledge, this is the largest and most comprehensive animal PTR at a national level providing a reliable tool for veterinary practitioners and researchers as well as a baseline from which further studies can be developed although being always aware of the aforementioned limitations of PRTs to perform surveillance strategies.

Given the importance of companion animals as sentinels and models of human health, this registry and its future developments could play a significant role in comparative studies with human cancer registries under a 'One Health' approach.

Methods

Sample collection and preparation. This project used anonymized diagnostic test results submitted to the Small Animal Veterinary Surveillance Network (SAVSNET) at University of Liverpool between April 2018 and June 2019 by three UK diagnostic laboratories (IDEXX Laboratories, the Veterinary Pathology Group (VPG) and Batt Laboratories Ltd). During the study period and based on matching of postcodes, this included data from 2196 (48%) of the 4573 UK small animal veterinary practices in the Royal College of Veterinary Surgeons practice database (as used in former publications[17]), and from 120 of 121 UK postcode areas (only missing Hebrides), as well as Jersey, Guernsey and the Isle of Man. Each test result includes assay codes, test methodologies, sample descriptors, results (e.g. pathologist microscopic description) and pathologist interpretation as well as patient details including species, age, sex and a geographical locator based on the UK postcode of the submitting veterinary practice.

For this study, assay codes for cytology and histopathology were used to extract relevant animal and test data for manipulation in Microsoft Excel. Additionally, data were filtered by species to only include EPRs from cats and dogs.

In most cases, each row represented a unique laboratory submission, with columns containing information about the animal (such as breed, sex, neuter status), the sample taken (unique reference, date of record, assay type and postcode of the veterinary practitioner) and a free text description of the pathology report including diagnosis, prognosis, clinical summary, histology and comments. From some laboratories, data for individual samples

	A	B	C	D	E	F	G	H
1	LABNO	RECD	SPECIES	BREED	GENDER	PRACTICE_ID	ASSAY_CODE	RESCOMMENT1 (Pathology report)
2	R.123	09/05/18	Canine	Labrador retriever	Female entire	XXXX XXX	HISTO	 DIAGNOSIS 1. Malignant mixed mammary gland tumour, gland three 2. Simple intratubular tubulopapillary carcinoma of the mammary gland, grade 2 - gland four 3.Consistent with MCT (second grade), forelimb 4. Low-grade cutaneous Lymphoma, highly likely> PROGNOSIS Cautious CLINICAL HISTORY Two mammary masses and a forelimb mass removed. Samples from skin lesions were also taken HISTOLOGY Four specimens are submitted and evaluated…

Table 1. Example of a typical electronic pathology report used in this study.

	A	B	C	D	E	F	G	H
1	LABNO	RESCOMMENT1 (Pathology report)	DIAGNOSIS	PROGNOSIS	CLINICAL HISTORY	HISTOLOGY	DIAGNOSIS INFO	LOCATION INFO
2	R.123	 DIAGNOSIS 1. Malignant mixed mammary gland tumour, gland three 2. Simple intratubular tubulopapillary carcinoma of the mammary gland, grade 2 - gland four 3.Consistent with MCT (second grade), forelimb 4. Low-grade cutaneous Lymphoma, highly likely> PROGNOSIS Cautious CLINICAL HISTORY Two mammary masses and a forelimb mass removed. Samples from skin lesions were also taken HISTOLOGY Four specimens are submitted and evaluated…	5	271	309	427	DIAGNOSIS 1. Malignant mixed mammary gland tumour, gland three 2. Simple intratubular tubulopapillary carcinoma of the mammary gland, grade 2 - gland four 3. Consistent with MCT (second grade), forelimb 4. Low-grade cutaneous Lymphoma, highly likely> 	CLINICAL HISTORY Two mammary masses and a forelimb mass removed. Samples from skin lesions were also taken

Table 2. Data extraction. Step 1: Tumour diagnosis (TD) and lesion location (LL) information were automatically extracted from the pathology free text based on key words used to delimit sections of the report.

(same sample reference) were supplied in a series of consecutive rows that required prior concatenation based on the sample reference number. Table 1 shows an example of a submission. For ease of manipulation, animal and lesion data were separated into two tables linked by the unique laboratory number.

Data extraction. Data extraction from the free text pathology report (column H, Table 1), was carried out in three steps as described below.

STEP 1: Diagnosis and lesion location. Key words were used to extract specific sections of the text related to diagnosis and location of reported lesions. These key words could be slightly different depending on the laboratory from which the results emanated or the assay type (histology or cytology). For instance, in a histology report, the diagnosis appeared after the word DIAGNOSIS, while on a cytology report it was written after the words CYTOLOGICAL INTERPRETATION.

In order to facilitate explanation of the extraction process, an example of pre-extraction data is shown in Table 1. Data concerning tumour diagnosis was located between the words 'DIAGNOSIS' and either 'PROGNOSIS' or 'CLINICAL HISTORY' (since sometimes, a prognosis section was not included). Data pertaining to lesion location (LL) was reported normally either close to the TD or between CLINICAL HISTORY and HISTOLOGY, as can be seen in Table 1. Less frequently LL was positioned between HISTOLOGY and COMMENTS.

Given that LL could be written in one of these three different sections of the EPR, we developed a search which looked for LL in each of the possible positions within it. A prioritization system was then established selecting LL positioned between DIAGNOSIS and CLINICAL DIAGNOSIS over a LL between CLINICAL HISTORY and HISTOLOGY which itself was prioritised over an LL written in the histology section between HISTOLOGY and COMMENTS.

The positions of each of these key words (DIAGNOSIS, PROGNOSIS, CLINICAL HISTORY, and HISTOLOGY) were identified using the Excel function SEARCH (columns C-F, Table 2). Subsequently, the MID function was used to extract the text potentially containing TD (between DIAGNOSIS and PROGNOSIS) and LL (as explained above) into separate columns (columns G and H, Table 2).

STEP 2: Separation into single lesions. In some cases, multiple lesions were recorded in a single submission so we decided to look for a maximum of six possible tumours in each animal since the frequency of report numbers repeated up to six times was small (approximately 1% of all the reports).

	G	H	I	J	K	L	M	N	O	P
1	**DIAGNOSIS INFO**	**LOCATION INFO**	1.	2.	3.	4.	1st tumour	2nd tumour	3rd tumour	4th tumour
2	DIAGNOSIS 1. Malignant mixed mammary gland tumour, gland three 2. Simple intratubular tubulopapillary carcinoma of the mammary gland, grade 2 - gland four 3.Consistent with MCT (second grade), forelimb 4. Low-grade cutaneous Lymphoma, highly likely> 	CLINICAL HISTORY Two mammary masses and a forelimb mass removed. Samples from skin lesions were also taken 	14	70	165	215	1. Malignant mixed mammary gland tumour, gland three 	2. Simple intratubular tubulopapillary carcinoma of the mammary gland, grade 2 - gland four 	3.Consistent with MCT (second grade), forelimb 	4. Low-grade cutaneous Lymphoma, highly likely>

Table 3. Separation of single lesions in a case with four different tumours.

The vast majority of such cases were identified as an individual diagnosis preceded by a number or a letter, as a delimiter (as shown in column G, Table 3 for an animal suffering from four tumours). Data relating to each of these lesions was extracted using the SEARCH function to locate the separating characters ("1." to "6." or "A" to "F") and the MID function to extract the data pertaining to the individual lesion into a separate column (column M-P, Table 3).

STEP 3: Identification of single lesions and tumour classifiers. We next identified tumour types, locations and grades recorded within the now separated lesion free text. First, all unique lesional free texts from columns M to P in Table 3 were copied into a single column of a new spreadsheet (e.g Table 4 column B).

As we planned to make the PTR search and sortable by both TD and tumour characteristics, we parsed the data into individual columns for each data item. An iterative process was then used in Table 4 to identify text relating to each TD (column C), tumour grade (columns D and E), degree of differentiation (column F), the location of the tumour (column H) and probability terms related to the pathologist's confidence in the TD such as "highly likely", "probable" or "consistent with" (column G).

This was accomplished using a nested array operation in Excel to identify text within all these above columns (C-H, Table 4) that matched a series of curated lists[19] compiled iteratively as a series of six look up tables (columns A-F, Table 5).

In particular, the curated reference TD list (column A, Table 5) was created mainly from 'Tumors in Domestic Animals'[20] (a standard and comprehensive text in the field).

Each nested array formula took the following general format: = INDEX(**Primary_tumour**,MATCH(TRUE,ISNUMBER(SEARCH(Primary_tumour;$B2)),0)).

As an example, the above formula searches for text in a specific cell of Table 4, column B that matches any of the terms in Table 5, column A, starting from the top of this column. This function works downwards from the top of the column until it reaches a matching entry. Once a match is identified it is copied to a new cell (in this case Table 4, column C). Consequently, only a single match was recorded.

Each column of Table 5 was established iteratively using this approach. The reference tables were first populated with generic "capture terms" based on domain knowledge.

For example: Column A included words like tumour, carcinoma, neoplasia etc, whereas Column F included head, neck, mammary etc. Patterns found by these capture terms in the first search were checked and specific tumour names added back to the top of Table 5 column A as necessary. As an example of this, in a first search with only generic "capture terms" (such as "Tumour", "Carcinoma" or "Neoplasia") in column A Table 5, cells C2 and C3 of Table 4 would have shown the terms "Tumour" and "Carcinoma" instead of "Mixed mammary gland tumour" and "Simple intratubular tubulopapillary carcinoma" respectively. Eventually, however, once these more specific TD terms were added on the top of the general ones in column A Table 5, they were the ones assigned to the record (instead of the general ones) and shown in cells C2 and C3 of Table 4.

After each round of searching and augmenting the look up tables, 200 records from Table 4 that did not match on a specific column in Table 5 were read, and any newly identified terms added to Table 5. This process was repeated iteratively until no new terms were identified in 200 read texts.

Data entries possible in each column of Table 5 are as follows:

COLUMN A - Type of primary tumour: It includes 1808 general expressions of tumour types.
We used a case definition outlined in 'Tumors of Domestic Animals'[20] and former publications[12,13] in such a way that those tumours considered specifically as neoplasms or tumours in these texts were included in the PTR while other lesions classified as hamartomas, cysts or tumour-like masses, were excluded.
COLUMN B - Grade 2 tier (Kiupel for MCT): here we have included the terms low-grade, intermediate-grade and high-grade where recorded.
COLUMN C- Grade 3 tier (Patnaik for MCT): here we have included the terms grade I, grade II and grade III where recorded.
COLUMN D - Differentiation: this list includes terms related to the differentiation of the tumour such as "Benign", "Malignant", "Undifferentiated" or "Well- differentiated".
COLUMN E - Uncertain terms: this category contains terms that may be added to the TD when the pathologist has any doubt about the diagnosis such as "highly likely" lymphoma or "consistent with" lipoma.

	A	B	C	D	E	F	G	H
1	**Tumour_ref**	**Lesion description**	**Primary_tumour**	**Grade_2_tier (Kiupel for MCT).**	**Grade_3_tier (Patnaik for MCT).**	**Differentiation**	**Uncertain terms**	**Location**
2	R.123-T.1	1. Malignant mixed mammary gland tumour, gland three 	Mixed mammary gland tumour			Malignant		Mammary gland
3	R.123-T.2	2. Simple intratubular tubulopapillary carcinoma of the mammary gland, second grade - gland four 	Simple intratubular tubulopapillary carcinoma		grade 2			Mammary gland
4	R.123-T.3	3.Consistent with MCT (second grade), forelimb 	MCT		second grade		Consistent with	Forelimb
5	R.123-T.4	4. Low-grade cutaneous Lymphoma, highly likely> 	Lymphoma	Low-grade			Highly likely	Skin

Table 4. A new column with all the individual lesions.

	A	B	C	D	E	F
1	**Primary_tumour**	**Grade_2_tier**	**Grade_3_tier**	**Differentiation**	**Uncertain terms**	**Tumour_location**
2	**N = 1808**	**N = 14**	**N = 9**	**N = 22**	**N = 39**	**N = 398**
3	(hepatoid gland) adenocarcinoma	Low grade		Malignant transformation	Compatible with	Anal region
4	(hepatoid gland) adenoma			Benign	Consistent with	Anal sac
5	(hepatoid gland) carcinoma	Low-grade		Malignant	Favoured	Anal gland
6	(hepatoid, circumanal) gland adenocarcinoma	High-grade		Poorly differentiated	Follow	Perianal
7	(hepatoid, circumanal) gland adenoma			Moderately differentiated	Highly likely	Anal
8	(hepatoid, circumanal) gland carcinoma	Intermediate-grade		Well differentiated	Highly suggestive	Hindlimb
9	adenocarcinoma (anaplastic)			Poorly differentiated	Inconclusive	Forelimb
10	Adenocarcinoma arising in mixed gland mammary			Moderately-differentiated	Indicative of	Axilla
11	Adenocarcinoma arising in mixed mammary			Well-differentiated	Keeping with	Brain
12	adenocarcinoma of the anal sac apocrine glands			Undifferentiated	Likely	Chest
13	adenocarcinoma of the apocrine glands					
14	adenocarcinoma of the mammary gland, tubulopapillary		Grade II			Mammary gland
15	adenocarcinoma of the Parathyroid gland					
16	Apocrine ductal carcinoma					

Table 5. A sample of the six look up tables[19] created to search for specific text in the pathology free text (*grades in the dataset have been kept for the tumours indicated in the Data records section, columns J and K. In this table they are shown just as an example).

	A	B	C
1	**Results from the tumour types**	**Number of times each term has been counted**	**Unique term for a certain tumour.**
2	(hepatoid gland) adenoma	5	Adenoma of the hepatoid glands
3	(hepatoid, circumanal) gland adenoma	5	
4	Perianal (hepatoid) adenoma	4	
5	Hepatoid adenoma	10	
6	Hepatoid gland adenoma	3	
7	Adenoma of the hepatoid glands	6	

Table 6. The same kind of tumour counted with different denominations.

COLUMN F - Location: In this category we have included anatomical terms related to the tumour location although some caution must be considered since sometimes this is not technically the tumour location but rather the location where a first lesion was detected in the animal and motivated the first visit to the vet.

Data normalization. As a result of applying the aforementioned methodology, different ways of referring to the same kind of data were obtained, as exemplified by Table 6 Column A, where an adenoma of hepatoid glands

Report Ref	Tumour Ref	Result Date	Species	Breed	Gender	Anomymous_ PracticeID	Histo_ Cyto	Tumours_ in_the_ report	Primary_ tumour	Grade_2_ tier (Kiupel for MCT).	Grade_3_ tier (Patnaik for MCT).	Differentiation	Location	Uncertain terms
R.123	R.123-T.1	09/05/18	C*	LR*	FE*	XXXX XXX	H*	4	Mixed tumour			Malignant	MG*	
R.123	R.123-T.2	09/05/18	C*	LR*	FE*	XXXX XXX	H*	4	Simple tubulo-papillary carcinoma		2		MG*	
R.123	R.123-T.3	09/05/18	C*	LR*	FE*	XXXX XXX	H*	4	Mast cell tumour		2		Forelimb	Consistent with
R.123	R.123-T.4	09/05/18	C*	LR*	FE*	XXXX XXX	H*	4	Lymphoma	Low-grade			Skin	Highly likely

Table 7. Basic structure of the dataset after merging tumour and animal data.

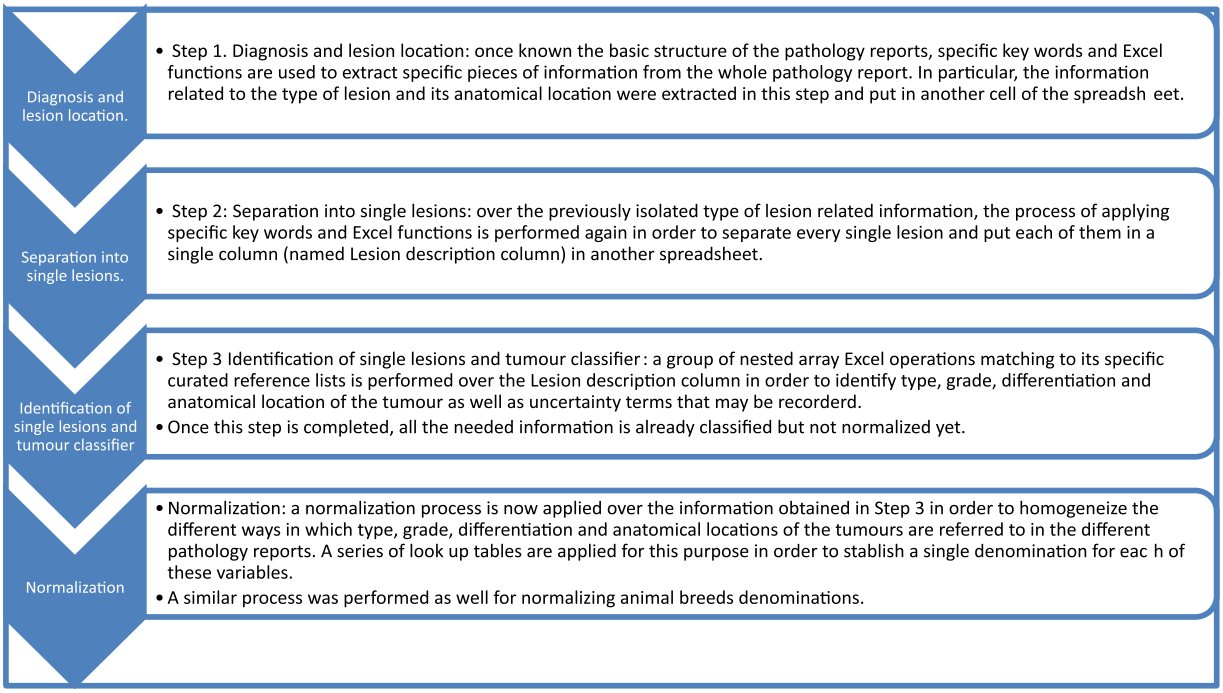

Fig. 2 Schematic overview of Data extraction (three steps) and normalization processes.

has been referred to by the pathologists in six different ways. Similar problems were identified for LL (e.g. leg and limb), degree of differentiation (e.g. "grade 1" and "grade I"), as well as dog and cat breeds from the animal data spreadsheet (e.g. "Labrador Retriever" and "Retriever, Labrador"). These were mapped to'preferred' terms[19] using the VLOOKUP Excel function. The preferred terms were themselves either based on domain expertise, or for tumour types using the different tumour lists found in 'Tumors of Domestic Animals'[19,20]. An alternative would have been to use WHO ICD-O terms, but these are not fully compatible with veterinary tumours at this time. Once a veterinary ICD-O has been finalised it would be relatively straightforward to code the PTR data to that format. Dog breeds were mapped to those recognised by the Fédération Cynologique Internationale (FCI) and the American Kennel Club (AKC) while cat breeds were mapped to those recognised by the Fédération Internationale Féline (FIFE) and the International Cat Association (TICA) augmented by recent additions based on popular hybrids (e.g. labradoodle).

Once both tumour and animal dataset were completely processed and normalised separately, they were merged using functions OPENXLSX and MERGE with RStudio software (RStudio Version 1.2.1335) by using a bespoke R script[19].

The end result is a dataset with an easy to read structure as shown in Table 7 although additional details of the actual dataset are described in the Data Records section.

Figure 2 shows an over-arching explanation of both the data extraction (three steps) and normalization processes. Additionally, for an easier understanding of the whole process, a spreadsheet containing a sample of reports with the formulas performing the aforementioned tasks is available online[19].

Data Records

The final SAVSNET PTR dataset[19] consists of 109895 rows tumours and 15 columns (columns A to O) which are described below.

A. ReportRef: N = 93941 pathology reports ("R." stands for report). It indicates the number of the pathology report (linked anonymised from the submitting laboratory report number). This value if repeated in different rows indicates those cases where reports contain multiple tumours.
   From the original 180232 pathology reports, 93941 reported at least one tumour while the other 86291 reports with no tumour were discarded.
B. TumourRef: N = 109895 tumour references within the 93941 pathology reports. It indicates the reference of the tumour, so for example tumours R.10000-T.1 and R.10000-T.2 means that there are two different tumours in report R.10000.
C. ResultDate: the date the tumour was reported by the lab.
D. Species: 180 canine breeds or 39 feline breeds.
E. Breed: breed of the dog (N = 180, top 5 unknown, Crossbreed, Labrador Retriever, Staffordshire Bull Terrier, Cocker Spaniel) or cat (N = 39, top 5 Domestic Short Hair, unknown, Domestic Long Hair, British Blue, Maine Coon).
F. Gender: gender of the cat or dog including neuter status where known. From a total of 93941 pathology reports, 85435 were from dogs and 8506 from cats. Within dogs, 41570 female, 41574 male and 2291 unknown. Within cats, 4275 females, 3969 males and 262 unknown.
G. Anonymous_PracticeID: indicates the practice where the sample was taken. During the study period and based on matching of postcodes (these have been anonymized since real postcodes cannot be published under SAVSNET´s ethical approval; more details are explained in the Usage notes section), this included data from 2196 (48%) of the 4573 UK small animal veterinary practices in the Royal College of Veterinary Surgeons practice database (as used in former publications[17]).
H. Histo_Cyto: indicates whether the tumour was analyzed by cytology (N = 40252) or histology (N = 69643).
I. Tumours_in_the_report: the number of tumours a report contains. 1 tumour = 82479, 2 = tumours 16904, 3 = tumours 6066, 4 = tumours 2480, 5 = tumours 1210, 6 tumours = 756. Median 1 for both cats and dogs.
J. Primary_tumour: indicates the specific name of the tumour (121 in total). Top 3 cat (Lymphoma, Squamous cell carcinoma, Carcinoma_others) and dog (Lipoma, Mast cell tumour, Histiocytoma).
K. Grade_2_tier (Kiupel for MCT): indicates the 2 tiers grade for lymphomas and Kiupel for mast cell tumours.
L. Grade_3_tier (Patnaik for MCT): indicates the 3 tiers grade for mammary carcinomas and soft tissue sarcomas and Patnaik for mast cell tumours.
M. Differentiation: provides additional information about the diagnosis 12 terms used in total. Most common: "malignant", "benign", "well differentiated".
N. Location: indicates the tumour location on the patient. 88 locations in total. Top 3 cat (Mammary gland, Skin, Neck) and dog (Mammary gland, Skin, Thorax)
O. Uncertainty_terms: this category contains terms such as "highly likely" or "consistent with" that may be added to the TD when the pathologist has any doubt about the diagnosis. Most common: "Consistent with", "Possible", "Probable".

The final dataset describes a PTR that includes a list of 121 different types of tumours that appear at least 10 times in the database. However, within this 121 TD list there are six non-specific terms (Carcinoma_others, Adenoma_others, Epithelioma_others,Epithelial tumour_others, Mesenchymal_neoplasias_others and Neoplasia_Tumours_others) which, in turn, either include other specific tumour types appearing less than 10 times such as for example some Leukaemias (included within the term Neoplasia_Tumours_others) or some Islet cell carcinomas (included within the term Carcinoma_others) as well as other tumours reported only using general terms such as "Mammary gland carcinoma" or "Rectal Adenoma" without additional information about the type or tumour it consisted. Additionally, some types of tumour such as multiple myelomas and plasmacytomas were aggregated under the term "Plasma cell tumour". In all these cases, LL and differentiation may be particularly useful for indicating the tumour type. For example: from the 4838 "Epithelial tumour_others" found in the dataset, 42 are located in the liver. Further, one of them are said to be "Well differentiated" and one is said to be "Benign" further supporting the impression that they are both hepatocellular adenomas. Conversely, from these 1 of the 42 liver epithelial tumours, one is said to be "Malignant"; so this is more likely to be an hepatocellular carcinoma.

In regard to LL; this information is derived either from the histology or more commonly from a transposition of the lesion description on the submission form into the pathology report, and has certain limitations. Firstly, therefore, the location may indicate a region of the body rather than a precise anatomical location. Three examples are that several lipomas are said to be located in the mammary gland according to the dataset due to the fact that they are reported as "lipomas close to the mammary gland" or "Lipomas: mammary gland region". Given that these reports use the term "mammary gland" to set a LL instead of using other words such as "thorax" or "abdomen" some of these tumours are recorded in the PTR as LL 'mammary gland' when in fact they may have been overlying the gland or just in that general location. Secondly, when there are multiple tumours without a clear separation between them and their respective locations, an erroneous LL may rarely appear as, for example, "a seminoma in the head". Finally, the user should be aware that anatomical structure and LL are sometimes not differentiated in the report for the same reason, hence, a tumour affecting a limb could in principle be affecting

TYPE OF REPORT (n = 400)	SINGLE TUMOUR GROUP (n = 298)			MULTIPLE TUMOUR GROUP (n = 102)		
	Total	Results matched	Accuracy by type of report.	Total	Results matched	Accuracy by type of report.
Cytology (n = 216)	144	133	92%	72	63	88%
Histology (n = 184)	154	153	99%	30	28	93%
Overall accuracy by group (single and multiple).	96%			89%		

Table 8. A summary of the results obtained by the technical validation process.

any of the structures of the limb, although in practice it is often evident from the tumour type which the most likely structure is.

It must also be pointed out, concerning lipomas, that given the cells of these tumours are identical to those in normal adipose tissue, it is not possible to differentiate between lipoma and normal subcutaneous fat by cytology alone; this is a clinical decision. Consequently, readers are encouraged to check the Histo_Cyto column when it comes to considering such tumour type where the diagnostic procedure could impact the diagnostic accuracy.

Technical Validation

Checking for accuracy of our exploratory text-mining methodology in determining TD. The ultimate goal of this system is to automate the collation of groups of tumour types for further review (for example in epidemiological studies). With this in mind, we designed a technical validation to assess the accuracy of the text-mining procedure in identifying the correct TD from each EPR. To do this we compared the text mining results to a gold standard of expert opinion. Firstly, two experts, one a board-certified medical oncologist (DK) and the other a board-certified veterinary pathologist (LR), each reviewed a random sample of 200 unique EPRs with no overlap, recording their own TD; to avoid any possible bias, both reviewers were blinded to the results obtained by the text-mining procedure.

Secondly, the assessment of the 400 expertly reviewed rows was compared to the output from the text mining procedure by a third expert (AE), a Professor of Veterinary Pathology, who was also blinded to the origin of both groups of results in such a way that he was unaware which of the two results were from the expert, and which were derived by text mining.

Overall, for reports in which a single tumour was present (298 out of 400), 286 successful results were observed giving an accuracy of 96%. For the multiple tumour group (102 out of 400), the accuracy was 89% with 91 successful results observed.

However, when considered separately, cytology and histology reports showed some differences in accuracy.

In the single tumour group, which included 144 cytology and 154 histology reports, accuracy was 92% (133 successful results) and 99% (153 successful results) respectively.

In the multiple tumour group, which included 72 cytology and 30 histology reports, accuracy was 88% (63 successful results) and 93% (28 successful results) respectively. Table 8 provides a summary of the results obtained by the technical validation.

Overall, there were 23 reports, shown in Table 9, where the diagnosis provided by the data mining was incorrect according to the experts. In this regard, five reasons for this misdiagnosis were identified:

Reason 1- Lipomas. Reporting a lipoma was missed six times by text-mining because the original report did not include the word "lipoma" in the Cytological interpretation section but rather expressions such as "fat tissue aspiration" or "aspiration of lipid material". In these cases, the experts determined that the most likely diagnosis was a lipoma based on information in other sections of the report including the clinical summary, the cytological description and the comments.

Reason 2 - Missing tumours in reports with multiple tumours. In seven cases, reports containing multiple tumours were partially misclassified by text mining because delimiters between tumours were not used the usual way. For example, instead of using numbers as delimiters (1. Seminoma, 2. Seminoma), the report may have quoted "Seminoma in both testicles" or "All four sites: Lipoma". In these cases, the current text mining approach would only identify the first tumour type mentioned in the report.

Reason 3 – Not detecting provisional diagnoses. In six cases, an NT or inconclusive diagnosis were misclassified by text mining because the report included expressions such as "…cannot exclude a melanocytic neoplasm", "Lymphoma not excluded" or "Meibomian gland hyperplasia (DDx early Meibomian gland adenoma)". In these particular examples, a diagnosis of a Melanocytic tumour, a Lymphoma and a Meibomian adenoma respectively were given wrongly.

Reason 4 – Wrong location. Two reports were misdiagnosed because a wrong tumour location was pulled out. Firstly, a carcinoma in the perianal area (hepatoid carcinoma) was diagnosed by the data mining when the actual location were the anal sacs glands (apocrine glands), so the experts diagnosed an anal sac carcinoma instead of an hepatoid carcinoma. Secondly, in a multiple tumour report without a clear separation between the different lesions, a report containing an epithelial tumour in the thyroid gland and an inflammatory lesion in the abdomen was misdiagnosed as an epithelial tumour in the abdomen.

Reason 5 – Incomplete diagnosis. Two reports were partially misdiagnosed because the complete diagnosis was not written in the report. One report was given the diagnosis of an Epithelial tumour (without specifying if benign or malign) in the thyroid gland when the actual diagnosis was a Thyroid carcinoma. Equally, a diagnosis of a mesenchymal neoplasia was given when the correct diagnosis was a soft tissue sarcoma.

Histo_ Cyto	Diagnosis SAVSNET-PTR	Diagnosis Experts	Reason	Comment about misdiagnosis	Frequency (N = 23)
Cyto	NT*.	Lipoma.	1	The term "lipoma" was not written in the CI*.	5
Cyto	One lipoma.	Four lipomas.	2	No delimiters between the different tumours.	2
Histo	Four plasma cell tumours and a peripheral odontogenic fibroma.	Five plasma cell tumours and a peripheral odontogenic fibroma.	2	No delimiters between the different tumours.	1
Cyto	Lymphoma.	NT*.	3	Diagnosis mentions "Lymphoma not excluded".	1
Cyto	Thyroid epithelial neoplasia.	Thyroid carcinoma.	5	Specific diagnosis not written in the CI*.	1
Cyto	Melanocytic tumour.	NT*.	3	Diagnosis mentions "cannot exclude a melanocytic neoplasm".	1
Cyto	Lipoma.	Lipoma and Basal cell tumour.	2	No delimiters between the different tumours.	1
Cyto	One lipoma.	Tow lipomas.	2	No delimiters between the different tumours.	1
Cyto	One lipoma.	Three lipomas.	2	No delimiters between the different tumours.	1
Histo	One seminoma.	Two seminomas.	2	No delimiters between the different tumours.	1
Cyto	Neoplasia-Tumour_others.	Lipoma.	1	The term "lipoma" was not written in the CI*	1
Cyto	Hepatoid (perianal) carcinoma.	Anal sac carcinoma.	4	Wrong location.	1
Cyto	Epithelial tumour.	NT*.	3	Diagnosis mentions "Possible lymphoid or epithelial neoplasia"	1
Cyto	Lipoma in oral cavity.	NT*.	3	Diagnosis mentions "Consistent with aspiration of adipose tissue, lipoma highly likely"	1
Histo	Meibomian adenoma	Meibomian hyperplasia (NT*).	3	Diagnosis mentions "Early Meibomian adenoma" as a differential diagnosis.	1
Cyto	Mesenchymal neoplasia.	Soft tissue sarcoma.	5	Specific diagnosis not written in the CI*.	1
Cyto	Carcinoma	NT*.	3	Diagnosis mentions "Carcinomatosis effusion".	1
Cyto	Epithelial tumour in abdomen.	Thyroid neoplasia.	4	Wrong location.	1

Table 9. A list with the 23 misdiagnosed reports found in the technical validation. NT* = Non tumour, CI*: Cytological Interpretation.

Usage Notes

Limitations and proper uses of the SAVSNET PTR. In spite of the large amount of information provided by the SAVSNET PTR and the wide geographic area (nationwide) from which these data are received, it should be pointed out that in this paper we are not providing any data or estimation about the reference population or population at risk which has been a key limitation to former TRs in the veterinary field over the last decades. As mentioned earlier, the SAVSNET PTR has received data from just three veterinary diagnostic labs so, consequently, we are not providing data on all the tumours diagnosed in the UK since not all veterinary diagnostic labs submit data to SAVSNET. Indeed, others have shown that tumour registries based on this kind of data suffer both from underreporting (not all diagnosed tumours in the area under study are submitted) and underascertainment (not all tumours detected in a clinical examination have samples submitted for diagnosis)[21]. Because of this, the data from this dataset cannot be extrapolated to the entire populations of dogs and cats in the UK due to the potential for systematic bias in the reporting and ascertainment.

In other words, this is not a population-based tumour registry but a pathology-based tumour registry and, therefore, this data should not be used to calculate tumour incidence rates in the whole population nor should it be considered as a reliable resource to obtain conclusions or estimations about risks related to any breed or tumour type within the whole UK populations of dogs and cats. For example, within the total 93,941 reports presented in this dataset, 10,095 came from Labrador Retriever dogs. However, this breed is also considered the most common in the UK population of vet visiting dogs[22].

Clearly, in the absence of clear denominator, it cannot be inferred that Labrador Retrievers are the most at risk of cancer in the UK.

In this regard, the Small Animal Veterinary Surveillance Network is looking to produce population denominator surrogates using electronic health records of dogs and cats visiting first opinion veterinary practices and estimates of overall UK dog populations.

Taking these limitations into account, the information presented in the dataset could however provide descriptions of the proportional distribution of tumour types within breeds and\or different neuter status or sex among animals included in our dataset. Additionally, as others have done before in similar research projects[23], it would be possible to perform simple statistical analysis to analyze the influence of the different variables (breed, sex, neuter status) on the appearance of the different tumours within the dataset although with the caution of being always aware that any result obtained from this analysis would be referred and limited to the animals within the dataset and not to the whole population.

The final dataset can be fully manipulated in Excel, using simple functions like pivot tables, thereby allowing the association between factors such as sex or breed and tumour types to be readily explored within the cohort of animals included in the dataset.

Limitations from secondary data sources. The SAVSNET tumour registry relies on information provided by diagnostic labs. All the data related to sex, neuter status, breed, etc., should be considered secondary data showing a lot of diversity given the large amount contained in the dataset. For that reason, a normalization process was performed in the Methods section.

Readers should consequently consider that normalized secondary data may not be as accurate as primary data obtained directly from the researchers.

Multiple counting of the same tumour and how to work with pathology reports instead of tumours. Given that this is a tumour diagnosis-based database, and no unique ID for animals is provided, it may be possible that individual dogs or cats might have more than one sample of the same tumour in the database (for example because owners wanted a second opinion and decided to take another sample of the same tumour in a different veterinary practitioner). This would lead to multiple counting of the same tumour, breed, etc.

In some cases, users may be interested in data related to the animals or regions presented in this dataset rather than in the tumours themselves and so, for this purpose, users can work at the level of 93941 pathology reports (n = 93941), rather than at the level of individual tumours (n = 109895).

Raw data access. The histopathology reports on which the final published dataset is based cannot be made available in an open access format as they contain clinically and financially sensitive information relating to the diagnostic laboratory or veterinary practice, as well as rare references to animal names. However, access may be possible by reasonable request for use in line with SAVSNET´s overarching ethical approval from the University of Liverpool. Researchers wishing to access the raw data need to apply for access here https://www.liverpool.ac.uk/savsnet/using-savsnet-data-for-research/ where assessment will be made based on objectives, publication strategy and track record. In some cases, an access fee may be chargeable. Those successful in their application will need to complete a data user agreement[19] which details the necessary safeguards for these data.

Under SAVSNET's ethical approval, owner consent is not required as SAVSNET does not collect any data that could identify them. Postcodes of the submitting practice for each test performed are collected; under our ethical approval, these postcodes cannot be published. Instead, we have described in the text the percent of veterinary practices as an indicator of coverage provided in the existing PTR and provided an anonymised practice code for each sample in the PTR itself to allow researchers to explore clustering of tumours by practice.

Code availability

The bespoke R script can be accessed at SAVSNET TUMOR REGISTRY DOCUMENTS figshare collection[19] with no restriction to access.

Received: 4 December 2020; Accepted: 27 August 2021;
Published online: 15 October 2021

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Acknowledgements

J.R. received an Erasmus+ scholar for funding his stay in University of Liverpool. We are also grateful for the support and major funding from BBSRC and previously BSAVA for SAVSNET, and those veterinary diagnostic laboratories that routinely submit data to SAVSNET (Axiom, Veterinary Laboratories, Batt Laboratories, BioBest, Idexx, NationWide Laboratories, Microbiology Diagnostics Laboratory at the University of Liverpool, the Department of Pathology and Infectious Diseases at the University of Surrey, and the Veterinary Pathology Group), without whose support and participation such research would not be possible. Finally, we are especially grateful for the help and support provided by SAVSNET team members, Beth Brant, Susan Bolan and Steven Smyth.

Author contributions

A.R. and P.N. conceived the idea for generating a tumour database. C.F., J.M and S.B. provided data for the tumour database. J.R. designed the database with support from A.R., A.S., A.E., P.N., G.P. and D.S. J.R. wrote the initial draft and made figures with support from D.K. and A.R. D.K., L.R. and A.E. analyzed data for the technical validation. All authors participated in verifying the data and revising the manuscript.

Competing interests

Cian Francesco is a clinical pathologist for Batt Laboratories. Samuel Beck is Laboratory director at VPG. Jenny McKay is the Head of Anatomic Pathology at Idexx UK. Other authors declare no competing interests.

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6.2.- Paper 2: Epidemiology of canine mammary tumours on the Canary Archipelago in Spain.

BMC Veterinary Research

RESEARCH

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Epidemiology of canine mammary tumours on the Canary Archipelago in Spain

José Rodríguez[1*], Ángelo Santana[2], Pedro Herráez[1], David R. Killick[3] and Antonio Espinosa de los Monteros[1]

Abstract

Background: Mammary gland tumours are the most frequently diagnosed tumours in the female dogs but just a few studies have analysed their epidemiology. Therefore, we set out to describe the epidemiology of canine mammary cancer in the Canary Archipelago, Spain. We analysed a pathology tumour registry (PTR) and identified 7362 samples obtained from 5240 female dogs resident on the Canary Archipelago during an 18-year period (2003–2020). Using a case–control study design, we compared mammary tumour affected dogs with the Canarian canine population registry in order to elucidate the breed associations for these tumours.

Results: The frequency of a diagnosis of mammary tumours relative to all tumour diagnoses in female dogs decreased during the study period from 62.7% to 48.9%. Contemporaneously, the proportion of dogs diagnosed with mammary tumours who were also neutered increased from 13.6% to 26.9%. There was a negative correlation ($R = -0.84$) between these changes. Additional findings were that: the proportion of female dogs diagnosed with multiple tumours increased by 23.5% and that the proportion of malignant tumours 89.2% diagnosed has remained stable through the period. Benign mammary tumours were diagnosed at younger ages (9.2 years old) than carcinomas (9.7 years old) and sarcomas (10.4 years old). Epithelial mammary tumours were diagnosed at younger ages in entire female dogs. Samoyed, Schnauzer, Poodle, German Pinscher and Cocker Spaniel were the breeds with the highest odds-ratios (OR) in comparison with the reference (crossbreeds) while Miniature Pinscher, American Staffordshire Terrier, English Pointer as well as some local breeds such as the Canary Warren Hound and the Majorero had the lowest ORs.

Conclusions: This study provides a description of the changing epidemiology of canine mammary cancer in the Canary Archipelago over the last two decades. We found high rates of CMT with a significant predominance of malignant tumours. Exact risk factors are uncertain, but a combination of environmental, regional socioeconomic affecting human and their pets, and animal management factors are likely to play a part. Specifically, neutering was negatively associated with the proportion of epithelial mammary gland tumours and breeds native to the region were at lower risk of mammary tumours. A deeper analysis of all these factors will facilitate a deeper understanding of the epidemiology of mammary gland tumours in both the canine and the human population.

Keywords: Canine, Mammary tumour, Female dog, Breast cancer, Pathology report, Breed, Epidemiology, Veterinary, Tumour, Cancer, Neoplasia

Plain English summary

In this study, we reviewed and described tumour and population data pertaining to 7362 canine mammary tumours (CMT) diagnosed over an 18-year period (2003–2020). The tumours affected 5240 female dogs (FD) from the Canary Archipelago, Spain. We compared

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data regarding the population of FDs affected by CMT with the Canary FD population recorded in a dog ownership registry in order to identify associations between breed and a diagnosis of CMT.

Over the course of the study period, the proportion of all tumours comprised by CMT decreased. However, the proportion of patients affected by more than one CMT increased. Contemporaneously, the neutering rate for female dogs increased significantly.

Our findings showed that almost 9 out of 10 CMT analysed were malignant epithelial tumours (carcinomas). Within this group, three types of carcinoma (complex-type carcinoma, tubulopapillary carcinoma and carcinoma arising in mixed benign tumour) accounted for almost 90% of all malignant CMT.

FD suffering from benign CMT were younger (9.2 years) that those suffering from malignant CMT (9.7 years for carcinomas and 10.4 years for sarcomas) and when the neuter status and age were considered, entire FD developed malignant epithelial CMT at younger ages (9.5 years) than neutered FD (10.2 years).

Samoyed, Schnauzer, Poodle, German Pinscher and Cocker Spaniel were shown to be at higher risk of being diagnosed with a malignant epithelial CMT compared with crossbreed dogs. Conversely, breeds such as the Miniature Pinscher, American Staffordshire Terrier, English Pointer, and some natively derived breeds such as Majorero and Canary Warren Hound were at lower risk.

Background

CMT are the most frequently diagnosed tumours in the FD population [1–5]. Epidemiological studies [1, 5, 6] focusing on these tumours have consistently found breeds such as Poodles and Cocker Spaniels to be at greater risk of developing a CMT than cross breeds. To date, the literature contains few epidemiological studies of CMT in dogs in Spain and none in the Canary Archipelago.

In addition to these epidemiological studies a number of others have reported patient signalment alongside tumour focussed data such as the proportion of malignant versus benign tumours, the distribution of the different histological types of tumours, and the frequency with which single versus multiples CMT lesions are diagnosed in the same patient. All these previous studies used CMT collections smaller than 3000 tumours and with study periods of less than a decade [7–11].

In this paper, we have conducted an epidemiological analysis of CMT in the Canary Archipelago in the period from 2003 to 2020. Here we provide data about patient breed, neuter status and histological tumour types diagnosed and longitudinal analysis showing changes in the proportion of CMT diagnosed over time. Using a data from a mandatory dog registration scheme we describe breed associations for CMT in this region.

To our knowledge, this would be the first veterinary cancer epidemiology study developed in the Canary Archipelago and one of the largest CMT datasets described in any paper.

Results

Description of study populations

Two data sets were used for this study:

a) The tumour dataset comprised of 7362 CMT affecting 5240 FD diagnosed from 2003 to 2020 was derived from the records of the Anatomical Pathology Diagnostic Service (APDS) of the Faculty of Veterinary Sciences of the University of Las Palmas de Gran Canaria (ULPGC), a recognized centre by the European College of Veterinary Pathologists [12]. Of the 5240 reports, 3891 (74.3%) FD presented a single CMT, while 869 (16.6%), 298 (5.7%) and 115 (2.2%) were diagnosed with 2, 3 and 4 different CMTs respectively. In 67 (1.3%) FD, 5 or more multiple mammary neoplastic nodules were identified. Table 1 shows the proportion of FD diagnosed with only benign or malignant histological types, both with single and multiple tumours, as well as FD simultaneously diagnosed with benign and malignant histological types.

Concerning the different histological types, Tables 2 and 4 show the proportion of malignant and benign histological types respectively diagnosed between 2003 and 2010 when the former CMT classification was used [13] while Tables 3 and 5 display the proportions of malignant and benign histological types diagnosed from 2011 to 2020 on when the current CMT classification [14] was published.

Tables 2 and 3 demonstrate that complex carcinoma, tubulopapillary carcinoma and carcinoma in benign

Table 1 Proportion of pathology reports containing single and multiple diagnosis with benign, malignant or a mix of both histological types

Reports by CMT nodules		Proportion
Multiple Benign	50	0.95%
Multiple Malignant	1020	19.47%
Multiple Mixed	279	5.32%
Single Benign	348	6.64%
Single Malignant	3543	67.61%

Table 2 Proportions of the different histological types of malignant CMT diagnosed between 2003 and 2010

Histological type	Proportion (CI95%)	Trend test *p*-value*
Complex carcinoma	42.30% (40.52%; 44.10%)	0.2150
Tubulopapillary carcinoma	29.74% (28.10%; 31.42%)	0.9030
Carcinoma in benign tumour	15.70% (14.40%; 17.05%)	0.3190
Solid carcinoma	5.42% (4.64%; 6.30%)	0.8990
Carcinosarcoma	2.22% (1.72%; 2.82%)	0.4360
Carcinoma NOS[a]	1.01% (0.68%; 1.44%)	0.2250
Anaplastic carcinoma	0.94% (0.63%; 1.36%)	0.3230
In situ carcinoma	0.94% (0.63%; 1.36%)	< 0.0001
Squamous cell carcinoma	0.74% (0.46%; 1.12%)	0.0133
Osteosarcoma	0.37% (0.19%; 0.66%)	0.5560
Fibrosarcoma	0.20% (0.07%; 0.44%)	0.0879
Haemangiosarcoma	0.17% (0.05%; 0.39%)	0.4260
Lipid-rich carcinoma	0.13% (0.04%; 0.34%)	0.7220
Cribriform carcinoma	0.03% (0.00%; 0.19%)	0.4760
Osteochondrosarcoma	0.03% (0.00%; 0.19%)	0.1300
Spindle cell carcinoma	0.03% (0.00%; 0.19%)	0.7890

[a] Carcinoma NOS refers to those diagnoses where the histological type of carcinoma has not been indicated

* A significant *p*-value (less than 0.05) implies some kind of trend (upward or downward) on the relative proportion of the histological type over the study period. Otherwise, the relative proportion has remained stable

tumour\carcinoma arising in a benign mixed tumour were the most commonly diagnosed malignant CMT over the whole study period with most of proportions being stable.

Concerning benign histological types, simple adenoma, benign mixed tumour and complex adenoma were the most usually reported as shown in Tables 4 and 5. Most of proportions kept sable during the study period.

b) The case–control dataset, consists of a subset of 1852 FD (used as cases) born between 2003 and 2013 selected from the 5240 FD included on the entire tumour dataset and of a subset of 79,100 FD born on the same period (2003–2013) obtained from a reference population (used as controls) derived from the Canary registry of animal identification (ZOOCAN). ZOOCAN is a centralized web-based registry through which veterinary practitioners from the whole Canary Archipelago have been required to register all companion animals under their care since 2011 [15].

Table 6 shows the distribution of individuals of each group (cases and controls) by year of birth while Fig. 1 shows the respective distribution of breeds.

Amongst the case group, 37.7% of dogs were crossbreed followed by Yorkshire Terriers (18.7%), other breeds group (18.3%), French Bulldogs, (5.1%), Cocker Spaniel (3.9%), Poodles (3.5%) and German Shepherd (2.5%). Within the control group, crossbreed dogs were also the largest breed group (33.6%) followed by the Canary Warren Hound (18.9%), the other breeds group (18.6%), Yorkshire Terrier (8.2%), Chihuahua (5.0%), French Bulldog (3.9%) and Labrador Retriever (2.3%).

The CMT tumour database. An overview

During the 2003–2020 period, the APDS diagnosed 13,816 tumours from 10,205 FD from the Canary Archipelago. A longitudinal assessment showed that the proportion of CMT diagnosed dropped from 62.7% in 2003 to 48.9% in 2020 (a decrease of 13.8% (95% CI 8.4–19.0%, $p < 0.0001$)). Due to this decline CMT was no longer the most frequent tumour diagnosis at the end of the study period. Contemporaneous with this, the neutered rate of FD suffering from any tumour tripled from 13.1% to 36.3% (95% CI 17.7%-28.4%, $p < 0.0001$) showing a marked negative correlation (Pearson's product-moment correlation: -0.84, 95% CI: -0.94 -0.60, $p < 0.0001$). Equally, the neutered rate of FD suffering from a CMT also increased significantly from 13.6% to 26.9% (95% CI 6.2%-20.6%, $p < 0.0002$) with a negative correlation of -0.84, (95% CI: -0.94 -0.61, $p < 0.0001$). These three tendencies are shown in Fig. 2.

Over the course of the study period 89.2% of the 7362 CMTs diagnosed were classified as malignant (95% CI 88.5%-89.9%). This proportion remained broadly stable across the study period as shown in Fig. 3.

Single and multiple CMT over the study period

Through the study period there has been an increase in the proportion of patients suffering from multiple CMT from 19.6% in 2003 to 43.0% in 2020, an overall increase of 23.5% (95% CI 15.4–31.6, $p < 0.0001$) as shown in Fig. 4.

Analysis of the age on the FD population affected by a CMT and its relationship with the neuter status and the presence of single and multiple tumours

The age of diagnosis in FD was analysed in comparison with the presence of single and multiple CMT, the histological type of CMT and the neuter status.

Table 7 shows the mean (± sd)) ages at diagnosis of FD depending on whether it is affected by single benign (SB), single malignant (SM), multiple benign (MB), multiple malignant (MM) or a combination of, at least, one benign CMT and one malignant CMT (MMB).

Table 3 Proportions of the different histological types of malignant CMT diagnosed between 2011 and 2020

Histological type	Proportion (CI95%)	Trend test *p*-value*
Complex carcinoma	40.16% (38.55%; 41.78%)	0.0014
Tubulopapillary carcinoma	24.72% (23.31%; 26.16%)	0.0131
Carcinoma arising in mixed benign tumour	20.80% (19.48%; 22.16%)	0.0044
Solid carcinoma	6.86% (6.06%; 7.73%)	0.0084
Carcinosarcoma	1.89% (1.47%; 2.39%)	0.0502
Anaplastic carcinoma	1.19% (0.87%; 1.61%)	0.1250
Squamous cell carcinoma	1.11% (0.79%; 1.51%)	0.4040
Carcinoma NOS[a]	0.61% (0.38%; 0.92%)	0.1450
Osteosarcoma	0.56% (0.34%; 0.86%)	0.4110
Fibrosarcoma	0.42% (0.23%; 0.69%)	0.0477
Ductal carcinoma	0.39% (0.21%; 0.65%)	0.0019
Haemangiosarcoma	0.39% (0.21%; 0.65%)	0.2370
Sarcoma NOS[b]	0.19% (0.08%; 0.40%)	0.0446
In situ carcinoma	0.17% (0.06%; 0.36%)	0.1560
Inflammatory carcinoma	0.17% (0.06%; 0.36%)	0.4150
Carcinoma and malignant myoepithelioma	0.11% (0.03%; 0.28%)	0.6620
Malignant myoepithelioma	0.11% (0.03%; 0.28%)	0.1430
Lipid-rich (secretory) carcinoma	0.08% (0.02%; 0.24%)	0.1610
Intraductal papillary carcinoma	0.06% (0.01%; 0.20%)	0.9460
Micropapillary invasive carcinoma	0.03% (0.00%; 0.15%)	0.8270

[a] Carcinoma NOS refers to those diagnoses where the histological type of carcinoma has not been indicated

[b] Sarcoma NOS refers to those diagnoses where the histological type of sarcoma has not been indicated

* A significant *p*-value (less than 0.05) implies some kind of trend (upward or downward) on the relative proportion of the histological type over the study period. Otherwise, the relative proportion has remained stable

Table 4 Proportions of the different histological types of benign CMT diagnosed between 2003 and 2010

Histological type	Proportion (CI95%)	Trend test *p*-value*
Simple adenoma	38.73% (33.32%; 44.35%)	0.9160
Benign mixed tumour	34.29% (29.05%; 39.82%)	0.1550
Complex adenoma	19.05% (14.86%; 23.83%)	0.2680
Duct papilloma	2.86% (1.31%; 5.35%)	0.0025
Fibroadenoma	2.86% (1.31%; 5.35%)	0.2770
Basaloid adenoma	1.90% (0.70%; 4.10%)	0.6720
Osteoma	0.32% (0.01%; 1.76%)	0.1590

* A significant *p*-value (less than 0.05) implies some kind of trend (upward or downward) on the relative proportion of the histological type over the study period. Otherwise, the relative proportion has remained stable

Significant differences were found between FD suffering SB CMT compared to FD suffering from either SM CMT ($p<0.0001$), MM CMT ($p<0.0001$) or MMB CMT ($p<0.0001$) as well as between FD with SM CMT and FD with either MM CMT ($p<0.0001$) or MMB CMT ($p=0.012$).

Considering the mean age at which different histological types were diagnosed, benign CMT were diagnosed at 9.2 years (sd: ± 2.57) whereas the mean age of FDs at diagnosis of carcinoma, sarcoma and carcinosarcomas was 9.7 years (sd: ± 2.50), 10.4 years (sd: ± 2.96) and 10.9 (sd: ± 2.53) respectively (Fig. 5).

Finally, neutered FD diagnosed with malignant epithelial tumours were significantly older than entire FDs diagnosed with these tumours (10.2 and 9.5 years respectively, 95% CI -0.8,-0.5, $p<0.0001$). A similar finding was noted for benign CMT (9.7 and 8.9 years respectively, 95% CI -1.3,-0.3, $p=0.0005$), but neither for sarcoma nor for carcinosarcoma, where *p*-value were not significant (0.9445 and 0.1921) (Table 8).

Breed as a risk factor for malignant epithelial mammary tumours

Fourteen breeds carried a significant higher risk of malignant epithelial CMT than crossbreeds. The five breeds with the highest odds ratios were Samoyed (OR 6.09, 95% CI 2.31–16.04), Schnauzer (OR 5.77, 95% CI 2.78–12.00), Poodle (OR 3.89, 95% CI 2.96–5.10), German Pinscher (OR 3.65, 95% CI 2.28–5.83) and Cocker Spaniel (OR

Table 5 Proportions of the different histological types of benign CMT diagnosed between 2011 and 2020

Histological type	Proportion (CI95%)	Trend test *p*-value*
Simple adenoma	33.54% (29.31%; 37.98%)	0.6770
Benign mixed tumour	31.66% (27.50%; 36.04%)	0.2350
Complex adenoma (adenomyoepithelioma)	28.51% (24.50%; 32.79%)	0.4350
Fibroadenoma	2.31% (1.16%; 4.09%)	0.2860
Myoepithelioma	2.31% (1.16%; 4.09%)	0.5920
Ductal adenoma (basaloid adenoma)	1.47% (0.59%; 3.00%)	0.0235
Fibrolipoma	0.21% (0.01%; 1.16%)	0.9990

* A significant *p*-value (less than 0.05) implies some kind of trend (upward or downward) on the relative proportion of the histological type over the study period. Otherwise, the relative proportion has remained stable

Table 6 Number of cases and controls by the year of birth

Year of birth	Control	Case
2003	271	215
2004	354	221
2005	601	194
2006	945	235
2007	1470	246
2008	2274	215
2009	4182	173
2010	12,312	151
2011	19,064	94
2012	18,667	81
2013	18,960	27
Total	79,100	1852

3.41, 95% CI 2.64–4.40). Ten breeds had a significant lower risk of malignant epithelial CMT than crossbreed, of which Canary Warren Hound (OR 0.09, 95% CI 0.06–0.13), Miniature Pinscher (OR 0.22, 95% CI 0.09–0.53), Majorero (OR 0.23, 95% CI 0.09–0.55), American English Pointer (OR 0.25, 95% CI 0.13–0.47) and American Staffordshire Terrier (OR 0.28, 95% CI 0.11–0.67) obtained the lowest OR. Table 9 and Fig. 6 show the OR by different breed.

Discussion

The aim of this study was to describe the epidemiological characteristics of CMT in the Canary Archipelago. To do this a sample of 7362 CMT diagnosed in 5240 female dogs between 2003–2020 was evaluated. Additionally, used the ZOOCAN population registry to explore the association between breed and a diagnosis of a malignant epithelial CMT within the Canarian dog population.

Over the study period, a diagnosis of CMT has become relatively less common in such a way that, at the end of the study period, tumours affecting the mammary glands were no longer the most frequently diagnosed cancer in FD. A similar downward tendency on the incidence for CMT in FD was observed in the late periods of the Animal Tumour Registry of Genoa [2].

Given the well-established protective effect of neutering on the development of CMT [16] a likely explanation for this change is an increase in the neutered rate within the FD population over the course of this period. Results of previous studies analysing animal tumour datasets and neutering rates are consistent with this hypothesis. In the Norwegian Canine Cancer Project [1], CMT were the most frequently (4223 CMT of a total of 9543) diagnosed tumours in the FD population which was described as sexually intact. On the contrary, when analysing data from a recent tumour database in the UK [17], the mammary gland was the location for just 22.5% of the tumours affecting the FD population with a neutering rate within this population of 66.4%. Additional factors that may play a part in the high rate of CMT in the Canary Archipelago include exposure to chronic and relatively high levels of contamination by Organochlorine pesticides (OCP) such as DDT and DDE found in the general population on the region given, they have been linked to breast cancer due to their role as environmental xenoestrogens [18, 19].

We found that complex carcinoma, tubulopapillary carcinoma and carcinoma arising in BMT were the most frequently ones diagnosed as has been the case in previous studies [7–11]. Despite the decrease in the diagnosis of CMT we found that the proportion of CMT that were malignant remained stable at a high level throughout the study period. The literature is somewhat discordant about the frequency of malignant CMT. Several publications found a malignancy rate of 40%-60% [6–10] conversely the Norwegian cancer register [1] and another recent study from Spain [11] found a malignant CMT rate of around 90%. Whether these results represent an

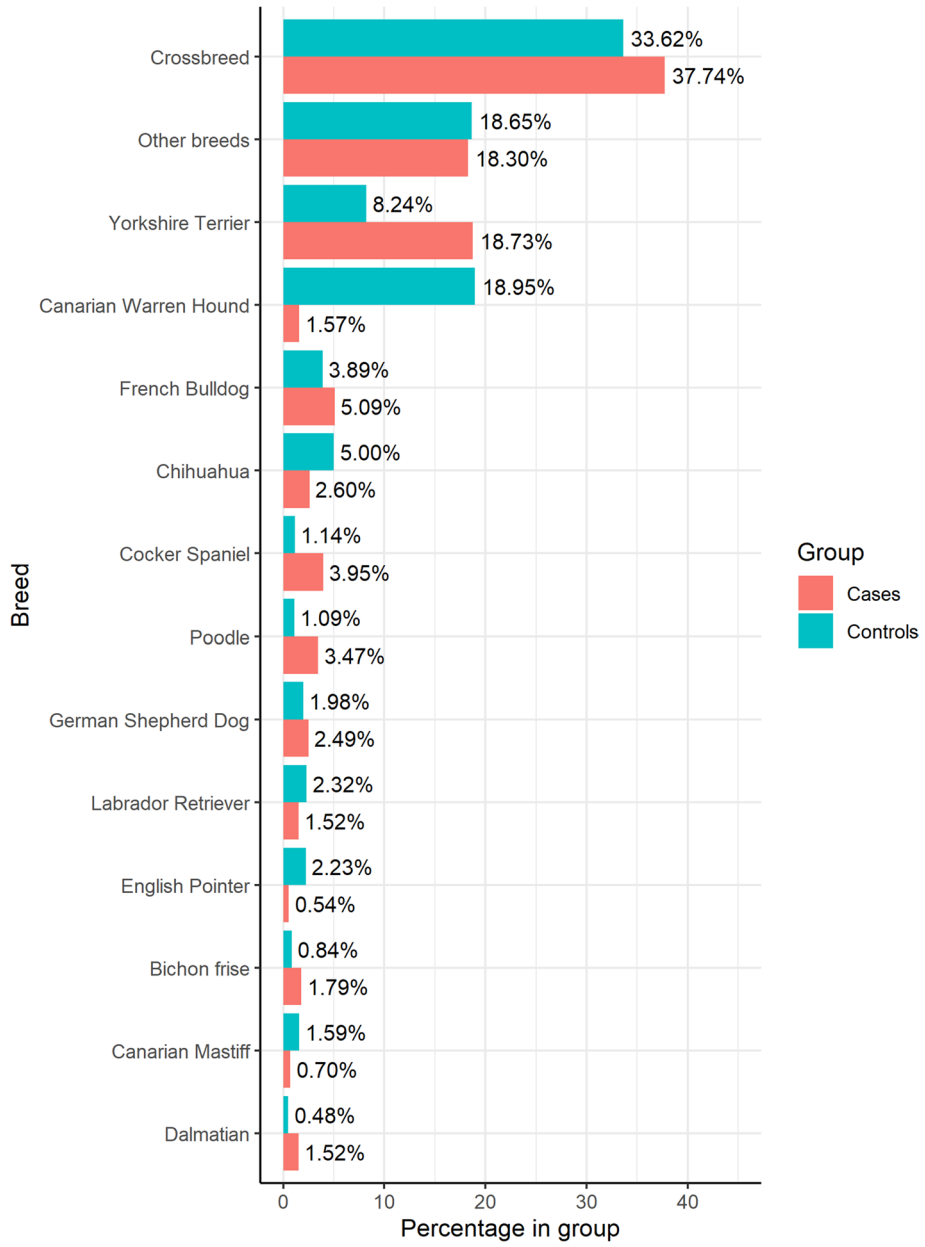

Fig. 1 Breed distribution in cases and controls

inherently great likelihood that a CMT is malignant in the Canaries or is a consequence of another factor cannot be determined from this study design. In line with findings from former studies [20, 21] suggesting a progression of CMT from a benign to a more malignant phenotype, one possibility is that FDs are presented for evaluation of mammary tumour later in the disease course in the Canaries than in some other countries. Another possibility is that this finding reflects regional difference in veterinary approach leading to a submission bias due to veterinary surgeons only submitting the more phenotypically concerning tumours as well as some degree of bias inherent to Pathologists.

Consistent with the previous literature we found that CMT was usually diagnosed in older FDs and that FDs with malignant CMT were on average older at diagnosis compared to those with benign CMT [6, 9, 10, 22, 23]. Interestingly we found that epithelial CMT was diagnosed at younger ages in entire dogs than in neutered ones. This is consistent with another study [6]

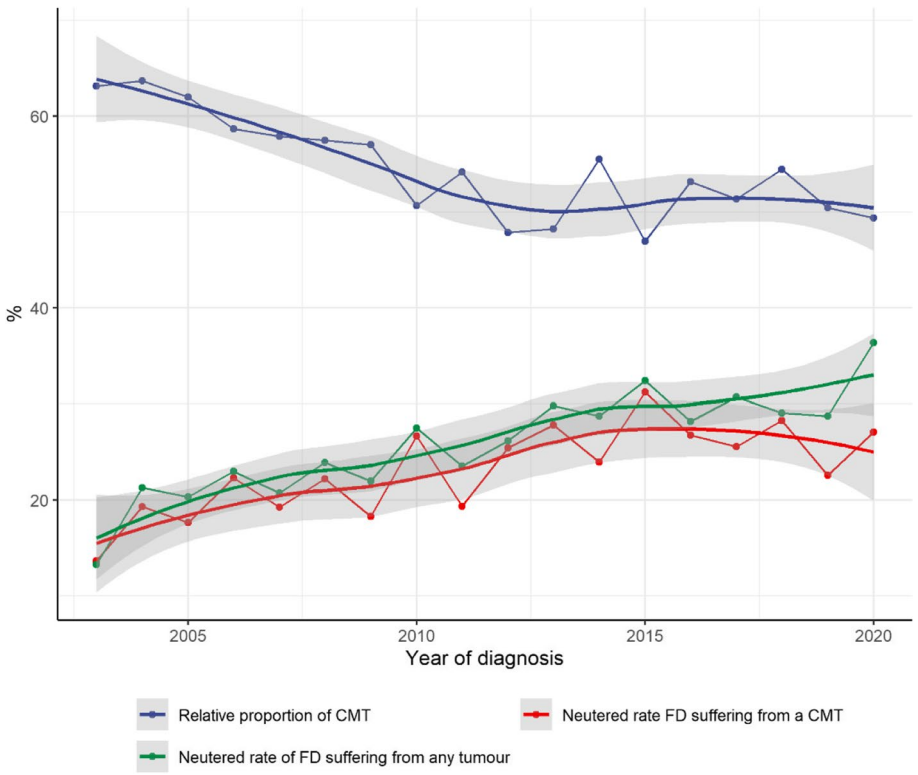

Fig. 2 Relative proportion of CMT and neutered rate evolution in FD

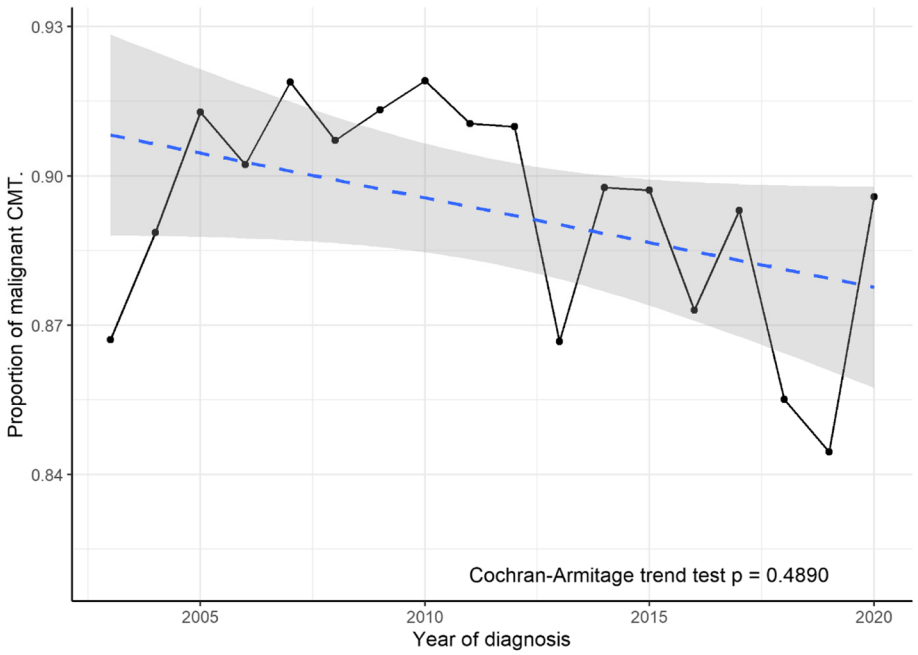

Fig. 3 Relative proportion of malignant CMT diagnosed by year

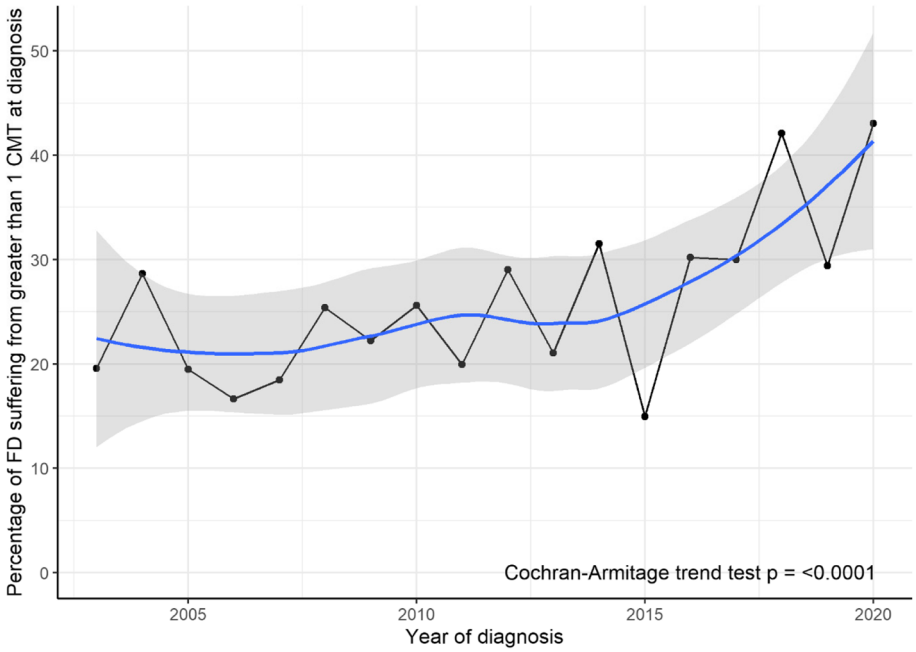

Fig. 4 Proportion of FD with multiple CMT

Table 7 Comparison of age at diagnosis by the presence of single and multiple CMT with benign, malignant or a mix of both histological types

Reports by CMT nodules	Age (mean ± sd)
Single Benign	8.57 ± 2.59
Multiple Benign	9.24 ± 2.45
Single Malignant	9.36 ± 2.63
Multiple Mixed	9.90 ± 2.23
Multiple Malignant	10.13 ± 2.32

and provides further evidence to the protective effect of neutering in relation to CMT. Age at neutering has been shown to impact on the strength of the protective effect of neutering on CMT development [16]. Another question is whether later neutering whilst not being as protective does lead to later CMT development. As we did not have access to age at neutering data, we could not determine this from this study design.

Concerning the frequency of FD suffering multiple CMT simultaneously, the literature is somewhat inconsistent with some studies [22] reporting a rate of around 25% while others [20] demonstrated a higher one of 66.7%. In our population, the proportion of FD affected by multiple tumours increased quite significantly throughout the study period. This upward trend may be attributable to a change in approach from veterinary practitioners due to greater awareness of the so-called hormonal field effect and histological continuum from benign to malignant [20] leading to more thorough examination and earlier intervention when assessing an FD already affected by a CMT.

In this study we found higher risk breed for malignant epithelial CMT included the Samoyed, Schnauzer, Poodle, German Pinscher, Cocker Spaniel, Dobermann, West Highland White Terrier, Dalmatian, Dachshund, Yorkshire Terrier and Boxer. Lower risk breeds included the Chihuahua, English Pointer, and Labrador Retriever as well as several local breeds such as the Canarian Warren Hound, Majorero and Canarian Mastiff. Several of these breeds including Poodle, Dachshund, Cocker Spaniel have also been found to be at greater risk in studies in Norway, the US, and Italy [1, 5, 6]. Similarly Chihuahua and Pointers have been found to be at lower risk in one or more of these studies. The finding that local breeds are at lower risk is interesting especially given their varied phenotype. Further investigation is indicated to determine if this relates to some unknown environmental adaptation or possibly that for some reason they are less likely to be presented for veterinary care.

Concerning the lower ORs obtained by the different Islands in comparison to Gran Canaria, this should be attributed to a selection bias on samples from Gran Canaria.

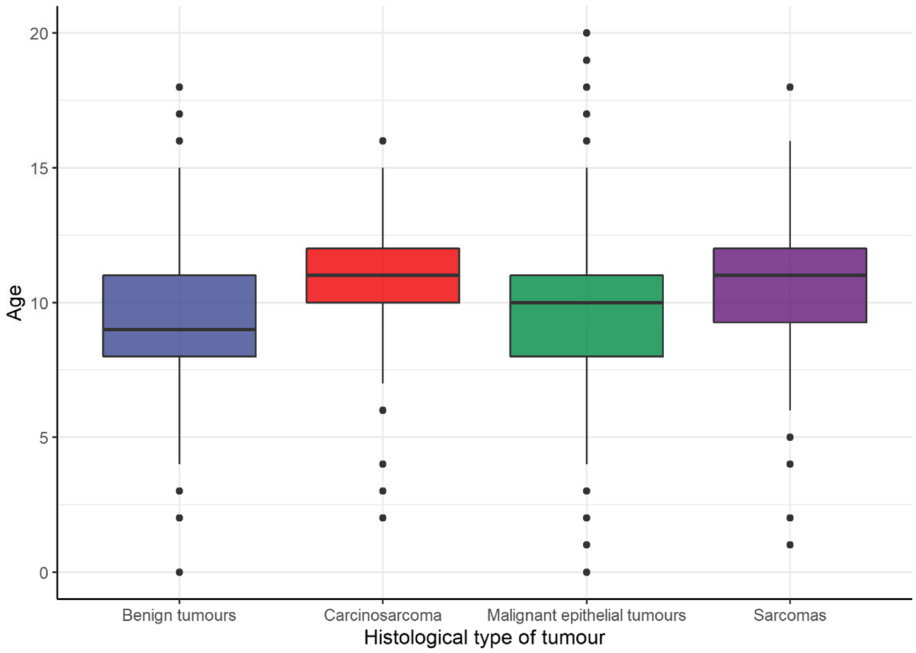

Fig. 5 Age distribution according to the histological type of CMT

Table 8 Ages of diagnoses of the different CMT in both neutered and entire FD

Histological type	Difference	CI95%	Entire mean age	Neutered mean age	*P*-value
Benign tumours	-0.7830332	(-1.28,-0.29)	8.949290	9.732323	0.0005
Carcinosarcoma	-0.8279570	(-2.03,0.38)	10.688172	11.516129	0.1921
Malignant epithelial tumours	-0.6577450	(-0.85,-0.47)	9.553555	10.211300	< 0.0001
Sarcomas	0.0500000	(-1.72,1.82)	10.400000	10.350000	0.9445

Limitations

Uncertainty of data provided by ZOOCAN

Dogs receiving veterinary care are registered on the ZOOCAN database. Typically, dogs are registered in the system when they receive their first rabies vaccination or when they are adopted from a shelter. However, follow-up information about the dog (i.e., related to changes in the neuter status or deaths) is known not to be recorded reliably on the ZOOCAN database in many cases [24]. For this reason, the only data provided by ZOOCAN used in this study was the one related to the year of birth and the breed of the dogs.

Breed and secondary data

The breed data used for this study was provided by veterinary practitioners and could not be checked consequently there is a degree of uncertainty accuracy of breed identification. For this reason, in this study we indicated that a dog belonged to a specific breed when that breed was clearly indicated in the report. Any combination of breeds was considered a crossbreed.

Selection bias in the tumour profile

As mammary tumours are superficial to the abdominal wall they are obvious to owners and vets and consequently, alongside other tumours of other superficial structures such as lymph nodes, their frequency will be overestimated relative to internal tumours [1–5]. A second likely cause of selection bias is that veterinary surgeons may be more likely to send tumours that are considered more concerning. Additionally, there might an interaction between socioeconomic factors and severity of tumour appearance such that worrisome tumours are submitted for analysis for both richer and poorer clients and less concerning ones only for wealthier clients.

Finally, geographic and logistics reasons may have led to an over-representation of cases from the island of

Table 9 OR among different breeds when compared with crossbreed dogs, adjusted by island

Effect	Value	OR	CI	*P*-value	Controls No. (%)	Cases No. (%)
Island	Gran Canaria	1.00			25,562 (34.4)	1229 (68.7)
	Fuerteventura	0.64	[0.54,0.75]	< 0.0001	5754 (7.7)	170 (9.5)
	La Gomera	0.29	[0.15,0.56]	0.0003	706 (1.0)	9 (0.5)
	Tenerife	0.21	[0.19,0.24]	< 0.0001	31,306 (42.1)	318 (17.8)
	Lanzarote	0.20	[0.15,0.26]	< 0.0001	5694 (7.7)	55 (3.1)
	El Hierro	0.17	[0.06,0.54]	0.0026	612 (0.8)	3 (0.2)
	La Palma	0.03	[0.01,0.08]	< 0.0001	4668 (6.3)	5 (0.3)
Breed	Crossbreed	1.00			26,549 (35.7)	697 (39.0)
	Samoyed	6.09	[2.31,16.04]	0.0003	38 (0.1)	5 (0.3)
	Schnauzer	5.77	[2.78,12.00]	< 0.0001	53 (0.1)	9 (0.5)
	Poodle	3.89	[2.96,5.10]	< 0.0001	862 (1.2)	64 (3.6)
	German Pinscher	3.65	[2.28,5.83]	< 0.0001	158 (0.2)	21 (1.2)
	Cocker Spaniel	3.41	[2.64,4.40]	< 0.0001	898 (1.2)	73 (4.1)
	Dobermann	3.09	[1.64,5.80]	0.0005	143 (0.2)	11 (0.6)
	West Highland White Terrier	2.61	[1.68,4.04]	< 0.0001	302 (0.4)	23 (1.3)
	Chow Chow	2.55	[1.01,6.44]	0.0478	67 (0.1)	5 (0.3)
	Dalmatian	2.45	[1.65,3.65]	< 0.0001	382 (0.5)	28 (1.6)
	Dachshund	2.36	[1.44,3.85]	0.0006	282 (0.4)	18 (1.0)
	Bichon frise	2.23	[1.55,3.21]	< 0.0001	663 (0.9)	33 (1.8)
	Bulldog	1.95	[1.20,3.17]	0.0071	358 (0.5)	18 (1.0)
	Yorkshire Terrier	1.92	[1.68,2.19]	< 0.0001	6505 (8.8)	346 (19.3)
	Boxer	1.68	[1.13,2.50]	0.0101	656 (0.9)	27 (1.5)
	Siberian Husky	1.56	[0.68,3.58]	0.2895	153 (0.2)	6 (0.3)
	Rottweiler	1.43	[0.80,2.57]	0.2305	330 (0.4)	12 (0.7)
	Shih-Tzu	1.28	[0.75,2.21]	0.3683	434 (0.6)	14 (0.8)
	Pomeranian	1.25	[0.64,2.45]	0.5172	281 (0.4)	9 (0.5)
	German Shepherd Dog	1.14	[0.84,1.54]	0.4053	1560 (2.1)	46 (2.6)
	French Bulldog	1.07	[0.86,1.33]	0.5534	3071 (4.1)	94 (5.3)
	Jack Russell Terrier	1.00	[0.54,1.83]	0.9903	417 (0.6)	11 (0.6)
	Golden Retriever	0.94	[0.56,1.58]	0.8128	737 (1.0)	15 (0.8)
	Bull Terrier	0.93	[0.56,1.54]	0.7803	547 (0.7)	16 (0.9)
	Beagle	0.79	[0.39,1.61]	0.5253	376 (0.5)	8 (0.4)
	Schnauzer (Miniature)	0.70	[0.29,1.70]	0.4265	264 (0.4)	5 (0.3)
	Staffordshire Bull Terrier	0.55	[0.32,0.96]	0.0369	777 (1.0)	13 (0.7)
	Labrador Retriever	0.54	[0.37,0.80]	0.0017	1831 (2.5)	28 (1.6)
	Andalusian Ratter	0.52	[0.23,1.17]	0.1157	419 (0.6)	6 (0.3)
	Pit Bull Terrier	0.42	[0.24,0.73]	0.0023	1126 (1.5)	13 (0.7)
	Canarian Mastiff	0.40	[0.23,0.70]	0.0013	1253 (1.7)	13 (0.7)
	Chihuahua	0.35	[0.26,0.47]	< 0.0001	3946 (5.3)	48 (2.7)
	American Staffordshire Terrier	0.28	[0.11,0.67]	0.0043	628 (0.8)	5 (0.3)
	English Pointer	0.25	[0.13,0.47]	< 0.0001	1760 (2.4)	10 (0.6)
	Majorero	0.23	[0.09,0.55]	0.0011	615 (0.8)	5 (0.3)
	Miniature Pinscher	0.22	[0.09,0.53]	0.0008	895 (1.2)	5 (0.3)
	Canarian Warren Hound	0.09	[0.06,0.13]	< 0.0001	14,966 (20.1)	29 (1.6)

Gran Canaria when compared with the other Canary islands. APDS is located in the Faculty of Veterinary Sciences in Gran Canaria, about thirty minutes away from the biggest urban area of the Canary Archipelago therefore the ease of submitting to APDS is clearly higher on Gran Canaria compared to the other islands and this has

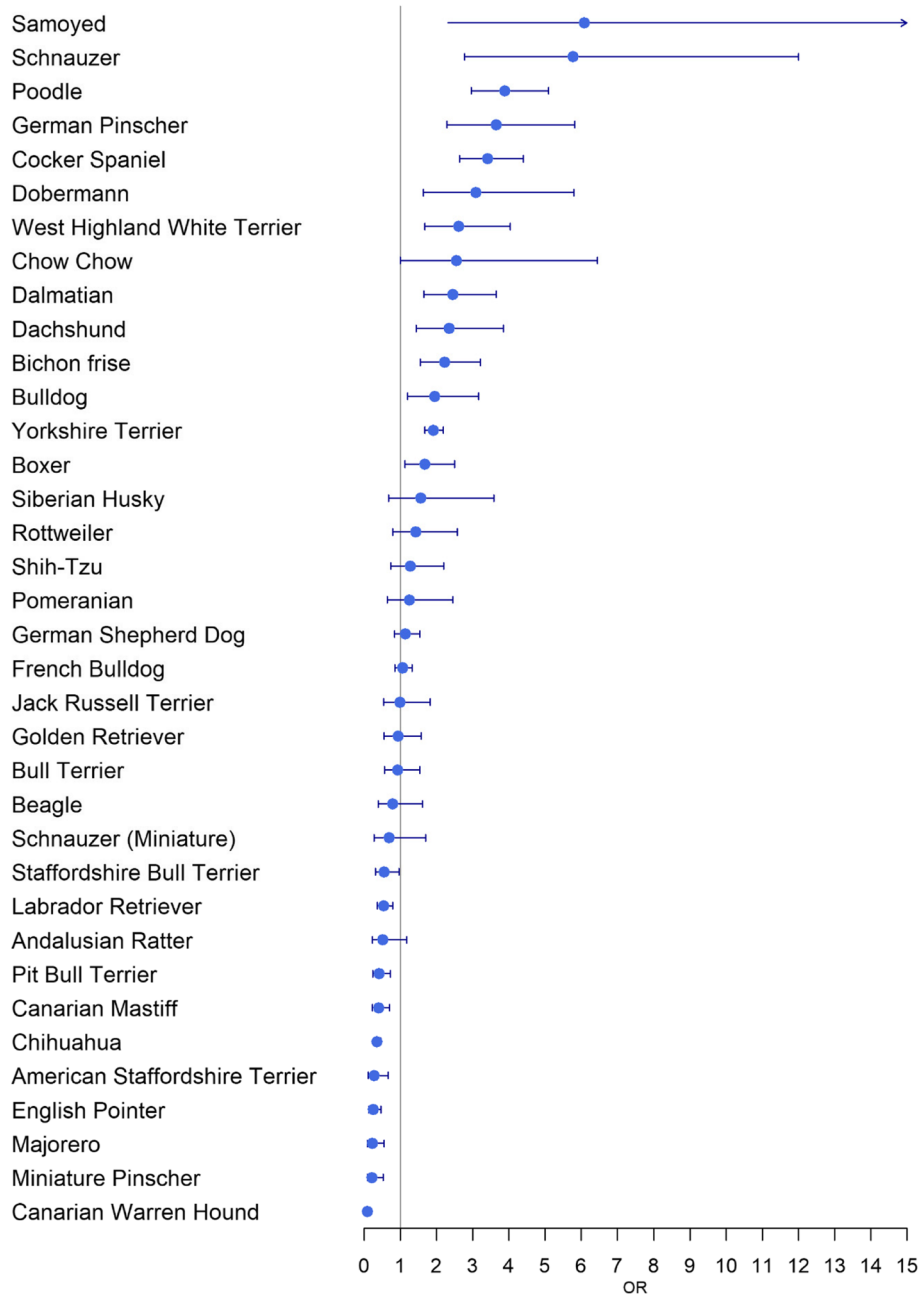

Fig. 6 OR among different breeds when compared with crossbreed dogs

undermined our options to provide an island-by-island analysis on this paper due to the lack of uniformity of the submitters situation across the Canaries.

Consequently, the tumour profile obtained from the APDS service may not complete accurately recapitulate the situation in the general population. Standing against this is the nature of APDS which is a diagnostic service integrated in an academic institution, the University of Las Palmas de Gran Canaria (ULPGC) and is an affordable not for profit service aimed at teaching veterinary pathology to students. Finally, a pathological diagnosis is somewhat subjective and there is therefore a risk of pathologist bias.

Conclusion

This study provides the first epidemiological description of FD affected by CMT on the Canary archipelago. We identified a high frequency of CMT compared to other tumours and a high rate of malignant CMT relative to most other CMT datasets. Our results support earlier observations that the age of presentation of epithelial CMT is later in neutered animals. Some local breeds like the Canary Mastiff, Majorero and Canary Warren Hound showed a lower tendency to suffer from malignant epithelial mammary tumours when compared to the crossbreed dog suggesting a possible genetic resistance adaptation developed by these breeds. A deeper analysis of all these factors could provide major insights on the epidemiology of mammary gland in the canine population.

Methods

The area under study

The Canary Archipelago is one of the 17 Spanish Autonomous Communities and is comprised of an archipelago of eight islands located in the Atlantic Ocean 1600 km southwest of the Spanish mainland with a population of 2.172.944 people [25] of which 80% live on the islands of Gran Canaria and Tenerife, where the two main metropolitan areas, Las Palmas de Gran Canaria and Santa Cruz de Tenerife are located.

Data sources

Data for conducting this study come from two main sources: The APDS and ZOOCAN database.

The APDS

The APDS receives about 1450 animal tissue samples annually. These are submitted by veterinary practitioners and official veterinarians from the whole Canary Archipelago. Along with each sample, a submission form is filled out by the vet with information about the animal from which the sample was taken (species, bred, sex, neuter status, age and location of the lesion) and about the veterinary facility where the sample was taken and submitted from.

After arriving on the APDS, samples are processed and prepared to be checked by the attending Pathologist. A diagnosis (tumour or not; in case of a tumour, the type of tumour and the grade) for each sample is indicated on the same case-report document used to submit the sample generating a diagnostic report.

Finally, both the diagnostic report and the processed sample are stored on the ADPS archives for further review if needed. For this study, tumour-related data came from the archived diagnostic reports covering the period 2003 to 2020.

Over the study period, there have been three main Pathologists working the samples with the role of Veterinary Pathology Professors of the University of Las Palmas de Gran Canaria these have been supported by occasional residents.

The ZOOCAN database

The ZOOCAN database is a centralized web-based registry in which veterinary practitioners from the whole Canary Archipelago must register all companion animals under their care. In the Canary Archipelago, it is mandatory to identify dogs once they are three months old [13]. Rabies vaccination is also mandatory from this age and this vaccination should be always preceded by the registration of the animal on the database. It is also possible to register dogs younger than three months of age.

The database is managed by the Regional College of Veterinary Surgeons who provided the anonymized dataset used in this study in the form of a.*csv* file containing 369,083 rows and 6 rows concerning to breed, gender, date of birth, neuter status, island of residence and date of registration.

As explained previously, a subset of this whole collection of data (female dogs born between 2003 and 2013) was selected as controls (non-cases) to shape the study population.

Data preparation

The tumour database

Initially, all APDS archives were in paper format so it was necessary to create a digital database (a pathology tumour registry) for its further analyses.

For this purpose, all diagnostic reports generated on the 2003–2020 period were individually read by a single author (JRT). Data from records describing a tumour in a dog or a cat was extracted and added to the registry.

Data was recorded in an MS Excel spreadsheet in a two steps process from which two subsets of data were created: a first one with only tumour related data (histological type, grade, location and whether cytology or histology) and a second one with animal data (species, breed, neuter status, age and place of living). Both subsets were merged afterwards using R by making use of the unique reference number.

At the end of the process, from 25,957 reviewed reports, 12,330 included at least a tumor lesion in a FD.

Case-definition: gland mammary histological type subset and multiple tumour cases

The CMT subset from the whole cancer registry was obtained by firstly filtering by species and gender (canine, female), next by the tumour location (mammary gland). When selecting the control cases for comparison with the reference population, a third filter was applied by the year of birth (2003–2013).

Given the long period of time, classification systems applied for the CMT have changed over the years so, for this study, mammary gland tumours were described according to either the current [24] or the former [25] CMT classification system.

Concerning multiple tumour cases, these refer to pathology reports including more than one CMT.

Breed name standardization

Given the high diversity of ways used to indicate the breeds of the dogs (for instance "Labrador Retriever", "Retriever, Labrador", "Lab", "L. Retriever", it was necessary to standardize all these terms by mapping to the lists of the Fédération Cynologique Internationale (FCI) and the Royal Spanish Canine Society augmented by recent additions based on popular hybrids (e.g. El Hierro Wolfdog, American Bullie).

Study design

In a case–control study, cases (animal with a disease) and controls (animals known to be free of the disease) are selected from the population of interest in such a way that both groups have similar characteristics that make them comparable to each other. In this kind of study, it is ideal to have at least as many controls as cases, in order to improve the efficiency of statistical analysis.

In this sense, both cases and controls consisted of individuals coming from the same Autonomous Community (Canarias) presumably from dogs living in this Autonomous Community (given the geographical separation of the Canaries from mainland Spain), while animals registered in ZOOCAN are those living in any city or town of the Canary Archipelago.

However, given that CMT affect elderly female dogs, it was necessary to choose a subset of individuals from the ZOOCAN database that also met this age-related requirement.

So, to meet the requirement of having so many controls as cases individuals, we chose a subset of FD born within the 2003–2013 period in which the number of animals on the control group was higher than of the one on the case group as shown in Table 6.

Statistical analysis

After performing an internal validity check and data cleaning with Microsoft Office Excel 2013, all analysis were performed with the R Language and Environment for Statistical Computing, version 4.1.2 [26]. Categorical variables were expressed as numbers and percentages, and continuous symmetric distributed variables (age) were expressed as the mean and standard deviation. Differences in the age at presentation for the different histological type of tumours depending on the neuter status were assessed by using t-test. The Cochran-Armitage trend test was used for assessing the presence of increasing or decreasing trend in proportions. Difference in proportions was assessed by chi-square tests and corresponding 95% confidence intervals were computed. Linear regression analysis was used to assess the annual growth rate in the proportion of various tumour types. Kruskal Wallis test was used to assess the differences between ages at diagnosis for different combinations of benign and malignant and single and multiple tumours. Additionally, when Kruskall Wallis provided significant results, a post hoc Conover test was used to find out which groups were significantly different.

Linear association between continuous variables was assessed by Pearson correlation coefficient. Logistic regression analysis was used to evaluate the association of particular breed with the risk of malignant epithelial CMT adjusted by island, and odds ratios with 95% confidence intervals were reported. Only malignant epithelial tumours (carcinomas) were evaluated given that this was the largest homogeneous histological type group. Crossbreed dogs were used as the reference group. Additionally, given the overrepresentation of cases in the island of Gran Canaria, the logistic model was adjusted by island.

In all tests, p-values lower than 0.05 were considered as statistically significant. The R script used to execute the above analyses is available at https://doi.org/10.6084/m9.figshare.19688721

Acknowledgements

We are grateful to the Canary College of Veterinary Surgeons for providing us with data from ZOOCAN and for all the veterinary practitioners who submitted samples to the APDS and for the technicians at the APDS laboratory who prepare these samples to be analysed and diagnosed.

Authors' contributions

A.E. and P.H. provided data for the tumour database. J.R. reviewed the pathology reports and created the digital database with support from and A.E. and A.S. J.R. wrote the initial draft with support from A.E. and A.S. A.S. wrote the R script for the data analysis, figures, tables, and the logistic regression model with support of J.R. A.E., A.S., P.H., D.K. reviewed the first draft and provided suggestions and ideas that contributed to improve the first draft. All authors participated in verifying the data and revising the manuscript. The author(s) read and approved the final manuscript.

Funding
No funding has been provided for this study.

Availability of data and materials
The datasets generated and/or analysed during the current study are available at https://doi.org/10.6084/m9.figshare.19688721

Declarations

Ethics approval and consent to participate
This study did not require official or institutional ethical approval as it was not experimental.

Consent for publication
Not applicable.

Competing interests
A.E. and P.H. are veterinary pathologist at the APDS.
The rest of the authors declare that they have no competing interests.

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Received: 3 March 2022 Accepted: 15 June 2022
Published online: 11 July 2022

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Publisher's Note

6.3.- Paper 3: Epidemiology of canine cutaneous round cell tumours on the Canary Archipelago in Spain.

Received: 29 October 2022 | Revised: 15 March 2023 | Accepted: 11 April 2023

DOI: 10.1111/vco.12899

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

Epidemiology of canine cutaneous round cell tumours on the canary archipelago in Spain

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Abstract

In this study we undertook a comprehensive analysis of a Pet Tumour Registry of the Canary Archipelago (PTR-CA) in Spain to investigate the epidemiology of canine cutaneous round cell tumours. From a database of 2526 tumours collected from 2003 to 2020, we conducted a longitudinal analysis of the main trends in diagnosis, age, multiplicity and anatomical distribution as well as a case–control study comparing these cases with the contemporaneous canine population of the Canary Archipelago to analyse breed distribution. In line with former studies, we found histiocytomas mostly affect young dogs (2, IQR 1–5) and mast cell tumours affect middle-to-old dogs (8, IQR 6–10) with grade 1 affecting at younger ages (6.5, IQR 6–8) than both grade 2 (8, IQR 6–10 years) and grade 3 (9, IQR 7–11). Histiocytomas and plasmacytomas showed a similar anatomical distribution appearing mainly on the face, head and neck regions while mast cell tumours occur mainly on limbs and trunk. Higher risk for mast cell tumours and histiocytomas were found for Bulldog-related breeds such as Boxer ($OR_{MCT} = 23.61$, CI95%: 19.12–29.15, $OR_{HCT} = 10.17$, CI95%: 6.60–15.67), Boston Terrier (OR_{MCT} 19.47, CI95%: 7.73–49.05, OR_{HCT} 32.61, CI95%: 11.81–90.07) and Pug (OR_{MCT} 8.10, CI95%: 5.92–11.07, OR_{HCT} 7.87, CI95%: 4.66–13.28) while Chihuahua dogs showed significantly less risk (OR_{MCT} 0.18, CI95%: 0.09–0.33, OR_{HCT} 0.41, CI95%: 0.21–0.78). Notably, the Canarian Mastiff, a local breed, had a low risk of suffering from a mast cell tumour which raises the question of whether this relates to a genetic peculiarity of this breed or some husbandry and environmental factor.

KEYWORDS

breed, cancer, cutaneous neoplasia, epidemiology, histiocytoma, mastocytoma, mast cell tumour, neoplasia, pathology report, plasmacytoma, round cell, skin tumour, tumour, veterinary

1 | INTRODUCTION

Skin tumours alongside mammary tumours are the most frequently diagnosed cancers of the canine population.[1–10] The cutaneous round cell tumour (CRCT) group commonly includes canine cutaneous histiocytoma (HCT), cutaneous lymphoma (LYM), plasmacytoma (PLA) and mast cell tumours (MCT). Less commonly reported round cell histotypes include transmissible

venereal tumour, histiocytic sarcoma melanoma and neuroendocrine tumours.[11]

Previous studies conducted on several countries such as the United States,[12,13] Denmark,[14] Romania,[15] Switzerland,[16] Portugal,[17] Korea[18] and Japan[19] have described the epidemiology of several canine skin tumours including MCT and, to a lesser extent, HCT and PLA. Additionally, other studies in the United States,[20–23] the United Kingdom,[24–26] Italy,[27] Poland,[28,29] Portugal,[30] Austria,[31] Croatia[32] and Australia[33,34] have focused specifically on different aspects of MCT epidemiology such as the association of breeds, age or anatomical distribution and the presence of simultaneous MCT. In the case of PLA, five studies from the United States[35–39] and one conducted on The Netherlands[40] have covered different aspects of its epidemiology in dogs. However, less attention has been paid to HCT with just one single specific study.[41]

Nonetheless, with the exception of the Norwegian Canine Cancer Project,[2] the Swiss canine cancer registry[8,16] and the study of Villamil et al,[12] all studies analysed samples of less than 600 MCT, less than 300 HCT and less than 100 cases of PLA.

The main paper of the Swiss canine cancer registry[8] covered a period from 1955 to 2008. The same group subsequently published a paper focused on skin tumours which covered a 5-year period from 2008 to 2013.[16] The Villamil et al study[12] also analysed data obtained from a larger period of time (1964 to 2002) but an important limitation reported by the authors was that the method of diagnosis was not always reported so tumour diagnosis could represent anything from a histologic diagnosis to a clinical impression.

Mochizuki et al[23] included a larger sample of MCT and the SAVSNET tumour registry[9] included larger samples of MCT, HCT and PLA but neither provided a denominator suitable for assessment of the risk for MCT, HCT and PLA in the whole population.

This study aims to enrich the existing literature with a new epidemiology paper conducted in Spain, where no study of similar characteristics has been conducted so far, in order to obtain a clear depiction of the epidemiology of these CRCT in the Spanish region of the Canary Archipelago.

The aims of this study were to: (a) evaluate the frequency of CRCT in the whole collection of tumours diagnosed by the Anatomical Pathology Diagnostic Service (APDS) of the Faculty of Veterinary Sciences of the ULPGC as well as the main trends followed by this CRCT over the study period; (b) analyse age at diagnosis of the different tumour histotypes and its relation with grade in the case of MCT; (c) assess anatomical distribution of the different tumour histotypes; (d) analyse the presence of simultaneous MCT; (e) analyse the relation of the different breeds and different grades of MCT; (f) analyse variable 'breed' adjusted by sex and island as a risk factor for MCT; (g) analyse variable 'breed' adjusted by sex and island as a risk factor for HCT; (h) analyse variable 'sex' adjusted by island as a risk factor for PLA.

2 | METHODS

2.1 | Study design and description of the study populations

The focus of this study is MCT, HCT, and PLA. LYM and other less frequently diagnosed CRCT cases were excluded as the numbers of these groups were not considered large enough to conduct an appropriate analysis of these histotypes.

The study case definition was: any tumour from a dog with a histopathological diagnosis (light microscopy examination of samples processed by the haematoxylin and eosin protocol) of a MCT, HCT or PLA affecting the skin and / or subcutis of a dog. For MCT, cases in which toluidine blue and Giemsa stains were required to make a diagnosis were included. Both cutaneous and subcutaneous MCT were included in the study although no differentiation was made between them. Tumours without a confirmed diagnosis by the histological examination process previously described (for instance those requiring immunohistochemistry to be confirmed) were excluded. Similarly, reports with a diagnosis of MCT, HCT or PLA obtained by cytological interpretation or affecting locations different from the skin and/or subcutis were excluded.

Data for this work was obtained from the diagnostic pathology reports from the APDS of the Faculty of Veterinary Sciences of the ULPGC[42] during the study period 2003 to 2020 as well as from the Canary registry of animal identification (ZOOCAN), a centralized web-based registry, managed by the Regional College of Veterinary Surgeons, in which veterinary practitioners are legally required to register all companion animals under their care.[43]

This study was structured in two parts for which different subsets of data were used as explained below.

In the first part, a longitudinal analysis of the relative proportions of the different CRCT within the case cohort was carried out. For each tumour type, the breed, age at diagnosis and anatomical distribution was evaluated and compared.

Additionally, for MCT, we studied the distribution and trend of multiplicity (dogs with more than one simultaneously diagnosed MCT) as well as the different MCT grades reported according to Patnaik's 3-tier histopathological approach.[44] In particular, we analysed the distribution of MCT of different grades on different breeds, for which we only considered those breeds with, at least, two cases of grade 1 MCT or grade 3 MCT or both. HCT and PLA were not analysed for multiple tumours given the small number of cases.

Specifically, this part of the study included a total of 2526 CRCT distributed as shown in Table 2.

The second part of the study consisted of a case–control study where we compared a selection of dogs with a diagnosis of, at least, one the three aforementioned histotypes (MCT case group, HCT case group and PLA case group) with a reference population (control group) obtained from ZOOCAN with the goal of evaluating the effect of the variables breed, sex and island as risk factors for these

TABLE 1 Number of cases and controls by the year of birth. For the case–control study, dogs were selected by year of birth. Different periods (in bold) were chosen for the analysis of the different histotypes in order to obtain a larger number of controls than cases as well as to have at least 10 cases by histotype, except for Plasmacytoma (PLA), on a yearly basis. In this sense, Mast cell tumours (MCT) were analysed in dogs born on the period 2000–2015, Histiocytoma (HCT) in dogs within the period 2000–2018 and Plasmacytoma (PLA) were studied on dogs born between 2000 and 2012. Note that this is not the number of tumours diagnosed but the number of dogs born in these years that developed any of these tumours later in life

Year of birth	Dogs from the control group	Dogs with an HCT	Dogs with a MCT	Dogs with a PLA
2000	191	10	66	6
2001	201	13	59	6
2002	326	12	53	4
2003	491	26	46	8
2004	660	29	68	6
2005	1101	35	68	4
2006	1723	36	78	11
2007	2652	40	79	12
2008	4148	43	90	6
2009	7371	46	86	5
2010	21 873	61	78	6
2011	34 560	44	63	7
2012	33 830	35	46	9
2013	34 447	32	43	3
2014	34 801	27	26	2
2015	35 249	10	11	2
2016	35 009	23	4	0
2017	32 400	12	2	0
2018	30 221	12	2	0
Total	311 254	546	968	97

TABLE 2 Relative proportions of the different cutaneous round cell tumours diagnosed over the study period 2003–2020

Histological type	*n*	Proportion (CI95%)	OR per year (CI95%)[a]	Trend test *p*-value
Histiocytoma	668	26.44% (24.73%; 28.21%)	0.94 [0.93, 0.96]	<0.0001
Mast cell tumour	1712	67.78% (65.91%; 69.60%)	1.05 [1.03, 1.06]	<0.0001
Plasmacytoma	146	5.78% (4.90%; 6.76%)	1.03 [1.00, 1.07]	0.0841

[a]An OR >1 means an increasing frequency of diagnosis while a value of OR <1 implies a decreasing frequency. OR with a CI95% including one means a stable tendency with no significant changes either downwards or upwards.

histotypes. Crossbreed dogs were the base category for breed analysis while female dogs and the island of Gran Canaria were the ones used for analysing sex and island as risk factors.

In order to facilitate a comparison that reduced age related bias, dogs in the control group were selected in such a way that their birth years were in the same range as the birth years of the cases.

Additionally, as can be seen in Table 1, we chose a study period for this part of the study ranging from 2000 to 2018 given that dogs born later (in 2019 onwards) were considered to be at low risk from any of the histotypes under study (MCT, HCT or PLA). Taking into consideration prior literature regarding the age at diagnosis of each tumour we used slightly different study periods for each tumour type. The precise study periods were chosen based on having at least 10 cases for the HCT group and the MCT group as well as breeds with at least four cases for either MCT and HCT. On the PLA case group, we included years with at least 4 cases given that the study for this histotype was limited to the sex as a risk factor. Therefore, the study period for the MCT, HCT and PLA cases groups run from 2000 to 2015, 2000 to 2018 and 2000 to 2012, respectively.

It should be emphasized that Table 1 shows number of dogs by year of birth instead of number of tumours by year of diagnosis. For instance, in 2005, there were 1101 dogs from the control group born that year as well as 35, 68 and 4 dogs born also in 2005 that ended up suffering from an HCT, a MCT or a PLA, respectively, later in life.

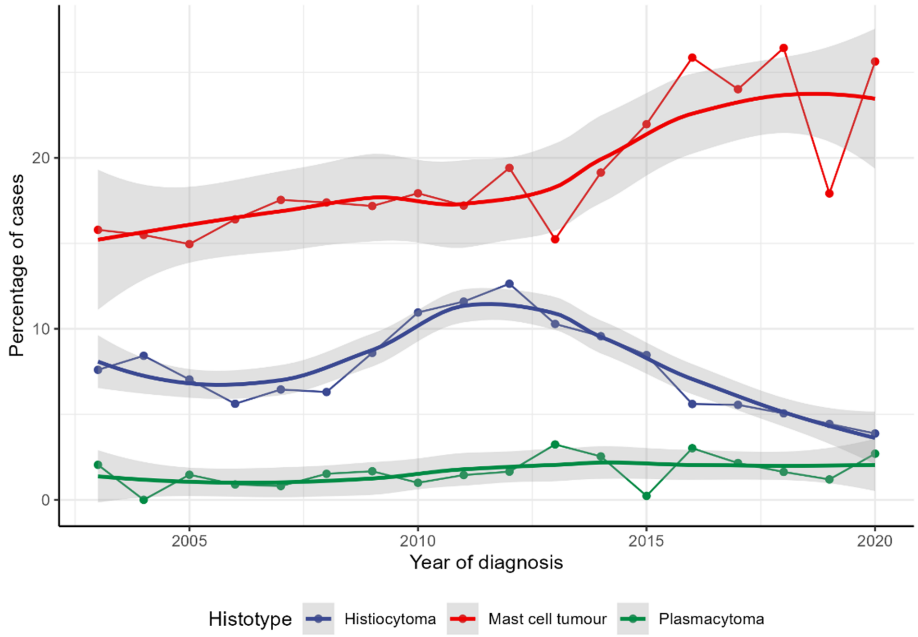

FIGURE 1 Percentage of the different histotypes with respect to the total number of tumours over the study period.

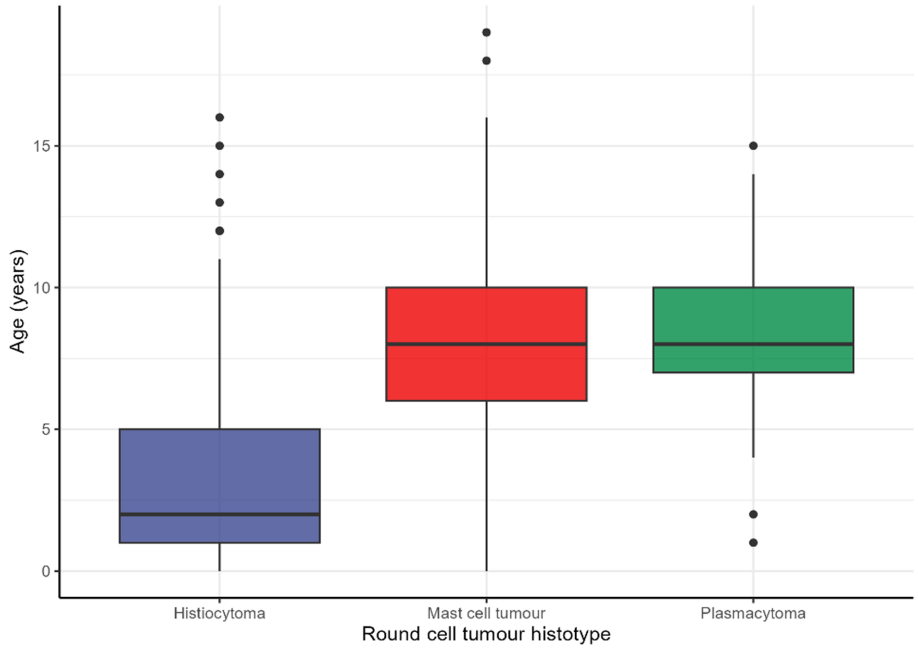

FIGURE 2 Median (IQR) of age by histotype. Median (IQR) was 2^{1-5} for Histiocytoma, 8^{6-10} for Mast cell tumour and 8^{7-10} for Plasmacytoma. Differences between these ages were significant (Kruskal-Wallis test Chi square = 753.4, $p < 0.001$, df = 2), and post hoc pairwise multiple comparisons showed that the age at diagnosis for Histiocytoma was significantly lower than that of Mast cell tumour and Plasmacytoma (Conover test $p < 0.001$ in both cases). Between Mast cell tumour and Plasmacytoma there was no significant difference ($p = 0.478$).

Also, for this reason, numbers on the three right columns in Table 1 tend to descend as the year of birth gets close to 2018 given that, the younger the animal, the less is the chance of being affected by a tumour.

Concerning the area under study, the Canary Archipelago is an Autonomous Community in Spain located in the Atlantic Ocean about 1500 km southwest of the mainland. There are eight islands with a total population in 2021 of 2.172.944 people[45] with 80% of people living in the islands of Gran Canaria and Tenerife where the two main metropolitan areas, Las Palmas de Gran Canaria and Santa Cruz de Tenerife are located.

2.2 | Statistical analysis

Both exploratory and statistical analysis were performed with the R Language and Environment for Statistical Computing, version 4.1.2.[46] Categorical variables were summarized as numbers and percentages; age was expressed as median and interquartile range. The Cochran-Armitage trend test was used for assessing the presence of increasing or decreasing trend in proportions. Increase (or decrease) of odds-ratio per year with its 95% confidence interval was reported to assess the magnitude of the trend. Association in contingency tables was also assessed by chi-squared test.

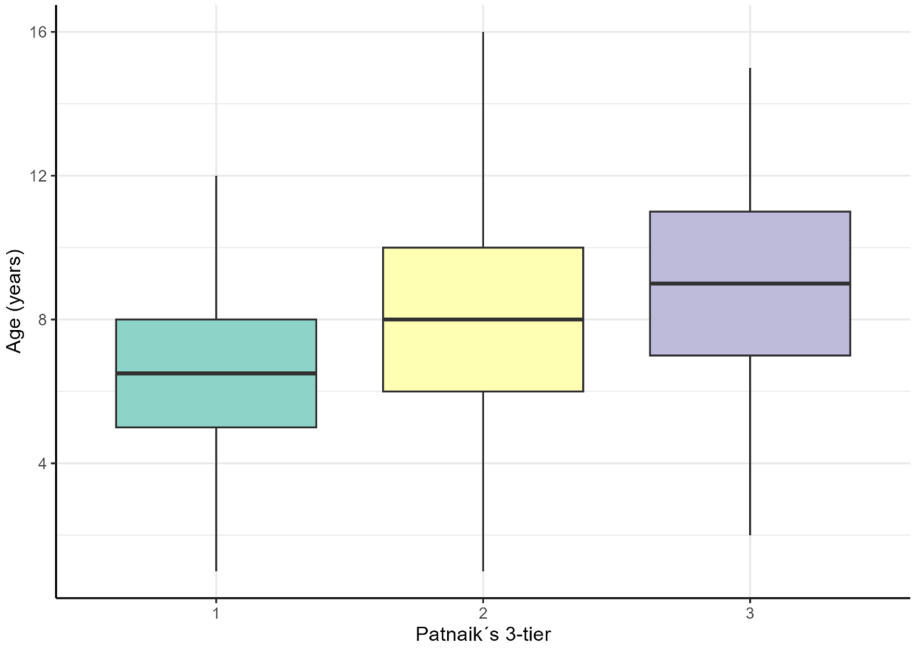

FIGURE 3 Median (IQR) of age by Patnaik's 3-tier histopathological approach. Median (IQR) was 6.5[5–8] for Grade 1, 8[6–10] for Grade 2 and 9[7–11] for Grade 3. Differences between these ages were significant (Kruskal-Wallis test Chi square = 21.4, $p < 0.001$, df = 2), and Conover post hoc pairwise multiple comparisons resulted in significant differences between all groups ($p = 0.0004$ for grade 2 vs. grade 1, $p < 0.0001$ for grade 3 vs. grade 1 and $p = 0.0097$ for grade 3 vs. grade 2).

TABLE 3 Anatomical distribution of Mast cell tumours, Histiocytomas and Plasmacytomas. Anatomical distribution of Mast cell tumours, Histiocytomas and Plasmacytomas. Firstly, an overall analysis of the frequency distribution of histotypes between the different anatomical regions showed significant differences between them (chi-squared = 344.7, df = 10, $p < 0.0001$). Secondly, multiple comparison post-hoc chi-squared analysis revealed that differences between the anatomical distributions of HCT and PLA were not significant ($p = 0.38$), but both distributions differed significantly from that of MCT ($p < 0.0001$)

Location	Mast cell tumour	Histiocytoma	Plasmacytoma
Limbs	602 (36.4%)	205 (31.8%)	41 (28.9%)
Trunk	632 (38.2%)	148 (22.9%)	26 (18.3%)
Face, head and neck	221 (13.3%)	282 (43.7%)	68 (47.9%)
Perianal and scrotum region	177 (10.7%)	4 (0.6%)	2 (1.4%)
Tail	24 (1.4%)	6 (0.9%)	5 (3.5%)

TABLE 4 Anatomical distribution of Mast cell tumours by grade according to Patnaik's 3-tier histopathological approach. An overall analysis of the frequency distribution of the different grades of Mast cell tumour between the different anatomical regions showed no significant differences between them (chi-squared = 14.60, df = 10, $p = 0.14$)

Location	Grade 1	Grade 2	Grade 3
Trunk	25 (54.3%)	452 (38.8%)	52 (37.7%)
Limbs	9 (19.6%)	423 (36.3%)	41 (29.7%)
Face, head and neck	8 (17.4%)	151 (13.0%)	25 (18.1%)
Perianal and scrotum region	4 (8.7%)	119 (10.2%)	19 (13.8%)
Tail	0 (0.0%)	19 (1.6%)	1 (0.7%)

Post-hoc chi-square tests with Bonferroni adjustment were used for pairwise comparisons when significant differences were detected between groups. Shapiro–Wilk test was used for testing normality. Kruskal-Wallis test was used for testing if age was equally distributed in several groups. Multiple post-hoc comparisons after Kruskal-Wallis test were performed using Conover-test. Logistic regression analysis was used to evaluate the association of dog breed with the risk of MCT and HCT, adjusted by island and sex, and odds ratios with 95% confidence intervals were reported. For PLA, logistic regression was used to analyse sex, adjusted by island, as a risk factor. In all tests, p-values lower than 0.05 were considered as statistically significant.

3 | RESULTS

3.1 | Results from the longitudinal study

(a) Cutaneous round cell tumour distribution and evolution over the study period

Over the study period 2003–2020, 2526 CRCT, diagnosed by histology, were analysed. The whole tumour histotypes (MCT, PLA, HCT), distributed as shown in Table 2, comprised for 28.83% of all skin and subcutis tumours diagnosed by the APDS over the same period.

Table 2 shows the tendencies to the changes in the relative proportion of MCT, HCT and PLA diagnosed over the study period. For HCT, we found a significant downward tendency (OR <1, p-value <0.0001) while, for MCT, the tendency was also significant but upward (OR >1, p-value <0.0001). No significant trend (p-value = 0.084) was detected for PLA.

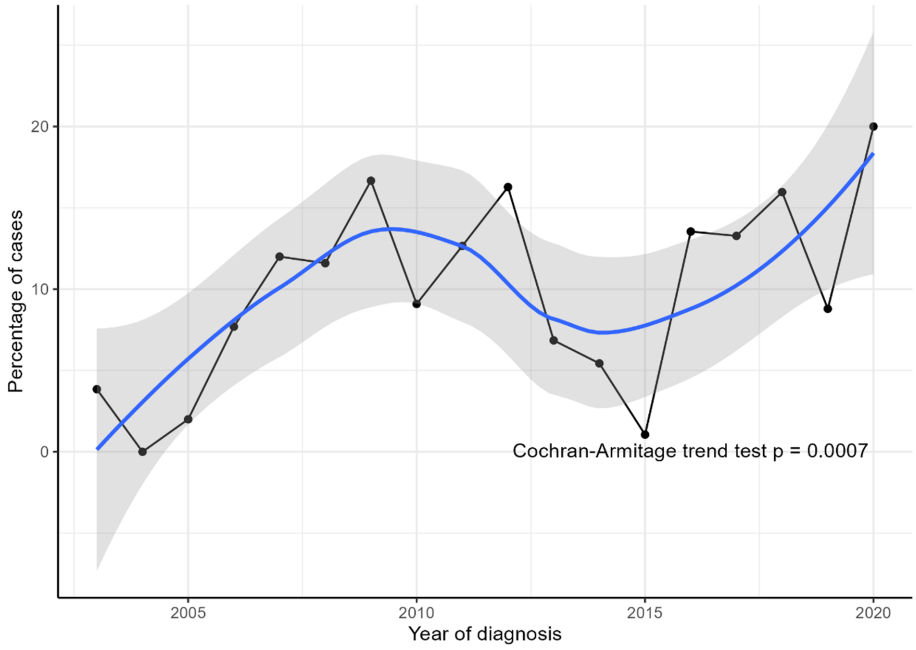

FIGURE 4 Percentage of dogs with multiple simultaneously diagnosed Mast cell tumours.

The relative proportions of each histotype (MCT, HCT and PLA) was compared with the whole collections of tumours diagnosed every year on the APDS. The tendency for MCT went from 15.9% in 2003 to 25.9% in 2020. On the contrary, diagnosis of HCT followed a downward tendency of −3.72% (from 7.6% to 3.9%). Finally, the difference for PLA went from 2.1% in 2003 to 2.7% in 2020. It should be noted that the relative proportions shown in Table 2 were related to the total of skin and subcutis tumours different from MCT, HCT and PLA while the relative proportions shown in Figure 1 were calculated over all the different kinds of tumours (not only skin and subcutis tumours) diagnosed on the APDS over the study period.

Concerning the different grades of MCT, this information was available on 1394 reports from which 47 (3.4%) were described as grade 1, 1206 (86.5%) as grade 2 and 141 (10.1%) as grade 3.

(b) Age at diagnosis

As shown in Figure 2, MCT and PLA were diagnosed at 8-year-old dogs (IQR 6–10 and 7–10 respectively) while HCT were diagnosed at younger ages (2, IQR 1–5). Significant differences were detected (Kruskal-Wallis test Chi square = 753.4, $p < 0.001$, df = 2) and post-hoc Conover test was applied for pairwise multiple comparisons resulting in significant differences between MCT and HCT ($p < 0.001$), and between PLA and HCT ($p < 0.001$) but with no significant differences between MCT and PLA ($p = 0.478$).

Concerning age and MCT grade, Figure 3 shows that grade 1 MCT were diagnosed at younger ages (6.5, IQR 6–8) than both grade 2 (8, IQR 6–10 years) and grade 3 (9, IQR 7–11) MCT.

These differences were statistically significant (Kruskal-Wallis test Chi square = 21.4, $p = 0.000023$, df = 2) and post-hoc Conover test was applied for pairwise multiple comparisons resulting in significant differences between all groups ($p = 0.0004$ for grade 2 vs grade 1, $p < 0.0001$ for grade 3 vs grade 1 and $p = 0.0097$ for grade 3 vs grade 2).

TABLE 5 Breed distribution of different Mast cell tumours grades according to Patnaik's 3-tier histopathological approach. For this analysis, only breeds with at least two cases of Grade 1 or Grade 3 Mast cell tumour or both were included

Breed	Grade 1	Grade 2	Grade 3
Crossbreed	14 (3.1%)	381 (84.7%)	55 (12.2%)
Boxer	14 (6.2%)	198 (87.6%)	14 (6.2%)
French Bulldog	3 (2.2%)	122 (89.7%)	11 (8.1%)
Pug	2 (3.1%)	60 (92.3%)	3 (4.6%)
Labrador Retriever	4 (6.8%)	53 (89.8%)	2 (3.4%)
Yorkshire Terrier	1 (2.3%)	35 (79.5%)	8 (18.2%)
Staffordshire Bull Terrier	1 (2.3%)	39 (90.7%)	3 (7.0%)
Shar-Pei	1 (2.6%)	27 (71.1%)	10 (26.3%)
Bull Terrier	1 (5.3%)	15 (78.9%)	3 (15.8%)
Canarian Mastiff	0 (0.0%)	14 (82.4%)	3 (17.6%)
Rottweiler	0 (0.0%)	6 (66.7%)	3 (33.3%)
Total	41 (3.7%)	950 (85.9%)	115 (10.4%)

(c) Anatomical distribution of the different tumour histotypes

The three types of tumours histotypes showed different frequency distributions across the anatomical locations considered (Table 3, chi-squared = 344.69, df = 10, $p < 0.0001$) and the multiple comparison post-hoc chi-squared analysis revealed that the differences between the anatomical distributions of HCT and PLA were not significant ($p = 0.38$), but both distributions were significantly different from that of MCT ($p < 0.0001$). Table 3 shows that the highest proportion

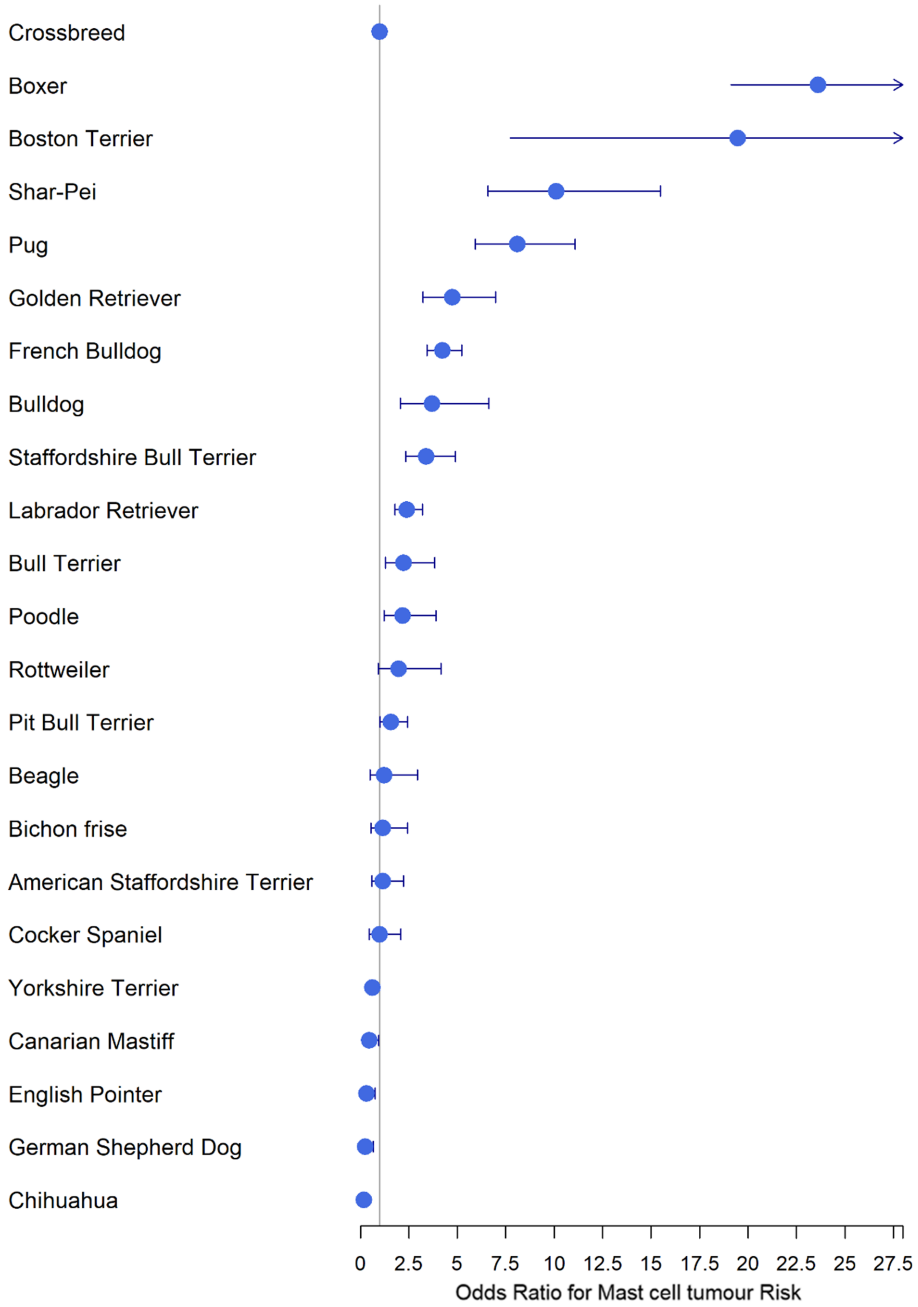

FIGURE 5 Odds Ratios (± 95% confidence intervals) for Mast cell tumour risk by dog breed when compared with crossbreed dogs (baseline), adjusted by island and sex.

of HCT and PLA (43.7% and 47.9% respectively) occurs on the face, head and neck regions, compared to only 13.3% of MCT. MCTs, in turn, occur more frequently than HCT and PLA on the limbs, trunk and perianal and scrotum regions.

Finally, we evaluated whether there were differences in the anatomical distribution of MCT by grade but none were identified (chi-squared = 14.60, df = 10, p-value = 0.14), as shown in Table 4.

(d) Multiple MCT

Longitudinal changes in the frequency of dogs being diagnosed with more than one MCT simultaneously evolution is shown in Figure 4. The proportion of animals affected by more than one MCT simultaneously increased markedly over the study period from 3.8% in 2003 to 20% in 2020 (Supplementary Table 1) in such a way that the odds of having multiple MCTs increased by 6% on average each year (OR = 1.062, CI95% [1.03, 1.1])

(e) Breeds and MCT grade

We found an association between breed and MCT grade (p-value = 0.0106), but post-hoc pairwise comparisons between breeds were not carried out due to the insufficient number of cases to perform this test.

As shown in Table 5, for grade 3 MCT, Shar-Pei and Rottweilers showed the highest proportion and French Bulldog, Staffordshire Bull Terrier, Boxer and Pug were diagnosed least frequently. Concerning grade 1 MCT, Boxer and Golden Retrievers obtained the highest proportions.

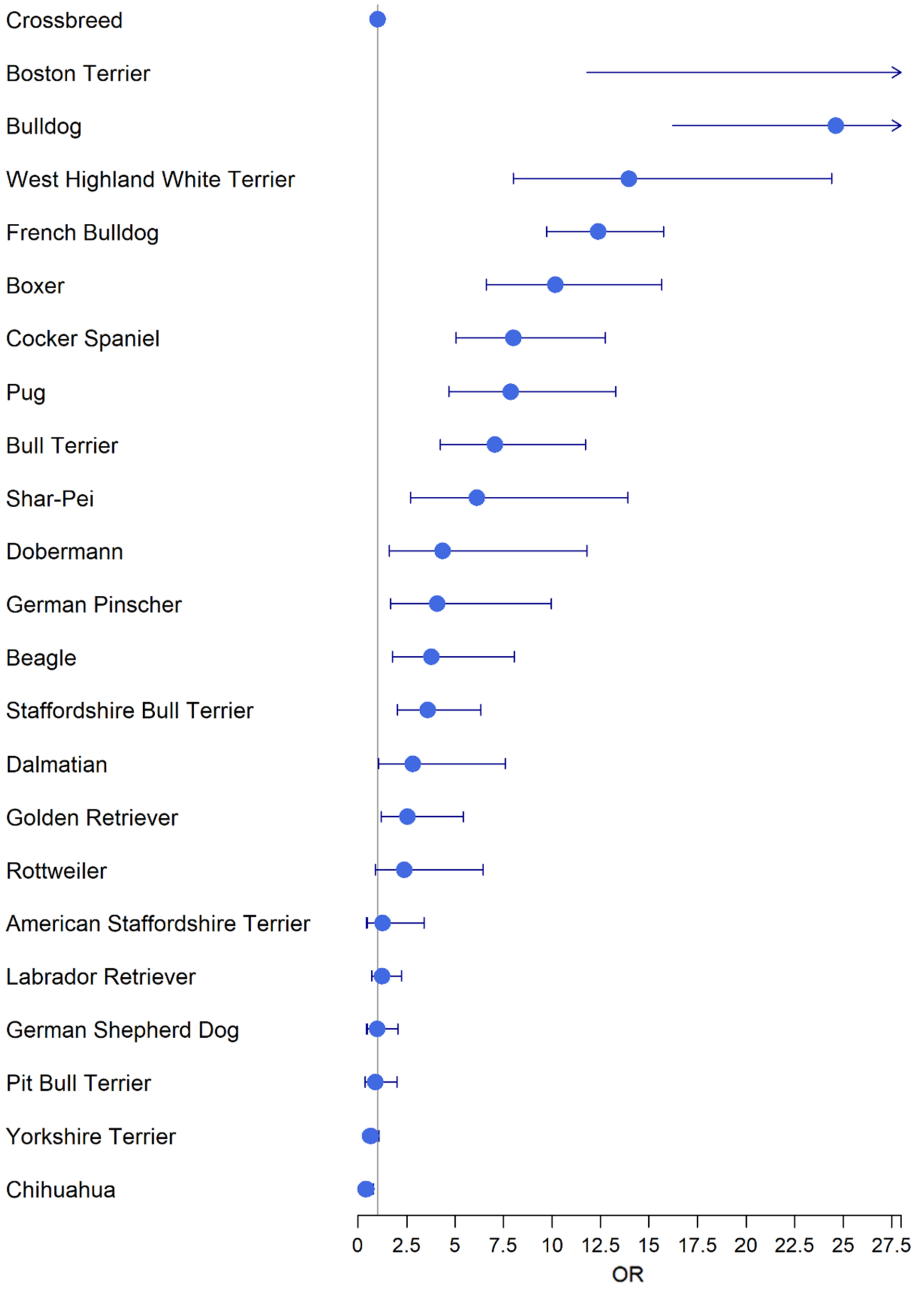

FIGURE 6 Odds Ratios (± 95% confidence intervals) for Histiocytoma risk by dog breed when compared with crossbreed dogs (baseline), adjusted by island and sex.

3.2 | Results from the case–control studies

This section describes the results of the case–control studies. Variable breed adjusted by sex and island was analysed for MCT (Figure 5 and Supplementary Table 2) and HCT (Figure 6 and Supplementary Table 3) cases while only sex, adjusted by island, was analysed for PLA cases (Supplementary Table 4).

Concerning the risk associated with the island upon which the dogs lived, all islands showed a lower risk than Gran Canaria (base category) for MCT. For HCT, the islands of Tenerife, Lanzarote and La Palma showed a lower risk while, for PLA, only the island of Tenerife showed a lower OR than Gran Canaria.

(f) MCT: Breed adjusted by sex and island

Twelve breeds had an increased odds of being diagnosed with an MCT compared to crossbreed dogs. In particular, Boxer (OR 23.61, 95% CI 19.12–29.15), Boston Terrier (OR 19.47, 95% CI 7.73–49.05), Shar-Pei (OR 10.09, 95% CI 6.59–15.47), Pug (OR 8.10, 95% CI 5.92–11.07) and Golden Retriever (OR 4.74, 95% CI 3.22–6.98) were the five breeds with the highest OR. On the contrary, only five breeds obtained a significant OR below one. Chihuahua (OR 0.18, 95% CI 0.09–0.33), German Shepherd (OR 0.24, 95% CI 0.09–0.66), English Pointer (OR 0.31, 95% CI 0.13–0.76), Canarian Mastiff (OR 0.45, 95% CI 0.21–0.95) and Yorkshire Terrier (OR 0.61, 95% CI 0.44–0.86).

Male dogs showed lower odds of developing a MCT compared to female dogs (OR 0.81, 95% CI 0.71–0.93).

(g) HCT: Breed adjusted by sex and island

Fourteen breeds had higher odds of being diagnosed with an HCT compared with crossbreed dogs. Higher risk was observed for Boston Terriers (OR 32.61, 95% CI 11.81–90.07), Bulldog (OR 24.6, 95% CI 16.23–37.30), West Highland White Terrier (OR 13.97, 95% CI 8.00–24.40), French Bulldog (OR 12.38, 95% CI 9.71–15.76) and Boxer (OR 10.17, 95% CI 6.60–15.67).

On the contrary, Chihuahua was the only breed showing decreased odds (OR 0.41, 95% CI 0.21–0.78).

Male dogs had higher odds than female dogs (OR 1.27, 95% CI 1.07–1.52).

(h) PLA: Sex adjusted by island

Sex was analysed as a risk factor for developing PLA but no significant differences were observed between male and female dogs (OR 1.50, 95% CI 0.99,2.28).

4 | DISCUSSION

This study describes the epidemiology of a large sample of CRCT (MCT, HCT and PLA) diagnosed on the Canary Island region in Spain from 2003 to 2020 and it is one of the longest studies of this kind within the published literature.

Our results showed a longitudinal increase in the proportion of MCT compared to all tumours as well as a decrease in the proportion of HCT diagnosed over the study period. A plausible reason for these tendencies could be related, at least in part, to a change in the age distribution of the canine population of the Canary Archipelago resulting in a slightly older population. In this sense, neutered rate on the group of dogs suffering from any tumour tripled from 10.3% in 2003 to 32.8% in 2020 (Supplementary Table 5) which would lead to a decrease in new litters and new puppies in the canine population. Additionally, a recent study[46] has shed light over the increasing popularity of importing rescue dogs (overseas adoptions), especially crossbreed young dogs (up to 2 years old) into the UK from different countries like Spain. Consequently, along with a higher neutered rate, the removal of these young animals from the Spanish canine population could be playing a role on the aging of this population favouring an increase in the diagnosis of tumours that more commonly affect middle-to-old dogs such as MCT[6,16,18,23,26,27,29–31] and also a decrease in tumours typically diagnosed in young dogs such as HCT.[6,16–18,41]

Another reason could relate to a better education or the public being more engaged with cancer diagnosis and treatment generally. In this sense, the availability of two specifics drugs for MCT treatment (masitinib and toceranib) since 2009[47,48] could have encouraged veterinary practitioners to diagnose and treat these kinds of tumours more than they used to. Additionally, a better knowledge of HCT behaviour and their tendency to regress spontaneously[49] as well as the high chance of being diagnosed by cytology could be reasons for the downward tendency of HCT diagnosed over the study period.

Concerning age at diagnosis, in our study, MCT were diagnosed on an average age of 8.38 years old which is consistent with former studies[6,16,18,23,26,27,29–31] although one study found average age being older (around 11 years old).[15]

Also, our results analysing differences in age depending on the grade of MCT were similarly consistent with other studies[23,29] in that the proportion of high grade MCT increases with advancing age. In this regard, the previously described aging of the canine population in our region could explain, at least partially, why we obtained smaller proportion of grade 1 MCT (3.4%) and higher ones of grade 2 MCT (86.5%), when compared with previous works[6,14,19,23,30,34] that obtained results ranging from 12.9% to 33.3% for grade 1 and from 33.6% and 76.3% for grade 2. However, our results are in this case consistent with the recent SAVSNET tumour registry[9] which obtained a proportion of grade 1 and 2 MCT of 4.7% and 92.1% respectively.

When it comes to age at diagnosis of HCT, our study also found similar results with prior literature[6,16–18,41] although two studies reported an average age of greater than 5 years,[1511] at diagnosis. Finally, our result for average age for PLA diagnosis was also in line with former publications.[11,35–37,39,40]

Concerning anatomical distribution, our results showed that around 75% of MCT were located evenly on the trunk region and on the limbs while the other 25% were located either on the face, neck and head and on the perianal and scrotum region with similar relative proportions between the different parts of the trunk (thorax and abdomen) but with a clear difference in favour of the hindlimbs vs forelimbs. The reasons for these locational differences are still unknown.

In this sense, in spite of the different ways in which former studies[9,14,16,19,21,27–29,32,33] show results of anatomical distribution for MCT, a general pattern can be observed; most studies found around 30% of MCT affecting both limbs with hindlimbs found to be consistently more frequently affected except in one study from Korea[19] which found extremities to be the first anatomical location with a proportion of 40.9% and another from Italy[27] where only 15.2% of MCT were located on the limbs. Additionally, former studies found MCT to be located on the trunk region with a frequency ranging from 19%[16] to more than 50% of cases[21,29] without a clear difference of any particular region (thorax and abdomen). Finally, all studies showed a frequency of less than 20% for the head-face-neck area although one paper from Austria[32] emphasized than 70% of MCT were located on the trunk and the head.

In our study we did not find strong evidence that any particular anatomical locations are associated with MCT tumours grade. This is discordant with the prior literature which suggests a greater risk of high-grade MCT for the head, inguinal and perigenital areas.[30,33]

When it comes to HCT and PLA, we found more than 40% of cases located on the head-face-neck area, a clear preponderance for abdomen vs thorax and an equal distribution on the hind vs fore limbs. Other studies found HCT[16,18,41] to be located on the head-face-neck area on a frequency ranging from 28.4% to 53% and PLA[19,35,36,38–40]

from 27.8% to 45.6%. Lower frequencies for these tumours on this location were obtained however in a Korean study[19] (18.2 for HCT on the head–neck area) and on the SAVSNET tumour registry[9] (17.8% for HCT and 13.9% for PLA) although up to 45% of the location for these tumours on this database was reported with the generic term ("skin").

We found that the following breeds are pre-disposed to MCT including Bulldog-related breeds such as Boxer, Boston Terrier, Pug, French Bulldog, Staffordshire Bull Terrier, Bull terrier and Bulldog to have the greatest risk of suffering from a MCT when compared with crossbreed dogs along with Retrievers (Golden and Labrador), Shar-Pei and Poodle. These results are consistent with most of literature[12,16,17,19,20,22,23,25–27,29,32] and in line with the hypothesis formulated by a former 1969 study[20] stating that a common ancestry may be behind the predisposition of these breeds when it comes to be affected the MCT.

Low-risk breeds in our study were the Chihuahua, German shepherd, English Pointer and Yorkshire terrier which have been also described previously as low-risk breeds by the same studies.[12,16,19,22,23,25,26,28,30–32]

We also found Canarian Mastiff, a local breed from this region to be a low-risk breed for MCT. Interestingly, a recent study describing the epidemiology of canine mammary tumours on the Canary Archipelago[50] also found local breeds such as the Canary Warren Hound, Majorero and Canarian Mastiff to be low-risk breeds for these kinds of tumours. Reasons for these low-risks-related findings are still unclear due to the lack of studies covering Canary Islands dog breeds. However, at least in part, the high genetic variability found in the Canary Island breeds[51] and their recent origin from mixed ancestral stock could be playing a role in this regard.

Concerning breed and grade of MCT, different studies tend to confirm the fact that certain breeds predisposed for MCT tend to suffer from low-grade MCT while other breeds less frequently affected by this tumour tend to have high-grade MCT. In our case, we found differences on the distribution of the different grades of MCT and the different breeds although our data was not large enough to conduct more in-depth analyses between the particular breeds. However, we found that among our cases of high-grade MCT, Rottweilers and Shar-Pei were the most frequently diagnosed as was the case in three previous studies[23,28,33] while Boxer, and Labrador Retriever were the most frequent breeds among dogs with a low-grade MCT being these observations also consistent with former studies.[17,23,28] In our case, also Canarian Mastiff, a low-risk breed for MCT showed a slightly higher proportion of grade 3 MCT. As pointed out by Mochizuki et al,[23] the discrepancies in proportions of low and high grade among different breeds may indicate that genetic alterations responsible to MCTs may be different from those contributing to aggressive biological behaviour.

We found less risk of males than females developing an MCT. Former studies had mostly found no differences in this regard[15,19,26,27,30,33] while others found higher risk for females.[29]

Regarding breed and risk of HCT, we also found Bulldog-related breeds mentioned previously for MCT and Retrievers plus the addition of West Highland White Terrier (third breed with greater risk), as well as the Cocker, German Pinscher and Beagle to be at greater risk of developing HCT. Chihuahua was the only breed with a lower OR than crossbreeds.

These results for HCT are consistent with other studies[16,17,41] that found Boxer, French Bulldog and English Bulldog to be at a greater risk of suffering from a HCT while Chihuahua was found as the only breed at lower risk than cross-breeds.

We found male dogs to be at a greater risk of HCT which is adds to the discordance in the literature about the relevance of sex to this tumour type.[15,41]

Our results for sex as a factor risk for PLA revealed no differences in risk due to sex for this tumour type, although the only reference to compare with found a greater proportion of males versus female dogs in a sample of 49 dogs suffering from PLA.[36,37]

Finally, when comparing the results obtained in different islands, we saw a lower risk in all islands when compared with Gran Canaria although this is mostly due to an overrepresentation of cases submitted from Gran Canaria to the APDS as explained below.

Some limitations presented in this study should be mentioned. Firstly, concerning the ZOOCAN database, dogs are typically registered in the system on his first visit to the vet but follow-up information (for instance, changes in the neuter status) is usually not recorded. For this reason, the only data provided by ZOOCAN that we considered reliable enough to be used in this study were the variables year of birth and breed of the dogs. Secondly, it should be noted that breed data used for this study was reported by veterinary practitioners (secondary data) so we should expect some degree of uncertainty in the accuracy of breed identification. So, in order to minimize this error, we chose to indicate that a dog belonged to a specific breed when that breed was clearly specified in the report. Any combination of breeds was considered a crossbreed.

Thirdly, as a pathology-based tumour registry,[52] some degree of bias should be expected when a comparison is made between the cancer profile emerging from the APDS and the actual (unknown) one of the canine population. One reason for this is due to a selection bias by veterinary surgeons in such a way that tumours considered more concerning are more likely to be removed and submitted for diagnosis. In this sense, a selection bias in favour of skin tumours over internal tumours is likely given the easy detection by owners or veterinary practitioners. Additionally, socioeconomic factors could play a role in the sense that paying for an anatomopathological analysis may be impractical for less affluent clients. There may also be some effect of socio-economic status and choice of dog breed.

However, it should be noted that the APDS is a diagnostic service integrated in an academic institution, the ULPGC and is an affordable not for profit service aimed at teaching veterinary pathology to students and thus costs are comparatively low.

Additionally, geographic and logistical reasons have created an overrepresentation of cases from the island of Gran Canaria when compared with the other Canary Islands, due to the fact that the APDS is located in the Faculty of Veterinary Sciences in Gran Canaria,

30 min away from the largest city of the Canary Archipelago, Las Palmas de Gran Canaria, providing a clear advantage for cases submitted from this island when compared to the others hindering our options to conduct an island-by-island analysis on this paper due to the lack of uniformity and representativeness of the different islands.

Finally, a pathological diagnosis is somewhat subjective and there is therefore a risk of pathologist bias that should be taken into account.

In conclusion, this study provides the first epidemiological description of the cutaneous round cell tumours that affect canine population on the Canary Archipelago in Spain. Our findings confirm MCT to be one of the most commonly diagnosed tumour affecting middle-to-old dogs and canine cutaneous histiocytomas as the main tumour histotype of young dogs.

Bulldog-related breeds were the most at-risk breeds of developing non-high-grades MCT while others like Shar-Pei were the most affected by high-grade MCT. These breeds were also found be high-risk breeds for HCT while Chihuahua were found to be protected against both MCT and HCT. Specially interesting in our study was to find Canarian Mastiff, a local breed, to be protected against MCT which could suggest some kind of advantage due to the high genetic variability found in the Canary Island dog breeds.

CELL LINE VALIDATION STATEMENT

No cell lines were used in the current study.

ACKNOWLEDGMENTS

We are grateful to the Canary College of Veterinary Surgeons for providing us with data from ZOOCAN and for all the veterinary practitioners who submitted samples to the APDS and for the technicians at the APDS laboratory who prepare these samples to be analysed and diagnosed.

FUNDING INFORMATION

The authors have no financial support to disclose.

CONFLICT OF INTEREST STATEMENT

A.E. and P.H. are veterinary pathologist at the APDS. The rest of the authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.21407595.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rodríguez J, Santana Á, Borzollino MA, Herráez P, Killick DR, de los Monteros AE. Epidemiology of canine cutaneous round cell tumours on the canary archipelago in Spain. *Vet Comp Oncol*. 2023;1-13. doi:10.1111/vco.12899

7.- CONCLUSIONS.

First: The computer tool developed, based on data mining to automate the reading, extraction and normalisation of data from large volumes of digitised unstructured diagnostic reports, proved to be efficient and effective.

Second: This study represents the first epidemiological analysis of mammary neoplasms and cutaneous round cell tumours in the canine species, the result of the creation of a standardised database of tumours in companion animals in the Canary Islands.

Third: The canine population of the Canary Islands has a higher incidence of malignant mammary tumours than other geographical areas, which could be related to regional environmental and socio-economic factors, as well as to a greater awareness on the part of owners and veterinarians regarding the diagnosis and treatment of these pathologies.

Fourth: In the Canary Islands, concerning canine cutaneous round cell tumours, mast cell tumour is the most frequently diagnosed tumour histotype in middle-aged and elderly individuals, while histiocytoma is the most common tumour histotype in young dogs.

Fifth: In the Canary Islands, Samoyed and Schnauzer breeds have the highest risk of mammary tumours, whereas Miniature Pinscher, English Pointer and American Staffordshire Terrier have a lower risk. For round cell skin tumours, Boxer, Boston Terrier, Shar-Pei and Pug breeds have a higher risk of developing mast cell tumours than Chihuahua, German Shepherd and English Pointer. Finally, for histiocytomas, the Boston Terrier, Bulldog and West Highland White Terrier breeds are most at risk, while the Chihuahua is the only breed to show some protection.

Sixth: Local breeds such as Canarian Warren Hound, Majorero and Presa Canario show a lower tendency to be affected by both mammary carcinomas and cutaneous mast cell tumours, which could indicate some kind of resistance due to the high genetic variability of these breeds.

8.- NORMATIVA Y ADECUACIÓN A LAS TESIS POR COMPENDIO DE PUBLICACIONES.

El Reglamento 1/2023, de Estudios de Doctorado de la Universidad de Las Palmas de Gran Canaria (ULPGC) es el marco regulatorio que establece los contenidos y formatos de las tesis doctorales presentadas en dicha Universidad.

Así, a lo largo de la Sección Segunda (Del contenido y formato de las tesis) del Capítulo III (De la Tesis Doctoral), se establece que las La tesis doctoral consistirán en un trabajo original de investigación elaborado por el doctorando sobre un tema relacionada con el Programa de Doctorado en que se encuentre matriculado debiendo contener, al menos, una introducción o estado de la cuestión, los objetivos planteados, la metodología desarrollada, los resultados y la discusión sobre los mismos, así como las conclusiones más relevantes y la bibliografía utilizada.

Las tesis podrán ser desarrolladas y defendidas en los en los idiomas habituales para la comunicación científica en su campo de conocimiento si bien, en el caso de tesis doctorales escritas en una lengua distinta a la española se deberá aportar un resumen en español sobre el contenido de esta, de una extensión de entre 3 y 15 páginas, en el que se incluyan los objetivos y las conclusiones.

El artículo 24 describe los requisitos para las tesis por compendio de publicaciones que deberán incluir un mínimo de tres publicaciones, con unidad temática, indexadas en el Journal Citations Reports,Arts and Humanities Citation Index o equivalentes, de las que el doctorando sea el primer autor o autor principal. Así mismo, al menos una de estas publicaciones deberá haber sido publicada en una revista cuyo índice de impacto la sitúe dentro de la primera mitad en orden decreciente de índice de impacto entre las revistas del área.

Respecto a su contenido, las tesis por compendio de publicaciones deberán contener una introducción en la que se presenten los objetivos de la tesis, los trabajos publicados

y la justificación de la unidad temática de la tesis, una copia de los trabajos publicados y unas conclusiones finales.

En este sentido, esta tesis doctoral se ajusta a los requerimientos anteriormente mencionados en tanto en cuanto se presentan tres publicaciones publicadas en revistas científicas publicadas en revistas científicas del ámbito de conocimiento del programa de doctorado e indexadas en el Journal Citations Report, en las cuales el doctorando figura como primer autor, y cuyos indicios de calidad se indican a continuación:

1.- José Rodríguez, David R. Killick, Lorenzo Ressel, Antonio Espinosa de los Monteros, Ángelo Santana, Samuel Beck, Francesco Cian, Jenny S. McKay, P.J. Noble, Gina L. Pinchbeck, David A. Singleton & Alan D. Radford. "A text-mining based analysis of 100,000 tumours affecting dogs and cats in the United Kingdom". https://doi.org/10.1038/s41597-021-01039-x. Scientific Data. Impact Factor 2021: 8.501. Journal Rank in Multidisciplinary Sciences: 13/135 (Q1).

2.- José Rodríguez, Ángelo Santana, Pedro Herráez, David R. Killick & Antonio Espinosa de los Monteros. "Epidemiology of canine mammary tumours on the Canary Archipelago in Spain". https://doi.org/10.1186/s12917-022-03363-9. BMC Veterinary Research. Impact Factor 2021: 2.792. Journal Rank in Veterinary Sciences: 25/145 (Q1).

3.- José Rodríguez, Ángelo Santana, Marisa Andrada Borzollino, Pedro Herráez, David R. Killick & Antonio Espinosa de los Monteros. "Epidemiology of canine cutaneous round cell tumours on the Canary archipelago in Spain". DOI: 10.1111/vco.12899. Veterinary and Comparative Oncology. Impact Factor 2021: 2.385. Journal Rank in Veterinary Sciences category: 40/145 (Q2).

9.- RESUMEN EXTENDIDO DEL PROYECTO DE TESIS DOCTORAL.

9.1.- Introducción: El papel de la epidemiología para minimizar el impacto del cáncer.

El cáncer es uno de los problemas de salud pública más importantes de nuestro tiempo, tanto en la población humana como en la de animales de compañía, y quizá ningún otro diagnóstico sea más preocupante cuando se trata de nuestra propia salud o la de nuestros seres queridos[1,2] y mascotas[3,4].

Bajo nuestro cuidado, nuestros perros y gatos pasan toda una vida con nosotros, proporcionándonos una leal compañía y enriqueciendo nuestras vidas en diversos aspectos[5–8] hasta el punto de que su salud y bienestar se terminan convirtiendo en una parte importante de nuestras preocupaciones diarias.

Por este motivo, el estudio de los factores epidemiológicos asociados a los distintos tipos de cáncer se ha convertido en una importante rama de la ciencia veterinaria[9] con un importante papel que desempeñar en la prevención del cáncer y, por tanto, en garantizar un estado de salud óptimo de las distintas poblaciones de animales de compañía.

Además, desde una perspectiva *One Health*, la prevención del cáncer en perros y gatos lo es también para los humanos[10,11] dada la importancia de las mascotas como centinelas y modelos de la salud humana[12,13] ya que, tanto mascotas como sus dueños comparten un entorno común durante gran parte de sus vidas. Ambos respiran el mismo aire, beben la misma agua y se exponen a las mismas fuentes de alimentos y contaminantes ambientales.

Desde esta perspectiva, es evidente la necesidad de supervisar el estado de salud de nuestras poblaciones de animales de compañía, y aquí es donde entran en juego los diversos sistemas de vigilancia zoosanitaria en general, y los registros de tumores en perros y gatos en particular, entre cuyos cometidos se incluirán los de recabar adecuadamente los datos sobre las distintas patologías en la población, preferiblemente en tiempo real, así como los de ordenar, estructurar y clasificar dichos datos para que estos sean fácilmente accesibles y manipulables por veterinarios e investigadores que deseen conocer o caracterizar el estado de salud de dichas poblaciones animales.

9.2.- Objetivos.

El objetivo general de esta tesis fue sentar las bases para el estudio de la epidemiología del cáncer en la población de animales de compañía de las Islas Canarias.

Para alcanzar este objetivo general se plantearon tres objetivos específicos:

1- Desarrollar una metodología de extracción, clasificación y normalización de datos de tumores en animales de compañía (perros y gatos) para la generación de una base de datos a partir de documentos e informes no estructurados, concretamente los informes del Servicio de diagnóstico anatomopatológico de la Facultad de Veterinaria de la Universidad de Las Palmas de Gran Canaria.

2- Analizar las neoplasias mamarias y los tumores cutáneos de células redondas en la especie canina y las características de los animales que los padecen.

3- Realizar una investigación epidemiológica sobre el cáncer en animales de compañía en Canarias mediante la comparación de las distribuciones de razas, sexos e islas de residencia de los animales de la base de datos de tumores con los de una base de datos de animales de compañía (ZOOCAN).

9.3.- Material y métodos.

En el marco de este proyecto de tesis se han publicado tres estudios sobre bases de datos de tumores en animales de compañía. El primero se centró en el desarrollo de una herramienta para la generación de una base de datos de tumores a partir de informes no estructurados, y fue publicado como base de datos normalizada (data descriptor) utilizando datos de la Red de Vigilancia Veterinaria de Pequeños Animales (SAVSNET) en el Reino Unido[40]. El segundo y el tercer estudio se publicaron como artículos de investigación centrados en la epidemiología de grupos específicos de tumores en las Islas Canarias.

Por lo tanto, describiremos brevemente, en primer lugar, el material y los métodos utilizados en la primera publicación y, en segundo lugar, el material y los métodos utilizados en los artículos segundo y tercero, dadas sus similitudes. Debe tenerse en cuenta que en este apartado sólo se ofrece una descripción general de los materiales y métodos utilizados para llevar a cabo los estudios mencionados, los cuales ya describen con detalle los diseños y metodologías empleadas, así como los análisis estadísticos y las principales limitaciones en las secciones específicas de los mismos.

Para la **primera publicación** utilizamos datos de SAVSNET, una red de vigilancia que abarca todo el territorio del Reino Unido, con sede en la Facultad de Veterinaria de la Universidad de Liverpool, que recoge diariamente unos 10000 resultados de pruebas diagnósticas de los laboratorios participantes, incluidas pruebas de hematología, patología, bioquímica y enfermedades infecciosas, y los utiliza para desarrollar investigaciones y apoyar la vigilancia nacional de los animales de compañía[40]. En este estudio, desarrollamos una metodología de minería de textos para extraer, clasificar y normalizar un conjunto de datos original de 180232 informes electrónicos de diagnóstico anatomopatológico (EPR) de texto libre (no estructurados) para perros y gatos obtenidos de tres laboratorios de diagnóstico en el Reino Unido entre abril de 2018 y junio de 2019.

Como resultado, se identificaron 109895 tumores caninos y felinos, junto con una descripción de cada tumor y animal afectado, y toda esta información se codificó en una base de datos debidamente estructurada y ordenada

Esta base de datos, elaborada mediante Microsoft Excel y el software RStudio, está disponible en Figshare[42].

Con la **segunda y tercera publicaciones** iniciamos nuestra serie de artículos dedicados al análisis de la epidemiología del cáncer en animales de compañía en Canarias, con el estudio de los dos grupos de tumores más importantes desde una perspectiva poblacional: tumores mamarios (segundo artículo) y tumores cutáneos de células redondas (tercer artículo) en la población canina durante el periodo 2003-2020.

Estos estudios epidemiológicos se basan en datos procedentes de dos fuentes principales: Las bases de datos SDAP y ZOOCAN, que se describen a continuación.

1.- El Servicio de Diagnóstico Anatomopatológico (SDAP) recibe anualmente unas 1500 muestras para diagnóstico procedentes de veterinarios privados y oficiales de toda Canarias, junto con un informe de remisión en la que se describe el animal del que se ha tomado la muestra (especie, raza, sexo, estado de castración, edad y localización de la lesión). Estas muestras son procesadas y preparadas para su examen por personal del SDAP y valoradas por un patólogo veterinario (profesor de Anatomía Patológica Veterinaria de la FAVE-ULPGC), quien, finalmente, emite un diagnóstico de la muestra en un informe diagnóstico, que se conserva en los archivos de la SDAP.

2.- Nuestra segunda fuente de datos fue la base de datos ZOOCAN, un registro centralizado en formato de página web donde los veterinarios tienen la obligación de registrar todos los animales de compañía a su cargo[43]. Este registro está gestionado por el Colegio Regional de Veterinarios de Canarias, el cual nos proporcionó la base de datos anonimizada con el conjunto de animales registrados en ZOOCAN para emplearla como población de referencia (*baseline*) en nuestros estudios.

Ambos artículos de investigación se estructuraron en dos partes diferenciadas. En primer lugar, desarrollamos un estudio longitudinal de las principales variables, tales como la edad de aparición del tumor, la presencia de tumores únicos frente a múltiples o la proporción de casos malignos frente a benignos, así como las principales tendencias observadas, donde examinamos cómo han evolucionado las proporciones de los distintos tipos de tumores a lo largo del periodo de estudio.

En segundo lugar, realizamos un estudio de tipo caso-control comparando los datos de animales y tumores de la base de datos SDAP (casos) con animales de la población de referencia descrita en ZOOCAN (controles) para analizar las asociaciones de las variables raza (principalmente), sexo e isla con la aparición del tipo de tumor estudiado en cada una de las publicaciones.

Se desarrolló un script en R para cada uno de estos estudios y las bases de datos resultantes se alojaron en Figshare[44].

9.4.- Resultados y discusión: Publicaciones.

A continuación, se describen y se comentan los resultados de principales de los artículos publicados en el marco de esta tesis doctoral.

9.4.1.- Primera Publicación: Un análisis basado en minería de datos de 100.000 tumores en perros y gatos de Reino Unido.

Para la elaboración de este trabajo se utilizaron datos de tres laboratorios de diagnóstico anatomopatológico colaboradores de la red SAVSNET, los cuales fueron normalizados, dada su heterogeneidad inicial, para poder formar parte de una única base de datos posterior compuesta por 180232 informes de diagnóstico generados entre abril de 2018 y junio de 2019 de entre los cuales se identificaron 93941 informes de perros y gatos padeciendo algún tipo de tumor, así como 109895 tumores en total.

La base de datos publicada constaba de 109895 filas (una por cada tumor identificado) y por 15 columnas (variables) que describen diferentes características de los mencionados tumores, así como de los animales que los padecen.

Entre los datos más relevantes destacamos:

- El tipo de tumor: se identificaron 121 tipos diferentes de tumores entre los cuales destacaban los linfomas, carcinomas de células escamosas y otros carcinomas en gatos, así como los lipomas, mastocitomas e histiocitomas en perros.
- El método diagnóstico: histología (N=69643) o citología (N=40252).
- Número de tumores simultáneos en cada animal: observándose 82479 casos de animales con un único tumor, 16903 con 2 tumores, 6066 con 3 tumores, 2480 con 4 tumores, 1210 con 5 tumores y 756 animales con 6 tumores.
- Diferenciación de los tumores: recogiéndose hasta 12 términos utilizados por los patólogos redactores de los informes siendo los más frecuentes “maligno”, “benigno” y “bien diferenciado”.
- Términos de incertidumbre: donde se recogieron términos tales como "Consistente con", "Posible" o "Probable".
- Localización de los tumores: identificándose hasta 88 localizaciones, entre las que destacaban glándula mamaria, piel y cuello en los gatos, así como glándula mamaria, piel y tórax en los perros.
- Especie animal y sexo: identificándose 85435 informes de perros frente a 8506 informes de gatos, con una proporción similar de machos y hembras en ambas especies.
- Raza: diferenciándose hasta 180 razas caninas, entre las cuales destacaron los perros de tipo mestizo, Labrador Retriever, Staffordshire Bull Terrier y Cocker Spaniel y 39 razas felinas, entre las cuales cabría destacar al gato doméstico de pelo corto, doméstico de pelo largo, British Blue y Maine Coon.

- Establecimiento veterinario en el cual se tomó la muestra: identificándose 2196 clínicas veterinarias de pequeños animales (datos anonimizados).

Otro aspecto importante a destacar en este trabajo fue la validación técnica de los resultados obtenidos. En este sentido, dado que el objetivo último de este sistema era el de automatizar la identificación de los diferentes tipos de tumores reportados en los informes diagnósticos presentes en la base de datos utilizando minería de datos y por tanto obviando la necesidad de que dichos informes fueran leídos por una persona humana, se diseñó un procedimiento de validación para evaluar la precisión del procedimiento. Para ello, en primer lugar, se compararon los resultados obtenidos por minería de datos con los resultados de dos muestras de 200 informes obtenidos al azar y no solapados que fueron valorados por dos expertos. Cada experto valoró una de las muestras de 200 informes y emitió un dictamen diagnóstico sin conocer el diagnóstico que sobre dichos informes había obtenido previamente nuestro procedimiento de minería de datos.

En segundo lugar, un tercer experto examinó los resultados de los 400 informes evaluados (de las 2 muestras aleatorias de 200 informes) y comparó los dictámenes diagnósticos emitidos por los dos expertos anteriores con los obtenidos por la minería de datos en esos mismos 400 informes sin tener conocimiento de qué dictámenes diagnósticos habían sido obtenidos por los expertos y cuáles lo habían sido por la minería de datos.

Del resultado de esta validación, se obtuvo una precisión del 96% en informes reportando un solo tumor (298 de 400) mientras que la precisión fue del 91% en casos de tumores múltiples (102 de 400).

Sin embargo, cuando se consideraron por separado, los informes citológicos e histológicos mostraron algunas diferencias de precisión de tal forma que, en el grupo de informes reportando un único tumor, que incluía 144 informes citológicos y 154

histológicos, la precisión fue del 92% (133 resultados correctos) y del 99% (153 resultados correctos) respectivamente.

En el grupo de tumores múltiples, que incluía 72 informes citológicos y 30 histológicos, la precisión fue del 88% (63 resultados correctos) y del 93% (28 resultados correctos) respectivamente.

9.4.2.- Segunda Publicación: Epidemiología de los tumores mamarios caninos en el Archipiélago Canario en España.

Durante el periodo 2003-2020, el SDAP diagnosticó un total de 13816 tumores en 10205 perras del Archipiélago Canario, observándose que el diagnóstico de tumores de mama (TM) se ha vuelto relativamente menos común. Así, en el año 2003, los TM suponían el 62.7% del total de tumores diagnosticados en perras mientras que, en 2020, el porcentaje había descendido un 13.8% hasta al 48.9% (CI95%: 8.4–19.0%; $p < 0.0001$). Similar hallazgo se observó en el último periodo del Registro de Tumores Animales de Génova[28].

En paralelo a la tendencia decreciente anteriormente descrita, se observó un incremento marcado de la tasa de esterilización en las hembras afectadas por cualquier tumor pasando del 13.1% al 36.3% (CI95%: 17.7%-28.4%; $p<0.0001$), así como en las hembras afectadas por TM pasando del 13.6% al 26.9% (CI95%: 6.2%-20.6%; $p<0.0002$). Esta correlación inversa entre disminución relativa de tumores de mama y aumento de las esterilizaciones podría explicarse, dado el conocido efecto protector de la esterilización sobre el desarrollo de TM[45], por un aumento de la tasa de esterilización dentro de la población canina de Canarias durante el período de estudio en línea con estudios anteriores que encontraron que los TM eran los tumores diagnosticados con mayor frecuencia en poblaciones caninas sexualmente intactas[30] o bien encontraron

bajas frecuencias de TM (22.5%) diagnosticadas en poblaciones con altas tasas de esterilización (66.4%)[46].

En total, en nuestro trabajo se identificaron 7362 TM afectando a 5240 perras, de las cuales, el 74.3% presentaba un único TM mientras que 869 (16.6%), 298 (5.7%) y 115 (2.2%) animales presentaron 2, 3 y 4 TM, respectivamente, y en 67 casos (1.3%) se identificaron 5 o más TM, observándose un aumento significativo del porcentaje de animales padeciendo más de un tumor de forma simultánea del 19.6% en 2003 al 43.0% en 2020 (un aumento global del 23.5% (CI95%: 15.4-31.6; p<0.0001)).

En relación con este hallazgo, las publicaciones previas han obtenido resultados dispares. Por ejemplo, unos estudios reportaron una tasa de tumores múltiples en torno al 25%[47] a diferencia de otros que encontraron una tasa del 66.7%[48]. En nuestro caso, esta tendencia al alza podría estar motivada por una mayor concienciación de los veterinarios clínicos en lo que se refiere a la evolución de tumores benignos a malignos (el continuo histológico)[48] y la consecuente idoneidad de extirpar y muestrear tumores mamarios en estadios tempranos ante la potencial evolución de estos a sus versiones malignas.

Entre los TM diagnosticados, observamos que el 89.2% fueron clasificados como malignos, porcentaje que se mantuvo estable a lo largo del período de estudio (CI95%: 88.5%-89.9%; trend test p=0.4890). A este respecto, la bibliografía no es unánime sobre la frecuencia de TM malignos en las diferentes poblaciones estudiadas. Así, varias publicaciones encontraron una tasa de malignidad entre el 40% y el 60%[49–53] mientras que el registro de cáncer noruego[30] y otro estudio reciente llevado a cabo en nuestro país [54]encontraron una tasa de TM malignos de alrededor del 90%.

En nuestro caso, factores ambientales analizados en el Archipiélago Canario tales como las altas tasas de obesidad[55], así como la exposición a niveles crónicos y relativamente

elevados de contaminación por xenoestrógenos ambientales como el DDT y el DDE[56,57] podrían estar detrás de estas alta tasas de malignidad.

En cuanto a los histotipos malignos más frecuentemente diagnosticados, estos fueron el carcinoma complejo (40.16%; CI95%: 38.55%-41.78% y el carcinoma túbulo-papilar (24.72%; CI95%: 23.31%-26.16%), en coherencia con publicaciones anteriores[37,51–54], seguido del carcinoma en tumor mixto benigno (20.80%; CI95%: 19.48%-22.16%). Referente a los histotipos benignos, los más diagnosticados fueron los adenomas simples (33.54%; CI95%: 29.31%-37.98%), los tumores mixtos benignos (31.66%; CI95%: 27.50%-36.04%) y los adenomas complejos (28.51%; CI95%: 24.50%-36.04%). Acerca del análisis de la edad, y en línea con estudios anteriores[37,47,49,51,58], los TM benignos se diagnosticaron a los 9.2 años (sd: ±2.57), mientras que los histotipos malignos, carcinoma, sarcoma y carcinosarcomas, se diagnosticaron a edades más tardías (9.7 años (sd: ±2.50), 10.4 años (sd: ±2.96) y 10.9 (sd: ±2.53), respectivamente). Así mismo, al comparar la edad al diagnóstico de animales castrados y enteros, los tumores epiteliales malignos (carcinomas) se diagnosticaron a edades más avanzadas en perras castradas en comparación con los mismos tumores en perras enteras (10.2 y 9.5 años, respectivamente, CI95%: -0.8-0.5; p<0,0001), al igual que se observó para los tumores benignos (9.7 y 8.9 años, respectivamente, para perras castradas y enteras, CI95%: -1.3-0.3; p=0.0005). Resultados similares se observaron igualmente en un estudio anterior[49]

Finalmente, en cuanto al análisis de la raza, éste se llevó a cabo únicamente para tumores epiteliales malignos (carcinomas). Catorce razas presentaron un riesgo significativamente mayor de TM epitelial maligno que las perras de raza mestiza. Las razas con un mayor riesgo fueron Samoyedo (OR=6.09; CI95%: 2.31-16.04), Schnauzer (OR=5.77; CI95%: 2.78-12.00), Caniche (OR=3.89; CI95%: 2.96-5.10), Pinscher alemán (OR=3.65; CI95%: 2.28-5.83) y Cocker Spaniel (OR=3.41; CI95%: 2.64-4.40). Por el contrario, diez razas tenían un riesgo significativamente menor que la población mestiza,

destacando razas canarias como el Podenco Canario (OR=0.09; CI95%: 0,06-0,13) y Majorero (OR=0.23; CI95%: 0.09-0.55), así como Pinscher miniatura (OR=0.22; CI95%: 0.09-0.53), Pointer inglés (OR=0.25; CI95%: 0.13-0.47) y American Staffordshire Terrier (OR=0.28; CI95%: 0.11-0.67).

A este respecto, estudios realizados en Noruega, Estados Unidos e Italia[30,33,49] también señalaron que varias de estas razas, como el Caniche, el Teckel y el Cocker Spaniel, presentaron un mayor riesgo mientras que perros de raza Chihuahua y Pointer presentan un riesgo menor.

El hallazgo de que las razas locales tengan un riesgo menor es interesante, sobre todo teniendo en cuenta su variado fenotipo. Futuros estudios deberán investigar si efectivamente existe resistencia de estas razas locales debido a alguna adaptación ambiental desconocida o si, por el contrario, existe algún motivo por el cual estos animales no reciben atención veterinaria en la misma medida que otras razas.

9.4.3.- Tercera Publicación: Epidemiología de los tumores cutáneos de células redondas en la población canina del Archipiélago Canario en España.

En el tercer estudio analizamos la epidemiología de los tumores cutáneos de células redondas (TCR) en la población canina del Archipiélago Canario durante el período 2003-2020. En particular, nos centramos en 3 tipos histológicos: el mastocitoma (MCT), el histiocitoma cutáneo canino (HCT) y el plasmocitoma (PLA). Si bien existen otros tipos de TCR, como los linfomas cutáneos, nos limitamos al estudio de los tres tipos histológicos mencionados por ser aquellos cuyo volumen de datos nos permitió llevar a cabo este estudio con plenas garantías metodológicas.

Se analizó una muestra de 2526 TCR diagnosticados mediante técnicas histológicas (los TCR diagnosticados mediante citología fueron excluidos del estudio al objeto de

maximizar la fiabilidad de los diagnósticos), entre los cuales, 1712 (67.78%) correspondieron a mastocitomas, 668 a HCT (26.44%) y 146 a PLA (5.78%). Observamos que, a lo largo del período, los diagnósticos de MCT crecieron significativamente un 15.9% (OR=1.05; CI95%: 1.03-1.06; p<0.0001) en proporción relativa en comparación con el resto de TCR mientras que los HCT experimentaron la tendencia opuesta, con un descenso del 3.72% (OR=0.94; CI95%: 0.93-0.96; p<0.0001) y los PLA se mantuvieron estables. Estos cambios de tendencia podrían estar relacionados, al menos en parte, con un cambio en la distribución de edades de la población canina del Archipiélago Canario resultando en una población ligeramente más envejecida. En este sentido, observamos que el porcentaje de perros castrados en el grupo de perros que padecen algún tumor se ha triplicado, pasando del 10.3% en 2003 al 32.8% en 2020, lo cual favorecería una disminución de nuevas camadas y la consecuente introducción de perros jóvenes en la población. Así mismo, la creciente popularidad de adopciones en Reino Unido de perros procedentes de otros países, entre ellos España, especialmente de perros jóvenes (menos de 2 años de edad)[59], podría igualmente estar contribuyendo a este fenómeno de envejecimiento de la población canina y dando lugar a un aumento en el diagnóstico de tumores diagnosticados típicamente en perros de mediana a avanzada edad como el MCT[38,39,60–62] y por tanto a una disminución de tumores diagnosticados con mayor frecuencia en perros jóvenes como el HCT[38,39,60–62]

Otras razones para el incremento de los diagnósticos de MCT podrían relacionarse con un mayor compromiso con el diagnóstico del cáncer en general y, por ende, con la disponibilidad de fármacos específicos para el tratamiento de los MCT (Masitinib y Toceranib) desde 2009[63,64]mientras que una menor frecuencia de diagnósticos de HCT en nuestros registros podría deberse a un incremento de los casos diagnosticados mediante citología por los propios veterinarios en sus clínicas (y por tanto no reportados al SDAP) así como su tendencia a la regresión (curación) espontánea[65].

En cuanto a los diferentes grados de MCT, esta información estuvo disponible en 1394 informes de los cuales 47 (3.4%) se describieron como grado 1, 1206 (86.5%) como grado 2 y 141 (10.1%) como grado 3.

Respecto al análisis de la edad en el momento del diagnóstico, los MCT y PLA se diagnosticaron en perros de 8 años (IQR: 6-10 y 7-10 respectivamente), sin diferencias significativas entre ambos resultados, y en concordancia con estudios anteriores[34,35,39,60,61,66–74], mientras que los HCT se diagnosticaron a edades más tempranas (2 años, IQR: 1-5), hallazgo igualmente consistente con anteriores publicaciones[38,39,60–62]

En el estudio de la edad al diagnóstico de los MCT, estratificamos el análisis para observar la asociación entre edad y diversos grados de MCT. Observamos que los MCT de grado 1 eran diagnosticados a edades significativamente inferiores (6.5 IQR: 5-8), mientras que los grados 2 y 3 eran diagnosticados en perros de 8 años (IQR 6-10) y 9 años (IQR: 7-11), respectivamente, en coherencia con trabajos anteriores que ya constataban que la proporción de MCT de alto grado aumentaba con la edad[34,68].

Estos hallazgos serían igualmente compatibles con la hipótesis planteada previamente acerca del envejecimiento de la población canina del Archipiélago Canario ya que, entre nuestros hallazgos, obtuvimos una menor proporción de MCT de grado 1 (3.4%) y mayor de MCT de grado 2 (86.5%), al compararlos con trabajos previos[34,37,39,75–77]que obtuvieron resultados que oscilaban entre el 12.9% y el 33.3% para el grado 1 y entre el 33.6% y el 76.3% para el grado 2, si bien, nuestros resultados concuerdan en este caso con el reciente registro de tumores de SAVSNET[46] que obtuvo una proporción de MCT de grado 1 y 2 del 4.7% y 92.1% respectivamente.

El siguiente análisis versó sobre la distribución anatómica de los diferentes histotipos tumorales en las localizaciones anatómicas consideradas (1.- extremidades, 2.- tronco, 3.- cara, cabeza y cuello, 4.- escroto y región perianal y 5.-cola). HCT y PLA se

distribuyeron de forma similar, sin observar diferencias significativas, fundamentalmente en las regiones de cara, cabeza y cuello (43.7% y 47.9%, respectivamente) en comparación con los MCT que únicamente aparecieron en esta localización en un 13.3% de los datos. En cambio, los MCT se presentaron con mayor frecuencia en las extremidades (36.4%), tronco (38.2%) y región perianal y escroto (10.7%).

Estudios anteriores[37,60,61,68,75,78–81] encontraron que la mayoría de los MCT se localizaron en las extremidades, siendo los miembros posteriores los más frecuentemente afectados, excepto en un estudio realizado en Italia[35] donde sólo el 15.3% de los MCT se localizaban en esta región. Además, dichos estudios observaron que los MCT se localizaron en la región del tronco con una frecuencia que oscilaba entre el 19%[60] y más del 50% de los casos[68,78], sin una diferencia clara de ninguna región en particular (tórax y abdomen). Por último, todos los estudios mostraron una frecuencia inferior al 20% para la zona de la cabeza, la cara y el cuello, aunque un artículo realizado en Austria[69] destacaba que el 70% de los MCT se localizaban en el tronco y la cabeza.

En nuestro estudio no encontramos evidencias de que ninguna localización anatómica concreta estuviera asociada con el grado de dichos MCT, lo cual no concuerda con hallazgos previos en los cuales se sugería un mayor riesgo de MCT de alto grado para las áreas de la cabeza, inguinal y perigenital[76,81].

En lo que respecta a la distribución anatómica de los HCT y PLA, nuestros hallazgos son coherentes con estudios anteriores. En particular, en nuestro estudio encontramos que más del 40% de los casos de HCT y PLA se localizaron en la zona de la cabeza, cara y cuello, con una clara preponderancia del abdomen frente al tórax, mientras que trabajos anteriores[60–62] observaron que los HCT se localizaron en la zona de la cabeza-cara-cuello con una frecuencia que oscilaba entre el 28.4% y el 53% mientras que los PLA lo hacían entre el 27.8% y el 45.6%[37,70,71,73,74,82]. Sin embargo, en el Registro de tumores de SAVSNET[46] únicamente el 17.8% de los HCT y 13.9% de los PLA se

encontraron en esta localización, si bien, hasta un 45% de la localización de estos tumores se notificó con el término genérico ("piel").

Respecto a la presencia de MCT múltiples, el análisis longitudinal de nuestros datos encontró un aumento significativo de los casos de tumores múltiples, del 3.8% en 2003 al 20% en 2020 de tal forma que las probabilidades de tener múltiples MCT aumentaron un 6% de media cada año (OR=1.062; CI95%: 1.03-1.1].

En cuanto a la asociación de la variable raza y la aparición de TCR, doce razas mostraron un mayor riesgo de padecer MCT en comparación con la población canina mestiza (*baseline*). En particular, Bóxer (OR=23.61; CI95%: 19.12-29.15), Boston Terrier (OR=19.47; CI95%: 7.73-49.05), Shar-Pei (OR=10.09; CI95%: 6.59-15.47), Pug (OR=8.10; CI95%: 5.92-11.07) y Golden Retriever (OR=4.74; CI95%: 3.22-6.98) mostraron las OR más altas. Por otro lado, los perros de la raza Chihuahua (OR=0.18; CI95%: 0.09-0.33), Pastor alemán (OR=0.24; CI95%: 0.09-0.66), Pointer inglés (OR=0.31; CI95%: 0.13-0.76), Presa canario (OR=0.45; CI95%: 0.21-0.95) y Yorkshire Terrier (OR=0.61; CI95%: 0.44-0.86) obtuvieron un OR significativo inferior a uno.

Estos resultados están en línea con estudios anteriores[24,34,35,37,38,60,67,68,81,83–85] y con la hipótesis formulada en 1969[83], según la cual una ascendencia común podría estar detrás de la predisposición de ciertas razas de tipo Bulldog a padecer MCT.

Así mismo, las razas que mostraron un menor riesgo, como los perros de la raza Chihuahua, Pastor alemán, Pointer inglés y Yorkshire Terrier, han sido igualmente descritas previamente como razas de bajo riesgo por los mismos estudios[24,34,37,60,67,69,76,79,80,84,85]

Encontramos que el Presa canario, una raza local de esta región, presentó un bajo riesgo de MCT. Este hallazgo de razas locales obteniendo métricas de bajo riesgo de tumores ya lo observamos en nuestro estudio sobre la epidemiología de los tumores de mama donde se apreció cierto grado de protección en el Podenco canario, Majorero y

Presa canario. En este sentido, llama la atención como estas razas obtienen valores similares de riesgo en grupos de tumores no relacionados entre sí (TCR y TM). Las razones de estos hallazgos no están claras debido a la falta de estudios que abarquen las razas caninas canarias si bien, al menos en parte, la alta variabilidad genética encontrada en las razas canarias[86] y su reciente origen a partir de una población ancestral mixta podrían estar desempeñando un papel en este sentido.

Finalmente, con relación a la raza y el grado de MCT, diferentes estudios tienden a confirmar el hecho de que ciertas razas predispuestas para el MCT tienden a padecer MCT de bajo grado, mientras que otras razas menos frecuentemente afectadas por este tumor tienden a tener MCT de alto grado. A este respecto, Mochizuki et al[34], plantean que las discrepancias en las proporciones de bajo y alto grado entre las distintas razas pueden indicar que las alteraciones genéticas responsables de los MCT pueden ser distintas de las que contribuyen al comportamiento biológico agresivo. En nuestro caso, encontramos diferencias en la distribución de los distintos grados de MCT y las distintas razas, aunque nuestros datos no eran lo suficientemente amplios como para realizar análisis más profundos entre las razas concretas. Sin embargo, hallamos entre nuestros casos de MCT de alto grado, que los Rottweilers y Shar-Pei fueron los más frecuentemente diagnosticados al igual que en tres estudios previos[34,79,81] mientras que el Bóxer, y el Labrador Retriever fueron las razas más frecuentes entre los perros con un MCT de bajo grado, siendo estas observaciones también consistentes con estudios previos[34,38,79]. Asimismo, también el Presa Canario, una raza de bajo riesgo de MCT, mostró una proporción ligeramente superior de MCT de grado 3.

En cuanto al sexo, encontramos menor riesgo de que los machos desarrollaran un MCT (OR=0.81; CI95%: 0.71-0.93) en comparación con las hembras. Este hallazgo genera discrepancia con estudios previos que no encontraron diferencias a este respecto[35,37,67,76,81,87]y con otro que observó un mayor riesgo en las hembras[68].

Respecto al HCT, en línea con trabajos anteriores[38,60,62], observamos que hasta catorce razas obtuvieron un mayor riesgo de padecer un HCT en comparación con los perros cruzados. Las métricas más altas se obtuvieron para la raza Boston Terrier (OR=32.61; CI95%: 11.81-90.07), Bulldog (OR=24.6; CI95%: 16.23-37.30), West Highland White Terrier (OR=13.97; CI95%: 8.00-24.40), Bulldog francés (OR=12.38; CI95%: 9.71-15.76) y Bóxer (OR=10,17; CI95%: 6.60-15.67), mientras que la más baja se observó para la raza Chihuahua que fue la única raza que obtuvo una OR inferior a la unidad (OR=0,41; CI95%: 0.21-0.78).

Se observó que los perros macho presentaban un mayor riesgo de padecer un HCT (OR=1.27; CI95%: 1.07-1.52), al contrario que lo reportado en estudios anteriores que no encontraron diferencias significativas entre animales de diferentes sexos en lo referente al padecimiento de estos tumores[62,87].

Finalmente, en cuanto al PLA, nuestros resultados para el sexo como factor de riesgo de PLA no revelaron diferencias en el riesgo debido al sexo para este tipo de tumor (OR=1.50; CI95%: 0.99-2.28).

9.5.- Conclusiones.

Primera: La herramienta informática creada, basada en la minería de datos para automatizar la lectura, extracción y normalización de datos de grandes volúmenes de informes diagnósticos digitalizados y no estructurados, se ha mostrado eficiente y eficaz.

Segunda: Este estudio representa el primer análisis epidemiológico sobre neoplasias mamarias y tumores cutáneos de células redondas en la especie canina, fruto de la creación de una base de datos normalizada de tumores en animales de compañía del Archipiélago Canario.

Tercera: La población canina del Archipiélago Canario está afectada por una mayor proporción de tumores mamarios malignos en comparación con otras localizaciones geográficas, hecho que pudiera estar relacionado con factores ambientales y socioeconómicos regionales, así como por una mayor sensibilización de propietarios y veterinarios en cuanto al diagnóstico y tratamiento de estas patologías.

Cuarta: En el Archipiélago Canario y con relación a los tumores cutáneos de células redondas en la especie canina, es el mastocitoma el histotipo tumoral más frecuentemente diagnosticado en individuos de mediana y avanzada edad, mientras que el histiocitoma es el histotipo tumoral más común en perros jóvenes.

Quinta: En Canarias, los perros de las razas Samoyedo y Schnauzer son los que tienen un mayor riesgo de padecer tumores de mama, al contrario que los de raza Pinscher miniatura, Pointer inglés y American Staffordshire Terrier, que tienen un riesgo reducido. En cuanto a los tumores cutáneos de células redondas, los perros de raza Bóxer, Boston Terrier, Shar-Pei y Pug presentan mayor riesgo frente al padecimiento de mastocitomas al contrario que los individuos de la raza Chihuahua, Pastor alemán y Pointer inglés. Finalmente, en cuanto a los histiocitomas, las razas Boston Terrier, Bulldog y West Highland White Terrier son las que presentan un mayor riesgo mientras que la raza Chihuahua es la única que muestra cierto grado de protección.

Sexta: Las razas caninas autóctonas, como el Presa Canario, Majorero y Podenco Canario, muestran una menor tendencia a verse afectadas tanto por carcinomas mamarios como por mastocitomas cutáneos, lo que podría sugerir algún tipo de resistencia debido a la alta variabilidad genética de estas razas.

10.- ACKNOWLEDGEMENTS/AGRADECIMIENTOS.

Esta tesis, imagino que, como todas, ha sido una auténtica carrera de fondo. Y las carreras están para correrlas, sufrirlas, caerse, levantarse…y ganarlas. En todos estos años en los que he desarrollado mi proyecto de tesis, he pasado por cada una de esas etapas. He sufrido, he llorado y me he sentido abrumado por todo lo que tenía que saber y no sabía…pero siempre que lo he necesitado, he tenido ayuda. Esa ayuda me sirvió siempre para levantarme, reponerme, y seguir adelante. Y a todos aquellos que me ayudaron, les doy las gracias.

Aquí están…

En el plano académico, el Profesor Antonio Fernández fue el que me convenció para iniciar esta aventura. Fue con él con quien tuve las primeras conversaciones sobre iniciar una tesis y sobre las posibilidades temáticas que podía desarrollar en el marco del IUSA y el que me canalizó hasta que di con una temática y unas personas que pudieran acompañarme en tal travesía. *¡¡Muchas gracias, Toño!!*

A mitad de camino, me tocó viajar a Reino Unido para hacer una estancia Erasmus y fue allí donde tuve la gran suerte de conocer al Profesor Alan Radford de la Universidad de Liverpool y ser recibido por el resto de del equipo de investigación de SAVSNET. Esa estancia de tres meses en el acogedor pueblo de Neston, en la Península de Wirral, marcó un claro punto de inflexión en mi carrera como investigador. *Alan Radford, David Killick and all the members of the SAVSNET team…thank you guys for your amazing support!!*

Sin embargo, este ha sido un trabajo de pico y pala y de ninguna manera hubiera sido posible terminar esta tesis sin el compromiso, la profesionalidad, el talento y, sobre todo, la calidad humana, de mis dos directores, los profesores Antonio Espinosa de los Monteros y Ángelo Santana. Dos personas absolutamente fundamentales en esta travesía en la que me han acompañado, codo con codo, durante innumerables horas,

viendo muestras al microscopio, escribiendo código en R o simplemente conversando sobre patología, tumores, estadística, programación…y otros tantos temas.

Para ellos, Antonio y Ángelo, va la mayor de mis gratitudes por su entrega y dedicación, pero sobre todo por la cercanía y el apoyo (por momentos, crítico) que me han dado durante todos estos años. *One for all and all for one!! GRACIAS!!*

No me olvido de Miguel Rivero, a quien agradezco siempre su disposición para aclararme las dudas relacionadas con el Doctorado y por su gran ayuda con los informes cuando estábamos en plena pandemia. *¡Miguel, muchas gracias!*

Y también debo agradecer a Ana Sofía Ramírez, que mandó ese mail crucial al profesor Alan Radford para ponerme en contacto con él y terminar haciendo mi Erasmus en la Universidad de Liverpool. *Ana, gracias!*

En el plano familiar, sencillamente no tengo palabras para describir el apoyo que he tenido porque ha sido, cuanto menos, inmenso.

Mis padres, mamá y Gus, han sido siempre una fuerza de empuje, entrega y confianza que me ha permitido alcanzar siempre mis metas. "*Jose, si lo ves claro, adelante*". Y así llegué a la Universidad, así estudié Veterinaria y así aprobé las oposiciones al Cuerpo Nacional Veterinario. Mi agradecimiento y amor infinito a mis padres. *This one's for you, ma, let me come right out and say it (Bruce on The Wish).*

Mi hermano Dani siempre ha sido una fuente de inspiración a la hora de no ponernos límites en cuanto a lo que queremos hacer. Su creatividad desbordante y su entrega total para con sus ideas siempre me han enriquecido. *Love u, bro!*

Mi segunda familia, Sissi, Pedro, David y Gabriela, han sido siempre un gran apoyo por su cariño y bondad y mi cuñado, David, ha sido la persona que durante todos estos años más veces me ha preguntado "*¿Qué tal llevas el PhD*?". ¡Mi enorme agradecimiento para ellos!

Mis hijos, Ana y Eloy me han ayudado a descubrir mi verdadera fuerza interior. Ellos me han enseñado a valorar mucho más la vida y el tiempo que tenemos y han sido y serán siempre mi mayor experiencia de aprendizaje vital. *¡Mis chicos, my living proof, les quiero de aquí a la Luna…y vuelta!*

Mi gran amiga Olivia, además de mi veterinaria favorita. Ella ha cuidado de mi perro, Ron, todos estos años y ha compartido su experiencia como veterinaria clínica en los diagnósticos de tumores. Siempre han sido muy enriquecedoras nuestras conversaciones en las cuales contrastábamos los hallazgos que yo recababa de las revisiones bibliográficas con lo que ella veía día a día en su clínica. *¡Tita Oli, te queremos!*

Y mi amigo Luis me ha acompañado, si bien desde la lejanía, todos estos años y ha sido un gran apoyo al compartir alegrías y darme ánimos cuando las cosas se ponían cuesta arriba. *Love u, man!*

No obstante, y aquí viene la parte realmente complicada de expresar con palabras, la persona que ha marcado la diferencia en mi vida y me ha ayudado a llegar a ser quien soy, como hombre, padre, profesional, investigador…el apoyo fundamental y básico ha sido siempre, mi mujer, Patricia.

Mi vida cambió aquella tarde de Julio y desde entonces no nos hemos separado. Prestándonos apoyo mutuo durante todos estos años, creciendo juntos, aprendiendo juntos, criando a nuestro perro, a nuestros hijos… con la fuerza y el empuje infinito de nuestro amor. Esta tesis es gracias a ella, como todas muchas cosas importantes en mi vida. Ella lo es todo y mi mayor agradecimiento y amor van para ella. Por todo lo vivido y todo lo que nos queda por vivir. *Oh, you always have, you always will. TQKx.*

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