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"DEVELOPMENT OF AN ANIMAL CANCER REGISTRY FOR THE MARCHE REGION AS A TOOL FOR PREVENTIVE HEALTH CARE"

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SUMMARY

Cancer registries are a key feature of any epidemiological study or prevention and control strategy. Moreover, companion animal tumor registries are intended to assist in different aspects of research on tumor development, pathogenesis, genetics and treatment. Traditionally, comparative cancer research is based on murine models, which lack many features that define human cancer, including growth over longer time periods, genomic instability, function of the immune system and a significant heterogeneity of tumor cells and tumor microenvironments. To fill this gap, spontaneous tumors in dogs and cats reflect more features of human cancer. Furthermore, sharing the living environment with humans, they are exposed to similar risk factors, therefore acting as sentinels for recognition of environmental factors implicated in oncogenesis.

Comparison of data from canine tumor registries has recently gained increasing interest in the context of the 'One Medicine-One Oncology' concept, part of the 'One Health Initiative'. The One Health concept is a worldwide strategy for expanding collaborations and communications of multiple disciplines in all aspects of health care for humans, animals and the environment. It is believed that an achieved synergism will improve public health, scientific knowledge as well as biomedical research. To learn more about tumors in companion animals, such as cancer development and risks, knowledge on the occurrence of tumors in pets needs to be expanded because statistics on the incidence of cancer in pet animals are very rare. As part of 'One Health curriculum' of the School of Advanced Studies of the University of Camerino, this thesis was based on establishment of a canine cancer registry of the Marche region, so far lacking, focused on extensive data collection and interpretation about spontaneous tumors occurring in dogs living in the Marche region as a tool for preventive health care.

Tumors were classified according to the tumor type, malignancy and physical location following the guidelines of the International Classification of Oncology for Humans (ICD-O), which subsequently allows comparisons with human cancer registries. Being a newborn cancer registry, the collected data were still insufficient to carry out an adequate statistical analysis, therefore a descriptive examination of the first available data was performed, pending further implementation in order to have a more truthful panorama of the oncological cases of dogs of the Marche region.

Moreover, this dissertation describes a similar study carried out in Cuba. The aim was to collect data from a country with socio-economical, cultural and climatically characteristics completely different from ours, and to investigate 'if' and 'how' these differences could influence tumors onset in canine population. In the same manner of canine cancer registry of the Marche region, data collected of the city of Havana were analyzed as descriptive statistic and represent a groundwork to implement further.

PREFACE

This thesis was conducted at the School of Biosciences and Veterinary Medicine of the University of Camerino, in a trans-disciplinary collaboration with IZSUM, and thanks to the School of Advanced Studies, that opened doctoral positions in 'One Health curriculum', and to the Marche Region, that courageously decided to take part to the 'One Health Initiative'.

The model of this research project was initiated by the Umbria Region, that successfully set up an Animal Cancer Registry 2 years early, managed by IZSUM in collaboration with the University of Perugia. In 2015, the Marche Region launched its own challenge with the goal of establish its Animal Cancer Registry that could, over time, become a useful tool to pursuit the Public Health.

My involvement in this ambitious project developed throughout my whole doctoral course and represented for me a professional and personal growth. This topic suited me really good and allowed me to move in the field I love more: pathology. At the same time, it opened my horizons, both scientifically and geographically. The trans-disciplinarily of the project introduced me to disciplines before unknown, like informatics and epidemiology, while comparative research led me to apply my new and old skills in a country diametrically opposite to ours, 9000 km far from Italy.

I would like to thank my tutor, Prof. Giacomo Rossi, who always believed in me and pushed me beyond my limits… what I am today is only his merit!
My thanks go also to my work team and co-authors for their support, help and inputs.
I am grateful to the School of Advanced Studies of the University of Camerino and its examiners, who choose my project 3 years ago and gave me the opportunity to go on until now.
Thanks to the Marche Region that, relying on the School of Biosciences and Veterinary Medicine of the University of Camerino and on my tutor, allowed me to work and manage its worth project.
Immensely thanks to the staff of the Laboratory of Experimental Pathology and Surgery of the National Institute of Oncology and Radiobiology (INOR) of the city of Havana, in particular to my Cuban tutor Prof. Juan Carlos Rodriguez Aurrecochea, that welcomed me and support in many aspects of my not easy foreign experience.
Further, I would like to thank the Experimental Animal Prophylaxis Institute (IZS) of Umbria and Marche, both the Histopathology Laboratory and Epidemiologic Observatory, for the valuable contribution.
Many thanks to my family, friends and colleagues outside the University, who always understood and supported me along the way.

1. INTRODUCTION

1.1 'ONE MEDICINE – ONE HEALTH' CONCEPT

The origin of the One Medicine concept has been linked to the 19th century German physician and pathologist Rudolf Virchow (1821-1902) who created the field of comparative pathology. During his study on human and animal pathogens, he noted the similarity in disease processes among animals and humans stating that differ only in details and not in kind. Dr. Virchow proclaimed "between animal and human and medicine there is no dividing line, nor should there be. The object is different, but the experience obtained constitutes the basis of all medicine". Although the One Medicine theme was continued by William Osler (1849-1919), Virchow's student and father of modern medicine, who taught it to his medical and veterinary students, human and animal medicine were practiced separately until the latter half of the 20th century. The One Medicine concept was revived and bolstered by the American veterinarian Calvin W. Schwabe (1927-2006) who coined the term 'One Medicine' in his textbook *Veterinary Medicine and Human Health* in 1964.

Today, the early term 'One Medicine' is commonly referred to as 'One Health' worldwide. This terminology evolution occurred during the first decade of the 21th century. One Health was born out of, and fueled by, fear. In 2004, there was global anxiety that a zoonotic disease, HPAI H5N1, could cause a pandemic in the human population, rivaling, and possibly exceeding, the estimated 50 million human deaths associated with Spanish influenza at the end of the First World War[20]. The introduction of the One Health initiative provided international agencies (FAO, OIE, WHO and the World Bank) with a vehicle for interinstitutional and interdisciplinary collaboration to address the threat of emerging zoonotic diseases, and it enabled these international agencies and national authorities to come to the table as equal partners in the search for solutions to the threats posed by this highly virulent strain of influenza[21].

The expression 'One Health' was proposed as a concept to foster interdisciplinary collaboration between physicians and veterinarians, but also wildlife specialists, environmentalists, anthropologists, economists and sociologists, among others, required to prevent and control zoonosis. One Health recognizes that humans do not exist in isolation, but are a part of a larger whole, a living ecosystem, and that activities of each member affect the others. Thus, One Health considers health as a whole, the humans, the animals, and the environment they exist on.

'*One Health is the collaborative effort of multiple health science professions, together with their related disciplines and institutions – working locally, nationally, and globally – to attain optimal health for people, domestic animals, wildlife, plants, and our environment.*' **One Health Commission**

Health experts worldwide met on September 29, 2004 for a symposium organized by the Wildlife Conservation Society (WCS). At the conference, the WCS introduced the term 'One

World-One Health'™ to embrace both medicine and ecosystem health, and listed 12 recommendations for establishing a more holistic approach to preventing epidemic/epizootic disease and maintaining ecosystem integrity for the benefit of humans, their domesticated animals, and the foundational biodiversity that supports us all (www.oneworldonehealth.org). This series of recommendations became known as the Manhattan Principles, in recognition of the fact that the meeting was hosted by Rockefeller University in New York.

The Manhattan Principles exhort the world's leaders, civil society, the global health community and institutions of science to:

1. Recognize the essential link between human, domestic animal and wildlife health and the threat disease poses to people, their food supplies and economies, and the biodiversity essential to maintaining the healthy environments and functioning ecosystems we all require.

2. Recognize that decisions regarding land and water use have real implications for health. Alterations in the resilience of ecosystems and shifts in patterns of disease emergence and spread manifest themselves when we fail to recognize this relationship.

3. Include wildlife health science as an essential component of global disease prevention, surveillance, monitoring, control and mitigation.

4. Recognize that human health programs can greatly contribute to conservation efforts.
5. Devise adaptive, holistic and forward-looking approaches to the prevention, surveillance, monitoring, control and mitigation of emerging and resurging diseases that take the complex interconnections among species into full account.

6. Seek opportunities to fully integrate biodiversity conservation perspectives and human needs (including those related to domestic animal health) when developing solutions to infectious disease threats.

7. Reduce the demand for and better regulate the international live wildlife and bush meat trade not only to protect wildlife populations but to lessen the risks of disease movement, cross-species transmission, and the development of novel pathogen-host relationships. The costs of this worldwide trade in terms of impacts on public health, agriculture and conservation are enormous, and the global community must address this trade as the real threat it is to global socioeconomic security.

8. Restrict the mass culling of free-ranging wildlife species for disease control to situations where there is a multidisciplinary, international scientific consensus that a wildlife population poses an urgent, significant threat to human health, food security, or wildlife health more broadly.

9. Increase investment in the global human and animal health infrastructure commensurate with the serious nature of emerging and resurging disease threats to people,

domestic animals and wildlife. Enhanced capacity for global human and animal health surveillance and for clear, timely information-sharing (that takes language barriers into account) can only help improve coordination of responses among governmental and nongovernmental agencies, public and animal health institutions, vaccine / pharmaceutical manufacturers, and other stakeholders.

10. Form collaborative relationships among governments, local people, and the private and public (i.e.- non-profit) sectors to meet the challenges of global health and biodiversity conservation.

11. Provide adequate resources and support for global wildlife health surveillance networks that exchange disease information with the public health and agricultural animal health communities as part of early warning systems for the emergence and resurgence of disease threats.

12. Invest in educating and raising awareness among the world's people and in influencing the policy process to increase recognition that we must better understand the relationships between health and ecosystem integrity to succeed in improving prospects for a healthier planet.

In the Manhattan Principles, the importance of education about the One Health concept is introduced, and public and private participation is encouraged. Several Universities worldwide have accepted the challenge and decided to contribute to this cause by offering dedicated didactical courses to their students. Most of them, like the Royal Veterinary College in London and the Royal (Dick) School of Veterinary Studies at the University of Edinburgh, set up specific master degrees in One Health, while very few Universities have gone beyond by offering PhD degree specifically in One Health. One of these is the Italian University of Camerino, and the present thesis is the product of such opportunity.

1.2 ONE HEALTH - ONE ONCOLOGY

During the period when the focus of international agencies was on avian influenza, there was recognition that the One Health approach had a wider application[21]. One Health activities do not involve only zoonotic diseases, but many other topics considered to be relevant for the promotion of health in a wider context (Figure 1).

Cancer in humans and animals is one of these. If we look back over the last 100 years, we realize how much studies in animals have contributed to the global health. For example, the study of avian leukosis virus led to a fundamental understanding of oncogenes in cancer[82]. The vaccine for cervical cancer, the second most fatal cancer in women, can be directly attributed to study done in the last fifty years on cattle infected with papillomavirus[42].

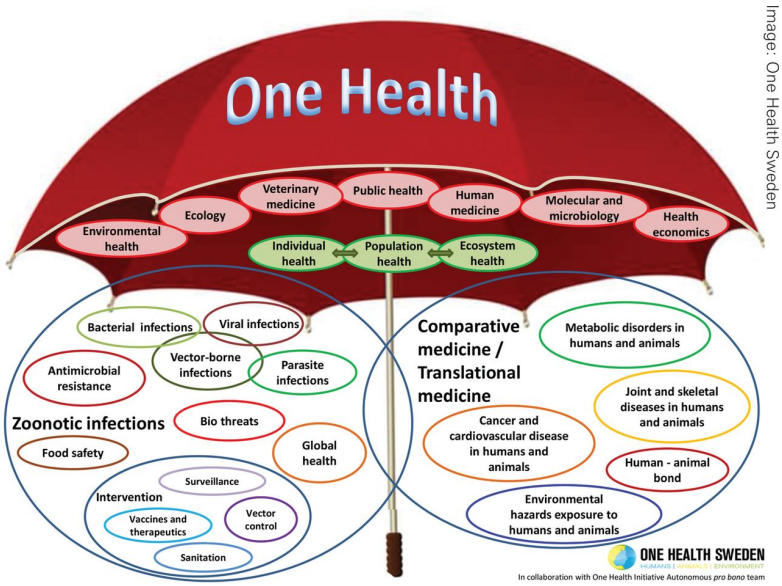


Image: One Health Sweden

Figure 1: Scope of One Health according to the One Health Initiative (www.onehealthinitiative.com). Image: One Health Sweden

Translation of this findings into human field is called comparative oncology. Comparative oncology shifts the occurring cancers seen in animals into more general study of cancer biology.

A great deal of work in comparative oncology is based on rodent models because of their low cost, short average lifespan and easiness in handling. However, despite of the unquestionable importance of rodent models in advancements in cancer research and preclinical tests, much of the data obtained from them rarely translate into human clinical practice due to the limitations of these models to better reflect the complexities of human tumors[37]. Mice have different anatomical, cellular and molecular features similar to humans that are known to have critical properties and functions in cancer. In addition, the percentage of murine genes with a human orthologue is 80%[79], thus providing an excellent experimentally tractable model system as a research tool to investigate the basic mechanisms of cancer development and treatment responses[43]. Although mouse models remained a valuable tool for examining the molecular mechanisms of carcinogenesis, the low degree of heterogeneity in mouse tumors compared to very heterogeneous human tumors is an important limitation[75].

Similarities in tumors expression are significantly closer between human and dog than those between human and mouse. Firstly, the recent deciphering of the canine genome provided evidence of strong similarities with humans[35, 46]; secondly, many gene families of canine genome, especially associated with cancer, show a greater homology to human genome than murine[48] (Figure 2).

Furthermore, canine tumors share evolutionarily conserved genomic changes that are found in their human counterparts[63]. In addition, tumors naturally occur in dogs, their initiation and progression are influenced by similar factors in both human and canine cancers, including age, nutrition, sex, reproductive status and environmental exposures, and show histological and

clinical features closely parallel to the corresponding tumors in humans[63, 84]. All these reasons place dogs in a unique position to better reflect tumor development and progression than traditional rodent models. Again, the range of tumors occurring in dogs are as diverse as tumors occurring in humans. This is given by a biological complexity of canine cancers[47], based on the intra-tumor heterogeneity (ITH)[19], which captures the essence of tumor in humans and shares the same features.

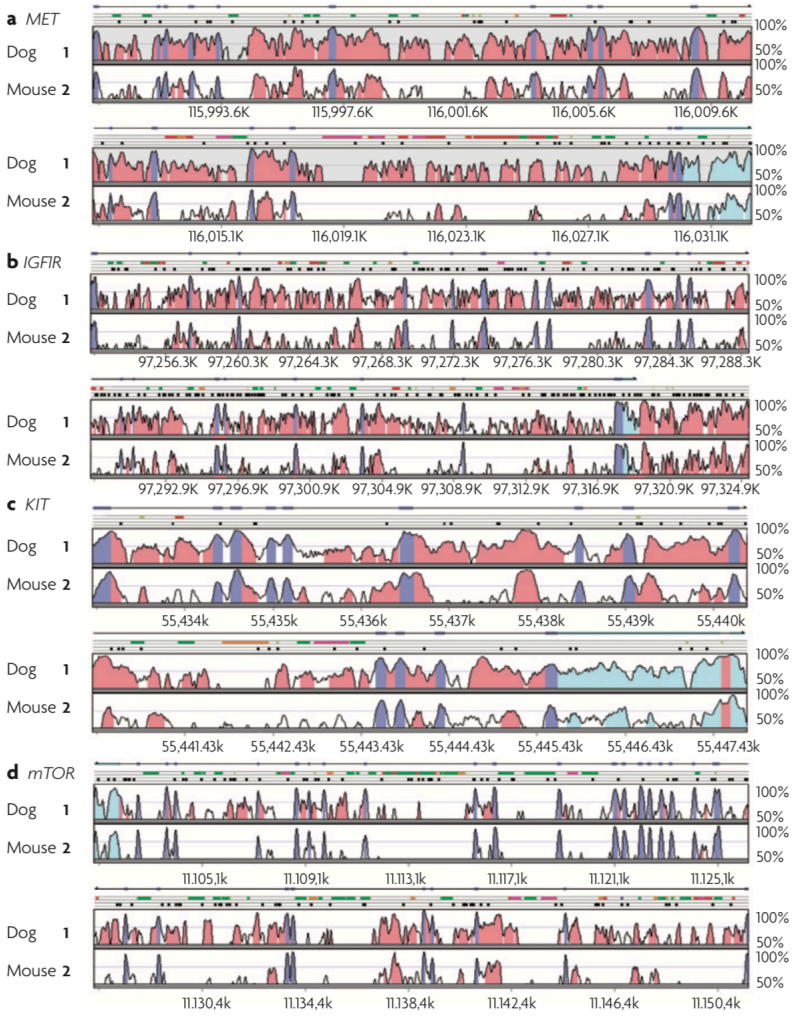


Fig. 2: Homology between dog, human and mouse for recognized cancer genes. Recent completion of the canine genome draft sequence has allowed demonstration of the strong similarities between canine and human cancer genes. VISTA graph displays (http://genome.lbl.gov/vista/index.shtml) visually compare aligned canine and murine genes with their human orthologues. The y-axis of the graph represents the percent conservation of the canine or murine sequence against the human gene target. The y-axis ranges from 50% to 100% conservation with a threshold line drawn at 70%, which is a level denoting significant similarity. The colors of the peaks describe the function of the sequence, dark blue representing exons, pink non-coding regions and light blue untranslated regions. The plots compare the entire human sequence for the following cancer-associated genes with their respective canine and mouse orthologues. **a** | *MET*, an oncogene activated in canine and human sarcomas. **b** | Insulin-like growth factor 1 receptor (*IGF1R*), a receptor for IGF, which is an important growth factor in various tumors. **c** | *KIT*, a causative oncogene in human gastrointestinal stromal tumors (GISTs) and canine mast-cell tumors and GISTs. **d** | Mammalian target of rapamycin (*mTOR*, also known as *FRAP1*), an integral regulator of protein translation in various tumors and a therapeutic target of rapamycin. The graphs indicate that the dog nucleotide sequences are more highly conserved with human sequences than mouse sequences are for all four candidate genes. This is especially evident at the level of similarity within the non-coding regions. Image and caption: Nature Reviews Cancer (2008) 8:150 (see reference 48).

Human disease is polygenic. Genetic manipulation on mice, exalting one or few genes, increases the gap between rodent models and humans[64]. On the contrary, dogs have strong analogies with humans in genetic molecular alterations that drive cancers[84]. Most, if not all, of the cancer associated genetic alterations that influence cancer progression in humans have been identified in canine cancer. For example, similar mutations in KIT, a tyrosine kinase growth factor receptor, have been identified in both gastrointestinal stromal tumors (GIST) in humans and mast-cell cancers in dogs[54]. Additionally, statistical analysis of genomic alterations in human and dog colorectal tumors showed the same genetic pathway in tumorigenesis in both species, and that species-specific alterations tend to localize to evolutionarily unstable genome regions as irrelevant mutation hotspots[70]. This suggests that alterations common to both species are more likely to cause tumor than those found in only one.

The germline genetic diversity (frequency of single nucleotide polymorphism) of a population of dogs with a given cancer is similar to the diversity observed in a well-balanced population of human patients with a given cancer[35, 48]. This relatively rare nature of dogs compared to most rodent cancer models further contributes to designating the dog as an ideal animal model in comparative oncology.

Next to humans, dogs have the most phenotypic diversity and known naturally-occurring diseases of all land mammals[67]. For example, in the same species coexist members that differ in size by 65-fold, as between Chihuahuas and English Mastiffs. Dogs share over ~650 Mb of ancestral sequence in common with humans, absent in mice, and DNA and protein sequence are more similar between human and dog than human and mouse[35]. Dogs and humans share approximately 400 similar inherited diseases, such as tumors, heart disease, and neurological disorders[49, 62]. Indeed, more than 40 naturally occurring canine diseases have mutations in a homologous human gene associated with a similar disease[45].

The greatest advantage of dog models is the result of their evolutionary history which led to selection of breeds on the basis of morphological and behavioral traits. Today there are ~400 isolated populations or breeds. Breed creation inadvertently selected "founder" mutations that are associated with specific traits and diseases; this translates into reduced disease and genetic heterogeneity, consistent with the fact that most breeds are predisposed to a distinct set of diseases[59]. It has been known for many years that there are some breeds that have a high incidence and a high risk of specific cancer subtypes, sometimes even more than one subtype (Table 1). Predisposed breeds provide the platform to readily identify genes known to be linked to cancer development (i.e. oncogenes) and those whose loss trigger cancer development or progression (i.e. tumor suppressor genes). Since several genetic alterations and molecular signaling pathways are the same in human and dog cancers, studying breeds with increased cancer incidence may allow more rapid progress in the identification of new cancer-associated genes than the study of human or mouse cancers alone.

Table 1: Tumors associated with specific dog breeds (data from reference 63).

cancer subtype	dog breed
lymphoma (unspecified)	Old English sheepdog, boxer, pointer, golden retriever, Rottweiler, St Bernard, Scottish terrier, bulldog
— B-cell lymphoma	— Irish wolfhound, Siberian husky, shih tzu, Airedale terrier, Cavalier King Charles spaniel, Yorkshire terrier
— T-cell lymphoma	— boxer, cocker spaniel, basset hound
osteosarcoma	large and giant breeds, such as Irish wolfhound, Scottish deerhound, Great Dane, BMD, mastiff, St Bernard, Irish setter, golden retriever, Rottweiler, Dobermann pinscher, greyhound
soft tissue tumours	larger dogs, such as boxer, BMD, Airedale terrier, Great Dane, St Bernard, basset hound, golden retriever—all with twice as many as the general canine population
hemangiosarcoma	German shepherd, BMD, golden retriever, flat-coated retriever, Portuguese water dog, Labrador retriever, boxer, Skye terrier, Australian shepherd
hs/malignant histiocytosis	BMD, flat-coated retriever, Rottweiler, golden retriever
mast cell tumours	boxer, pug, Labrador retriever, golden retriever, vizsla
meningiomas	mesocephalic (medium) and dolichocephalic (long)-nosed breeds, e.g. Labrador, golden retriever, collies
gliomas (including glioblastoma multiforme)	brachiochephalic (short-nosed) breeds, including boxers, bulldogs and terriers
testicular seminoma	Norwegian elkhound
nasal cavity carcinoma	golden retriever, beagle, Boston terrier, rough collie, Belgian shepherd
UC	Scottish terrier, beagle, West Highland white terrier, Shetland sheepdog, American Eskimo dog, standard schnauzer
lower urinary tract carcinoma	Airedale terrier, beagle
squamous cell carcinoma (digit)	STPO, giant schnauzer
melanoma	
— oral melanoma	— poodles
— cutaneous melanoma	— schnauzers, beauce shepherds

In summary, dogs are useful in multiple approaches to cancer investigation[5]: breed-specific risk can be used to discover disease pathways; human cancer pathways can be tested for roles, and targeted for treatment, in canine disease; and canine somatic mutations and genome alterations can be used to narrow down human mutations. Through these studies, comparative oncology confirms its value in the field of public health.

1.3 ANIMAL SENTINELS

The term “sentinel” is derived from the French *sentinelle*, “watch tower”. An animal sentinel system is one in which animal data are regularly and systematically collected, summarized, and analyzed in order to identify health hazard to either humans or the animals themselves from chemical or biological contaminants in the environment[87]. The familiar image of the canary in the coal mine remains relevant in the 21st century [8].

Just as miners carried caged canaries in the early 20th century to detect exceeding levels of carbon monoxide in the air, many animals have been used over time to warn of environmental contamination effects in human populations. Pets, in particular, share the environment and are exposed to many of the same agents as their human companions. Furthermore, they suffer a similar spectrum of disease as humans and, therefore, may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention. There are many historical examples that highlight animals’ usefulness as predictors of human illness[58].

Much of the work involving the use of sentinels to identify environmental hazards has focused on cancers in pet animals, particularly dogs, which share the environment intimately with humans but do not indulge in activities (i.e. smoking or working) that confound interpretation of human epidemiologic studies[84]. Naturally occurring canine tumors provide useful models for the study of the health effects of environmental hazards. Many canine cancers are similar to those in humans for biological behavior, histopathologic features, proportional morbidity, and recognized risk factors. A classic example of a canine cancer sentinel is the study of mesothelioma by Glickman[23]. The authors identified chrysotile asbestos bodies in lung tissue of dogs with spontaneous mesothelioma and linked this finding to asbestos exposure of their owner. The findings showed the importance of epidemiologic research to identify environmental health hazards for humans who share the environment with their pets. Thus, the diagnosis of canine mesothelioma is an early warning system for the human disease, because of the shorter latency period of mesothelioma in dogs than in humans, of about 8 and 30 years respectively [87].

The impact of these interactions can be appreciated only by studying population effects under natural conditions over time. Herein lies the strength of the epidemiologic method which, if rigorously applied, can bring closer to the truth and provide a clear picture of what happens [87].

1.4 CANCER REGISTRY

1.4.1 Introduction [29]

Cancer registry is one of the fundamental tools for epidemiological research. It has a pivotal role in cancer control. Its primary function is to record all cancer cases occurring in a defined population, collected continuously and systematically from various data sources. The registry analyses and interprets such data periodically and provides information on the incidence and characteristics of specific cancers in various segments of the resident population and on temporal variations in incidence. Such information is the primary resource not only for epidemiological research on cancer determinants but also for planning and evaluating health services for the prevention, diagnosis and treatment of the disease.

Cancer registries can also be used for monitoring occupational groups and cohorts of individuals exposed to various carcinogens and as a convenient source of subjects for clinical and epidemiological studies.

The value of a cancer registry depends on the quality of its data and the extent to which they are used in research and health services planning. It is obviously important that the registration of cancer cases should be as complete as possible. Epidemiological research, based on comprehensive cancer registration, remains the most valid and efficient way to plan and evaluate all aspects of cancer control.

The data collected by individual registries may vary according to local needs and availability of information but the nomenclature and definition of each item should be the same in all registries to give uniformity and facilitate international comparability of cancer data.

The main objective of the cancer registry is to collect and classify information on all cancer cases in order to produce statistics on the occurrence of cancer in a defined population and to provide a framework for assessing and controlling the impact of cancer on the community. Cancer registry information may be used in a multitude of areas, and the value of the data increases if comparability over time is maintained. The data become useful for more and more purposes as they are accumulated over longer periods of time.

The cancer registry's enumeration of cancer cases in a defined population permits assessment of the scale of the cancer problem in terms of the number of new cases and the computation of incidence rates. The type of statistics emerging from the cancer registry should be adapted to local needs and interests, bearing in mind the importance of international comparability. Ability to calculate rates depends on the availability of population denominators. Indeed, the information on cancer cases should be collected and classified so that it accords with the population statistics.

Comparison of cancer occurrence in various populations may provide clues to etiology, and the demonstration of variation in incidence has made an important contribution to the recognition of the environmental origin of many cancers, thus pointing to the possibilities for prevention. Such basic features of cancer incidence may not always be easily understood and explained, but they should provoke the epidemiologist's curiosity and are useful in the generation of etiological hypotheses.

The contribution of cancer registries to our knowledge of international variation in cancer incidence is an important purpose of registering cancer cases. The stimulation of etiological ideas from such geographical comparisons of cancer incidence may be enhances by correlation with statistics on potential risk factors. Cancer registries, through their mission to perform public health surveillance and research in oncology, contribute to the development of public health.

Just as human cancer registry, animal cancer registry takes part to epidemiological studies and represent a useful tool of comparative oncology. Quantitative comparison of tumor types may reveal unusual cancer frequencies, providing directions for research and generation of hypotheses of cancer causation in a specific area, and suggest leads for identifying risk factors.

While human cancer registries began for the first time in London in 1728[15], and now numbering over 400 individual registries, animal cancer registries are more recent, small in number and often short-lived and sporadic. A review of animal cancer registry from the beginning to recent time was conducted by Brønden and others[6]. The review explains that many animal registries are no longer existent, furthermore stresses the lack of communication and

collaboration between the registers, showing how their potential as information sources has not been fully exploited making them largely underutilized. The continuation of the registries, together with the collaboration between them, would increase the size of the database, allowing to evaluate temporary trends, fluctuations in cancer incidence and assessment of potential environmental and individual risk factors.

Inactive and active veterinary registries in Italy and worldwide are listed below. For inactive registries, period of passed activity was reported in brackets, while for active registries only the start year is reported:

- California 1968 School of Veterinary Medicine, Davis (Jul 1963- Jun 1966)
- Norway (1990-1998)
- Denmark Royal Veterinary and Agricultural University – 2005

In Italy:

- Genoa Animal Cancer Registry – 1985
- Ivrea Animal Cancer Registry – 2001
- Venice and Vicenza Animal Cancer Registry – 2005
- Sicily Animal Cancer Registry
- Tuscany Animal Cancer Registry (RoVeT) – 2006
- Campania Animal Cancer Registry – 2010
- Lazio Animal Cancer Registry – 2010
- Emilia Romagna Animal Cancer Registry – 2012
- Umbria Animal Cancer Registry – 2014

1.4.2 The concept of disease registries

A number of definitions have been suggested for the word "registry". Such definitions vary from author to author, but have the same perspective. Last[34] stated that "in epidemiology, the term register is applied to the file of data concerning all cases of a particular disease or other health-relevant condition in a defined population such that the case can be related to a population base". Bellows[3] defined registries as "a system of recording frequently used in the general field of public health, which serves as a device for the administration of progress

concerned with the long-term care, follow up or observation of individual cases". Solomon[66] defined a registry as "data base of identifiable persons containing a clearly defined set of health and demographic data collected for a specific public health purpose." Finally, a complete definition of a registry was presented by the World Health Organization[85] as follows: "a registry is a continuously updated file, set up for a specific purpose, of individuals with symptoms, health states disorders or diseases, or events in a defined population."

Weddell[80] classified all registries into seven types: registers used in preventive medicine, disease-specific registers, treatment registers, after-care registers, at risk registers, registers for prospective studies, and specific information registers. These classification systems are useful, but they are limited because they fail to recognize that potentialities of registry uses are related to their sources of registry data. Accordingly, Pedersen[51] classified registries by their sources of data. He proposed three types of registries (specifically for cancer): local hospital registries, central registries, and population-based registries.

1.4.3 Types of Registry

Registries are classified by their sources of data and the scope of coverage that can be achieved. A registry may be population-based, a central cancer registry or hospital-based[51].

A population-based registry covers the entire population in a defined geographic area[1]. A population-based cancer registry attempts to gather as much detailed information as possible on all new cancer cases diagnosed in a population of a known size and composition. The task of a population-based registry will obviously be much easier when there are collaborating hospital registries, which contribute in providing the information.

The central registry is analogous to the local hospital registry, but includes a selected group of hospitals in a region. Its chief function is to supply data on diagnosis and treatment of the involved hospital patients and submit to the central registry. A central cancer registry is a co-ordination facility of co-operating hospital registries in a specified geographic area, which collects information on cancer patients. Such kinds of cancer registries are particularly valuable for comparing end results among different therapeutic regimens[24].

Unlike a population-based registry, and a central cancer registry, a hospital-based registry covers only one hospital[51]. The purpose of a hospital-based cancer registry is to serve the needs of hospital administration, the hospital cancer program, and above all the individual patient[86]. Its main function is to ensure that the information in case records is detailed enough to enable statistical analysis. Thus, some of the hospital registry data items collected will be different from those collected by a population based registry. The hospital registry alone does not contribute to the epidemiology of cancer because it cannot provide the incidence of cancer *in* the population[51].

The key objective of a cancer registry is to produce statistics on the occurrence of cancer in a defined population, and to assess cancer survival. To perform such tasks, cancer registries need to have the capability, the computing facilities, and the statistical skill necessary for such analyses.

Based on its main function registries can be classified into three groups:

The first group are registries that are interested only in producing cancer incidence reports. Such reports represent basic presentation of the registry data. They allow feedback to reporting physicians, health authorities, and the public on the occurrence of cancer. The report could be annual, or based on incidence information for several consecutive years.

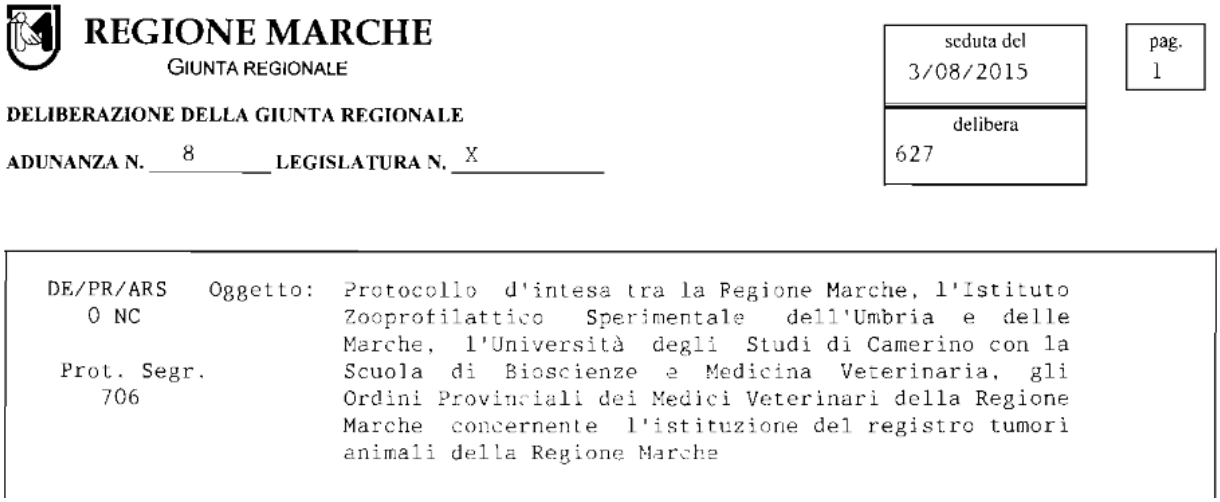
The second group are registries interested in numerous issues related to cancer survival. Such data once calculated can be used to represent the average prognosis in the population and provide theoretically at least, an objective index of the effectiveness of cancer care in the region concerned.

The third group are population-based cancer registries, whose main task is to perform incidence data reporting, but also to have the facilities and skill for follow-up reporting. If undertaken by a population-based cancer registry, such tasks include all those cases that reside in the registry area.

2. ANIMAL CANCER REGISTRY OF THE "MARCHE" REGION

2.1 INTRODUCTION

In a panorama of fragmented data on canine cancer epidemiology and following the need to acquire more and more useful tools to pursue the public health, with resolution n°627 of the 3rd August 2015 (Fig. 3), Marche Region created the Animal Cancer Registry of the Marche region (ACR-M).



REGIONE MARCHE
GIUNTA REGIONALE

DELIBERAZIONE DELLA GIUNTA REGIONALE

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Oggetto: Protocollo d'intesa tra la Regione Marche, l'Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, l'Università degli Studi di Camerino con la Scuola di Bioscienze e Medicina Veterinaria, gli Ordini Provinciali dei Medici Veterinari della Regione Marche concernente l'istituzione del registro tumori animali della Regione Marche

Fig. 3: Resolution of Marche Region, dated 3rd August 2015, that established the regional Animal Cancer Registry.

The ACR-M was created to enable exhaustive and continuous recording of all cases of cancer in dogs living in the Marche region. It is a population-based registry, for this reason it is currently dealing only with canine population, for which a mandatory registry based on identification by microchip exists. This registry has been participating in epidemiological surveillance and evaluation of cancer, through the analysis of incidence data over time including more than 700 cases since December 2015.

Its activity is based on the cooperation between Marche Region, School of Biosciences and Veterinary Medicine of the University of Camerino, Experimental Animal Prophylaxis Institute (Italian acronym IZS) of Umbria and Marche, and veterinary practitioners of provincial professional Orders of Ancona, Ascoli Piceno, Fermo, Macerata, and Pesaro/Urbino.

Veterinarians practicing on the regional territory are responsible for samples collection. Histopathology laboratories of the School of Biosciences and Veterinary Medicine of the University of Camerino and of the Experimental Animal Prophylaxis Institute of Umbria and Marche perform the histopathologic diagnosis of tumors through a double-blind mechanism. Epidemiologic Observatory of the Experimental Animal Prophylaxis Institute of Umbria and Marche deals with data analysis. Marche Region promotes its animal cancer registry and provides the digital platform on which this is based on.

2.2 MATERIALS AND METHODS

2.2.1 Data source

In human medicine, population-based cancer registries are maintained using hospital and death-certificate data as numerators and census data as denominators in morbidity and mortality rates. Animal cancer registries usually lack census data and so the denominators tend to be biased by non-response. Some registries reduce the non-response bias by utilizing demographic survey in specified areas.

The Animal Cancer Registry of the Marche region uses demographic census data of canine population based on the SIVA information system (http://siva.regione.marche.it). SIVA (Italian acronym for Veterinary Information System and Food) is a digital platform where canine regional demographic data, based on identification microchip number of each dog living in the Marche region, are collected.

In addition to the denominator, SIVA also provides numerator of incidence rates since it hosts not only the regional canine registry but also the canine cancer registry. The Animal Cancer Registry of the Marche region is entirely digitalized and developed in SIVA system. Indeed, veterinary practitioners have a dedicated SIVA section where insert exam requests and receive related histopathologic reports, and pathologists enter their diagnosis directly into the digital system.

In SIVA system, veterinarians are asked to fulfill a digital request form at the time of excisional surgery, for obtaining a numeric code identifying sample and patient and a histopathological diagnosis. The form's items concern animal data, some automatically caught by the system from the regional canine registry thanks to microchip number (i.e. date of birth, sex, breed, ovariohysterectomy or castration status, geographical area of residence) and others added by veterinarian (i.e. gross data on lifestyle – urban or rural, and on nutrition – commercial, home-made, etc.), and details about anatomical site of the lesion, date and type of surgical excision and clinical history of the patient.

Once received the sample, pathologists carry out diagnosis and enter it into the SIVA system. Diagnosis reliability is guaranteed by a double-blind mechanism: first and second pathologists perform microscopically evaluation and report separately and, only if there is coincidence of diagnosis, the SIVA system sends the report to the veterinarian electronically. When there is no coincidence, a third pathologist who completes diagnosis is involved.

Since diagnosis are entered into a computerized system, reports cannot be only descriptive but need of a classification and coding system. Classification and coding system also answers to problems that a cancer registry is always faced: internal comparability of long time tumor series and international comparability between registries. The underlying principles of coding are to bring together in classes cancers with common characteristics. Thus, ACR-M

adopted the International Classification of Diseases for Oncology (ICD-O)[16], an internationally accepted system, which easily allows to classify tumors in broad categories and to assign a code for each tumor type.

2.2.2 Sample collection and diagnosis

To promote participation of veterinary practitioners, a free courier service was established. Following the request of practitioners, courier took the sample directly from the veterinary facilities and delivered it to the pathology laboratory of the School of Biosciences and Veterinary Medicine of Camerino.

Once delivered, the sample was registered with a double code: the code assigned by the SIVA information system at the time of exam request, and the internal code of the university laboratory.

The tissue samples were processed routinely through graded alcohol and xylene in automatic tissue processor to obtain paraffin–embedded tissue blocks. The blocks were cut using manual microtome to obtain 3 um thick sections. The sections were stained by hematoxylin and eosin staining method and examined under the microscope. The diagnosis of various tumor conditions was made based on the characteristic histopathological features.

As previously reported in 'data source' paragraph, all histological slides from diagnosed tumors in the registry were examined independently by two experienced veterinary pathologists. The classification was according to the ICD-O codes. After reaching a diagnosis the results were compared and any disagreements between the two raters were solved by consensus of a third pathologist.

2.2.3 International Classification of Diseases for Oncology: ICD-O

Since it was first published in 1976, the International Classification of Diseases for Oncology (ICD-O)[26] has been internationally recognized as the definitive classification of neoplasms. It is used by cancer registries throughout the world to record incidence of malignancy and survival rates, and the data produced are used to inform cancer control, research activity, treatment planning and health economics.

The classification of neoplasms used in ICD-O links closely to the definitions of neoplasms used in the WHO/IARC Classification of Tumors series which are compiled by consensus groups of international experts and, as such, the classification is underpinned by the highest level of scientific evidence and opinion.

ICD-O consists of two axes (or coding systems), which together describe the tumor:

- the topographical code, which describes the anatomical site of origin (or organ system) of the tumor, and
- the morphological code, which describes the cell type (or histology) of the tumor, together with the behavior (malignant or benign).

By agreement with the College of American Pathologists, the morphology section of ICD-O is incorporated into the 'Systematized Nomenclature of Medicine' (SNOMED)[10, 11] classification as the neoplasm section of the morphology field.

The 'International Classification of Diseases for Oncology, Second Edition'[52] was published in 1990, followed by the 'International Classification of Diseases for Oncology, Third Edition'[16] in 2000. The topography section of the third edition remained the same as in the second edition, which was based on the neoplasm section of the 'International Statistical Classification of Diseases and Related Health Problems, 10th Revision' (ICD-10)[28]. However, the morphology section was revised. New classifications, especially for lymphomas and leukemias, were introduced, and new codes assigned to accommodate them. Although one of the prime commitments of the editors was to change as few terms as possible, to add new terms at empty spaces, and not to reuse previously assigned codes, this was not always possible. In order to keep groups of similar entities together, the codes for some terms had to be changed. Furthermore, the sequence or grouping of terms may not always be as logical as possible because of the limitations of available code numbers.

In developing the previous editions and the present third of ICD-O, a particular effort was made to use the nomenclature appearing in the World Health Organization 'International Histological Classification of Tumors' series (WHO "Blue Books")[27]. This series covers all the principal sites of cancer and includes the morphology codes of ICD-O for each neoplasm.

Since the initial publication of the third edition of ICD-O (ICD-O-3) in 2000, updates to the WHO Blue Book series have continued. During the development of the fourth edition of the Blue Book volumes, chapter authors worked with the International Agency for Research on Cancer/International Classification of Diseases for Oncology (IARC/ICD-O) Committee for ICD-O-3 to review recently identified neoplasm entities and assign morphology codes. This updated version of ICD-O-3 (ICD-O-3 First Revision, or ICD-O-3.1) (Figure 4) includes the new terms, codes, synonyms, related terms, morphology, and behavior code changes from the WHO Blue Books published between 2007 and 2010 on tumors of hematopoietic and lymphoid tissues[68], the central nervous system[36], and the digestive system[4].

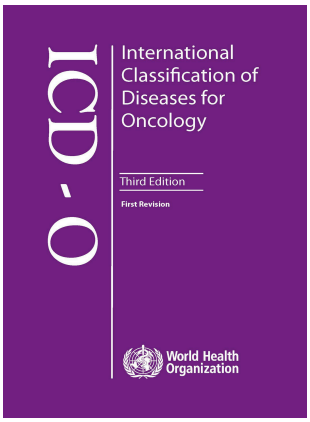


Figure 4: International Classification of Diseases for Oncology (ICD-O), Third Edition, First Revision. WHO, ISBN-13 978924158496

The International Classification of Diseases for Oncology (ICD-O) is a dual classification, with coding systems for both topography and morphology.

The 'topography' code describes the anatomical site of origin of the neoplasm and, while it uses the same categories as in the neoplasm section of Chapter II of the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10), some of the individual codes are different. The code always has a prefix of "C", followed by a three-digit number that indicates the site (two digits) and the subsite (one digit), separated by a decimal point. For example, in C18.4, the C18 indicates that the site is the colon and the 4 indicates that the subsite is the transverse colon.

The 'morphology' code describes the characteristics of the tumor itself, including its cell type and biological activity. The code is composed of four digits that indicate the cell type or histology and one digit that indicates the behavior. The first four digits are separated from the last (behavior) digit by a forward slash (/). The behavior digit can be 0 (benign), 1 (uncertain behavior), 2 (carcinoma in situ), 3 (malignant, primary site), 6 (malignant, metastatic site), or 9 (malignant, uncertain whether primary or metastatic site).

2.2.4 Data analysis

Data analysis was carried out by the Epidemiological Observatory of the Experimental Animal Prophylaxis Institute (IZS) of Umbria and Marche. Similar to what happens for human population-based registries, when possible, the data were evaluated on an annual basis.

Prevalence ratio (PR) was used to quantify the relationship between tumor and independent variable (sex, age, breed, lifestyle), and reported as percentage.

Given the low total number of cases collected in 32 months, crude incidence rates (CIR) and related 95% confidence intervals (CIs) of benign and malignant tumors per 100.000 dogs were calculated not per year but for the whole period of study. A still exiguous number of cases collected did not allow to have an adequate denominator for the calculation of incidence rates by race, sex, age, topography and lifestyle. In spite of this, CIR and 95% CIs calculation of tumors by sex and age was anyway carried out, forcing the calculation and obtaining results only partially comparable to reality.

For all the other variables, a proportional morbidity rate was introduced. A proportional morbidity rate is the number of cases of a specific disease in a specified population during a specified time period, divided by the total number of cases of all diseases in that population during that time period, and expressed by percentage.

A spatial analysis highlighting each municipality trend was also performed.

Data source for analysis of regional canine population was the canine registry of the Marche Region, contained in SIVA. Despite legal obligations for owners to register their dogs by an identification microchip and to quickly denounce the death, when occurs, data of the regional canine registry cannot be considered exhaustive. In order to obtain a more real dimension of the regional canine population, therefore a correct rates denominator, the starting data were cleaned. This screening led to exclusion from processing of:

- dogs without a residence reference or residing out of the region;
- dogs much older than expected average lifespan per breed. Expected average lifespan was calculated, per breed, on the basis of the available scientific literature[13, 73], while per mongrel, on the 95th percentile of dead dogs' distribution recorded in the regional canine registry. Age was categorized into 5 classes: 'very young', 'young', 'adult', 'senior', and 'very old' (Table 0).

Table 0: Categorization based on maximum life expectancy

Categories	Maximum life expectancy (years)								
	8	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17-18 and mongrels**
Very young	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Young	2-3	2-3	2-3	2-3	2-3	2-4	2-4	2-4	2-5
Adult	4-5	4-6	4-7	4-8	4-8	5-9	5-10	5-11	6-11
Senior	6-7	7-8	8-9	9-10	9-11	10-12	11-13	12-14	12-15
Very old	8	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-18

Statistical analysis was performed with the Stata 11.2 software (StataCorp, College Station, TX, USA), while for maps creation the freeware program QGIS 2.4.0-Chugiak.

2.3 RESULTS

2.3.1 Dataset

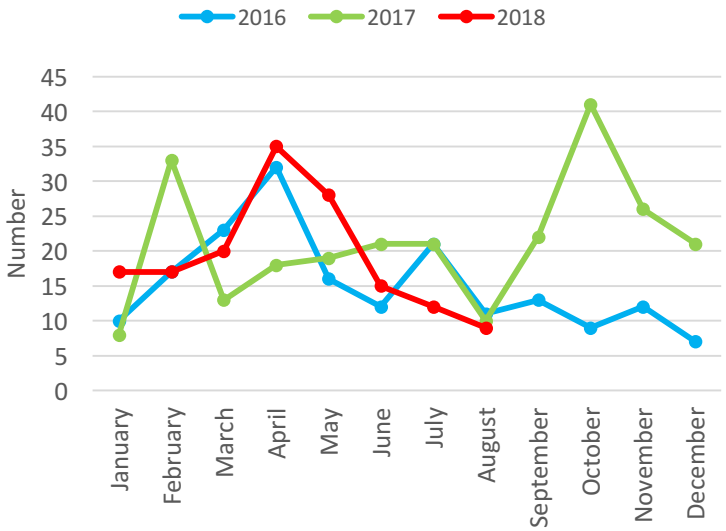
The dataset was based on extraction of data from 1st January 2015 to 31st August 2018 (32 months). During this period of time, the Animal Cancer Registry of the Marche region (ACR-M) received a total of 589 requests.

In the first year of activity of the ACR-M, 183 requests were recorded, in the second year the requests were 253 with a 38% increase compared to the previous year. The highest number of requests was recorded in the month of April in the first year and in October in the second; the monthly distribution during the year is shown in Table 1 and Figure 1. In 2018 the requests were 153, as partial data because involve requests received only from January 1st to August 31st. As for

the first year, the peak was recorded in April with 35 requests followed by the month of May with 28 (Figure 5 and Table 2).

Veterinary facilities conferring samples to the registry were 35 in the first year (Figure 6), of whom 17 stopped in the second year, and 26 in the second year (Figure 7), 8 of which sent samples for the first time. In 2018 (till August) participating facilities were 28 (Figure 8), of whom 5 had never collaborated to the ACR-M. Over the three years, only 15 veterinary facilities have continuously conferred. The most represented municipalities were Osimo, Arcevia, Ancona, Pesaro and Lunano (Table 3 and Figure 9). Many of 2017-2018 samples were got from private histopathology laboratories, not yet included into the ACR-M mechanism of SIVA request and diagnosis, so these data lack of some details like geographical origin and are indicated as "undetermined" in Table 3.

Figure 5 and Table 2: Requests distribution (number) per month and year



Month	2016	2017	2018	Total
January	10	8	17	35
February	17	33	17	67
March	23	13	20	56
April	32	18	35	85
May	16	19	28	63
June	12	21	15	48
July	21	21	12	54
August	11	10	9	30
September	13	22	-	35
October	9	41	-	50
November	12	26	-	38
December	7	21	-	28
Total	**183**	**253**	**153**	**589**

Table 3: Belonging municipalities of the veterinary facilities conferring to ACR-M

Municipality	2016	2017	2018	Total N°
Ancona	34	8	2	44
Arcevia	13	24	21	58
Ascoli Piceno	9	3	3	15
Auditore			1	1
Camerino	3	7		10
Carpegna		4	3	7
Castel Di Lama	4			4
Castelplanio			2	2
Cerreto D'esi	12			12
Civitanova Marche	3			3
Cupramontana		2	2	4
Esanatoglia	2	4	1	7
Fabriano		5	1	6
Fano	1			1
Grottammare	1			1
Jesi	1		1	2
Lunano	15	11	9	35
Macerata			1	1
Macerata Feltria	2	1		3
Monte Vidon Corrado			1	1
Montecosaro	1			1
Montefano	1			1
Montelparo		14	5	19
Napoli	1			1
Offagna	8	12	6	26
Osimo	32	35	39	106
Pesaro	14	22	8	44
Polverigi	7	8	6	21
Porto San Giorgio	2	5	2	9
Porto Sant'elpidio	1	11	9	21
Recanati	2			2
San Benedetto Del Tronto	2	2	2	6
Sassoferrato			1	1
Senigallia	8	9	10	27
Tolentino			1	1
Urbino	1	2		3
undetermined (private labs)	3	64	16	83
Total	**183**	**253**	**153**	**589**

Figure 6: Municipality thematic map of veterinary facilities conferring in 2016

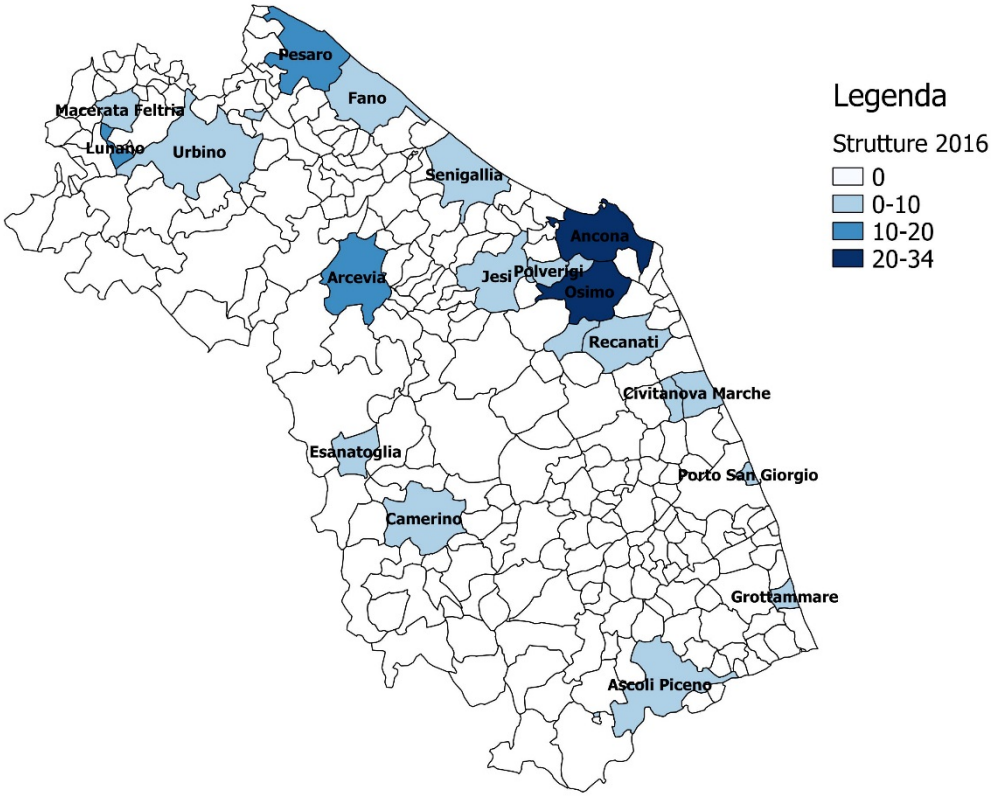


Figure 7: Municipality thematic map of veterinary facilities conferring in 2017

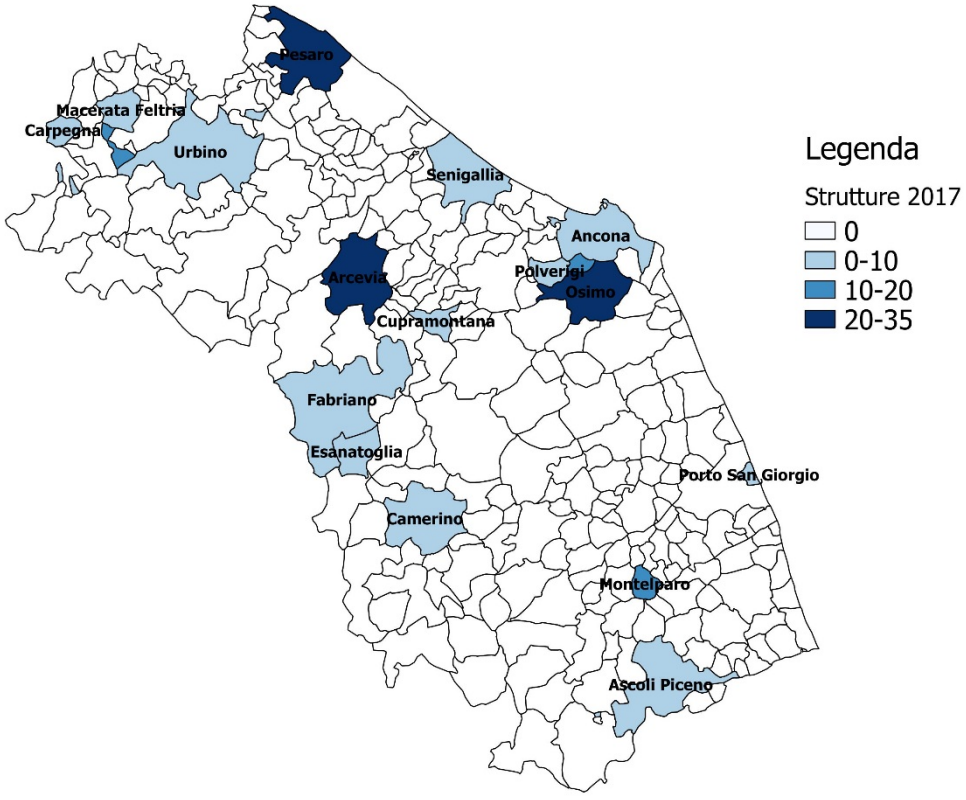


Figure 8: Municipality thematic map of veterinary facilities conferring in 2018

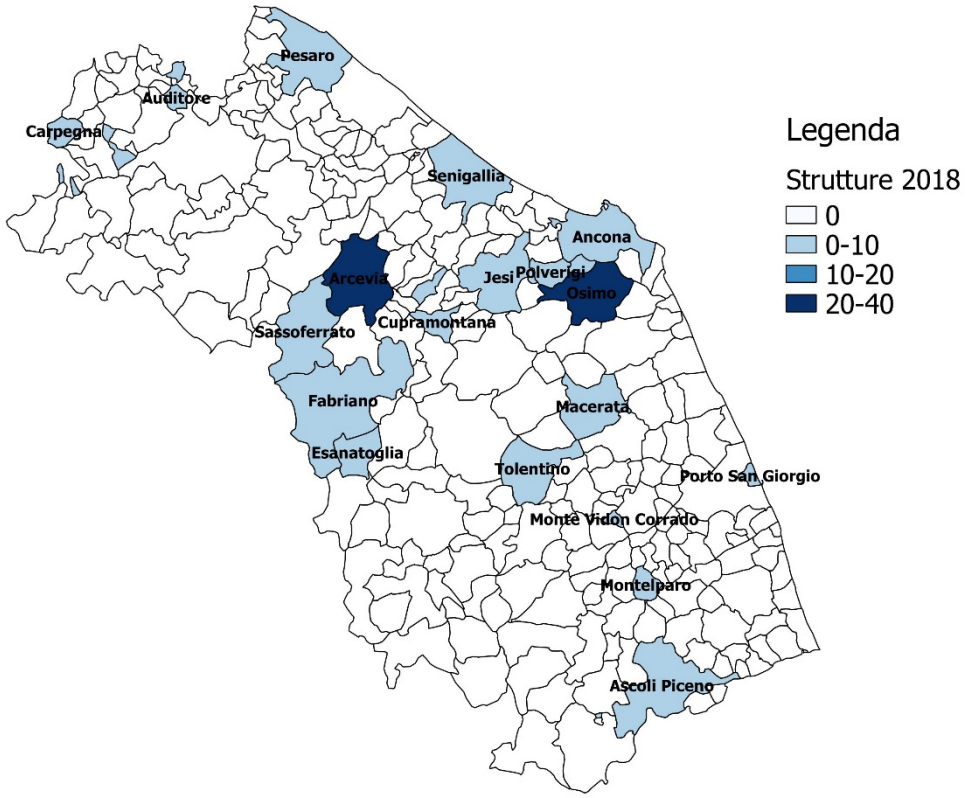
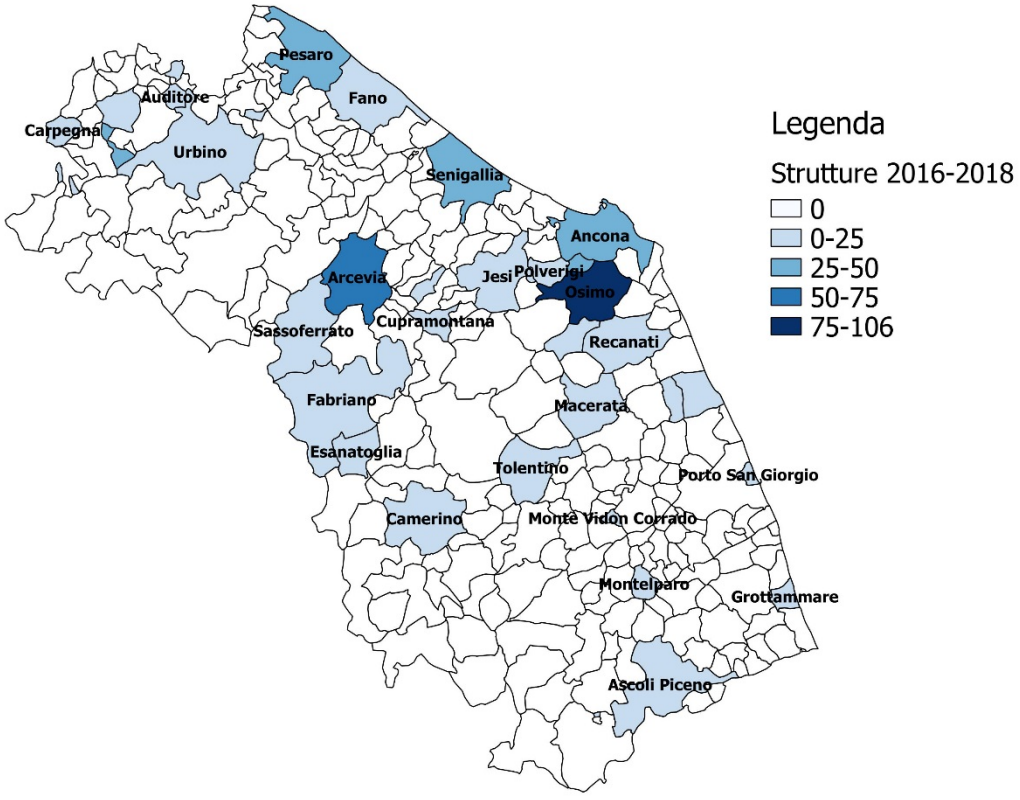
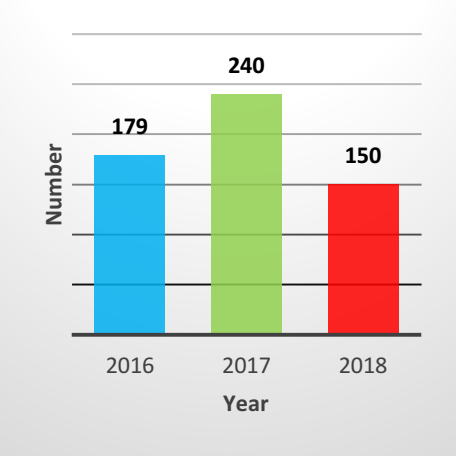


Figure 9: Municipality thematic map of veterinary facilities conferring in Jan 2016-August 2018 period



The total number of dogs involved was 569, most of them clustered in 2017 (Figure 10 and Table 4).

Figure 10: Dogs distribution (number) per year

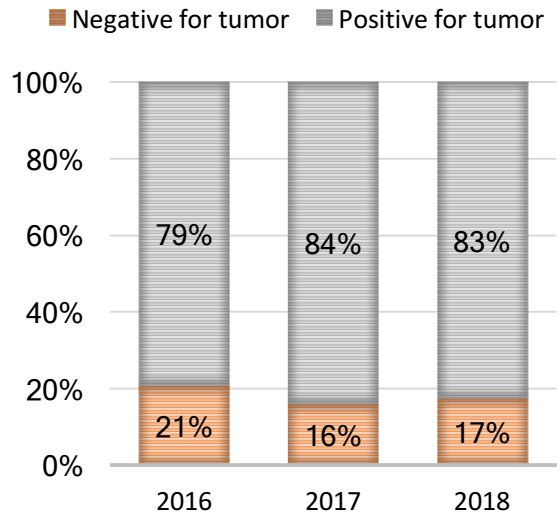


ACR-M	Total N°
2016	179
2017	240
2018	150
Total	569

Table 4: Dogs distribution (number) per year

The number of dogs with confirmed tumors was 468 (82%). Of these, in 2016 represented 79% (142/179), in 2017 about 84% (202/240), and in the first 8 months of 2018 were 83% (124/150). Figure 11 and table 5 show distributions of percentage and absolute frequency.

Figure 11: Percentage (%) distribution of negative/positive for tumor per year



Year	Negative for tumor	Positive for tumor	Total number
2016	37	142	179
2017	38	202	240
2018	26	124	150
Total	**101**	**468**	**569**

Table 5: Absolute frequency distribution of negative/positive for tumor per year

Given the low total number of cases collected in less than 3 years, crude incidence rates (CIR) and related 95% confidence intervals (CI) of benign and malignant tumors per 100.000 dogs were calculated not per year but for the whole period of study. Incidence rate for all tumors was

160,5/100.000, while for benign and malignant tumors were 81,9/100.000 and 78,5/100.000 respectively. These data and related 95% confidence intervals are reported in the table 6.

Table 6: Incidence rates of tumors per 100.000 dogs and related lower and upper limits 95% confidence intervals (CI) in the Marche region for the period 01/01/2016-31/08/2018.

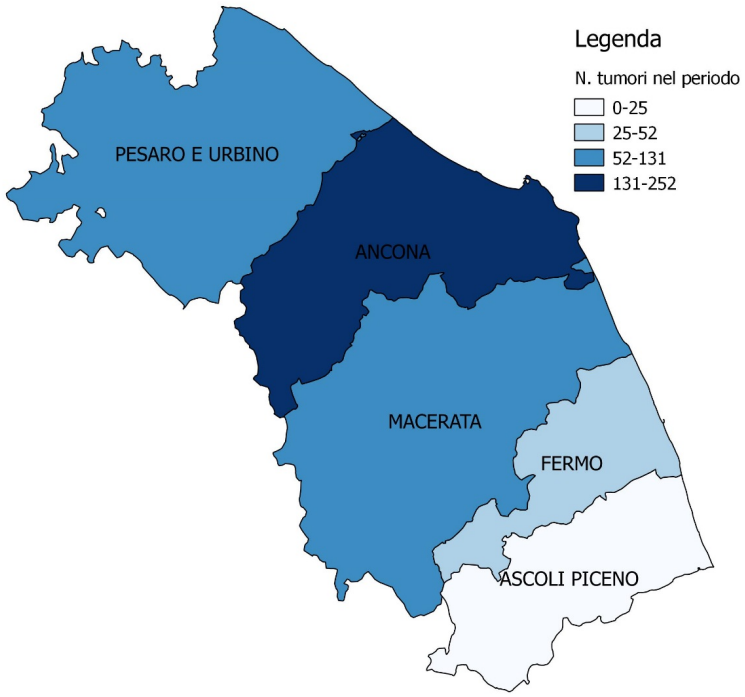
Tumors	Incidence rate	Lower limit 95% C.I.	Upper limit 95% C.I.
All tumors	160,5	147	174,9
Benign tumors	81,9	72,4	92,4
Malignant tumors	78,5	69,2	88,8

Spatial analysis indicated Ancona and Pesaro-Urbino as the provinces with the greatest number of conferred samples and of tumors (Table 7 and Figure 12).

Table 7: Distribution (number) of negative/positive cases for tumor by province and year

Year	2016		2017		2018		
Province	**Negative for tumor**	**Positive for tumor**	**Negative for tumor**	**Positive for tumor**	**Negative for tumor**	**Positive for tumor**	**Total**
Ancona	19	75	17	89	13	67	280
Ascoli Piceno	5	12	1	8	1	4	31
Fermo		5	4	29	2	13	53
Macerata	4	15	3	28		11	61
Pesaro-Urbino	9	35	13	48	10	29	144
Total	**37**	**142**	**38**	**202**	**26**	**124**	569

Figure 12: Thematic map of tumors (benign and malignant) by province in Jan 2016-August 2018 period



Proportional morbidity rates calculated by provinces and year were reported in Table 8.

Table 8: Proportional morbidity rates expressed in percentage (PMR 100) by province and year

Variable	PMR 100	2016	2017	2018	2016-2018
Province	Ancona	54%	36%	59%	46%
	Ascoli Piceno	9%	4%	2%	5%
	Fermo	5%	16%	9%	11%
	Macerata	11%	15%	9%	13%
	Pesaro Urbino	22%	29%	20%	25%

Municipalities conferring the greatest number of samples were Ancona (75), Osimo (53), Pesaro (33), Senigallia (21), Pergola (18), and Castelfidardo (15). The same municipalities, but in different order, had the largest number of diagnosis positive for tumor: Ancona (57), Osimo (42), Pesaro (25), Pergola (15), Senigallia (13), and Castelfidardo (13) (Table 9 and Figure 13).

Table 9: Distribution of negative/positive cases for tumor by municipality and year

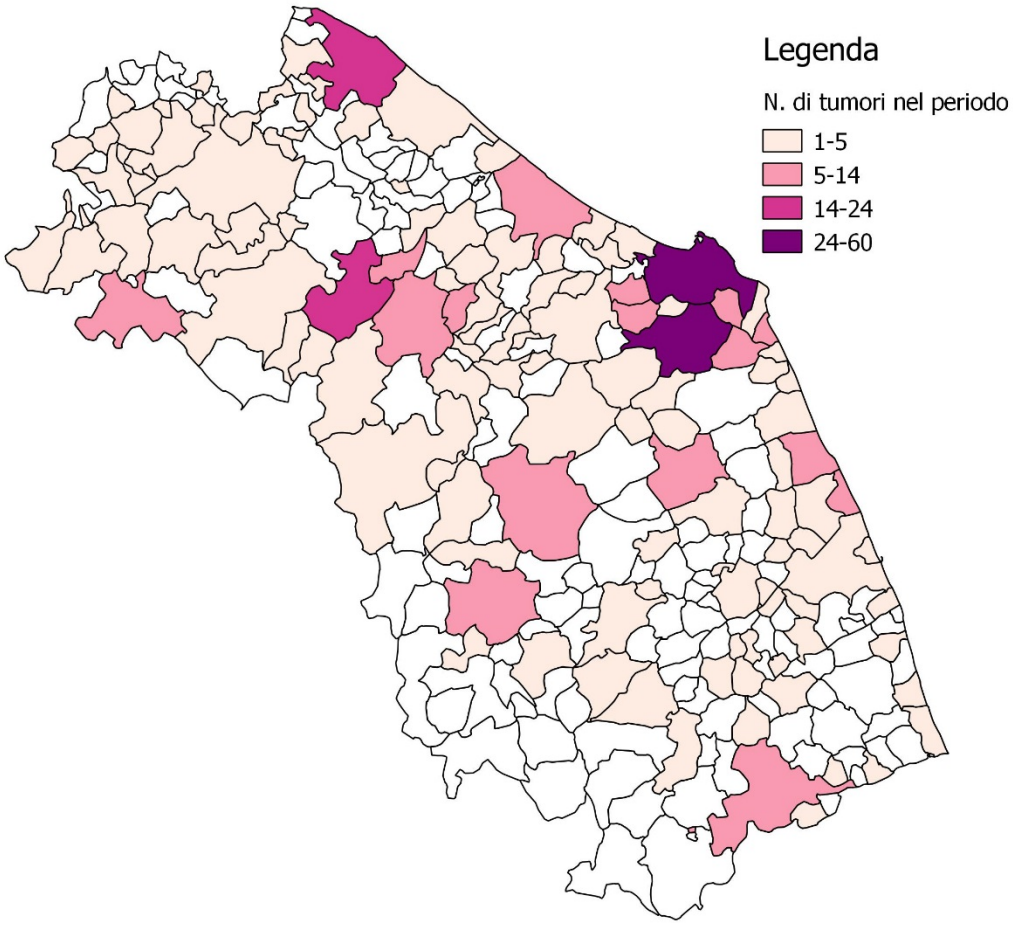
	2016		2017		2018		
Municipality	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	**Total**
ACQUALAGNA				1	1		2
AGUGLIANO		4		3		1	8
ALTIDONA						1	1
AMANDOLA				1			1
ANCONA	10	22	4	20	4	15	75
APECCHIO	1		2	4		2	9
APPIGNANO						1	1
ARCEVIA		1	1	8		1	11
ASCOLI PICENO	2	5		1		2	10
AUDITORE				1			1
BARBARA				1		1	2
BELFORTE ALL'ISAURO			2				2
BORGO PACE		1		2			3
CAGLI		1		1	2	1	5
CAMERANO	1	7		1	1	2	12
CAMERINO		3		5			8
CARPEGNA				2		2	4
CASTEL DI LAMA		3					3
CASTELBELLINO				1			1
CASTELFIDARDO	1	6	1	3		4	15
CASTELRAIMONDO		1		1			2
CERRETO D'ESI						1	1
CHIARAVALLE		1		1		1	3
CINGOLI	1	1					2
CIVITANOVA MARCHE	1	2	1	1		4	9
COMUNANZA				1			1
CORINALDO						1	1
CORRIDONIA		1				1	2
CUPRAMONTANA				1	1	1	3
FABRIANO				4		1	5
FALCONARA MARITTIMA	1	1		1		1	4
FANO		1		3		1	5
FERMIGNANO		1			1		2
FERMO				1		2	3
FIASTRA				1			1
FILOTTRANO		1	1				2
FOLIGNANO	2	1		2			5
FRONTONE				1			1
GROTTAMMARE		1					1
GROTTAZZOLINA				1			1

	2016		2017		2018		
Municipality	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	**Total**
JESI						2	2
LORETO						3	3
LUNANO		1		1			2
MACERATA			1	5		3	9
MACERATA FELTRIA		1		1			2
MAGLIANO DI TENNA		1	1				2
MAIOLATI SPONTINI		2	1	1		1	5
MASSIGNANO						1	1
MATELICA		1		1			2
MERCATELLO SUL METAURO	1	1				1	3
MONDAVIO				1			1
MONDOLFO				2			2
MONSAMPIETRO MORICO		1	1				2
MONSANO		2			1		3
MONTALTO DELLE MARCHE				1			1
MONTE CERIGNONE		1		1			2
MONTE URANO				1			1
MONTECALVO IN FOGLIA	1			1			2
MONTECAROTTO		1				1	2
MONTECASSIANO				2			2
MONTECICCARDO			1				1
MONTECOSARO						1	1
MONTEFANO		1					1
MONTEGIORGIO				1			1
MONTEGRANARO				2			2
MONTELABBATE				1			1
MONTELEONE DI FERMO				1			1
MONTELPARO			1	3			4
MONTEMARCIANO				1			1
MORRO D'ALBA				1			1
MUCCIA			1	1			2
NUMANA		2		3		1	6
OFFAGNA		3	2	1			6
ORTEZZANO				2		3	5
OSIMO	3	12	4	20	4	10	53
OSTRA						1	1
OSTRA VETERE				1		1	2
PERGOLA	1	5	1	5	1	5	18

	2016		2017		2018		
Municipality	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	**Total**
PESARO	2	11	2	11	4	3	33
PETRIANO		1					1
PETRITOLI				4			4
PIANDIMELETO		1	1		1		3
PIETRARUBBIA	1			1			2
PIOBBICO			1				1
POGGIO SAN MARCELLO				1			1
POLVERIGI		2		3		5	10
PONZANO DI FERMO				1		1	2
PORTO RECANATI				2			2
PORTO SAN GIORGIO		1		1			2
PORTO SANT'ELPIDIO		2	1	5	1	5	14
POTENZA PICENA				1			1
RIPE SAN GINESIO				1			1
ROSORA		1		1			2
ROTELLA				1			1
SAN BENEDETTO DEL TRONTO	1	1	1	2	1	1	7
SAN GINESIO				2			2
SAN LORENZO IN CAMPO	1	1		1		6	9
SAN MARCELLO		1					1
SAN PAOLO DI JESI				1			1
SAN SEVERINO MARCHE	2	5		1		1	9
SANTA VITTORIA IN MATENANO				1			1
SANT'ANGELO IN VADO		3				2	5
SANT'ELPIDIO A MARE				3	1	1	5
SARNANO				2			2
SASSOCORVARO				3			3
SASSOFERRATO				1			1
SENIGALLIA	3	5	3	2	2	6	21
SERRA DE'CONTI				3		3	6
SERRA SAN QUIRICO				2			2
SERRA SANT'ABBONDIO		1		1		1	3
SIROLO		1		1		3	5
SPINETOLI		1					1
TAVULLIA			2	1			3

Municipality	2016		2017		2018		Total
	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	Negative for tumor	Positive for tumor	
TORRE SAN PATRIZIO				1			1
TRECASTELLI				2			2
URBANIA		1		1		1	3
URBINO	1	1				2	4
URBISAGLIA				2			2
VALLEFOGLIA		2	1	1		2	6
Total	**37**	**142**	**38**	**202**	**26**	**124**	**569**

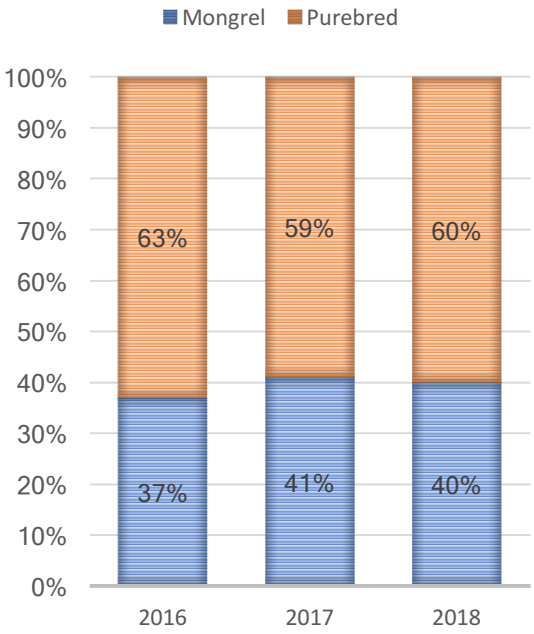
Figure 13: Thematic map of tumors (benign and malignant) by municipality in Jan 2016-August 2018 period



2.3.2 Breed distribution

The number of dogs belonging to a given breed varied. Most dogs were purebred (61%), while mongrels were 38% (Figure 14 and Table 10). The most represented breeds were: German shepherd, Labrador retriever, Golden retriever and Miniature pinscher (Table 15). This prevalence remained constant in the whole period of time.

Figure 14: Distribution in percentage (%) of mongrel and purebred dogs by year



Dog breeds	2016	2017	2018
Mongrel	66	98	60
Purebred	113	142	90
Total number	179	240	150

Table 10: Distribution in number of mongrel and purebred dogs by year

Table 15: Distribution (number) of dog breeds

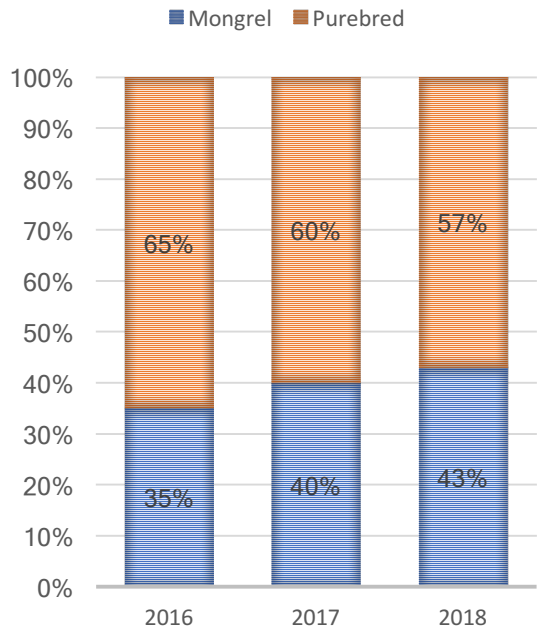
DOG BREEDS	2016	2017	2018	Total
Mongrel/mixed-breed	66	98	60	224
German shepherd	9	10	10	29
English setter	11	7	8	26
Labrador retriever	6	13	4	23
Golden retriever	3	10	6	19
Miniature pinscher	10	5	4	19
Boxer	5	6	2	13
Dachshund	1	8	3	12
Jack Russell terrier	3	2	4	9
English springer spaniel	4	2	3	9
Miniature poodle	3	2	2	7
Maremma sheepdog	2	3	2	7
American pit bull terrier	3	2	2	7
Italian short haired hound	2	3	2	7
West highland white terrier	1	6		7

DOG BREEDS	2016	2017	2018	Total
Beagle	2	2	2	6
French bulldog	2	3	1	6
English cocker spaniel	2	2	2	6
Italian coarse haired hound	1	3	2	6
Yorkshire terrier	1	4	1	6
Dobermann pinscher	3	2		5
Shih tzu	2	3		5
Staffordshire bull terrier	2	2	1	5
Poodle	1	1	2	4
Italian Bracco	1	1	2	4
American cocker spaniel	1	3		4
German short haired pointer dog - Kurzhaar		2	2	4
Maltese	2	1	1	4
Medium-size schnauzer	2	1	1	4
Giant schnauzer		4		4
Akita		3		3
Bichon à poil frisé	1	1	1	3
Border collie	1	2		3
Bernese mountain dog		1	2	3
Bulldog		1	2	3
Cane Corso	2		1	3
Australian kelpie	2	1		3
Pug	2		1	3
Dogo Argentino	2		1	3
Lagotto Romagnolo	1	2		3
Siberian husky	2	1		3
Volpino Italiano	1	2		3
Great Dane	1		1	2
Alaskan malamute		1	1	2
Boston terrier	1	1		2
German wired haired pointer dog - Drahataar	1		1	2
Chihuahua	2			2
Chow-chow	1	1		2
Dogue de Bordeaux		1	1	2
Brittany	2			2
White Swiss shepherd		2		2
Italian greyhound			2	2
Rottweiler	1	1		2
Old English sheepdog			1	1
Bolognese	1			1
Great Pyrenees			1	1
Briquet griffon vendeen			1	1
Bull terrier		1		1
Czechoslovakian wolfdog	1			1
Scottish short haired shepherd dog		1		1

DOG BREEDS	2016	2017	2018	Total
Cavalier king Charles spaniel		1		1
Chin		1		1
Gordon setter			1	1
Greater Swiss mountain			1	1
Brussels griffon	1			1
Pekingese	1			1
Miniature schnauzer		1		1
Irish setter	1			1
Spinone Italiano		1		1
Miniature German spitz			1	1
Terranova		1		1
Weimaraner			1	1
Whippet		1		1
TOTAL	179	240	150	569

Most dogs with cancer involved purebreds. Over the whole period of study, dogs with tumor were 468, of which 285 purebreds (61%) and 183 mongrels (39%). Observations by year were performed and the general prevalence always resulted similar, indeed in 2016 purebreds represented 65% (93/142) of tumor cases, in 2017 were 60% (121/202), and in 2018 were 57% (71/124) (Figure 16 and Table 11).

Figure 16: Distribution in percentage (%) of dogs with tumor by breed and year



Dog breeds	2016	2017	2018
Mongrel	49	81	53
Purebred	93	121	71
Total number	142	202	124

Table 11: Distribution in number of dogs with tumor by breed and year

Incidence rate for purebred dogs was 158,8/100.000, subdivided in 78,4/100.00 and 80,4/100.000 for benign and malignant tumors respectively. Incidence rate for mongrel dogs was 163,2/100.000, subdivided in 87,6/100.00 and 75,6/100.000 for benign and malignant tumors respectively. These data and related 95% confidence intervals are reported in the table 12.

Table 12: Incidence rates of tumors by breed per 100.000 dogs and related 95% confidence intervals (CI) for the period 01/01/2016-31/08/2018.

BREED	Benign	95% C.I.	Malignant	95% C.I.	Total	95% C.I.
Purebred	78,4	66,6-91,7	80,4	68,4-93,9	158,8	141,8-177,3
Mongrel/mixed-breed	87,6	71,9-105,7	75,6	61,1-92,5	163,2	141,5-187,2

Proportional morbidity rates calculated by breed and year were reported in Table 13.

Table 13: Proportional morbidity rates expressed in percentage (PMR 100) by breed and year

Variable	PMR 100	2016	2017	2018	2016-2018
Breed	Purebred	65%	63%	63%	63%
	Mongrel	35%	37%	37%	37%

The most represented breeds in dogs with tumor were English setter, Miniature pinscher and German shepherd in 2016 (Table 14); Labrador retriever, Golden retriever and German shepherd in 2017 (Table 15); German shepherd, English setter and Golden retriever in 2018 (Table 16). In the whole period since 1st January 2016 till 31st August 2018, English setter, German shepherd and Labrador retriever were the tumor highest frequency breeds (Table 17).

Table 14: Distribution (number) of dogs with tumor by breed in 2016

DOG BREED	Negative for tumor	Positive for tumor	Total
Mongrel/mixed-breed	17	49	66
English setter		11	11
Miniature pinscher	1	9	10
German shepherd	1	8	9
Labrador retriever	2	4	6
Boxer		5	5
English springer spaniel	1	3	4
Miniature poodle	1	2	3
Dobermann pinscher		3	3
Golden retriever	1	2	3
Jack Russell terrier	1	2	3

American pit bull terrier	1	2	3
Beagle		2	2
French bouledogue		2	2
Cane Corso		2	2
Australian kelpie	1	1	2
Maremma sheepdog	1	1	2
Pug		2	2
Chihuahua		2	2
English cocker spaniel	1	1	2
Dogo Argentino		2	2
Epagneul breton	1	1	2
Maltese	1	1	2
Medium-size schnauzer		2	2
Italian short haired hound		2	2
Shih tzu		2	2
Siberian husky		2	2
Staffordshire bull terrier	1	1	2
Great Dane		1	1
Poodle		1	1
Dachshund		1	1
Bichon à poil frisé		1	1
Bolognese		1	1
Border collie	1		1
Boston terrier		1	1
Italian Bracco		1	1
German wired haired pointer dog - Drahataar		1	1
Czechoslovakian wolfdog	1		1
Chow-chow		1	1
American cocker spaniel		1	1
Brussels griffon		1	1
Lagotto Romagnolo		1	1
Pekingese	1		1
Rottweiler		1	1
Italian coarse haired hound	1		1
Gordon setter		1	1
Volpino Italiano		1	1
West highland white terrier		1	1
Yorkshire terrier	1		1
Total	**37**	**142**	**179**

Table 15: Distribution (number) of dogs with tumor by breed in 2017

DOG BREED	Negative for tumor	Positive for tumor	Total
Mongrel/mixed-breed	17	81	98
Labrador retriever	1	12	13
Golden retriever	1	9	10
German shepherd	3	7	10
Dachshund		8	8
English setter		7	7
Boxer	1	5	6
West highland white terrier	3	3	6
Miniature pinscher		5	5
Giant schnauzer	2	2	4
Yorkshire terrier		4	4

Akita		3	3
French bouledogue		3	3
Maremma shepdog		3	3
American cocker spaniel		3	3
Italian coarse haired hound	1	2	3
Italian short haired hound	1	2	3
Shih tzu		3	3
Miniature poodle		2	2
Beagle		2	2
Border collie	2		2
German short haired pointer dog - Kurzhaar	1	1	2
English cocker spaniel	1	1	2
Dobermann pinscher		2	2
Jack Russell terrier		2	2
Lagotto Romagnolo		2	2
White Swiss shepherd		2	2
American pit bull terrier		2	2
English springer spaniel		2	2
Staffordshire bull terrier		2	2
Volpino Italiano		2	2
Alaskan malamute		1	1
Poodle	1		1
Bichon à poil frisé		1	1
Boston terrier		1	1
Bernese mountain dog		1	1
Italian Bracco	1		1
Bull terrier		1	1
Bulldog		1	1
Australian kelpie	1		1
Scottish short haired shepherd dog	1		1
Cavalier king Charles spaniel		1	1
Chin		1	1
Chow-chow		1	1
Dogue de Bordeaux		1	1
Maltese		1	1
Rottweiler		1	1
Medium-size schnauzer		1	1
Miniature schnauzer		1	1
Siberian husky		1	1
Spinone Italiano		1	1
Terranova		1	1
Whippet		1	1
Total	**38**	**202**	**240**

Table 16: Distribution (number) of dogs with tumor by breed in 2018

DOG BREED	Negative for tumor	Positive for tumor	Total
Mongrel/mixed-breed	7	53	60
German shepherd	2	8	10
English setter	2	6	8
Golden retriever		6	6
Jack Russell terrier	1	3	4
Labrador retriever		4	4

Miniature pinscher		4	4
Dachshund		3	3
English springer spaniel	1	2	3
Poodle	1	1	2
Miniature poodle	1	1	2
Beagle		2	2
Bernese mountain dog		2	2
Boxer		2	2
Bracco Italiano		2	2
Bulldog	1	1	2
Maremma shepdog		2	2
English cocker spaniel		2	2
Italian greyhound	1	1	2
American pit bull terrier	2		2
Italian coarse haired hound		2	2
Italian short haired hound	1	1	2
German short haired pointer dog - Kurzhaar	1	1	2
Great Dane		1	1
Alaskan malamute		1	1
Bichon à poil frisé		1	1
Old English shephdog	1		1
French bouledogue		1	1
Great Pyrenees	1		1
Briquet griffon vendeen		1	1
Cane Corso		1	1
German wired haired pointer dog - Drahataar	1		1
Pug		1	1
Dogo Argentino		1	1
Dogue de Bordeaux		1	1
Irish setter		1	1
Greater Swiss mountain		1	1
Maltese		1	1
Medium-size schnauzer		1	1
Miniature German spitz		1	1
Staffordshire bull terrier		1	1
Weimaraner	1		1
Yorkshire terrier	1		1
Total	**26**	**124**	**150**

Table 17: Distribution (number) of dogs with tumor by breed in the period 01/01/2016-31/08/2018

DOG BREED	2016	2017	2018	Total
Mongrel/mixed-breed	49	81	53	183
English setter	11	7	6	24
German shepherd	8	7	8	23
Labrador retriever	4	12	4	20
Miniature pinscher	9	5	4	18
Golden retriever	2	9	6	17
Dachshund	1	8	3	12
Boxer	5	5	2	12
Jack Russell terrier	2	2	3	7
English springer spaniel	3	2	2	7
Beagle	2	2	2	6

DOG BREED	2016	2017	2018	Total
French bulldog	2	3	1	6
Maremma shepdog	1	3	2	6
Miniature poodle	2	2	1	5
Dobermann pinscher	3	2		5
Italian short haired hound	2	2	1	5
Shih tzu	2	3		5
American cocker spaniel	1	3		4
English cocker spaniel	1	1	2	4
American pit bull terrier	2	2		4
Medium-size schnauzer	2	1	1	4
Italian coarse haired hound		2	2	4
Staffordshire bull terrier	1	2	1	4
West Highland white terrier	1	3		4
Yorkshire terrier		4		4
Akita		3		3
Bichon à poil frisé	1	1	1	3
Bernese mountain dog		1	2	3
Italian Bracco	1		2	3
Cane Corso	2		1	3
Pug	2		1	3
Dogo Argentino	2		1	3
Lagotto Romagnolo	1	2		3
Maltese	1	1	1	3
Siberian husky	2	1		3
Volpino Italiano	1	2		3
Great Dane	1		1	2
Alaskan malamute		1	1	2
Poodle	1		1	2
Boston terrier	1	1		2
Bulldog		1	1	2
Chihuahua	2			2
Chow-chow	1	1		2
Dogue de Bordeaux		1	1	2
White Swiss shepherd		2		2
Rottweiler	1	1		2
Giant schnauzer		2		2
German short haired pointer dog - Kurzhaar		1	1	2
Bolognese	1			1
Briquet griffon vendeen			1	1
Bull terrier		1		1
German wired haired pointer dog - Drahataar	1			1
Australian kelpie	1			1
Cavalier king Charles spaniel		1		1
Chin		1		1
Brittany	1			1
Gordon setter			1	1
Greater Swiss mountain			1	1
Brussels griffon	1			1
Italian greyhound			1	1
Miniature schnauzer		1		1
Irish setter	1			1
Spinone Italiano		1		1
Miniature German spitz			1	1
Terranova		1		1
Whippet		1		1

DOG BREED	2016	2017	2018	Total
Total	**142**	**202**	**124**	**468**

Prevalence ratio (PR) for purebreds and mongrels was calculated and reported in Table 18.

Table 18: Prevalence ratio (PR) by breed in the period 01/01/2016-31/08/2018.

DOG BREEDS	Prevalence ratio (PR)	Positive for tumor	Total number of dogs for breed
Purebreds	83%	285	345
Mongrel/mixed-breeds	82%	183	224

2.3.3 Age distribution

The highest number of samples per age group expressed in years were from dogs clustered into the 'adult' group, that resulted also to be the group with the highest number of tumor diagnosis over the whole period of study, followed by 'senior' group (Table 19 and Figure 17). Both for samples and tumors number, the poorest group was of dogs clustered into the 'very young' group (Table 19 and Figure 17).

Percentage calculation of cancer cases by age revealed a strong preponderance of 'adult' (52%) and 'senior' (29%) groups towards 'very young', 'young' and 'very old' that resulted into a 2%, 7% and 10% respectively (Figure 18).

'Adult', 'senior' and 'very old' groups showed the highest prevalence of tumor, calculated as tumor cases/total number ratio for each age group: 82% (245/298), 87% (138/159), and 85% (45/53) were the respectively results (Figure 17). Prevalence of tumor cases in 'young' and 'very young' groups were of 70% (32/46) and 61% (8/13) (Figure 17).

Table 19: Distribution (number) of dogs with tumor by age group and year

Year and Age Group	Negative for tumor	Positive for tumor	Total
2016	**37**	**142**	**179**
Very young	2	3	5
Young	1	12	13
Adult	23	74	97
Senior	8	37	45
Very old	3	16	19
2017	**38**	**202**	**240**
Very young		2	2
Young	5	13	18

Adult	19	105	124
Senior	10	71	81
Very old	4	11	15
2018	**26**	**124**	**150**
Very young	3	3	6
Young	8	7	15
Adult	11	66	77
Senior	3	30	33
Very old	1	18	19
Total	**101**	**468**	**569**

Figure 17: Distribution of dogs, total number and positive for tumor, by age group in the whole period of study (01 Jan 2016 - 31 Aug 2018).

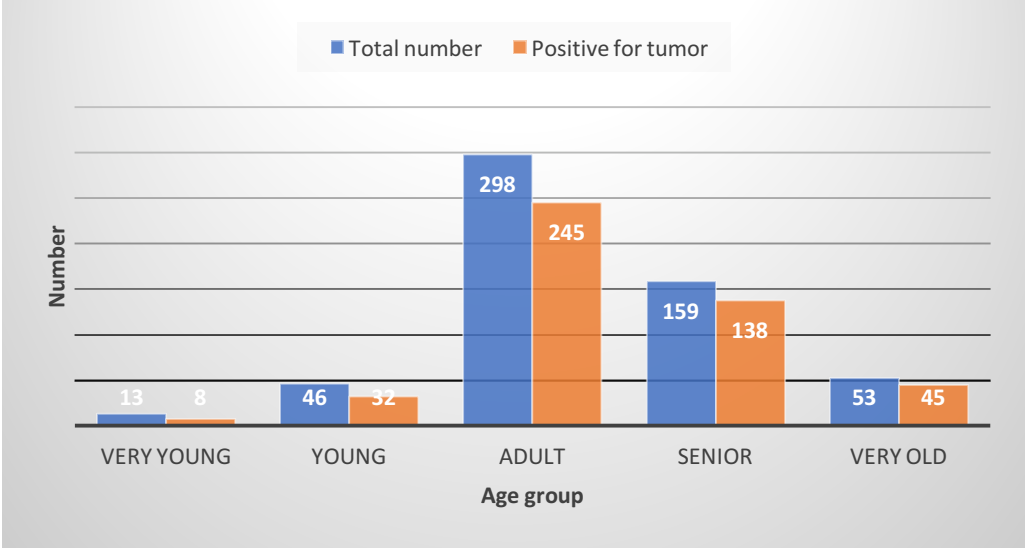
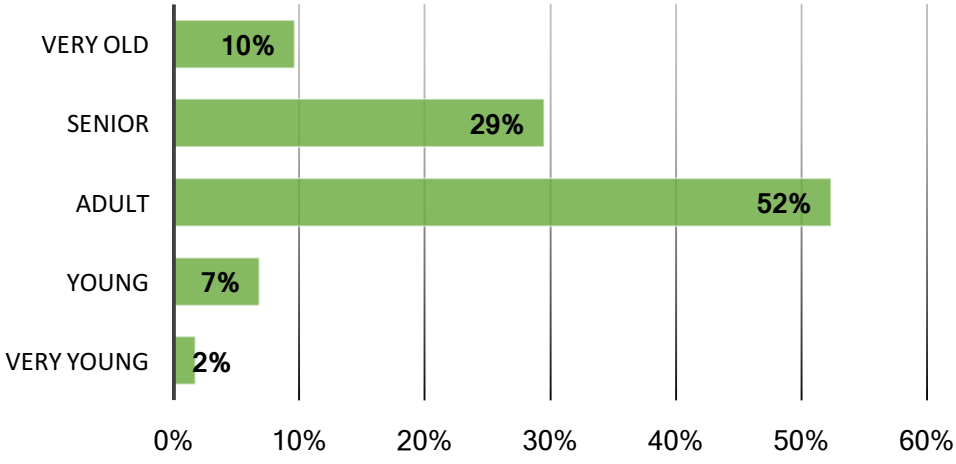


Figure 18: Distribution in percentage (%) of dogs with tumor by age group in the period of study (01 Jan 2016 – 31 Aug 2018)



Proportional morbidity rates calculated by age group and year were reported in Table 20.

Table 20: Proportional morbidity rates expressed in percentage (PMR 100) by age group and year

Variable	PMR 100	2016	2017	2018	2016-2018
Age group	Very young	-	-	2%	0,4%
	Young	5%	5%	2%	4%
	Adult	59%	47%	43%	50%
	Senior	26%	36%	33%	32%
	Very old	10%	5%	20%	10%

2.3.4 Sex distribution

Both in total number of dogs analyzed and in tumor cases, sex was not a discriminant. In the whole period of study, females registered were 289: 91 in 2016, 121 in 2017, and 77 in 2018; males were 280: 88 in 2016, 119 in 2017, and 73 in 2018 (Table 21).

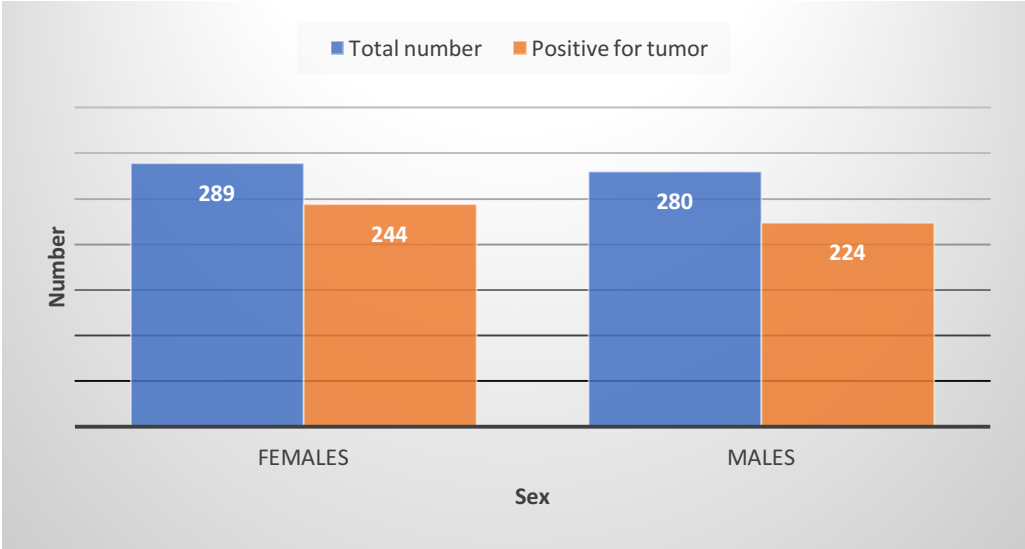
Tumors occurred most frequently in females (84%, 244/289), with a negligible difference compared to males (80%, 224/280) (Figure 19).

Reproductive status was not reported because veterinary practitioners cannot modify this variable in SIVA, so data are unreliable and the true number of neutered dogs is strongly underestimated.

Table 21: Distribution (number) of dogs with tumor by sex and year

Year and Sex	Negative for tumor (n)		Positive for tumor (n)	
	F	M	F	M
2016	**20**	**17**	**71**	**71**
2017	**13**	**25**	**108**	**94**
2018	12	14	65	59
Total	45	56	244	224

Figure 19: Distribution of dogs, total number and positive for tumor, by sex in the whole period of study (01 Jan 2016 - 31 Aug 2018).



Dogs were also evaluated by sex associated to age group. Both in females and males, 'adult' was the most represented age group over the whole period of study, followed by 'senior', without any significant differences between sexes (Table 22 and Figure 20-23). In 2016, there was a little difference between 'young' groups, indeed in males this age group was more represented (14%, 12/88) than in females (1%, 1/91) (Table 22 and Figure 20).

Percentage calculation of cancer cases by sex and age revealed a high frequency in 'adult' and 'senior' groups, both in females and in males, towards 'very young', 'young' and 'very old' (Figure 24).

Similar to analysis by age group, also evaluating males and females, 'Adult', 'senior' and 'very old' groups showed the highest prevalence of tumor, without any difference between sexes: 79% (111/140), 87% (63/72), and 81% (26/32) in males, and 85% (134/158), 86% (75/87), and 90% (19/21) in females were the respective results (Figure 25). Prevalence of tumor cases in 'young' and 'very young' groups were of 50% (4/8) and 71% (20/28) in males, and 80% (4/5) and 67% (12/18) in females (Figure 25).

Table22I: Distribution (number) of dogs with tumor by sex, age group and year

Year, age group and sex	Negative for tumor		Positive for tumor	
	F	M	F	M
2016	**20**	**17**	**71**	**71**
Very young	1	1	2	1
Young	0	1	1	11
Adult	12	11	43	31
Senior	5	3	18	19

Very old	2	1	7	9
2017	**13**	**25**	**108**	**94**
Very young	0	0	0	2
Young	1	4	5	8
Adult	8	11	57	48
Senior	4	6	41	30
Very old	0	4	5	6
2018	**12**	**14**	**65**	**59**
Very young	0	3	2	1
Young	5	3	6	1
Adult	4	7	34	32
Senior	3	0	16	14
Very old	0	1	7	11
Total	**45**	**56**	**244**	**224**

Figure 20: Age pyramid of dog with tumor in 2016

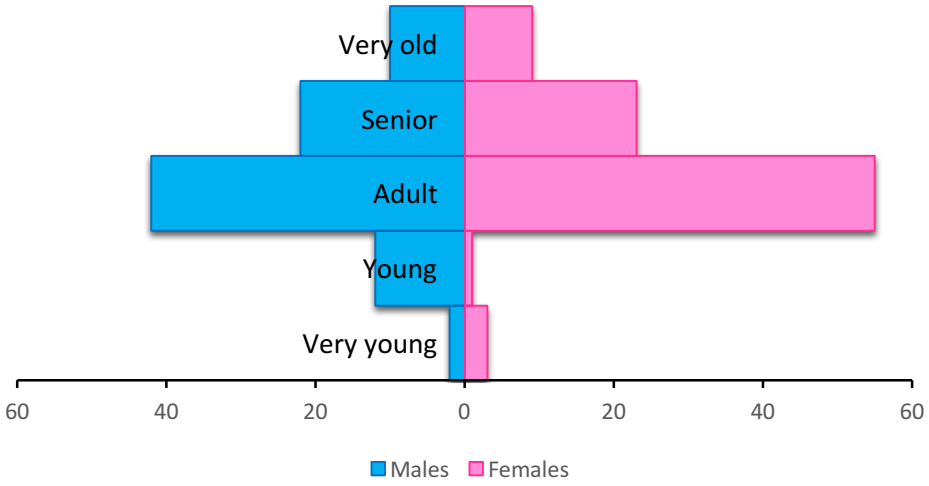


Figure 21: Age pyramid of dog with tumor in 2017

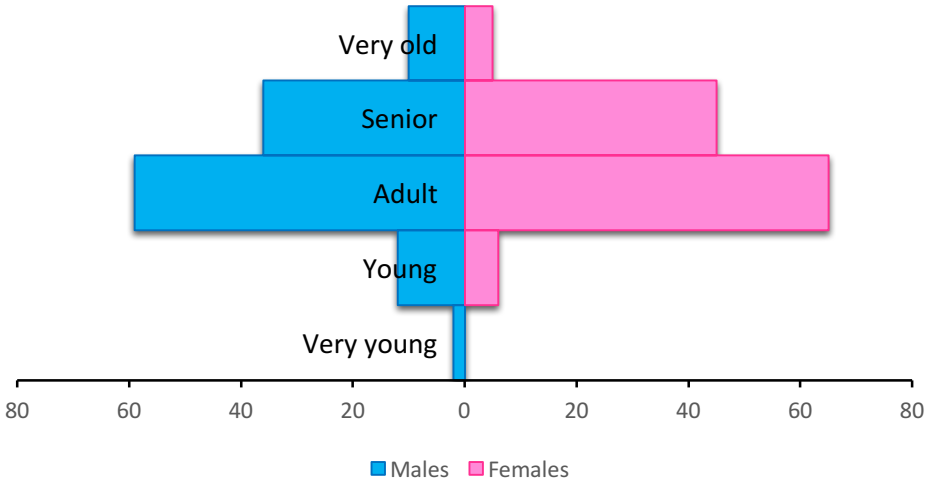


Figure 22: Age pyramid of dog with tumor in 2018

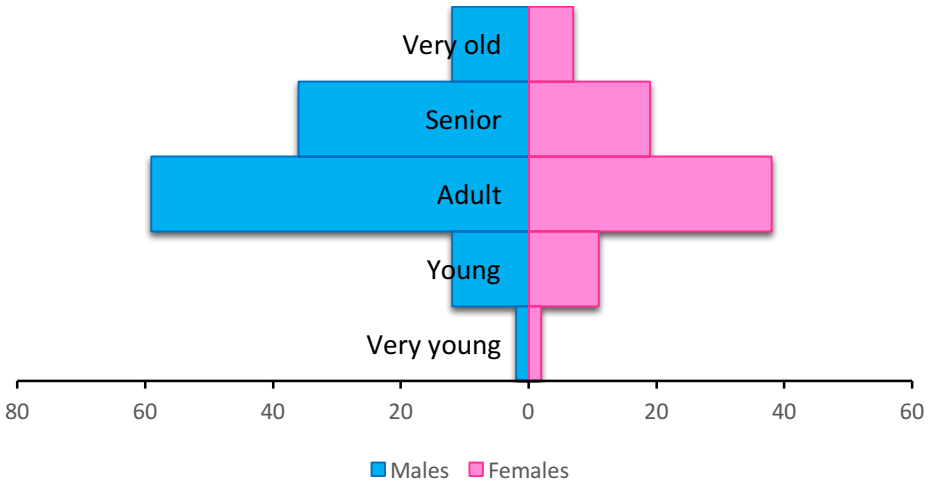


Figure 23: Age pyramid of dog with tumor in the whole period of study (01 Jan 2016 - 31 Aug 2018)

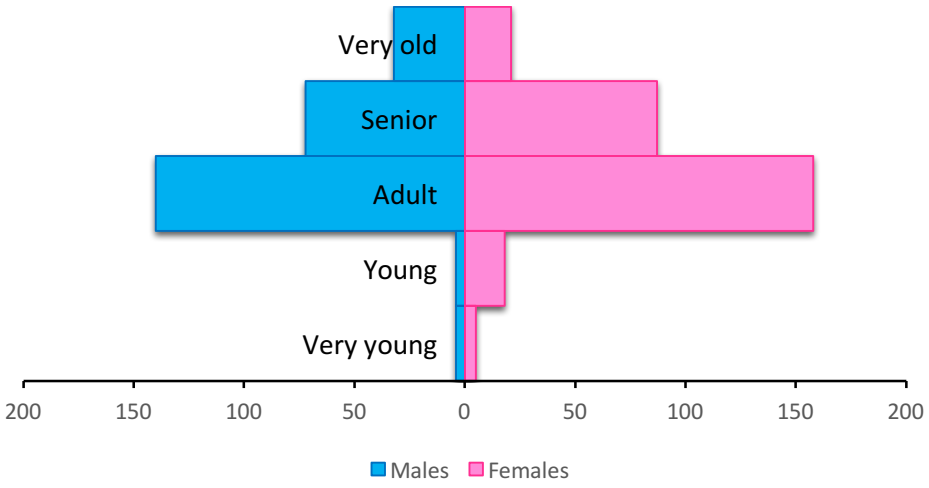


Figure 24: Distribution in percentage (%) of dogs with tumor by sex and age group in the period of study (01 Jan 2016 – 31 Aug 2018)

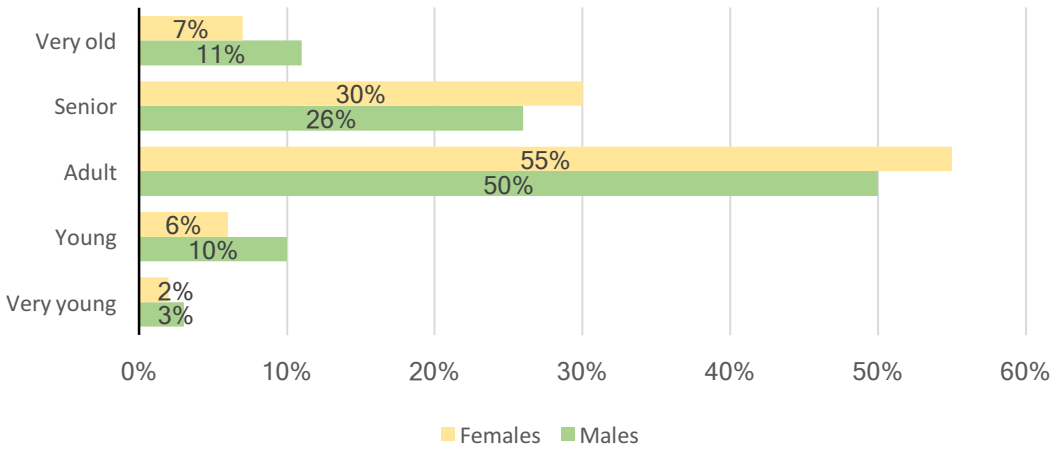
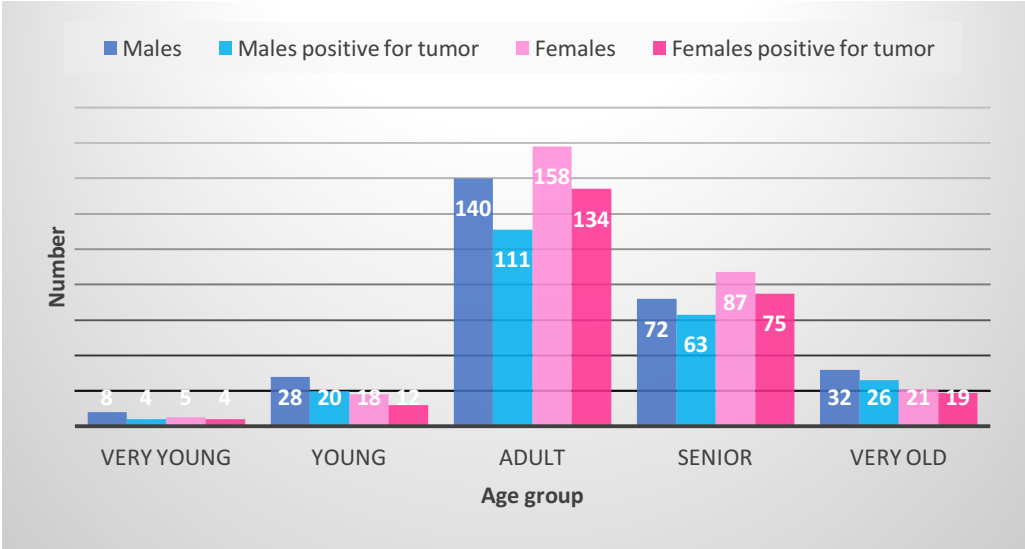


Figure 25: Distribution of dogs, total number and positive for tumor, by sex and age group in the whole period of study (01 Jan 2016 - 31 Aug 2018)



2.3.5 Lifestyle

The highest number of examined samples by habitat was from dogs living in urban areas, both per each year of study (Table 23) and in the whole period as final total count (Figure 26). Urban habitat resulted also to be the group with the higher number of tumor diagnosis over the whole period of study (Table 23). For a discrete number of dogs, extracted data could not determine living habitat and was reported as 'undetermined' group.

Percentage calculation of cancer cases by habitat revealed a strong preponderance of 'urban' (56%) group towards 'rural', that resulted into a 32% (Figure 26).

On the contrary, there is no difference in prevalence of tumor, calculated as tumor/total number ratio for each group, since for both urban (261/325) and rural (152/187) habitat, was about 80% (Table 23 and Figure 26).

Proportional morbidity rates calculated by habitat and year were reported in Table 24.

Table 23: Distribution (number) of dogs with tumor by habitat and year

Habitat and year	Negative for tumor	Positive for tumor	Total
2016	**37**	**142**	**179**
Rural	9	42	51
Urban	27	90	117
Undetermined	1	10	11
2017	**38**	**202**	**240**
Undetermined	1	41	42

Rural	16	58	74
Urban	21	103	124
Undetermined	1	41	42
2018	**26**	**124**	**150**
Rural	10	52	62
Urban	16	68	84
Undetermined		4	4
Total	**101**	**468**	**569**

Figure 26: Distribution in percentage (%) of dogs, total and with diagnosis of cancer, by habitat in the period of study (01 Jan 2016 – 31 Aug 2018)

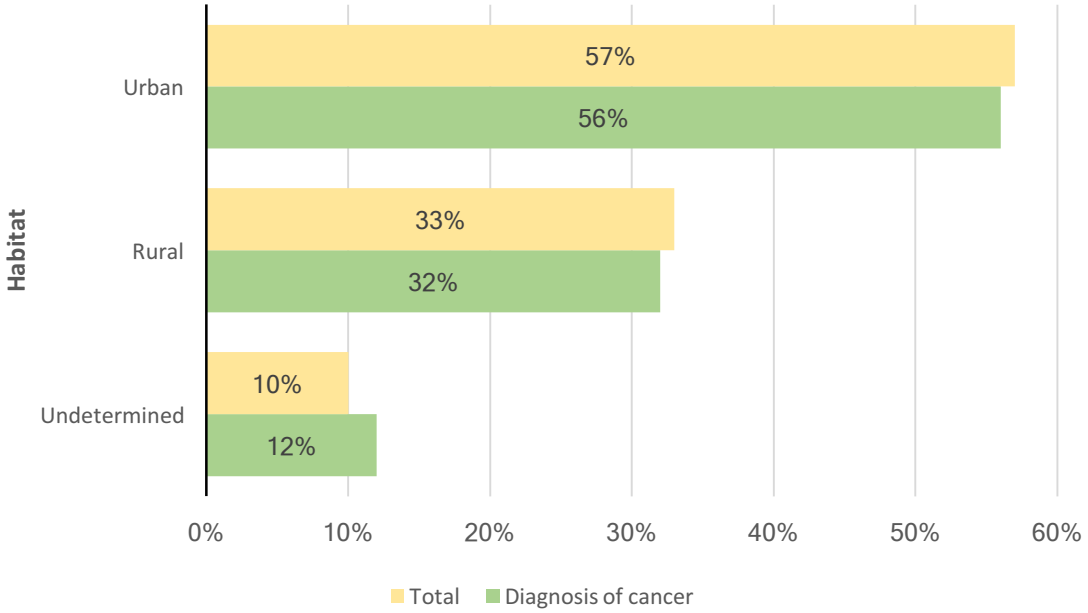


Table 24: Proportional morbidity rates expressed in percentage (PMR 100) by habitat and year

Variable	PMR 100	2016	2017	2018	2016-2018
Habitat	Urban	65%	53%	52%	57%
	Rural	28%	25%	44%	30%

Most of the examined dogs resulted to live in an indoor/outdoor mixed housing, followed by indoor and outdoor housing; only a little number of samples came from dogs housed in kennel (Table 25 and Figure 27). Similarly, tumors occurred most frequently in dogs housed in indoor/outdoor mixed (38%, 176/468) and indoor housing (28%, 130/468), followed by outdoor housing (18%, 84/468) and kennel (5%, 23/468) (Figure 27). Like for habitat, data on a certain number of dogs did not indicate housing type, for this reason a group named 'undetermined' was set up.

Prevalence of tumor, always calculated as tumor/total number ratio for each group, resulted higher in mixed I/O (86%, 176/205) and outdoor (81%, 84/104) housed dogs. For indoor and kennel was 76% (130/170) and 72% (23/32) respectively (Table 25 and Figure 27).

Proportional morbidity rates calculated by housing and year were reported in Table 26.

Table 25: Distribution (number) of dogs with tumor by housing and year

Year and housing	Negative for tumor	Positive for tumor	Total
2016	**37**	**142**	**179**
Indoor (I)	17	47	64
Outdoor (O)	8	27	35
Mixed I/O	10	59	69
Kennel	2	7	9
Undetermined		2	2
2017	**38**	**202**	**240**
Indoor (I)	15	46	61
Outdoor (O)	9	37	46
Mixed I/O	8	65	73
Kennel	3	6	9
Undetermined	3	48	51
2018	**26**	**124**	**150**
Indoor (I)	8	37	45
Outdoor (O)	3	20	23
Mixed I/O	11	52	63
Kennel	4	10	14
Undetermined		5	5
Total	**101**	**468**	**569**

Figure 27: Distribution in percentage (%) of dogs, total and with diagnosis of cancer, by housing in the period of study (01 Jan 2016 – 31 Aug 2018)

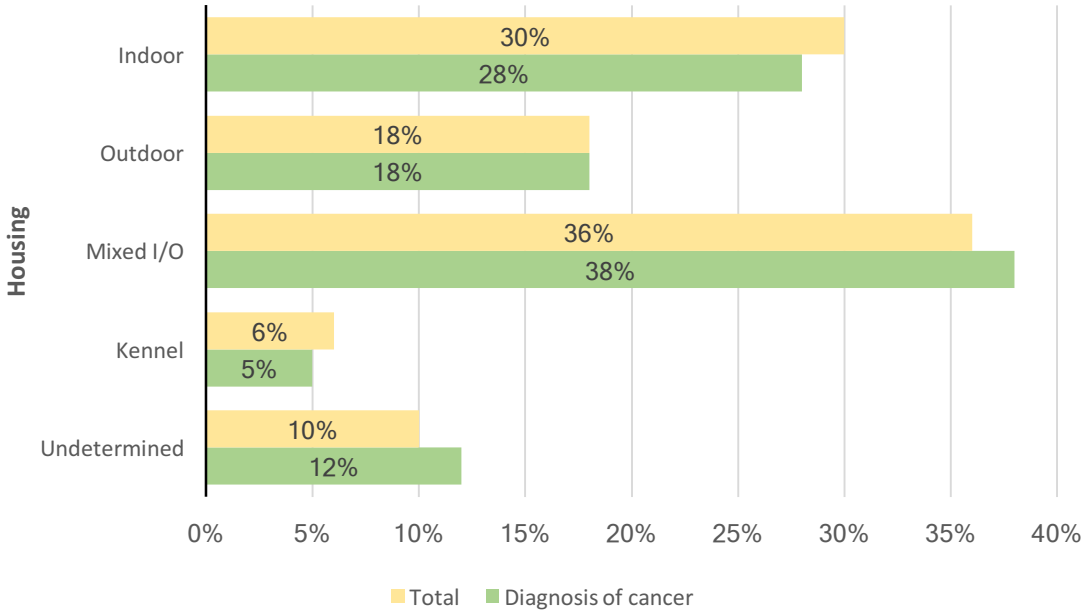
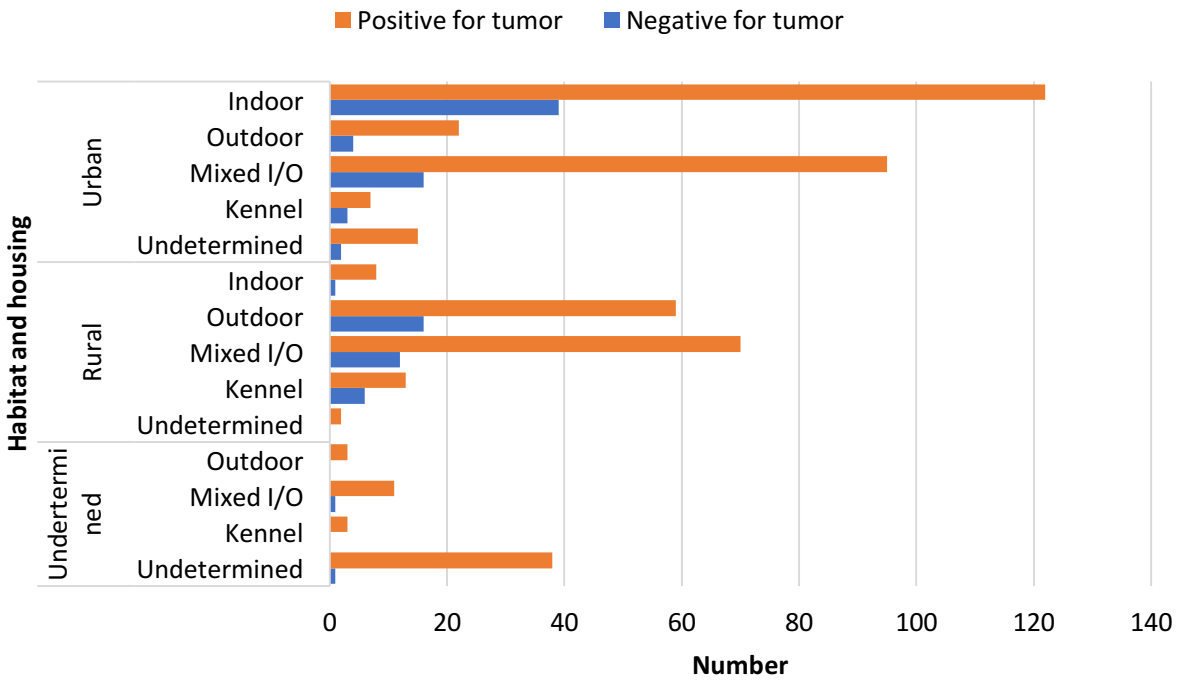


Table 26: Proportional morbidity rates expressed in percentage (PMR 100) by housing and year

Variable	PMR 100	2016	2017	2018	2016-2018
Housing	Indoor	37%	20%	26%	27%
	Outdoor	15%	19%	13%	17%
	Mixed I/O	45%	27%	50%	38%
	Kennel	2%	4%	9%	5%

Crossing habitat and housing data, tumors occurred more frequently in indoor (47%, 122/261) and indoor/outdoor mixed (36%, 95/261) housing for dogs living in urban areas, and in indoor/outdoor mixed (46%, 70/152) and outdoor (39%, 59/152) housing for dogs living in rural habitat (Figure 28).

Figure 28: Distribution (number) of dogs by diagnostic outcome, habitat and housing in the whole period of study (01 Jan 2016 – 31 Aug 2018)



Data on feeding showed commercial dry food (38%, 216/569) as the most chosen diet, followed by homemade/dry food (18%, 67/569), homemade/canned food (11%, 65/569) and homemade/canned/dry food (11% 62/569) mixed diet. For a good number of dogs (15%, 88/569), feeding type was not known ('undetermined' group) since not reported by the veterinary practitioner at the moment of histopathology analysis request (Table 27 and Figure 29).

Commercial dry food diet showed the highest frequency of tumor (38%, 177/468), followed by homemade/canned/dry food (12%, 55/468), homemade/dry food (11%, 53/468), and homemade/canned food (11%, 50/468) mixed diet (Table 27 and Figure 29).

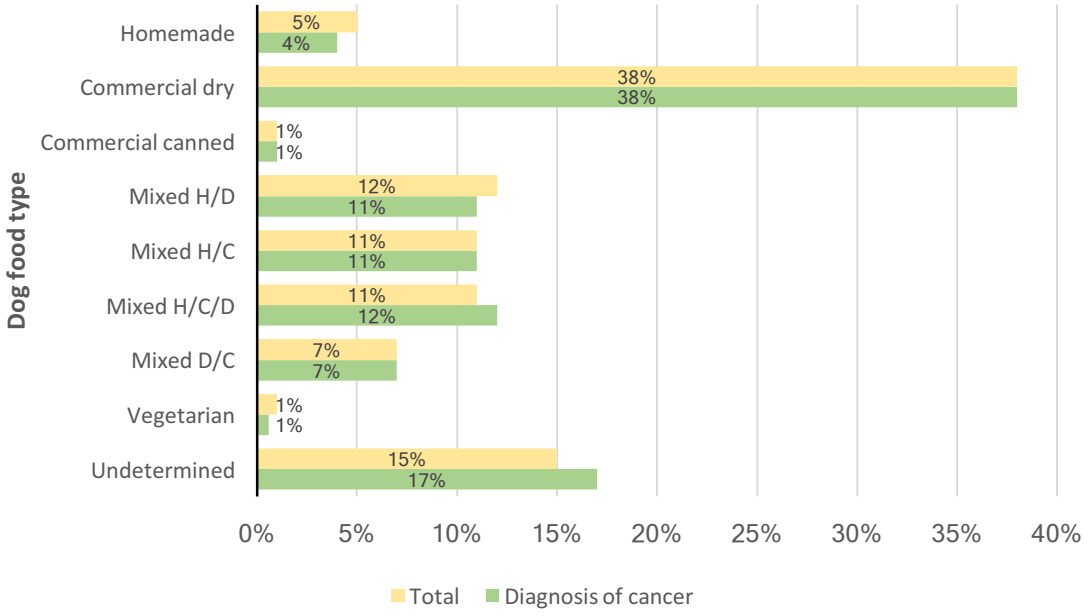
Prevalence of tumor, calculated as tumor/total number ratio for each group, did not show any significant difference given fluctuations of only few percentage points in a range from 77% to 89% (Table 27 and Figure 29).

Table 27: Distribution (number) of dogs by diagnostic outcome and diet

Dog food type	Negative for tumor	Positive for tumor	Total
Homemade (H)	6	20	26
Commercial dry (D)	39	177	216
Commercial canned (C)		3	3
Mixed H/D	14	53	67

Mixed H/C	15	50	65
Mixed H/C/D	7	55	62
Mixed D/C	9	32	41
Vegetarian	1		1
Undetermined	10	78	88
Total	**101**	**468**	**569**

Figure 29: Distribution in percentage (%) of dogs, total and with diagnosis of cancer, by food diet in the period of study (01 Jan 2016 – 31 Aug 2018)

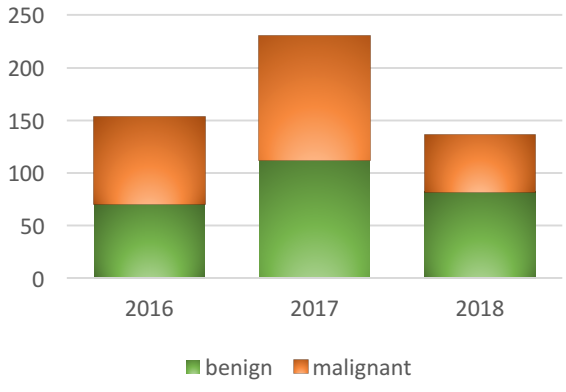


2.3.6 Most common tumor types

The total number of diagnosed tumor was 519, with a clear prevalence in 2017. Both in 2016 and 2017, there was no significant difference between benign and malignant tumors. In 2018, malignant were proportionally more represented than benign tumors (Table 29 and Figure 30).

Table 29 and Figure 30: Tumor distribution (number) per year and diagnostic outcome

Year	Benign	Malignant	Total
2016	71	82	153
2017	112	118	230
2018	82	54	136
Total	**265**	**254**	**519**



Analysis of most common tumor types in the examined dogs revealed that cutaneous adnexal and mast cell tumors (both 13,5%) were the most common, followed by adenomas and adenocarcinomas (13,1%), blood vessel tumors (7,9%), and gonadal tumor /7,1%). Other tumor types showed a lower frequency, appearing by a percentage below 6%. The frequency of tumor types is shown in Table 30.

Table 31 shows in detail which tumors were diagnosed for each broad group, based on the ICD-O classification. Among cutaneous adnexal tumors, one of the most frequent broad group (13,5%), sebaceous gland adenoma (20/70) and hepatoid gland adenoma (17/70) and carcinoma (11/70) were the most diagnosed tumors. In mast cells tumors group (13,5%), mast cells tumor code used for subcutaneous and visceral tumors was the most frequent (40/70). Among adenomas and adenocarcinomas (13,1%), mammary tumors, such as simple mammary adenoma (19/68) and carcinoma (14/68), complex mammary adenoma (11/68) and solid mammary carcinoma (10/68), occurred mostly. Hemangiosarcoma (16/41), followed by hemangiopericytoma (9/41) and cavernous (9/41) and capillary (6/41) hemangioma, represented the most frequent neoplasia among blood vessels tumors (7,9%). Gonadal tumors (7,1%) occurred mostly in males, indeed testicular tumors, such as Leydig cell (17/37) and Sertoli cell (13/37) tumors, were the most numerous of the tumor group.

The highest percentage of malignant tumors was found among mast cells tumor (100%, 70/70), soft tissue tumors and sarcomas (100%, 17/17), lymphomas (100%, 5/5), papillomas and transitional cells carcinomas (100%, 5/5), epithelial tumors (96%, 26/27), nevi and melanomas (88%, 15/17), germ cells tumors (80%, 8/10) (Table 30 and Figure 31).

Table 30: Distribution, in number and percentage, of tumor types by diagnostic outcome

Morphologic broad group	Benign tumor	Malignant tumor	Total (n)	Total (%)
Cutaneous adnexal tumors	50	20	70	13,5%
Mast cells tumors		70	70	13,5%
Adenomas and adenocarcinomas	32	36	68	13,1%
Blood vessels tumors	24	17	41	7,9%
Gonadal tumors	36	1	37	7,1%
Fibromatous tumors	26	5	31	6,0%
Epithelial tumors NOS*	1	26	27	5,2%
Complex, mixed, and stromal tumors	17	8	25	4,8%
Basal cells tumors	17	2	19	3,7%
Lipomatous tumors	18	1	19	3,7%
Soft tissue tumors and sarcomas NOS*		17	17	3,3%
Odontogenic tumors	16	1	17	3,3%
Nevi and melanomas	2	15	17	3,3%
Papillary and spinocellular tumors	5	6	11	2,1%
Germ cells tumors	2	8	10	1,9%
Myomatous tumors	7		7	1,3%

Lymphomas NOS* or diffused		5	5	1,0%
Papillomas and transitional cells carcinomas		5	5	1,0%
Peripheral nerve tumors	3	1	4	0,8%
Histiocytic tumors	1	3	4	0,8%
Cystic, mucinous and serous tumors	3		3	0,6%
Mature B-cell lymphomas		2	2	0,4%
Ductal, lobular and medullary tumors	1	1	2	0,4%
Neoplasms NOS*	1	1	2	0,4%
Mieloproliferative and lymphoproliferative disorders (miscellaneous)	1		1	0,2%
Fibroepithelial tumors	1		1	0,2%
Myxomatous tumors		1	1	0,2%
Neuroepitheliomatous tumors		1	1	0,2%
Plasm cells tumors	1		1	0,2%
Osteomas and osteosarcomas		1	1	0,2%
Total	**265**	**254**	**519**	**100%**

*NOS - Not Otherwise Specified - is used when a topographic or morphologic term has an adjective that does not appear elsewhere or when a term is used in a general sense.

Table 31: Distribution (number) of tumor types, described by morphologic diagnosis, and diagnostic outcome.

	Benign	Malignant	Total
Cutaneous adnexal tumors	**50**	**20**	**70**
Infundibular keratinizing acanthoma	6		6
Hepatoid gland adenoma	16	1	17
Meibomian gland adenoma	6		6
Sebaceous gland adenoma	20		20
Apocrine gland adenoma		2	2
Apocrine gland carcinoma (mixed and complex)		1	1
Ceruminous gland carcinoma (mixed and complex)		1	1
Hepatoid gland carcinoma		11	11
Metastatic hepatoid gland carcinoma		1	1
Eccrine carcinoma		2	2
Sebaceous gland carcinoma		1	1
Meibomian epithelioma	1		1
Sebaceous epithelioma	1		1
Mast cells tumors		**70**	**70**
Mast cells tumor		40	40
Canine mast cells tumor grade 1		11	11
Canine mast cells tumor grade 2		17	17
Canine mast cells tumor grade 3		2	2
Adenomas and adenocarcinomas	**32**	**36**	**68**
Follicolar adenocarcinoma NOS*		1	1
Adenocarcinoma NOS*		5	5
Tubulo-papillary adenocarcinoma		1	1
Complex mammary adenoma	11		11

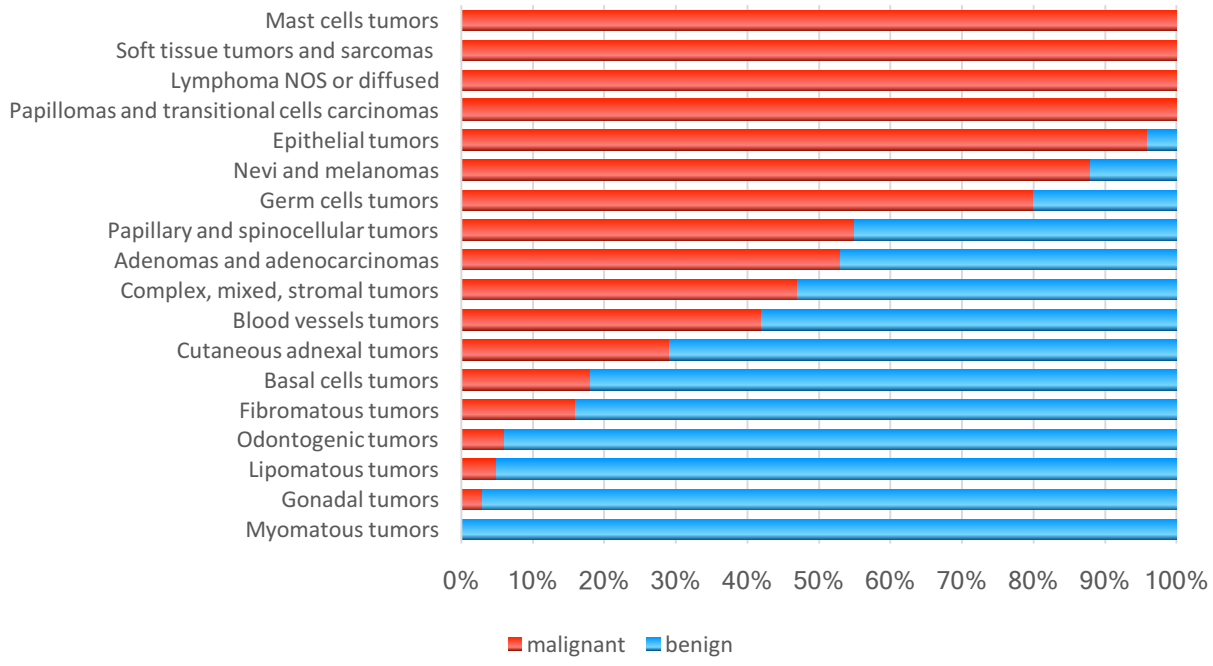
Simple mammary adenoma	19		19
Tubulo-papillary adenoma	1		1
Carcinoid (neuroendocrine tumor)		1	1
Cholangiocellular carcinoma		1	1
Simple carcinoma		1	1
Simple mammary carcinoma	1	13	14
Solid mammary carcinoma		10	10
Metastatic solid carcinoma NOS*		1	1
Simple tubulo-papillary mammary carcinoma		2	2
Blood vessels tumors	**24**	**17**	**41**
Capillary hemangioma	6		6
Cavernous hemangioma	9		9
Hemangiopericytoma NOS*	9		9
Hemangiosarcoma		16	16
Metastatic hemangiosarcoma		1	1
Gonadal tumors	**36**	**1**	**37**
Luteoma cell tumor NOS*	2		2
Sertoli-Leydig cell tumor	2		2
Leydig cell tumor	17		17
Sertoli cell tumor	13		13
Granulosa cell tumor		1	1
Mixed germ cell-sex cord-stromal tumor	2		2
Fibromatous tumors	**26**	**5**	**31**
Fibroma	1		1
Fibrosarcoma NOS*		4	4
Canine cutaneous histiocytoma	25	1	26
Epithelial tumors NOS*	**1**	**26**	**27**
Complex mammary carcinoma		25	25
Carcinoma In-situ NOS*	1		1
Carcinoma NOS*		1	1
Complex, mixed and stromal tumors	**17**	**8**	**25**
Carcinoma in polymorphous adenoma	1	3	4
Myoepithelioma	6		6
Malignant myoepithelioma (myoepithelial carcinoma)		2	2
Benign mixed tumor	10		10
Malignant mixed tumor NOS*		2	2
		1	1
Neoplasie lipomatose	**18**	**1**	**19**
Infiltrating lipoma	1		1
Lipoma NOS*	17		17
Well differentiated liposarcoma		1	1
Basal cells tumor	**17**	**2**	**19**
Basosquamous carcinoma		2	2
Trichoblastoma	1		1
Trichoblastoma ribbon type	8		8
Trabecular trichoblastoma	2		2
Trichoepithelioma	2		2

Trichofolliculoma	4		4
Odontogenic tumors	**16**	**1**	**17**
Canine acanthomatous ameloblastoma (acanthomatous epulis)	2		2
Ameloblastoma NOS*		1	1
Odontogenic fibroma NOS*	14		14
Soft tissue tumors and sarcomas NOS*		**17**	**17**
Round cell sarcoma		5	5
Sarcoma NOS*		11	11
Metastatic sarcoma NOS*		1	1
Nevi and melanomas	**2**	**15**	**17**
Melanocytoma NOS*	2		2
Malignant spindle cell melanoma NOS*		4	4
Amelanotic malignant melanoma		2	2
Amelanotic malignant melanoma mixed type		1	1
Malignant epithelioid and spindle cell melanoma		8	8
Papillary and spinocellular tumors	**5**	**6**	**11**
Squamous cell carcinoma		1	1
Subungual squamous cell carcinoma		1	1
Squamous carcinoma		4	4
Dyskeratosic papilloma	1		1
Spinocellular papilloma	1		1
Squamous papilloma	1		1
Papillomatosis NOS*	2		2
Germ cell tumors	**2**	**8**	**10**
Seminoma diffuse type	1	4	5
Seminoma intratubular type	1	4	5
Myomatous tumors	**7**		**7**
Angioleiomyoma	3		3
Leiomyoma NAS	4		4
Papillomas and transitional cells carcinomas		**5**	**5**
Transitional cells carcinoma NOS*		4	4
Papillary transitional cells carcinoma		1	1
Lymphomas NOS* or diffuse		**5**	**5**
Diffuse well-differentiated lymphocytic lymphoma		1	1
Lymphomas NOS*		4	4
Peripheral nerve tumors	**3**	**1**	**4**
Schwannoma NOS*	2	1	3
Peripheral sheath nerve tumor (neurofibroma, schwannoma)	1		1
Histiocytic tumors	**1**	**3**	**4**
Cutaneous histiocytosis	1		1
Histiocytic sarcoma / malignant histiocytosis		3	3
Cystic, mucinous and serous tumors	**3**		**3**
Papillary cystadenoma NOS*	3		3
Ductal, lobular and medullary tumors	**1**	**1**	**2**
Ductal adenoma NOS*	1		1
Metastatic inflammatory carcinoma		1	1
Tumors NOS*	**1**	**1**	**2**

Unclassified malignant tumor		1	1
Tumor NOS*	1		1
Mature B-cell lymphomas		**2**	**2**
Marginal zone lymphoma		2	2
Osteomas and osteosarcomas		**1**	**1**
Productive osteoblastic osteosarcoma		1	1
Plasm cells tumors	**1**		**1**
Plasmacytoma NOS*	1		1
Mieloproliferative and lymphoproliferative disorders (miscellaneous)	**1**		**1**
Chronic lymphoproliferative disorder	1		1
Neuroepitheliomatous tumors		**1**	**1**
Neuroblastoma NOS*		1	1
Myxomatous tumors		**1**	**1**
Mixosarcoma		1	1
Fibroepithelial tumors	**1**		**1**
Fibroadenoma	1		1
Total	**265**	**254**	**519**

*NOS - Not Otherwise Specified - is used when a topographic or morphologic term has an adjective that does not appear elsewhere or when a term is used in a general sense.

Figure 31: Percentage proportion of tumor types (above 5 cases) by malignancy



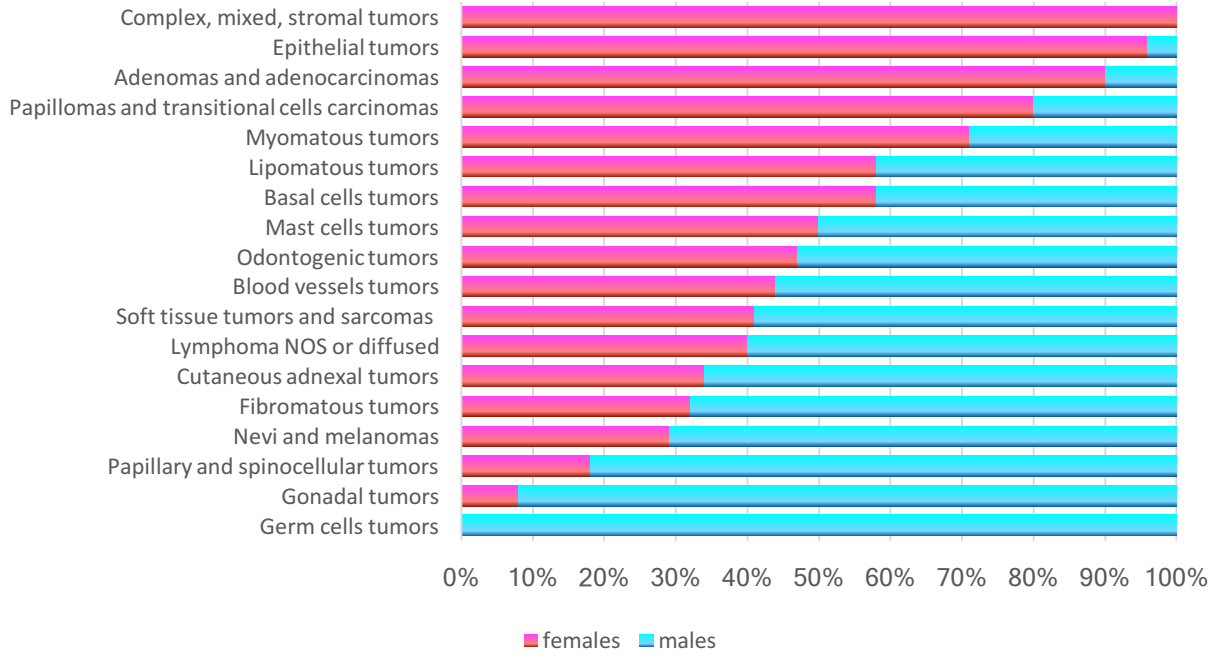
Analysis of tumor types by sex showed a highest frequency in females of complex, mixed, and stromal tumors (100%, 25/25), epithelial tumors (96%, 26/27), adenomas and adenocarcinomas (90%, 61/68), papillomas and transitional cells carcinomas (80%, 4/5), myomatous (71%, 5/7), lipomatous (58%, 11/19), and basal cells tumors (58%, 11/19). In mast cells tumors, a perfectly balance happened since both females and males showed a 50%

frequency (35/70). While for males, the most frequent tumors were germ cells (100%, 10/10) and gonadal (92%, 34/37) tumors, papillary and spinocellular tumors (82%, 9/11), nevi and melanomas (71%, 12/17), fibromatous tumors (68%, 21/31), cutaneous adnexal tumors (66%, 46/70), lymphomas (60%, 3/5), soft tissue tumors and sarcomas (59%, 10/17), blood vessels (56%, 23/41) and odontogenic (53%, 9/17) tumors (Table 32 and Figure 32).

Table 32: Distribution (number) of tumor types by sex

Morphologic broad group	Females	Males	Total (n)
Cutaneous adnexal tumors	24	46	70
Mast cells tumors	35	35	70
Adenomas and adenocarcinomas	61	7	68
Blood vessels tumors	18	23	41
Gonadal tumors	3	34	37
Fibromatous tumors	10	21	31
Epithelial tumors NOS*	26	1	27
Complex, mixed, and stromal tumors	25	0	25
Basal cells tumors	11	8	19
Lipomatous tumors	11	8	19
Soft tissue tumors and sarcomas NOS*	7	10	17
Odontogenic tumors	8	9	17
Nevi and melanomas	5	12	17
Papillary and spinocellular tumors	2	9	11
Germ cells tumors	0	10	10
Myomatous tumors	5	2	7
Lymphomas NOS* or diffused	2	3	5
Papillomas and transitional cells carcinomas	4	1	5
Peripheral nerve tumors	0	1	4
Histiocytic tumors	3	1	4
Cystic, mucinous and serous tumors	2	1	3
Mature B-cell lymphomas	2	0	2
Ductal, lobular and medullary tumors	1	1	2
Neoplasms NOS*	1	1	2
Mieloproliferative and lymphoproliferative disorders (miscellaneous)	1	0	1
Fibroepithelial tumors	0	1	1
Myxomatous tumors	1	0	1
Neuroepitheliomatous tumors	0	1	1
Plasm cells tumors	1	0	1
Osteomas and osteosarcomas	1	0	1
Total	**265**	**254**	**519**

Figure 32: Percentage proportion of tumor types (above 5 cases) by sex



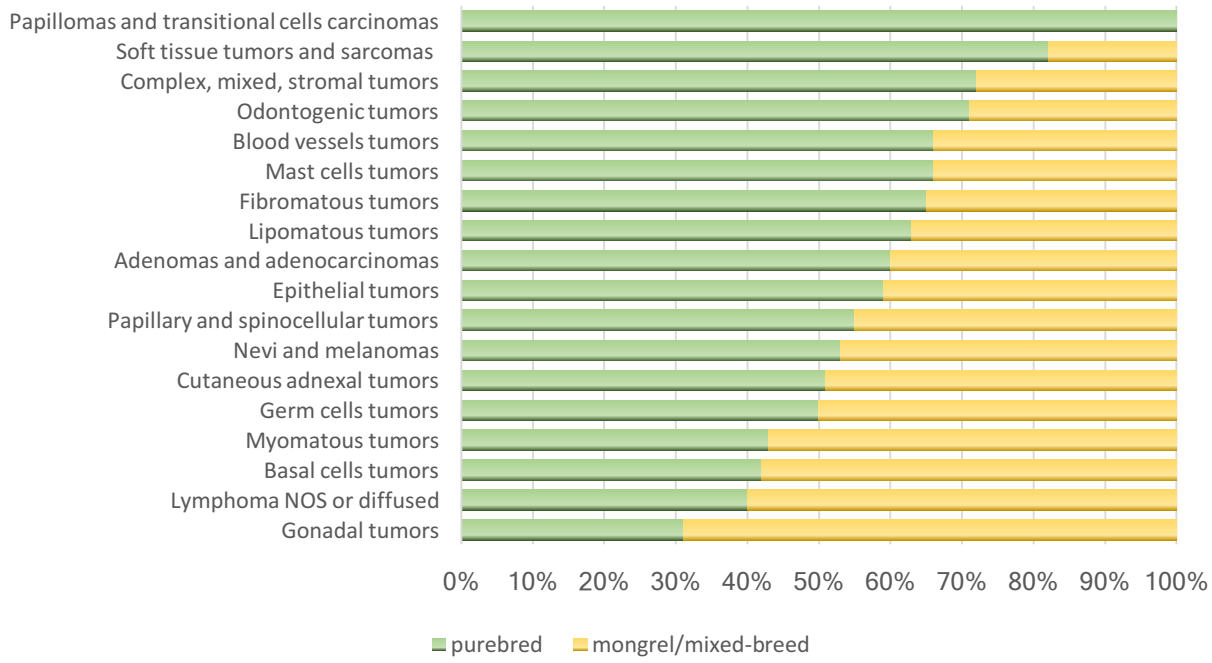
The most frequent tumor types mostly involved purebred dogs, which showed a percentage frequency always greater than in mongrels and mixed-breed dogs. Papillomas and transitional cells carcinomas occurred only in purebreds (100%, 5/5), and other tumor types, such as soft tissue tumors and sarcomas (82%, 15/17), complex, mixed and stromal tumors (72%, 18/25), and odontogenic tumors (71%, 12/17), resulted much more common in purebred dogs than in mongrels and mixed-breeds. Regarding breeds, point of balance was represented by the germ cells tumor since frequency was 50% (5/10) both in purebreds and mongrels/mixed-breeds. Gonadal tumors mostly involved mongrels/mixed-breed dogs, sharing this trend with lymphomas, and showing a frequency that did not overpass 69% (15/37) and 60% (3/5) respectively (Table 33 and Figure 33).

Table 33: Distribution (number) of tumor types by breed

Morphologic broad group	Mongrel/ mixed-breed	Pure bred	Total (n)
Cutaneous adnexal tumors	34	36	70
Mast cells tumors	24	46	70
Adenomas and adenocarcinomas	27	41	68
Blood vessels tumors	14	27	41
Gonadal tumors	15	22	37
Fibromatous tumors	11	20	31
Epithelial tumors NOS*	11	16	27
Complex, mixed, and stromal tumors	7	18	25
Basal cells tumors	11	8	19
Lipomatous tumors	7	12	19

Soft tissue tumors and sarcomas NOS*	2	15	17
Odontogenic tumors	5	12	17
Nevi and melanomas	8	9	17
Papillary and spinocellular tumors	5	6	11
Germ cells tumors	5	5	10
Myomatous tumors	4	3	7
Lymphomas NOS* or diffused	3	1	5
Papillomas and transitional cells carcinomas		5	5
Peripheral nerve tumors	2	2	4
Histiocytic tumors	2	2	4
Cystic, mucinous and serous tumors	2	1	3
Mature B-cell lymphomas	1	1	2
Ductal, lobular and medullary tumors	2	0	2
Neoplasms NOS*	2	0	2
Mieloproliferative and lymphoproliferative disorders (miscellaneous)		1	1
Fibroepithelial tumors		1	1
Myxomatous tumors		1	1
Neuroepitheliomatous tumors		1	1
Plasm cells tumors		1	1
Osteomas and osteosarcomas	1	0	1
Total	**265**	**254**	**519**

Figure 33: Percentage proportion of tumor types (above 5 cases) by breed



2.3.7 Most common anatomical tumor locations

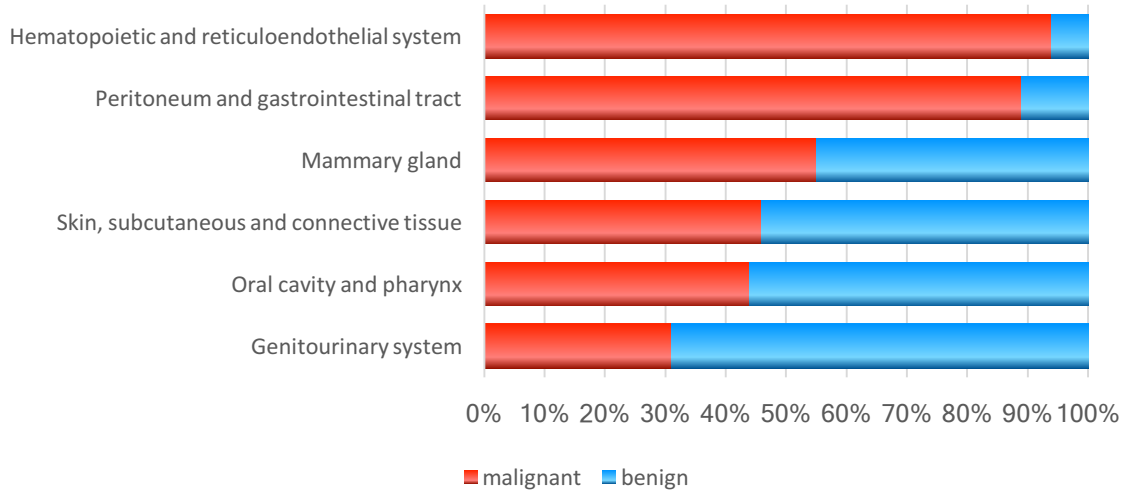
Skin, subcutaneous and connective tissue (51,6%, 263/519) was the most frequent location for tumors, followed by mammary gland (21,7%, 113/519), genitourinary system (12,4%, 65/519), oral cavity and pharynx (6,8%, 36/519), hematopoietic and reticuloendothelial system (3,2%, 17/519), and peritoneum and gastrointestinal tract (1,7%, 9/519). Other anatomical locations collected less than 5 cases, representing a percentage always lower than 1%. The frequency of the tumor anatomical locations is shown in Table34.

The highest percentage of malignant tumors was found among hematopoietic and reticuloendothelial system (94%, 16/17), peritoneum and gastrointestinal tract (89%, 8/9), and mammary gland (55%, 62/113). A lower malignancy rate was showed in genitourinary system (31%, 20/65), oral cavity and pharynx (44%, 16/36), and skin, subcutaneous and connective tissue (46%, 120/263). Number and proportion of malignant tumor per anatomical location are shown in Table 34 and Figure 34.

Table 34: Distribution, in number and percentage, of tumor anatomical location by diagnostic outcome

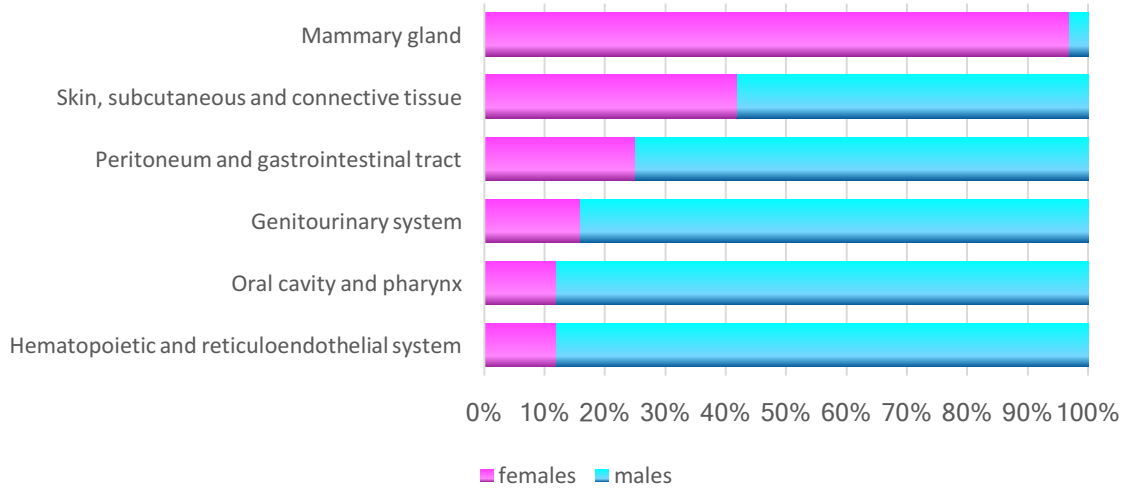
Anatomical location	Benign tumor	Malignant tumor	Total (n)	Total (%)
Skin, subcutaneous and connective tissue	143	120	263	51,6%
Mammary gland	51	62	113	21,7%
Genitourinary system	45	20	65	12,4%
Oral cavity and pharynx	20	16	36	6,8%
Hematopoietic and reticuloendothelial system	1	16	17	3,2%
Peritoneum and gastrointestinal tract	1	8	9	1,7%
Respiratory system and intrathoracic organs	1	3	4	0,7%
Eye and lacrimal gland	3	1	4	0,7%
Other undefined locations		3	3	0,4%
Lymph nodes		2	2	0,3%
Endocrine glands		2	2	0,3%
Musculoskeletal system		1	1	0,2%
Total	**265**	**254**	**519**	**100%**

Figure 34: Percentage proportion of tumor anatomical locations (above 5 cases) by malignancy



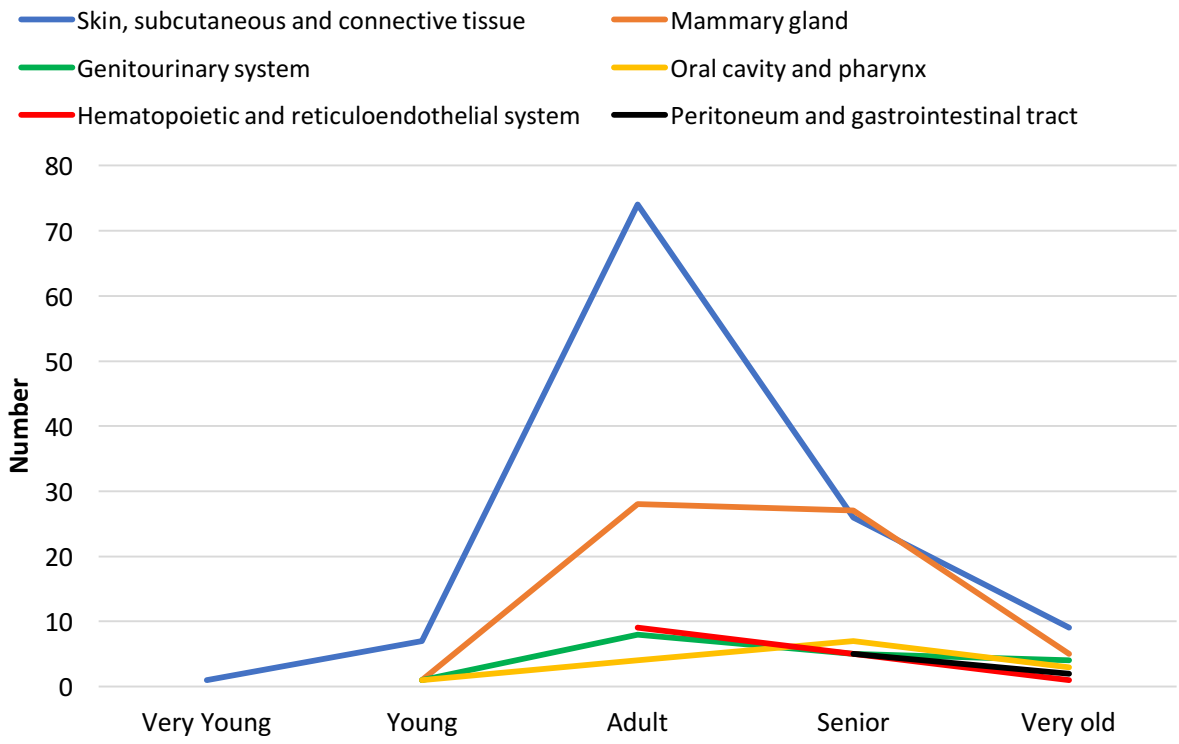
Analysis of tumor anatomical locations by sex showed that mammary gland tumors occurred almost totally in females (97%), while in the other anatomical locations, considering only groups with 5 cases at least, males prevailed. Indeed, hematopoietic and reticuloendothelial system (88%), oral cavity and pharynx (88%), genitourinary system (84%), peritoneum and gastrointestinal tract (75%), and skin, subcutaneous and connective tissue (58%) occurred with a higher frequency in male dogs (Figure 35).

Figure 35: Percentage proportion of tumor anatomical locations (above 5 cases) by sex



A combined analysis of the occurrence of tumors per anatomical location by age group was performed and revealed that skin, subcutaneous and connective tissue, hematopoietic and reticuloendothelial system, and genitourinary system were anatomical locations mostly represented in the ‘adult’ group age. Oral cavity and pharynx, together with peritoneum and gastrointestinal tract, involved in particular the ‘seniors’. Mammary gland location was most represented both in ‘adult’ and ‘senior’ age groups (Figure 36).

Figure 36: Distribution (number) of anatomical locations by age group



2.4 DISCUSSION

Animal cancer registries increase awareness of tumor occurrence and distribution in pets over time. They can also improve awareness of risk factors and allow improvement of prevention and treatment strategies. In addition, spontaneously originating pet tumors could serve as sentinels for human cancers. Unfortunately, there are few animal cancer registries and many of them only cover short periods of time, losing of purpose. In general, as in human field, animal cancer registry data are considered usable only after several years of collection. Early years are fundamental to set the basis of collection and to get a first amount of data, but the number of cases collected is usually still not sufficient to allow a statistically significant analysis that can delineate a real picture on the tumors of a given population. However, collected data can be valuable since the beginning even if still not usable in epidemiological studies related to public health. Veterinary practitioners can get data immediately available and apply information to their work. Trends emerging from data could direct practitioners to pay more attention forward a patient specific class, for example 'senior' age group because of a higher tumor frequency. To this, entrepreneurial considerations could be added, such as the choice to undertake, as professional perspective, an oncological surgery upgrade taking in care that mammary tumors are the most frequently diagnosed in female dogs in the Marche region.

The Animal Cancer Registry of the Marche region (ACR-M) is newborn and, at the moment of data analysis here reported, counted less than 3 years of activity (32 months). The number of collected requests was 589, an exiguous amount if compared to the 363.325 recorded dogs in the regional canine registry (data updated to 31 Dec 2017[78]). Compared to the first year of activity, in 2017 requests had an increase of 38%. In the third year, though data were partial because covering only 8 on 12 months of 2018, requests number seemed to be constant even if not further increased. Participating veterinary facilities showed fluctuations, with defections and new entries during the 32 months. At 31st August 2018, conferring veterinary facilities total number was slightly lower than the first year of activity. Geographical origin of requests corresponded to the conferring veterinary facilities locations and covered only 36 of 228 municipalities of the Marche region (ISTAT data updated to 1st Jan 2018). Part of the samples was referred from some private laboratories operating in the Marche region, without details about the conferring veterinary facilities and related geographical area, so a part of data was missing.

This low success of the Animal Cancer Registry of the Marche region could be related to different aspects. First of all, the lack of popularity due to a small investment in terms of publicity. Despite the Marche Region organized meetings in collaboration with the provincial professional orders to promote the ACR-M, the poor participation by veterinary practitioners limited its popularization. Later, no meetings were proposed again, trusting in 'word of mouth'. Even a widespread distribution of publicity materials or a presentation of the ACR-M project directly into the veterinary facilities was not carried out. Another aspect explaining participation failure could be a real difficulty to convince practitioners to change their referring histopathology laboratory, with whom a trust relationship often exists. In addition, by a practical view of point, most of private laboratories receive and process different sample types (histopathology, blood test, PCR, etc.), for this reason sending to a single laboratory rather than to different laboratories simplifies request preparation, shipment and correspondence. A strong incentive to obtain a greater participation could be the gratuitousness of service. For example, for the Umbria region, where the histopathological diagnostic service for the animal cancer registry is free, collected samples number is considerably higher than in the Marche region. The Marche Region decided to offer a paid service, although at a much lower price than market costs, to ensure the long-term survival of the animal cancer registry. Indeed, by self-financing, the ACR-M does not depend on public funds and can go on even if the Marche Region should be submitted to financial cuts. A strategy to increase participation by practitioners, and therefore the samples number, could be to realize a widespread divulgation, throughout publicity materials and direct contact with veterinary facilities, and to include the private laboratories working on the territory among the functional diagnostic units of the animal cancer registry.

About 80% of the samples received had diagnosis of cancer, without any significant difference between malignant and benign tumors. Obviously, this data does not mean that 8 out of 10 dogs in the Marche region are cancer patients, but there is an understandable bias considering that request for histopathological analysis of the samples occur only when a tumor lesion is suspected. Spatial analysis indicated Ancona and Pesaro-Urbino as the provinces with

the greatest number of tumors. This higher frequency of tumor cases than other provinces of the Marche region could be due to the highest population density of these areas. The crude incidence rate (CIR) for all tumors was about 160 cases per 100.000 dogs at risk, while for malignant and benign tumors CRI was about 78 and 82 per 100.000 dogs at risk respectively. Given the constraints derived from working with a canine population, considerable effort was expended to estimate the relevant denominators in terms of population size and structure. A higher cases number is requested to calculate the crude incidence rate per year and relative risk (RR), now missing. These first data were recorded but could not yet be discussed or compared, although CIR for malignant tumors appears smaller than in other population-based registries in Italy where the crude incidence rates for all malignant cancer were less than 200 cases[40, 76] till about 800[2] per 100.000 dogs at risk.

With regard to the cases of incidental tumors, a certain degree of bias could be occurred. In particular, a proportion of tumors could be undiagnosed (e.g., a deep organ tumor), or not reported because of a no required histological confirmation (e.g., osteosarcoma, lymphoma). Mammary gland, genital, and skin tumors are easier to recognize by physical examination than tumors of the respiratory and digestive tract, and other internal organs that require specific examinations such as X-ray, computed tomography scanning (CT), magnetic resonance imaging (MRI), and ultrasound examination. Supporting this hypothetical misdetection, and similar to other animal cancer registries[2, 40], data extracted from ACR-M revealed skin, mammary gland, genitourinary system and oral cavity as the most involved anatomical locations by tumor. Visceral organs showed a low number both in conferred samples and in tumors frequency.

The distribution of common types in the Animal Cancer Registry of the Marche region, although only as preliminary data, seemed fairly consistent with the literature. As reported elsewhere[12, 56, 76], the most prevalent malignant tumors were mammary carcinoma and mast cells tumor.

With regard to breed, purebreds were always more represented than mongrels/mixed-breed dogs and showed a higher frequency of tumor cases. In spite of this, there was no significant difference in prevalence ratio (PR) neither in crude incidence rates of tumors, differing from other works where a greater incidence in purebreds than in mongrels was reported, with particular regard to some breeds[2, 7, 14], considered particularly at risk of developing tumors because of genetic predispositions. In this registry, data were still premature to calculate crude incidence rate by specific breed.

Both in total number of examined dog samples and in tumor cases, sex was not a discriminant. In the female dogs, the observed tumor trend by age matched those seen in other studies, with an increase in adult and elderly females for all tumors[41] or specifically for mammary tumors[14]. Unfortunately, reproductive status data missing, for this reason a crossed analysis by sex, reproductive status and tumor types was not performed.

As highlighted by Kelsey et al.[30], the data from population-based canine cancer registries may facilitate the identification of a less select group of cases for case-control studies and allow examination of trends over time and geographic differences in cancer incidence. The findings from the current study provide still weak data on canine tumors. In the future, the incidence rates will be useful for assessing the impact of neoplastic diseases in the canine population of the Marche region and serve as a reference when setting up studies to detect excess risks in the incidence of malignant tumors in dogs used as sentinels for community exposure to environmental carcinogens.

3. THE CUBAN EXPERIENCE: ANIMAL CANCER REGISTRY OF HAVANA CITY

3.1 INTRODUCTION

Ten of thousands of dogs roam the streets of Havana. Condition of Cuban pets is a reflection of a society impoverished by constant economic crises. In Cuba, there is no a law for animal protection and dogs are barely considered pets. In most cases, dogs lead stray life. Cuban government does not impose a mandatory identification of dogs and, obviously, there is no an official canine registry. Canine population is constantly growing and demographic control is in the hands of foreign animalist associations (mainly Canadian), which voluntarily employ their resources, economic and professional, to face routinely campaigns to spay or neuter the Cuban dogs. Medical care is reserved only to dogs adopted by some public institutions (such as museums, schools, etc.), identifiable on the street by a tag attached to the collar, and dogs of owners able to afford veterinary expenses. Compared to the Cuban average salary, prices for veterinary care are very high, even if diagnostic and surgical tools are not high in quality because of the economic restraints of the country. Again, another big problem is unavailability of veterinary drugs. All these reasons let clearly guess how, in Cuba, veterinary field is complicated.

In Havana city exists a veterinary facility, the Laboratory of Pathology and Experimental Surgery of the National Institute of Oncology and Radiobiology (INOR), probably unique in the whole country, working as reference center for veterinary oncology, where dogs and cats receive free clinical and diagnostic consultations, surgeries and treatments. This is possible thanks to research projects in collaboration with some Research Centers in Human Medicine and the University of Agriculture of La Habana (UNAH). When there is no available medical or surgical treatment, and only after an informed consent of the owner, pet patients are included in experimental projects to test antitumor molecules in natural occurring cancer[77]. The Laboratory of Pathology and Experimental Surgery of INOR performs a big part of these studies, working both on laboratory animal models, mainly mice, and on spontaneous cancer animal models, mainly dogs.

The free veterinary services and the available of more advanced diagnostic and surgical tools than in other veterinary facilities of the municipality allow to the Laboratory of Pathology and Experimental Surgery of INOR to reach a good number of clinical cases. Most of them are oncological patients, because veterinary practitioners of Havana confer to the Laboratory of Pathology and Experimental Surgery of INOR, as cancer reference center, dogs and cats suspected of tumor. This veterinary facility contributes in minimal part to compensate for the lack of a canine registry thanks to its hospital-based canine registry collecting a municipality-wide audience. Thanks to its characteristics, it has been possible established an Animal Cancer Registry and start epidemiologic studies, in addition to already existing comparative clinical trials on spontaneous tumors.

3.2 MATERIALS AND METHODS

3.2.1 Data source

As hospital-based cancer registry, denominator of this study is represented by all canine patients recorded at the INOR Laboratory of Pathology and Experimental Surgery during 5 months, since 1st June to 31st October 2018.

Dogs brought to clinical consultation came from veterinary colleagues, after a suspecting cancer diagnosis, or by a spontaneous initiative of owners who had known about the INOR Laboratory of Pathology and Experimental Surgery and the gratuitousness of diagnostic and therapeutic services provided. Geographical provenance area involved the entire municipality of Havana.

Patients data were tabulated into Excel software and recorded into digital folders, associated to images of macroscopic lesions, radiology, ultrasonography, cytology and, obviously, final diagnosis.

Economic restraints of the country did not allow the histopathological examination of samples; therefore, diagnosis were based on cytological specimen evaluation. Diagnosis reliability was increased by a double-blind mechanism: first and second pathologists perform microscopically evaluation and report separately.

Due to the lack of a histopathological diagnosis, classification and coding system of tumors were not performed but, to compare data to other registries, even if with enormous limitations, tumors were classified according to broad categories similar to those belonging to the International Classification of Diseases for Oncology (ICD-O)[16].

3.2.2 Sample collection and diagnosis

From clinical and imaging evaluation to microscopically diagnosis, passing by samples collection and preparation, my work at the INOR Laboratory of Pathology and Experimental Surgery developed at 360 degrees.

Before collecting the samples, the first part of the work was to record data and clinical history of patients, take a picture of macroscopic lesions and perform a clinical examination, adding laboratory evaluation when needed (blood, urine, faecal tests). For internal lesions, radiographic or ultrasonography imaging was performed.

On the basis of lesion type, samples were collected by:

- Fine Needle Biopsy (FNB) / Fine Needle Aspirate Biopsy (FNAB)→ for sampling proliferative lesions, using a 22-25 gauges needle and a 2-5 ml syringe. For non-

aspiration procedure, FNB, a fine gauge needle was inserted into the mass and redirected within the lesion at several different angles. Once the needle was removed from the mass, was attached to an air-filled syringe and the material within the needle was gently expelled onto glass slides for smear preparation. This technique works better than aspiration (FNAB) for high vascular masses, as blood contamination is often reduced. For aspiration procedure, FNAB, once the needle was inserted into the mass, negative pressure was applied to the plunger/syringe. This procedure was repeated 3-4 times at different angles within the lesion to obtain a representative cell population from the mass. Once released negative pressure, needle was removed from the mass, detached from syringe and, after having drawn air into the syringe, newly re-attached to the air-filled syringe to expelled the material within the needle onto a glass slide.

For sampling from visceral organs, FNB and FNAB were performed as percutaneous biopsy by ultrasound-guide.

- Imprints → for sampling crusted and ulcerative skin/mucosal lesions or deep surgical biopsy gently rolled onto a glass slide after excision. Dry scabs/crusts were removed manually prior to impression smears being made, as cells in these scabs/crusts generally reveal poor cellular morphological preservation and poor staining characteristics. The tissue was blotted dry to remove surface fluid or blood as these may impairs adhesion of cells to the slide and dilute the cytological material. Following this, the biopsy sample or lesion being examined was firmly pressed several times onto a clean glass slide. Imprints from ulcerative lesions often only yield superficial inflammation and infection and any underlying/primary neoplastic process may be missed. This was a good technique for investigating superficial neoplasia such as squamous cell carcinoma and transmissible venereal tumor.
- Scrapings → this method has similar uses to imprinting, but was used where imprinting was likely to yield too few cells for complete assessment, such as in mesenchymal neoplasia. The back (blunt edge) of a scalpel blade or edge of a glass slide was used to gently scrape across the lesion or tissue biopsy until a small amount of material was collected. This material was then gently spread across a slide.
- Swabs → this technique was useful for the sampling of fistulous tracts, ear canals, exudates and for vaginal cytology. Once the area to be investigated was sampled by using swab, smears were prepared by gently rolling the swab over a glass slide to avoid cell rupture or poor cell preservation.

Smears were air-dried and stained by May-Grünwald Giemsa (MGG) quick stain. Similar to other methods in histology, MGG stain is based on the electrostatic interaction between dye and target molecules. The May-Grünwald stain composed of an acidic stain (eosin) and a basic stain (methylene blue), while the Giemsa stain composed of eosin and another metachromatic basic stain: azure of methylene. The first stain induces an orthochromatic staining on cell components (pink or orange dye for acidophilic components and blue or purple for basophilic and neutrophil components). The second induces a metachromatic staining: red dye for azurophil

components. After a rapid air-drying of cell smears and fixation in methanol for 5 minutes, two-step staining was performed: 50% May-Grünwald in buffer pH 6.8 v/v for 3-5minutes, followed by 10% buffered Giemsa solution for 10-30 minutes, and running water for 1-3 minutes[53]. After air-dried, stained slides were mounted by Canada balm and observed to microscope at different magnifications.

Samples evaluation was always performed by two different pathologists, without any comparing before final diagnosis (double-blind), indeed each cytological diagnosis I carried out was followed or preceded by a diagnosis of an INOR staff pathologist. When a diagnostic disagreement happened, a third pathologist was involved to reach a final diagnosis. Trying to according to the International Classification of Diseases for Oncology (ICD-O)[16] and to fitting the data to the Animal Cancer Registry of the Marche region, primarily theme of this thesis, tumors were classified into broad categories.

3.2.3 Data analysis

Given the low number of total cases involved and the short period of study, data were obviously considered of categorical (discrete) kind and analysis was carried out as descriptive statistic.

Data distribution concerning examined dogs, included analysis by breed, sex, age, and diagnostic outcome, subdivided by malignancy, tumor types and anatomical locations, was expressed as number and percentage. Furthermore, crossed analyses were carried out to evaluate most variables contemporarily, such as tumor type and sex or anatomical location and age group.

Age years were clustered into age groups related to expected average lifespan. Expected average lifespan was calculated, per breed, on the basis of the available scientific literature[13, 73]. Age was categorized into 5 classes: 'very young', 'young', 'adult', 'senior', and 'very old' (Table 0).

Table 0: Categorization based on maximum life expectancy

Categories	**Maximum life expectancy (years)**								
	8	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17-18 and mongrels**
Very young	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Young	2-3	2-3	2-3	2-3	2-3	2-4	2-4	2-4	2-5
Adult	4-5	4-6	4-7	4-8	4-8	5-9	5-10	5-11	6-11
Senior	6-7	7-8	8-9	9-10	9-11	10-12	11-13	12-14	12-15
Very old	8	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-18

Prevalence ratio was used to quantify the relationship between tumor and independent variable (sex, age, breed), and reported as percentage.

The low total number of cases collected did not allow to calculate crude incidence rates (CIR) and related 95% confidence intervals (CIs).

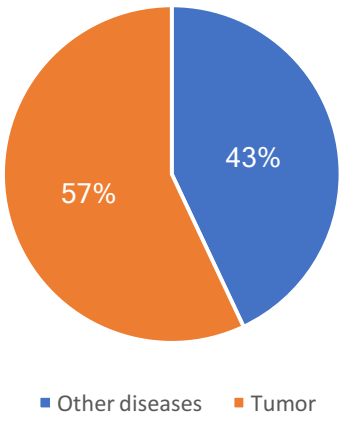
Data analysis was performed with Microsoft Excel 2016, version 15.29.1.

3.3 RESULTS

3.3.1 Dataset

The dataset was based on collected data from 1st June to 31st October 2018 (5 months). During this period of time, INOR Laboratory of Pathology and Experimental Surgery received a total of 233 dog patients, referred by veterinary practitioners working throughout the whole territory of the municipality of Havana. The number of dogs with confirmed tumors was 133 (57%) (Figure 37).

Figure 37: Distribution (%) of total examined dogs by diagnostic outcome



3.3.2 Breed distribution

Most dogs were purebreds (56%, 130/233), while mongrel/mixed-breed dogs were 44% (103/233). Calculation of tumor frequencies resulted in the same percentages, thus 56% (75/133) of dogs diagnosed with cancer were purebred and 44% (58/133) were mongrel (Figure 38).

Prevalence ratio (PR) for purebreds and mongrels, calculated as tumor cases/total number ratio for each group, was reported in Table 35.

Figure 38: Distribution in percentage (pie chart) and in number (histogram) by breed

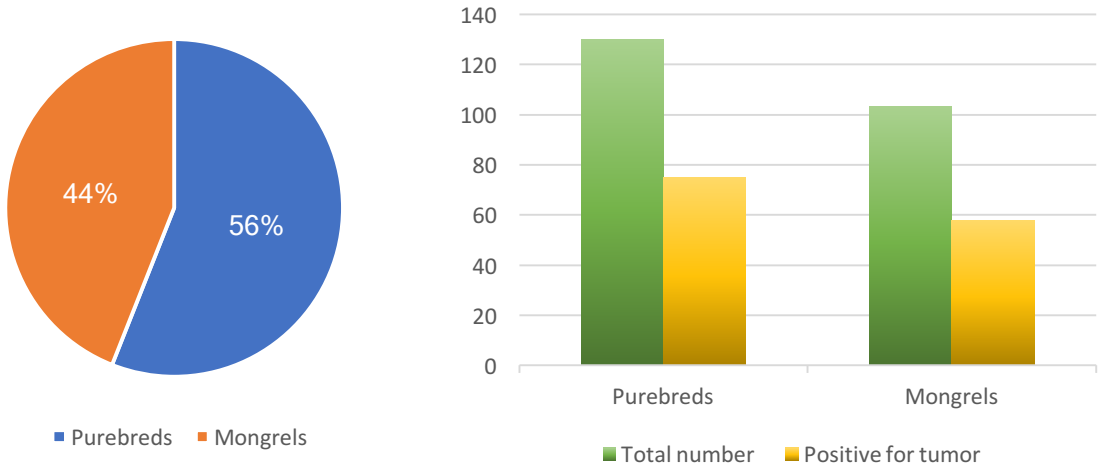


Table 35: Prevalence ratio (PR) by breed

DOG BREEDS	Prevalence ratio (PR)	Positive for tumor (n)	Total number (n)
Purebreds	58%	75	130
Mongrel/mixed-breeds	56%	58	103

The most represented breeds were: mongrel/mixed-breeds, followed by Dachshund, Pekingese, English cocker spaniel, Rottweiler and Chihuahua (Table 36). Mongrel/mixed-breeds were also the dogs with the highest frequency of tumor, followed by dachshunds, Pekingese, English cocker spaniel, and Siberian husky (Table 36).

Table 36: Distribution (number) of dog breeds

DOG BREED	Negative for tumor	Positive for tumor	Total
Mongrel/mixed-breed	45	58	103
Dachshund	7	15	22
Pekingese	11	9	20
English cocker spaniel	4	9	13
Rottweiler	7	5	12
Chihuahua	5	4	9
Siberian husky	1	6	7
Dalmatian	1	5	6
German shepherd	2	3	5
American pit bull terrier	2	3	5
American Staffordshire terrier	2	2	4
Miniature pinscher	2	1	3
Shar-pei		3	3
Labrador retriever		2	2
Boxer		2	2
Chow-chow		2	2
Golden retriever	2		2

Chinese crested dog		2	2
Miniature schnauzer	2		2
Great Dane	2		2
Dobermann pinscher		1	1
Jack Russell terrier	1		1
White Swiss shepherd	1		1
Beagle	1		1
French bouledogue	1		1
Bassett hound		1	1
Bichon à poil frisé	1		1
Total	**100**	**133**	**233**

3.3.3 Age distribution

Dogs were categorized into age groups based on maximum life expectancy as reported in the paragraph 3.2.3. The highest number of examined cases was of dogs clustered into the 'senior' group, that resulted also to be the group with the highest number of tumor diagnosis, followed by the 'adult' group in both cases. Both for examined cases and tumor diagnosis, the poorest group was of dogs clustered into the 'very old' group (Table 37 and Figure 39).

Percentage calculation of cancer cases by age revealed a strong preponderance of 'senior' (48%) and 'adult' (40%) groups towards 'very old', very young' and 'young', that resulted into a 1%, 5% and 6% respectively (Figure 40).

'Senior' and 'adult' groups showed the highest prevalence of tumor, calculated as tumor cases/total number ratio for each group: 70% (64/91) and 65% (53/84) were the respectively results. Prevalence of tumor cases in 'young' and 'very old' groups was similar, being of 35% (8/23) and 33% (2/6) respectively, followed by the 'very young' group whose prevalence percentage was 21% (6/29) (Table 37 and Figure 39).

Table 37: Distribution (number) of dogs by age group

Age Group	Negative for tumor	Positive for tumor	Total
Very young	23	6	29
Young	15	8	23
Adult	31	53	84
Senior	27	64	91
Very old	4	2	6
Total	**100**	**133**	**233**

Figure 39: Distribution (number) of dogs, total number and positive for tumor, by age group

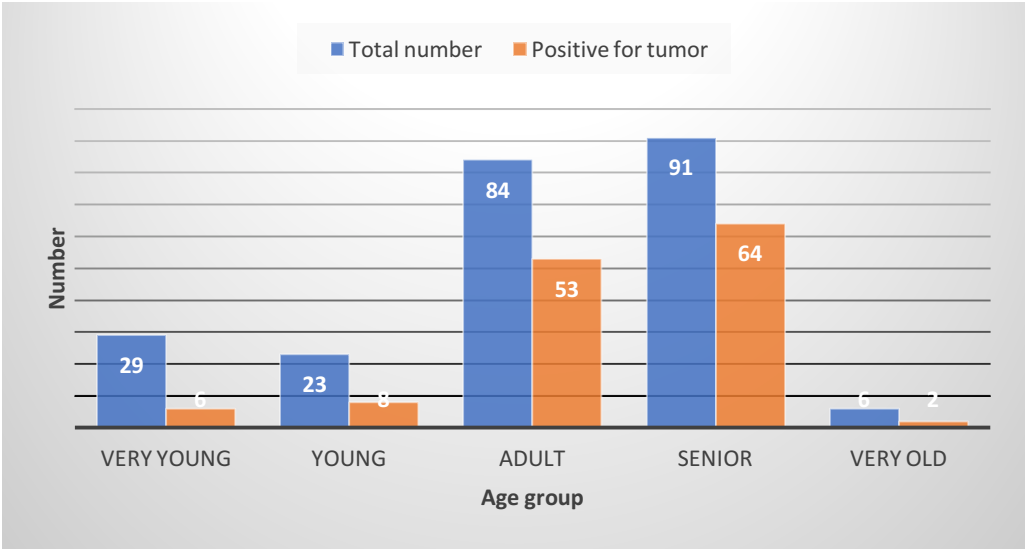
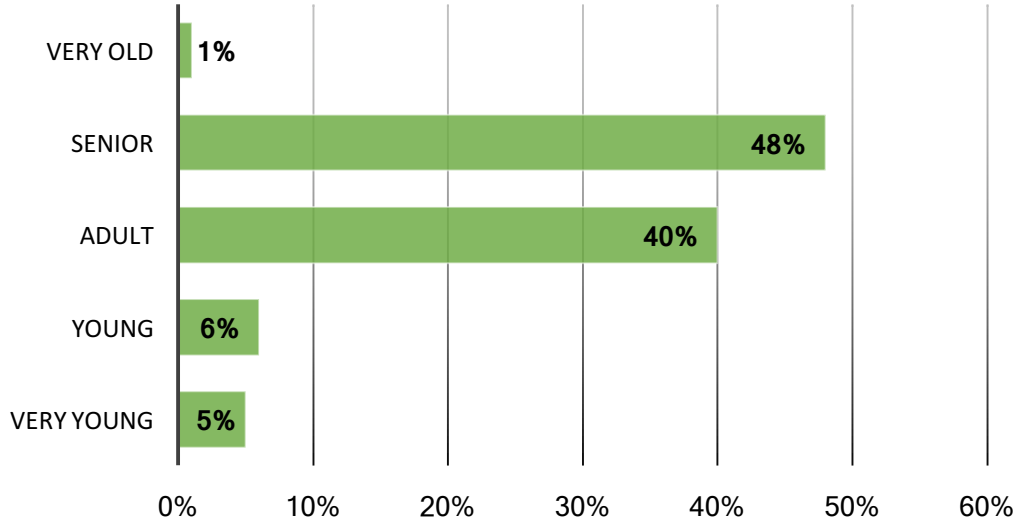


Figure 40: Distribution in percentage (%) of dogs with tumor by age group



3.3.4 Sex distribution

Females showed always the highest frequency both as examined dogs and as patients with cancer diagnosis. This prevalence was particularly pronounced in the last case, where females represented 67% of dogs with cancer while males were 33%. Data about sex distribution are shown in Table 38 and Figure 41.

About reproductive status, recorded data reported only 1 neutered male and 2 spayed female dogs in a total number of 98 and 135 respectively (Figure 42).

Table 38: Distribution (number) of dogs by sex

Sex	Negative for tumor (n)	Positive for tumor (n)	Total
Females	46	89	135
Males	54	44	98
Total	**100**	**133**	**233**

Figure 41: Distribution (%) of dogs, total number and positive for tumor, by sex

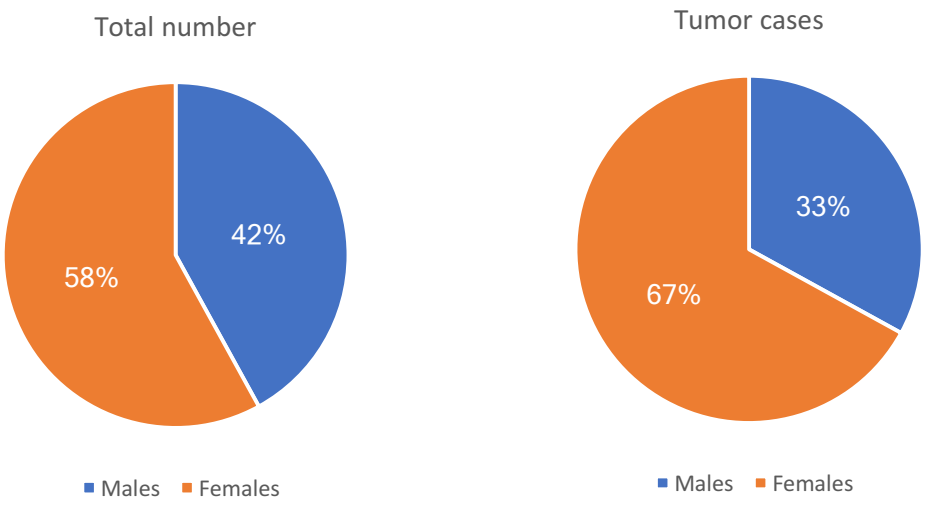
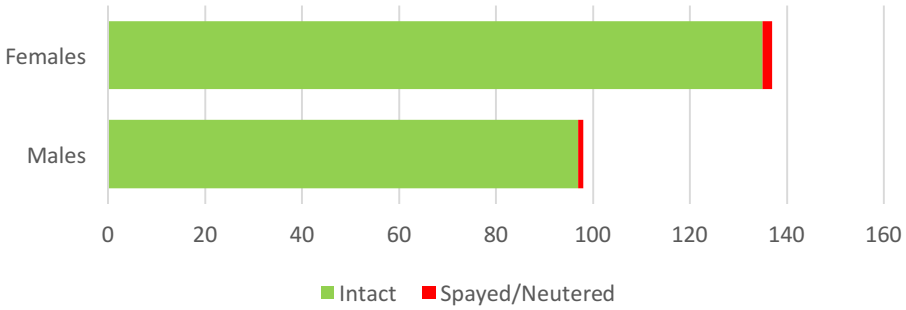


Figure 42: Distribution (number) of dogs by reproductive status



Dogs were also evaluated by sex associated to age group. In females, 'senior' was the most represented group, followed by 'adult' group. 'Young' and 'very young' group showed a similar frequency. 'Very old' group, as for males, was the less represented group. In males, 'adult' group was the most represented, even if only for a minimal difference was distanced from 'senior' group. 'Very young' and 'young' groups showed a similar frequency than in females (Table 39 and Figure 43).

Percentage calculation of cancer cases by sex and age revealed a high frequency in 'senior' and 'adult' groups, both in females and in males, toward 'young', 'very young' and 'very old' (Figure 44).

Similar to analysis by age group only, in a crossed evaluation with sex, 'senior' and 'adult' groups showed the highest prevalence of tumor: 66% (23/35) and 50% (18/36) in males, respectively, and 73% for both age groups in females ('senior' 41/56, 'adult' 35/48). Prevalence ratio by sex and age showed a significant difference in 'very young' and 'young' groups, because percentage results in females were much higher than in males: 50% (7/14) and 35% (5/13) were tumor prevalence in female 'young' and 'very young' groups, respectively, and 11% (1/9) and 6% (1/16) in male counterparts (Figure 45).

Table 39: Distribution of dogs with tumor by sex and age group

Age group and sex	Negative for tumor		Positive for tumor		Total
	F	M	F	M	
Very young	8	15	5	1	29
Young	7	8	7	1	23
Adult	13	18	35	18	84
Senior	15	12	41	23	91
Very old	3	1	1	1	6
Total	**46**	**54**	**89**	**44**	**233**

Figure 33: Age pyramid of dog with tumor

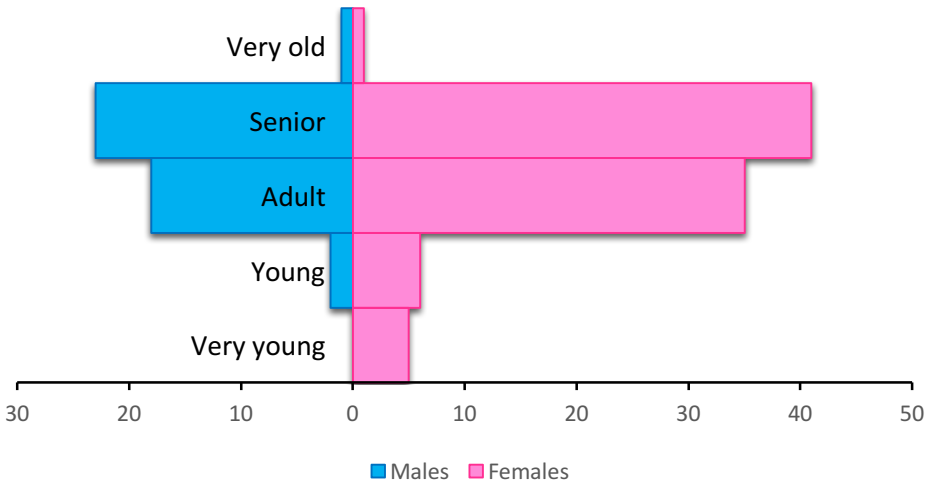


Figure 44: Distribution in percentage (%) of dogs with tumor by sex and age group

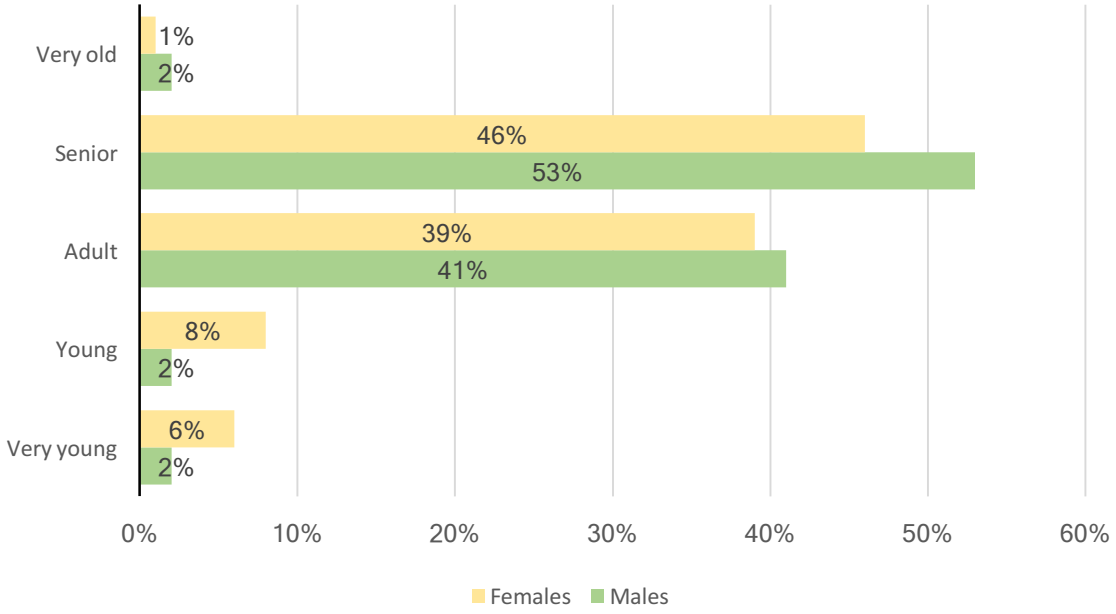
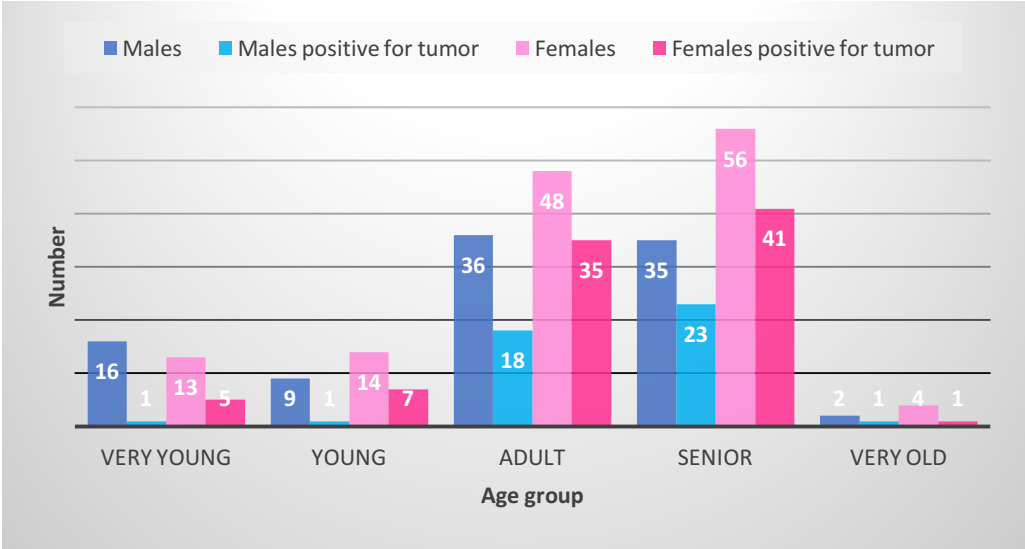


Figure 45: Distribution of dogs, total number and positive for tumor, by sex and age group



3.3.5 Most common tumor types

The total number of cancer diagnoses was 133, with a significant difference on the basis of diagnostic outcome, since malignant tumors were proportionally much more represented than benign tumors (Table 40).

Analysis of most common tumor types revealed that histiocytic tumors were the most represented (18%), followed by adenomas and adenocarcinomas (14%), papillary and spinocellular tumors (12%), nevi and melanomas (7,5%), lymphomas (7,5%). Other tumor types showed a lower frequency and are shown in Table 40.

Table 41 shows in detail which tumors were diagnosed for each broad group, based on the same categorization extracted from International Classification of Diseases for Oncology (ICD-O) codes and previously utilized for the Marche region Animal Cancer Registry diagnoses. Histiocytic tumors, the largest group, were almost exclusively represented by the transmissible venereal tumor (TVT) (23/24). Among adenomas and adenocarcinomas, none tumor occurred more frequently than others, but the broad category was fragmented in different tumor morphotypes affecting breast. Papilllary and spinocellular group coincided with a single tumor type, squamous cell carcinoma (16/16), which therefore corresponded to 12% of the tumor total number not only as a category but as a specific morphotype. Malignant melanoma leaded the nevi and melanomas broad group (9/10), while lymphomas were divided into diffuse lymphomas, mainly large B-cell one (4/10), and cutaneous lymphoma (3/10).

The highest percentage of malignant tumors were found among papillary and spinocellular tumors (100%, 16/16), mast cell tumors (100%, 6/6), histiocytic tumors (96%, 23/24), and nevi and melanomas (90%, 9/10) (Table 40 and Figure 46).

Table 40: Distribution, in number and percentage, of tumor types by diagnostic outcome

Morphologic broad group	Benign tumor	Malignant tumor	Total (n)	Total (%)
Histiocytic tumors	1	23	24	18%
Adenomas and adenocarcinomas	2	17	19	14%
Papillary and spinocellular tumors		16	16	12%
Nevi and melanomas	1	9	10	7,5%
Lymphomas	3	7	10	7,5%
Cutaneous adnexal tumors	6	3	9	7%
Lipomatous tumors	8	1	9	7%
Complex, mixed, and stromal tumors	3	4	7	5%
Mast cells tumors		6	6	4,5%
Gonadal tumors	3		3	2%
Fibromatous tumors	1	2	3	2%
Blood vessels tumors	2		2	1,5%
Epithelial tumors		2	2	1,5%
Odontogenic tumors	2		2	1,5%
Myomatous tumors	2		2	1,5%
Papillomas and transitional cells carcinomas		2	2	1,5%
Fibroepithelial tumors	2		2	1,5%
Osteomas and osteosarcomas		2	2	1,5%
Peripheral nerve tumors	1		1	1%

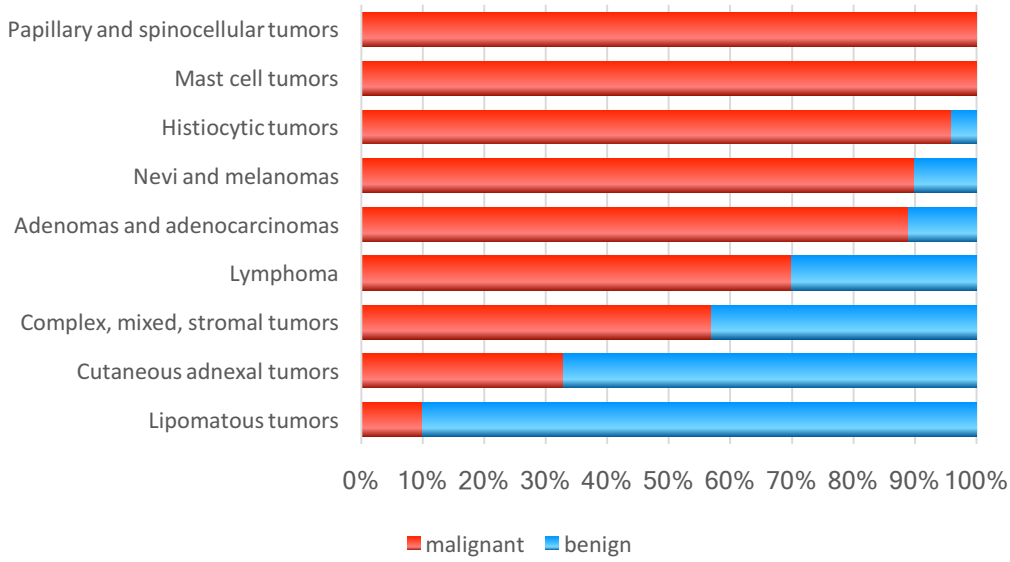
Germ cells tumors		1	1	1%
Tumors (unclassified)		1	1	1%
Total	**37**	**96**	**133**	**100%**

Table 41: Distribution (number) of tumor types, described by morphologic diagnosis, and diagnostic outcome

	Benign	Malignant	Total
Cutaneous adnexal tumors	**6**	**3**	**9**
Hepatoid gland adenoma	2		2
Meibomian gland adenoma	1		1
Sebaceous gland adenoma	1		1
Apocrine gland adenoma	1		1
Ceruminous gland adenoma	1		1
Hepatoid gland carcinoma		2	2
Eccrine carcinoma		1	1
Mast cells tumors		**6**	**6**
Cutaneous/subcutaneous mast cells tumor		5	5
Visceral mast cell tumor		1	1
Adenomas and adenocarcinomas	**2**	**17**	**19**
Adenocarcinoma		5	5
Mammary adenocarcinoma		3	3
Tubulo-papillary adenocarcinoma		3	3
Simple mammary adenoma	2		2
Simple mammary carcinoma		1	1
Solid mammary carcinoma		3	3
Prostate adenocarcinoma		2	2
Blood vessels tumors	**2**		**2**
Hemangioma	1		1
Hemangiopericytoma	1		1
Gonadal tumors	**3**		**3**
Leydig cell tumor	1		1
Sertoli cell tumor	2		2
Fibromatous tumors	**1**	**2**	**3**
Fibroma	1		1
Fibrosarcoma		2	2
Epithelial tumors		**2**	**2**
Mammary carcinoma		2	2
Complex, mixed and stromal tumors	**3**	**4**	**7**
Mammary benign mixed tumor	3		3
Mammary malignant mixed tumor		4	4
Lipomatous tumors	**8**	**1**	**9**
Lipoma	8		8
Well differentiated liposarcoma		1	1
Odontogenic tumors	**2**		**2**
Fibromatous epulis	2		2

Nevi and melanomas	**1**	**9**	**10**
Melanocytoma	1		1
Malignant melanoma		8	8
Amelanotic malignant melanoma		1	1
Papillary and spinocellular tumors		**16**	**16**
Squamous cell carcinoma		16	16
Subungual squamous cell carcinoma			
Germ cell tumors		**1**	**1**
Seminoma		1	1
Myomatous tumors	**2**		**2**
Angioleiomyoma	1		1
Leiomyoma	1		1
Papillomas and transitional cells carcinomas		**2**	**2**
Transitional cells carcinoma		2	2
Lymphomas	**3**	**7**	**10**
Diffuse well-differentiated lymphocytic lymphoma		1	1
Diffuse large B-cell lymphoma		4	4
Mixed cell lymphoma		2	2
Cutaneous lymphoma	3		3
Peripheral nerve tumors	**1**		**1**
Schwannoma	1		1
Histiocytic tumors	**1**	**23**	**24**
Cutaneous histiocytosis	1		1
Histiocytic sarcoma / malignant histiocytosis		1	1
Transmissible venereal tumor (TVT)		22	22
Tumors		**1**	**1**
Unclassified malignant tumor		1	1
Osteomas and osteosarcomas		**2**	**2**
Osteosarcoma		2	2
Fibroepithelial tumors	**2**		**2**
Fibroadenoma	2		2
Total	**37**	**96**	**133**

Figure 46: Percentage proportion of tumor types (above 5 cases) by malignancy



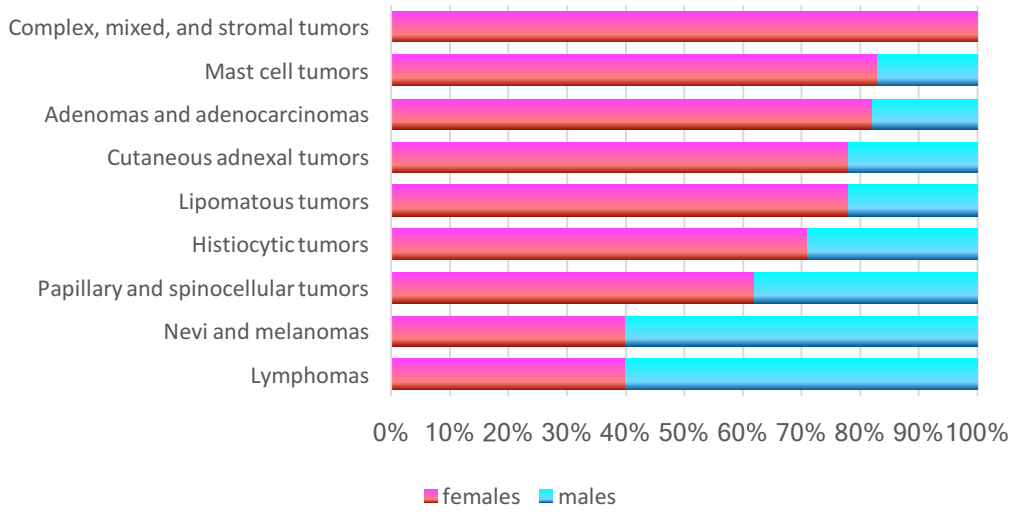
Analysis of tumor types by sex showed a highest frequency in females of complex, mixed, and stromal tumors (100%, 7/7), mast cell tumors (83%, 5/6), adenomas and adenocarcinomas (82%, 16/19), cutaneous adnexal and lipomatous tumors, both 78% (7/9), histiocytic tumors (71%, 17/24), and papillary and spinocellular tumors (62%, 10/16). In male dogs, the most frequent tumors were nevi and melanomas (60%, 6/10), and lymphomas (60%, 6/10) (Table 42 and Figure 47).

Table 42: Distribution (number) of tumor types by sex

Morphologic broad group	Females	Males	Total (n)
Histiocytic tumors	17	7	24
Adenomas and adenocarcinomas	16	3	19
Papillary and spinocellular tumors	10	6	16
Nevi and melanomas	4	6	10
Lymphomas	4	6	10
Cutaneous adnexal tumors	7	2	9
Lipomatous tumors	7	2	9
Complex, mixed, and stromal tumors	7	0	7
Mast cells tumors	5	1	6
Gonadal tumors	0	3	3
Fibromatous tumors	1	2	3
Blood vessels tumors	0	2	2
Epithelial tumors	2	0	2
Odontogenic tumors	2	0	2
Myomatous tumors	1	1	2
Papillomas and transitional cells carcinomas	1	1	2
Fibroepithelial tumors	2	0	2

Osteomas and osteosarcomas	2	0	2
Peripheral nerve tumors	0	1	1
Germ cells tumors	0	1	1
Tumors (unclassified)	1	0	1
Total	**89**	**44**	**133**

Figure 47: Percentage proportion of tumor types (above 5 cases) by sex



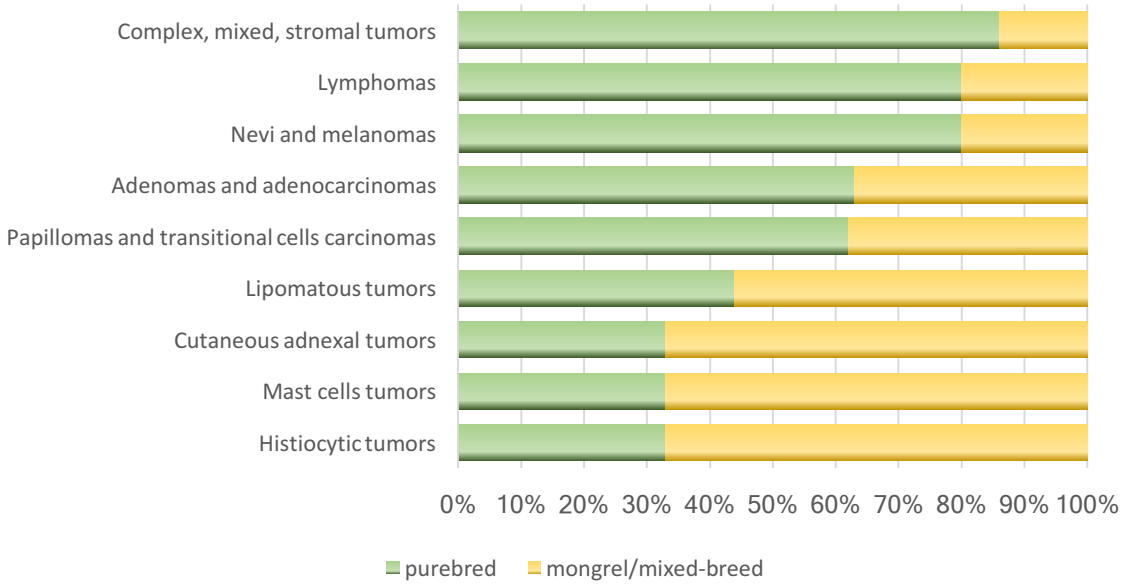
The most frequent tumor types mostly involved purebred dogs, which showed a percentage frequency greater than in mongrels and mixed-breed dogs. Complex, mixed, and stromal tumors occurred mainly in purebreds (86%, 6/7). Nevi and melanomas, together with lymphomas (both 80%, 8/10), resulted much more common in purebred dogs than in mongrels and mixed-breeds, followed by adenomas and adenocarcinomas (63%, 12/19) and papillary and spinocellular tumors (62%, 10/16). While mongrel/mixed-breed dogs showed a high frequency of histiocytic tumors (16/24), cutaneous adnexal tumors (6/9) and mast cell tumors (4/6), with a percentage of 66% for all, followed by lipomatous tumors (5/9) where 56% were mongrels/mixed-breeds (Table 43 and Figure 48).

Table 43: Distribution (number) of tumor types by breed

Morphologic broad group	Mongrel/ mixed-breed	Pure bred	Total (n)
Histiocytic tumors	16	8	24
Adenomas and adenocarcinomas	7	12	19
Papillary and spinocellular tumors	6	10	16
Nevi and melanomas	2	8	10
Lymphomas	2	8	10
Cutaneous adnexal tumors	6	3	9
Lipomatous tumors	5	4	9

Complex, mixed, and stromal tumors	1	6	7
Mast cells tumors	4	2	6
Gonadal tumors	1	2	3
Fibromatous tumors	1	2	3
Blood vessels tumors	2	0	2
Epithelial tumors	0	2	2
Odontogenic tumors	0	2	2
Myomatous tumors	0	2	2
Papillomas and transitional cells carcinomas	1	1	2
Fibroepithelial tumors	1	1	2
Osteomas and osteosarcomas	1	1	2
Peripheral nerve tumors	1	0	1
Germ cells tumors	0	1	1
Tumors (unclassified)	1	0	1
Total	**58**	**75**	**133**

Figure 48: Percentage proportion of tumor types (above 5 cases) by breed



3.3.6 Most common anatomical tumor locations

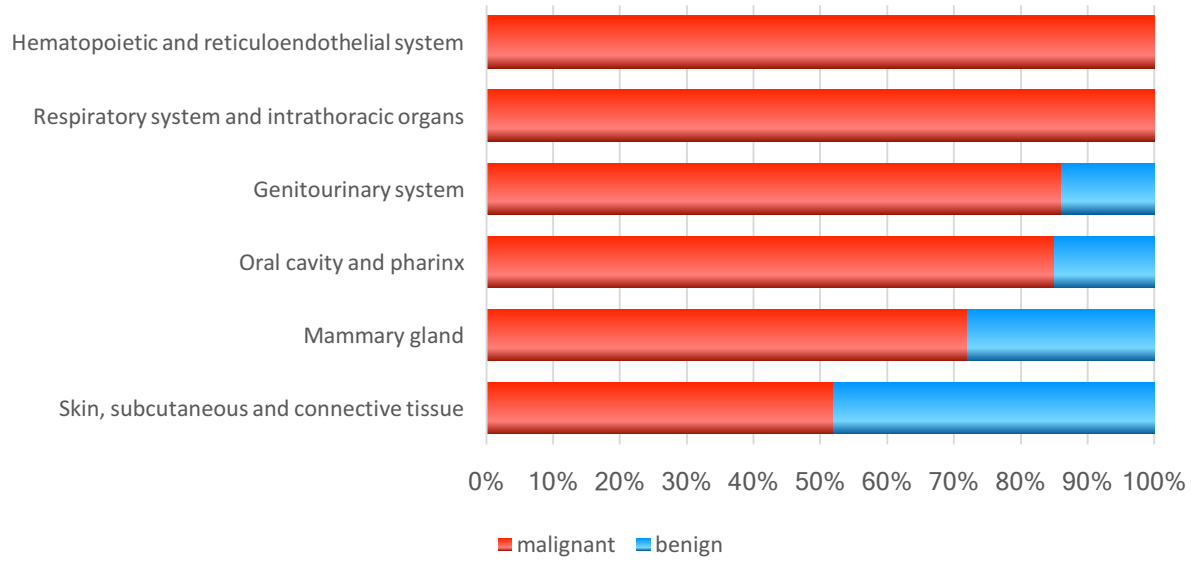
Skin, subcutaneous and connective tissue (32%, 42/133) was the most frequent location for tumor, followed by genitourinary system (21%, 28/133), mammary gland (19%, 25/133), oral cavity and pharynx (10%, 13/133), respiratory system (5%, 7/133), and hematopoietic and reticuloendothelial system (4%, 5/133). Other anatomical locations collected less than 5 cases, representing a percentage always lower than 3%. The frequency of the tumor anatomical locations is shown in Table 44.

The highest percentage of malignant tumors was found among hematopoietic and reticuloendothelial system (100%, 5/5), respiratory system and intrathoracic organs (100%, 7/7), genitourinary system (86%, 24/28), oral cavity and pharynx (85%, 11/13), and mammary gland (72%, 18/25). Number and proportion of malignant tumor per anatomical location are shown in Table 44 and Figure 49.

Table 44: Distribution, in number and percentage, of tumor location by diagnostic outcome

Anatomical location	Benign tumor	Malignant tumor	Total (n)	Total (%)
Skin, subcutaneous and connective tissue	20	22	42	32%
Genitourinary system	4	24	28	21%
Mammary gland	7	18	25	19%
Oral cavity and pharynx	2	11	13	10%
Respiratory system and intrathoracic organs	0	7	7	5%
Hematopoietic and reticuloendothelial system	0	5	5	4%
Lymph nodes	0	4	4	3%
Musculoskeletal system	2	2	4	3%
Eye and lacrimal gland	1	2	3	2%
Peritoneum and gastrointestinal tract	1	1	2	1%
Total	**37**	**96**	**133**	**100%**

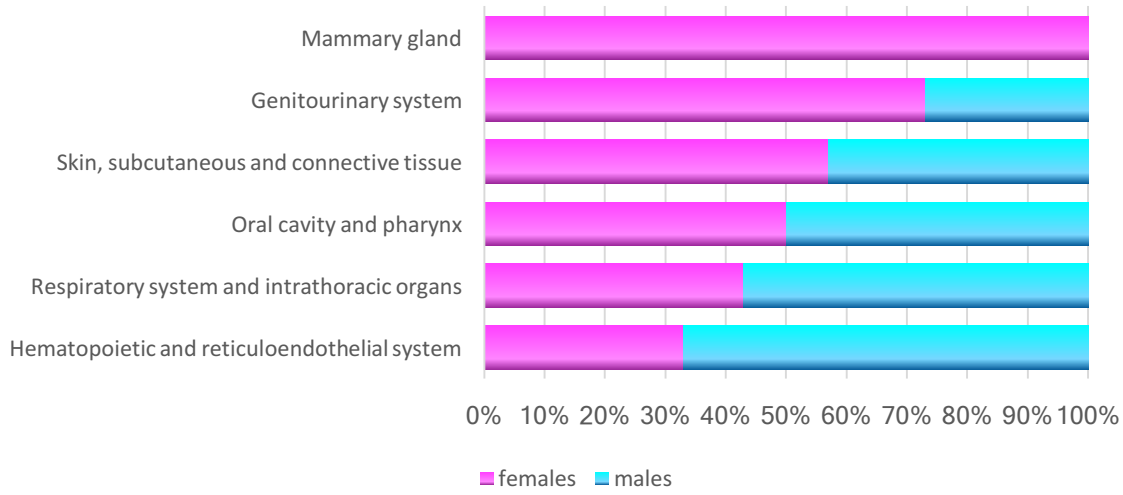
Figure 49: Percentage proportion of tumor anatomical locations (above 5 cases) by malignancy



Analysis of tumor anatomical locations by sex showed that mammary gland tumors occurred exclusively in females (100%), followed by genitourinary system (73%) and skin, subcutaneous and connective tissue (57%). In oral cavity and pharynx tumors, a perfectly balance happened since both females and males showed a 50% frequency. Hematopoietic and

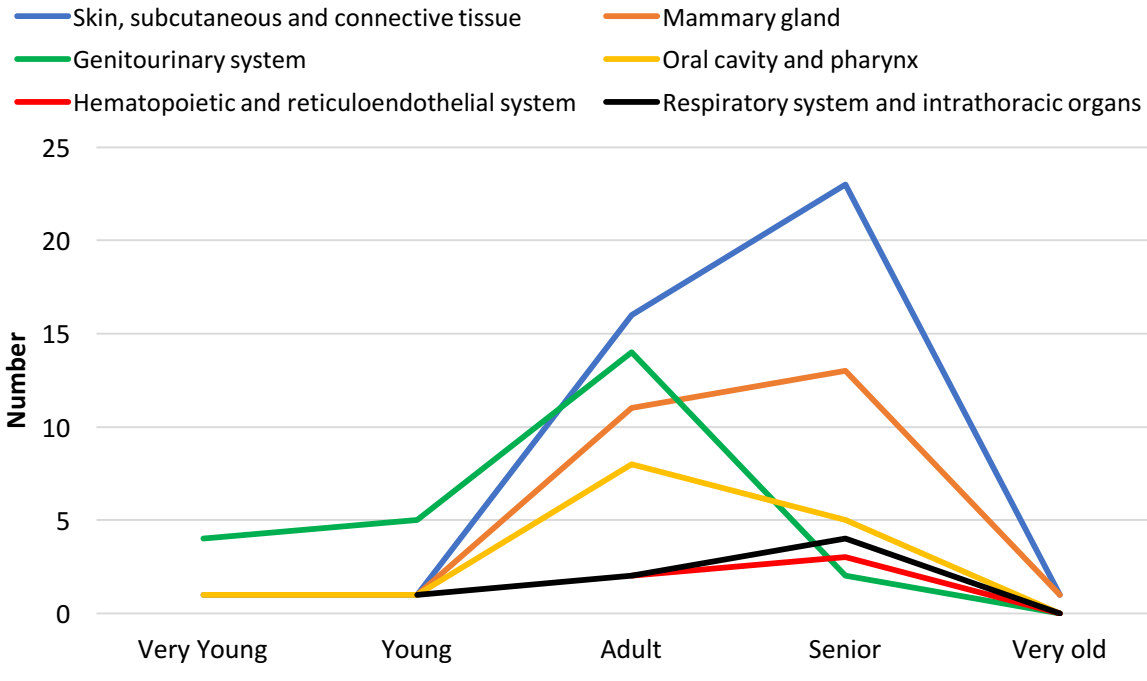
reticuloendothelial system (67%), and respiratory system and intrathoracic organs (57%) occurred with a higher frequency in male dogs (Figure 50).

Figure 50: Percentage proportion of tumor anatomical locations (above 5 cases) by sex



A combined analysis of tumors occurrence per anatomical locations by age group was performed and showed that skin, subcutaneous and connective tissue, respiratory system and intrathoracic organs, and hematopoietic and reticuloendothelial system were anatomical locations mostly represented in the 'senior' group age. Genitourinary system and oral cavity and pharynx involved in particularly the 'adults'. Mammary gland was most represented both in 'adult' and 'senior' groups (Figure 51).

Figure 51: Distribution (number) of anatomical locations by age group



3.4 DISCUSSION

In Cuba, small animals veterinary medicine has been taking its first steps only a few years ago. Also, the concept of dogs and cats as pets is taking hold only in recent years. While the cat is still mostly considered a 'street animal', the dog has been beginning to take part to the family and therefore, despite stray-life is strongly widespread, a good number of dogs lives a domestic life.

Most of adopting people choose purebred dogs, but sometimes the luckiest stray dogs are taken off from the street and become pets. As often happens in many fields, also the dog breed choice follows a trend, and Cubans are particularly attracted by dachshunds, Pekingeses, English cocker spaniels, Rottweilers, chihuahuas and Siberian huskies, that is, the most frequently listed breeds in the results of this study.

In Cuba, pet dogs live mostly promiscuous life, in fact, only a small part of them is managed indoor and controlled when taken outdoor, while most pets live in the neighborhood, constantly in contact with stray dogs. This explains the great 'success' of transmissible venereal tumor (TVT) which, being contagious and transmitted not only through coitus but also during social interactions such as grooming or maternal behavior[17], is the most common tumor type in Cuba. Because of its promiscuous nature, TVT involves 'street dogs' and this explains why it was the only tumor type showing a greater frequency in mongrel/mixed-breed dogs than in purebreds.

Another feature of Cuban pet dogs is their reproductive status. Unlike western countries, such as United States or United Kingdom where spaying/neutering is promoted and spayed/neutered dogs are about 86% [31] and 57% [61] respectively, in Cuba most dogs are intact. Attitude not to spay/neuter pets is linked mainly to a pair of factors:

- benefits awareness missing, such as unintended reproduction prevention and stray dogs population control, prevention of risks related to pregnancy (dystocia, pregnancy toxemia or transient diabetes mellitus, etc.), prevention of mammary or prostatic neoplasms and reproductive organs (uterus, ovaries, testes), prevention of sexually transmitted diseases (TVT, Brucella infection, etc.), prevention of pyometra[39];
- economic restraints of the country for which pet owners can rarely afford veterinary expenses such as the cost of a surgery, and the government cannot control stray dog population.

This explains the high frequency of tumors linked to sexual behavior, such as transmissible venereal tumor, and to fertile status, such as mammary tumors. Mammary tumors represent the most frequently diagnosed neoplasm in intact female dogs[33]. It is widely known that females are predisposed to present this disease because of the tropism of natural estrogens in relation to the mammary gland that are capable of stimulating cell proliferation and generating carcinogenesis

through potential cells. Likewise, it represents stimulation in transformed or pre-initiated cells in order to promote its expression[72]. Canine mammary tumor (CMT) frequency reported in different countries may vary according to the place where the study was conducted. The study performed in Mexico (16% CMT frequency) was similar to the one conducted in Venezuela[9] (17.1% CMT frequency). These data are very similar to mammary tumors frequency (19% CMT frequency) found in Cuba, even if the low number of cases did not allow to perform a significant statistical analysis. On the contrary, in developed countries, the frequency of female dog mammary tumors has diminished due to reproductive health policies. These programs are responsible for spaying animals at an early age, and as a result, there are fewer chances of developing the disease[60].

In female dogs, mammary cancer is a heterogeneous group in terms of morphology and biological behavior[25]. Mammary tumors represent 50% of all malignancies affecting intact female dogs[44]. A study in Norway, where almost all female dogs are intact because spaying was illegal till 2010 due to a Norway's Animal Welfare Act, found a crude incidence of malignant mammary tumors of 53.3%[41]. Although calculation of crude incidence rates was not possible, this information support our findings, which showed a high malignancy rate in mammary tumor cases.

A high frequency of mammary tumors in intact females, together the sexual activity predisposing to TVT, explains also why in this study there was a big difference of tumors by sex, since 67% of tumor cases involved females, and mammary gland and genitourinary system were among the most frequent recorded tumor anatomical locations.

About tumor types topic, high frequency of squamous cell carcinomas (12%) and melanomas (7,5%) in Cuba deserves consideration. Primary risk factor of squamous cell carcinoma (SCC) is sun exposition, since SCC often arises on a pre-cancerous condition called actinic keratosis, caused by cumulative UV exposure from sunlight[55]. The mechanism frequently proposed for cutaneous SCC and its association with UV light involves the tumor suppressor gene p53. This gene encodes a protein (p53) that arrests the cell cycle when DNA damage is present, giving the cell time to repair the damage before continuing mitosis. If the damage cannot be repaired, p53 will induce apoptosis of the cell. UV light is a common carcinogen that can mutate the p53 gene. Cells in which the p53 gene is mutated continue replication even if DNA damage is present, leading to the accumulation of other mutations and a greater chance of neoplasia[71]. The high frequency of squamous cell carcinomas revealed in this study could be explained by the tropical climate condition of Cuba. As a measure of ultraviolet ray intensity, World Health Organization adopted the Ultraviolet (UV) Index. UV index is a scale that represents the intensity of UV radiation produced by the sun, designed as an open-ended linear scale, directly proportional to the intensity of UV radiation that causes sunburn. UV index ranges of risk of 7-8, 8-11 and above 11 indicate respectively 'high', 'very high' and 'extreme' harm from sun exposure[83]. Unlike Italy where average UV index is often under 7, in Cuba average UV index is 9-12 for most of the months[81], thus harm risks from sun exposure are almost always 'very high' and 'extreme'.

The same considerations could be applied to the high frequency of melanomas recorded in this study. Even if pathogenesis of malignant melanoma is not totally clear, the sunlight was one of the first agents recognized to be carcinogenic. There is convincing evidence from epidemiologic studies that exposure to solar radiation is the major cause of melanoma in light-skinned and light-pigmented dogs. The reason is because the animal's low pigment in the skin and hair is more likely to increase the skin's cellular mutations from ultraviolet light. The molecular mechanisms by which UV radiation exerts its varied effects are not completely understood, however, it is considered that UVA and UVB are equally critical players in melanoma formation. Whereas UVA can indirectly damage DNA through the formation of reactive oxygen radicals, UVB can directly damage DNA causing the apoptosis of keratinocytes by forming the sunburn cells. Besides action through mutations in critical regulatory genes, UV radiation may promote cancer through indirect mechanisms, e.g. immunosuppression and dysregulation of growth factors[65].

Another tumor type frequently diagnosed in this study was lymphoma. Similar to results recorded by the Italian Genoa Animal Cancer Registry[40], this exceptionally higher frequency could be due to diagnoses performed by cytology or because of exposure to unknown risk factors present in a large city like Havana. Although no definitive cause for canine lymphoma has been established, living in industrial areas and exposure to (household) chemicals[18, 69], living near waste incinerators, radioactive or polluted sites[38, 50], and exposure to magnetic fields[57] were all shown to increase the risk of developing lymphoma. Further evidence that environmental toxins might play a role in carcinogenesis comes from the observation that defective genotypes of the detoxifying enzyme glutathione-S-transferase (GST), and GST theta 1 (GSTT1) in particular, are overrepresented in human cancers. Of the 27 GSTT1 variants identified in the dog, one genotype was found to be present in 18% of all lymphoma cases and the observed mutation was predicted to affect mRNA splicing and, as a result, enzyme expression and activity[22]. Failure to repair DNA damage, resulting, for instance, from oxidative stress or radiation, increases the risk for developing neoplastic diseases and it was found that golden retrievers with lymphoma had a lower capacity for DNA damage repair compared to golden retrievers without lymphoma or mixed-breed dogs[74].

What so far discussed is the result of mere indications emerged from a simple descriptive analysis of first data, because the low number of cases and the strong technical limitations of this study cannot result in a statistically strong conclusion. To the low case series, the bias determined by an altered denominator must be added, being represented by a cohort of dogs deviated from the real diagnostic variability, because of spoiled by the fact to be patients with high probability of tumor. Moreover, the diagnostic method, based on cytological and non-histopathological evaluation of tumors as in other animal cancer registries, greatly increases both the possibility of incurring incorrect diagnoses and performing a wrong tumor classification.

The strength is undoubtedly represented by having started an epidemiological study design in a country that, considering the enormous geo-climatic, social and political differences,

could represent an excellent term for future comparisons. The final part of this study resulted in a presentation of collected data to the research directors of the National Institute of Oncology and Radiobiology, both in veterinary and human field, where the potential of Cuba for scientific research in comparative oncology was highlighted, since for many of the most of represented tumors in this study, international scientific literature considers the dog as spontaneous animal model for researches in human field.

4. GENERAL CONCLUSION

An animal cancer registry has the potential to increase our knowledge of the distribution of neoplasia in the companion animal population while at the same time being an instrument with which we are able to monitor fluctuations in cancer occurrence. In addition, animals suffering from specific neoplastic diseases may be selected and utilized as spontaneous animal models for human cancers. Veterinary practitioners may benefit from a registry by obtaining data for specific breeds, age groups or geographic areas, as these may vary considerably from surveys and registries quoted in books and journals.

An important contribution that veterinary cancer registries can provide is data on identification of geographical differences and high or low animal risk groups, providing insight into the etiology of neoplasia, as clearly suggested in this study.

A major challenge in the creation of a registry is to communicate its existence to the veterinarians and to create incentives for the participants to join and maintain this valuable research tool.

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6. LIST OF ACTIVITIES AND PUBLICATIONS

Scientific communications:

- **Scarpona S**., Berardi S., Mari S., Magi G.E., Mariotti F., Rossi G. An uncommon case of clear cell odontogenic carcinoma (CCOC)in a dog: immunomorphological characterization and literature review. LXX Convegno SISVet, 13-16 June 2016, Palermo
- **Scarpona S.**, Berardi S., Bordicchia M., Rossi G. Th1 paradigm: Redirect the cell-mediated immune response in a typical Th2 disease such as Leishmaniasis. International Veterinary Immunology Symposium - IVIS 2016, 16-19 August 2016, Gold Coast, Queensland (Australia)
- **Scarpona S.**, Berardi S., Bordicchia M., Rossi G. Th1 paradigm: Redirect the cell-mediated immune response in a typical Th2 disease such as Leishmaniasis. International Congress of Immunology - ICI 2016, 21-26 august 2016, Melbourne, Victoria (Australia)
- **Scarpona S.**, Berardi S., Eleuteri A.M., Suchodolski J.S., Gavazza A., Bordicchia M., Rossi G. Gut microbiota modulation enhances amyloid-β uptake by macrophages of an Alzheimer's disease triple transgenic mice model. 34° Meeting of the European Society of Veterinary Pathology - ESVP 2016, 7-10 September 2016, Bologna
- **Scarpona S.** I giusti alimenti per ogni stadio: guida ragionata all'alimentazione del paziente nefropatico. 56° Congresso annuale AIVPA (Associazione Italiana Veterinari Piccoli Animali), 5-7 May 2017, Piacenza
- **Scarpona S.**, Gioacchini G., Cerquetella M., Gavazza A., Rossi G., Carnevali O., Bassotti G., Bonfili L., Marini M.C., Berardi S. Probiotic modulation of the microbiota-gut-brain axis: some evidence at different evolutionary levels. The lesson of the endocannabinoid system. 2° Convegno a cura delle piattaforme tematiche di Ateneo su "Alimenti e Nutrizione" e "Salute Umana e Animale", 4 July 2017, Camerino (MC)

Publications:

- **Scarpona S.**, Berardi S., Bordicchia M., Rossi G. Th1 paradigm: redirect the cell-mediated immune response in a typical Th2 disease such as leishmaniasis. European Journal of Immunology. August 2016, 46(1):801.
- **Scarpona S.**, Berardi S., Eleuteri A.M., Suchodolski J., Gavazza A., Bordicchia M., Rossi G. Gut microbiota modulation enhances amyloid-B uptake by macrophages of an Alzheimer's disease triple transgenic mouse model. J Comp Path, 2017, vol. 156,54-141, pg. 109
- D'Ettorre G., Rossi G., Scagnolari C., Andreotti M., Giustini N., Serafino S., Schietroma I., Scheri G.C., Fard S.N., Trinchieri V., Mastromarino P., Selvaggi C., **Scarpona S.**, Fanello G., Fiocca F., Ceccarelli G., Antonelli G., Brenchley G.M., Vullo V. Probiotic supplementation promotes a reduction in T-cell activation, an increase in Th17 frequencies, and a recovery

of intestinal epithelium integrity and mitochondrial morphology in ART-treated HIV-1-positive patients: Probiotic and HIV-1-infected patients. Immunity, Inflammation and Disease, 2017 Apr 20. doi: 10.1002/iid3.160

- Rossi G., Cerquetella M., **Scarpona S.**, Pengo G., Fettucciari K., Bassotti G., Jergens A.E., Suchodolski J.S. Effects of probiotic bacteria on mucosal polyamines levels in dogs with IBD and colonic polyps: a preliminary study. Beneficial Microbes; 2017 Oct 12:1-10. doi: 10.3920/BM2017.0024
- Bonfili L., Cecarini V., Berardi S., **Scarpona S.**, Suchodolski J.S., Nasuti C., Fiorini D., Boarelli M.C., Rossi G., Eleuteri A.M. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. Scientific Reports, 2017 May 25;7(1):2426. doi: 10.1038/s41598-017-02587-2.
- Bonfili L., Cecarini V., Cuccioloni M., Angeletti M., Berardi S., **Scarpona S.**, Rossi G., Eleuteri A.M. SLAB51 Probiotic Formulation Activates SIRT1 Pathway Promoting Antioxidant and Neuroprotective Effects in an AD Mouse Model. Molecular Neurobiology; October 2018, Volume 55, Issue 10, pp 7987–8000

Participation to projects:

- FAR University research project of the University of Camerino. Years 2015-2017. Title "GUT TLR4 overstimulation by probiotics administration, associated to an immunomodulatory protocol as potentially safe and effective new farmacological tool in a transgenic mouse model of Alzheimer's Disease". Principal investigator: Prof. Giacomo Rossi, University of Camerino. Participation to the WP4 Task.

Courses:

- Research ethics and integrity – School of Advanced Studies (SAS) activities 2016
- La valutazione tecnico scientifica dei progetti sperimentali con animali - OPBA Unicam. 25 May 2016
- English for writing research papers - School of Advanced Studies (SAS) activities 2016. May 2016
- How to get published - School of Advanced Studies (SAS) activities 2016. June 2016
- Course on confocal microscopy – Unicam. September 2016
- Scientific English - School of Advanced Studies (SAS) activities 2016. October 2016
- Executive Master in Clinical Pathology – Unisvet. November 2015 – June 2018