# **Original Article** Clinicopathological Diversity and Epidemiological Aspects of Canine and Feline Mammary Gland Tumors in Tehran: A Survey (2020-2022)

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# ABSTRACT

**Background:** Mammary tumors are common in dogs and cats. They are models for investigating carcinogenesis and designing treatment protocols that benefit human beings. Senescence, sex, and reproductive status affect the development of such neoplasms.

**Objectives:** In Iran's absence of a national animal tumor registry, the present study determined clinicopathological and epidemiological aspects of canine and feline mammary tumors in referral cases of four veterinary practices across Tehran from 2020 to 2022. Here, the incidence and types of canine and feline mammary tumors are described, in addition to sex, reproductive status, age, breed, the affected mammary gland(s), grades, lymphatic invasion, and lymph node metastases.

**Methods:** All canine and feline patients with masses in the mammary gland region were considered in this study. The resected tumors and occasional lymph nodes were macroscopically scrutinized. Hematoxylin-Eosin slides were reviewed by light microscopy and immunohistochemistry was utilized when necessary.

**Results:** Of the 76 dogs and eight cats, 100% were females, and the majority were intact. Most patients were 10-11 years old. Purebreds were the most frequent referrals. In some patients, more than one mammary gland had developed neoplasm, i.e. 141 affected glands in 76 bitches and nine affected glands in eight queens. Tumors were presented in both chains and even on the ventral midline, with an increased preponderance of caudally located glands. Thus, the caudal-most glands, i.e. inguinal and caudal abdominal glands, constituted 31.2% and 66.7% of the affected glands in bitches and queens, respectively. Intraductal papillary carcinoma (17%) and complex carcinoma (16.3%) had the highest incidence in dogs. The most commonly observed lesions in cats were lobular hyperplasia with atypia, and duct ectasia, each encompassing 22.3% of the affected glands. Most canine neoplasms were grade I (90.3%), while grade II neoplasms had the highest incidence in cats (50%). Lymphatic invasion and lymph node metastases were seen in canine anaplastic carcinoma, solid carcinoma, and complex carcinoma, as well as feline comedocarcinoma and tubular carcinoma.

**Conclusion:** The risk of developing malignant tumors increases as the individual ages, and generally, caudally located mammary glands in intact purebred females are at increased risk. Moreover, anaplastic carcinomas must be precisely examined, both clinically and histopathologically, for lymph node metastases.

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# **1. Introduction**

n recent years, improved health care and hygiene have increased longevity in humans and animals, hence increasing the risk of neoplasms, as they are most commonly seen in middle-aged to old individuals (Goldschmidt et al., 2017; Nadhiya et al., 2020). Mammary gland tumors are

one of the most frequently detected neoplasms in companion dogs, cats, and humans (Vascellari et al., 2016; Goldschmidt et al., 2017; Noury et al., 2020; Hameed et al., 2022; Abdulhussain Fadhil et al., 2022). They encompass roughly more than half of the neoplastic diseases in female dogs, making them the most frequent neoplasm in bitches (Goldschmidt et al., 2011; Varallo et al., 2019; Nadhiya et al., 2020; Devarathnam et al., 2021). In addition to senescence, sex, and reproductive status also significantly affect the development of these particular neoplasms as they are most common in intact females, with a higher incidence in bitches than in queens (Argyle et al., 2008; Goldschmidt et al., 2017). After the third heat cycle, delayed ovariectomy does not remarkably inhibit mammary tumor development (Goldschmidt et al., 2017; Varallo et al., 2019). Additional risk factors, such as genetic predisposition of certain breeds, progestin prescription, imbalanced diet, obesity, environmental pollution, and radiation, significantly affect tumor development (Goldschmidt et al., 2017; Nadhiya et al., 2020). Mammary gland tumors' heterogeneity implies histopathological and behavioral diversity (Varallo et al., 2019, Devarathnam et al., 2021). Tumors of this glandular structure are composed of hyperplastic, dysplastic, benign, or malignant conditions of its epithelial and mesenchymal components (Goldschmidt et al., 2011).

In Iran's absence of a national animal tumor registry, this study provides information on the incidence and types of canine and feline mammary gland tumors in a defined population of pet cats and dogs in Tehran. Other epidemiological aspects, such as sex, reproductive status, age, breed, and the affected mammary gland(s), will also be described.

# 2. Materials and Methods

Four veterinary clinics and hospitals were chosen across Tehran. All canine and feline patients presenting tumors of the mammary gland region were included in this study from 2020 to 2022. The tumors were carefully inspected for their site, size, shape, structure, color, consistency, and involvement of the underlying tissues and sentinel lymph node(s) if excised. The resected mammary masses and occasional lymph nodes were also scrutinized and photographed by the pathologists. Then, samples were fixed in 10% neutral buffered formalin. Following the routine tissue processing, 5  $\mu$ m sections were obtained and stained with hematoxylin and eosin (H&E). When necessary, immunohistochemistry was utilized for a more precise evaluation. The malignant tumors were graded using a modified Elston and Ellis grading system for canine mammary gland tumors (CMTs) (Peña et al., 2012). A novel grading system introduced by Mills et al. (2015) was utilized for feline mammary carcinomas.

# 3. Results

Samples of 76 canine and eight feline patients were received throughout 2020-2022. All tumors were examined histologically.

## Sex

Although no gender restriction was considered in this study, all 84 referred patients were females.

## **Reproductive status**

Of the 76 canine patients, the majority (43, 56.6%) were intact, 32 cases (42.1%) were spayed after the third heat cycle, and only one case (1.3%) was ovariectomized before the third heat cycle. Among the feline patients, seven patients (87.5%) were intact, and one queen was spayed after 6 months of age (12.5%) (Table 1).

## Age

The age group of 10-11 years had the highest incidence of mammary gland tumors in both canine and feline patients (Figure 1), with 15(19.7%) and two (25%) affected individuals, respectively. The mean age of malignancy was 10 years in dogs and 11 years in cats.

## Breed

Among the various canine breeds (68 purebreds and eight mongrels), Terriers had the highest incidence, encompassing almost %31.5 of the patients (Figure 2). In the feline group, most referred patients were Persian cats, with %75 of the referred cats.

## Involved glands

A total of 141 glands were received from the 76 dogs, and nine glands were excised from the eight feline patients. All specimens revealed macroscopic and/or microscopic lesions.

Reproductive Status —	No. (%)		Total
	Dogs	Cats	IUtai
Intact	43 56.6)	7(87.5)	50
Spayed after the 3 <sup>rd</sup> cycle	32(42.1)	1(12.5)	33
Spayed before the 3 <sup>rd</sup> cycle	1(1.3%)	0	1

Table 1. Frequency and percentage of cases according to their reproductive status

#### Location of tumor

Within the canine group, 29 patients (38.2%) had developed tumors on the right side, 32 cases (42.1%) on the left side, and 14 cases (18.4%) on both sides. Only in one case (1.3%), the tumor was presented on the ventral midline. The highest involvement incidence was noted in the inguinal pair (31.2%), followed by the caudal abdominal (27.7%), cranial abdominal (19.1%), caudal thoracic (12.8%), and cranial thoracic (9.2%) pairs. In the feline patients, four cases (50%) had developed tumors on the right side, two cases (25%) on the left side, one case (12.5%) on both sides and one case (12.5%) on the ventral midline. The maximum incidence of lesions was noted in the caudal abdominal pair (66.7%), followed by the cranial abdominal pair (22.2%), while cranial thoracic was less affected (11.1%), and no tumor was observed in caudal thoracic glands (Figure 3).

## Number of involved glands in each individual

In the canine group, the number of submitted and affected glands in each individual ranged from one to eight, with the preponderance of involvement of only one gland (47 dogs). The number varied from one to two glands in the cats, with a similar preponderance of a single gland involvement (seven cats) (Figure 4). Interestingly, multiple tumors were seen exclusively in patients that were either intact or spayed after their third heat cycle/six months of age, i.e. 29/75 dogs (38.6%) and 1/7 cats (14.2%).

#### Histologic characteristics

A total of 32 various pathologic conditions were diagnosed in the 150 submitted mammary glands (Figure 5). Some glands showed more than one distinct histopathologic diagnosis. The lesions were composed of malignant neoplasms (113 in dogs (80%) and six in cats (66.7%)), benign neoplasms (ten in dogs (7%) and one in cats (11.2%)), and hyperplastic/dysplastic conditions (25 in dogs (17.8%) and five in cats (55.6%)). Of the 30 different lesions diagnosed in 141 glands in canine patients, intraductal papillary carcinoma (Figure 6) had the highest incidence with 24(17%). Complex carcinoma (Figure 7) had the secondhighest incidence with 23(16.3%) affected glands, followed by carcinoma-mixed type with 20(14.1%) neoplastic glands (Figure 8). In feline patients, the most observed lesions



Figure 1. Age-wise frequency of canine and feline patients



Figure 2. Breed-wise frequency of canine patients

were lobular hyperplasia with atypia, and duct ectasia, each with 2(22.3%) affected glands. In five dogs and two cats, more than one histologic lesion was seen within each gland. A combination of lobular hyperplasia with atypia and epitheliosis was diagnosed in a 12-year-old intact Terrier. Concurrent tubulopapillary carcinoma and suppurative mastitis were seen in a nine-year-old intact Siberian Husky. Intraductal papillary carcinoma was associated with lobular hyperplasia with atypia in a five-year-old Docker spayed after its third heat cycle. Duct ectasia and intraductal papillary adenoma were simultaneously observed in an 11-year-old Terrier spayed after its third heat cycle. An eight-year-old Terrier, spayed after its third heat cycle, suffered from duct ectasia with dysplastic alterations. In two cats, a three-yearold Persian and a 15-year-old domestic shorthair (DSH), duct ectasia was seen accompanied by lobular hyperplasia



Figure 3. Frequency of canine mammary gland tumors by anatomic location of the gland



Figure 4. Frequency of canine and feline mammary gland tumors by the number of affected glands in each individual

with atypia and tubulopapillary carcinoma, respectively. This heterogeneity was also observed between different glands of the same individual (13 dogs and one cat), i.e. neoplastic glands presented various neoplasms.

# Grades

Within the diagnosed neoplasms in the canine group, 102 malignancies were grade I (90.3%), seven were grade II (6.2%), and four were grade III (3.5%). Within



Figure 5. Frequency of the diagnosed lesions in canine and feline patients



**Figure 6.** Intraductal papillary carcinoma. Intraductal invaginations of neoplastic epithelial cells supported by fibrovascular stroma (arrows) with remarkable lymphoplasmacytic infiltration (asterisk) (H&E. 40x)

the feline patients, two neoplasms were grade I (33.4%), three were grade II (50%), and one was grade III (16.6%).

Lymphatic invasion and lymph node metastases

# 4. Discussion

In the canine patients, all three anaplastic carcinomas (grade III), one solid carcinoma (grade III), and one complex carcinoma (grade I) had developed lymphatic invasions and lymph node metastases. Within the feline

There are several known risk factors for mammary gland tumors. Epidemiological studies help determine the importance of these factors in different geographical areas and their direct or indirect role in tumor development. The provided data can also benefit tumor pre-

patients, lymphatic invasion was seen in one comedocar-

cinoma (grade III) and one tubular carcinoma (grade II).



**Figure 7.** Complex carcinoma. Foci of invasive malignant epithelial cells (arrows) surrounded by proliferative myoepithelial cells (arrowheads). (H&E staining, 40x)



Figure 8. Carcinoma- mixed type. Foci of invasive malignant epithelial cells (arrows), with osseous (arrowhead) and cartilaginous (asterisk) metaplasia (H&E staining, 40x)

vention and decrease tumor incidence by establishing reproductive health guidelines for domestic animals. Moreover, the frequency of mammary gland tumors may differ depending on the geographical location and time of the study. Breed popularity, dietary habits, environmental factors, and carcinogen exposure levels may be unique to each geographical area (Devarathnam et al., 2021).

The well-known mitogenic role of estrogen and progesterone predisposes females to mammary gland neoplasms (Salas et al., 2015; Nadhiya et al., 2020, Devarathnam et al., 2021). In the present study, all tumors were received from bitches and queens. This observation is in agreement with earlier reports (Zatloukal et al., 2005; García et al., 2019; Nadhiya et al., 2020; Noury et al., 2020; Devarathnam et al., 2021). Patel et al. (2019), Šoštarić-Zuckermann et al. (2013), and Saba et al. (2007) recorded few mammary gland tumors, being usually benign, in male dogs. It has now become clear that ovariectomy significantly decreases the risk of mammary gland neoplasm development if performed before the first heat cycle. This beneficial effect can still be attained, but to a much lesser extent, if dogs are ovariectomized before their second and third heat cycles. Ovariectomy after the third heat cycle does not decrease the risk of mammary gland neoplasm development compared to intact individuals (Salas et al., 2015; Goldschmidt et al., 2017). Ovariohysterectomy of cats before six months of age has proven to reduce the risk of mammary gland malignancies by 91%. Spaying before one year of age is also

protective by 86% (Overley et al., 2005). In both canine and feline patients, intact animals had the highest incidence of mammary gland neoplasms, followed by those spayed after their third heat cycle. Only one dog, spayed before its third heat, had developed a mammary gland tumor, and the neoplasm was benign. This finding is in harmony with the results of previous studies (Hemanth et al., 2015; Sahabi et al., 2015; Salas et al., 2015; Goldschmidt et al., 2017; Nadhiya et al., 2020; Devarathnam et al., 2021; Gedon et al., 2021). Additionally, Nadhiya et al. (2020) recorded mammary gland tumors exclusively in intact bitches. In the present study, only intact individuals or those who were spayed after the appropriate age had developed multiple mammary gland tumors and this is in agreement with the findings of a recent study on 625 dogs (Gedon et al., 2021).

The malignancy rate has not been the same in different studies on CMTs, with a wide range of 20-80% (Goldschmidt et al., 2017). In the present study, 80% of the submitted canine specimens were malignant. This higher incidence is in harmony with that estimated previously (Zatloukal et al., 2005; Šoštarić-Zuckermann et al., 2013; Pastor et al., 2018; Patel et al., 2019; Silva et al., 2019; Nadhiya et al., 2020). In contrast, a study conducted in Korea reported a higher incidence of benign CMTs compared to malignant ones (Kim et al., 2009). The incidence of benign and malignant CMTs was seen almost equally in a study (Salas et al., 2015).

The incidence of hyperplastic/ dysplastic changes was higher than benign neoplasms and lower than malignant ones, which is in contrast to the findings reported by Zatloukal et al. (2005). Intraductal papillary carcinoma had the highest incidence in dogs, followed by carcinomamixed type. Nadhiya et al. (2020) reported carcinomamixed type as the third most frequent malignant neoplasm. In a study, complex carcinoma was reported as the second most frequent mammary gland neoplasm (Ežerskytė et al., 2011), precisely the same as the present study's findings. Conversely, benign mixed mammary gland tumor was the most frequently observed neoplasm in a previous study (Kim et al., 2009). In the present study, only one case of benign mixed mammary gland tumor was recorded. Also, most malignant neoplasms were of epithelial origin, which is consistent with previous works (Sorenmo et al., 2009).

Intra-glandular and inter-glandular heterogenicity were noted in the canine and feline patients in the present study, which is consistent with the findings of the aforementioned researchers (Sorenmo et al., 2009; Hemanth et al., 2015). In a study conducted in Poland, cats were mostly affected by malignant mammary tumors (Ciaputa et al., 2013), which is in contrast to our findings, where duct ectasia and lobular hyperplasia with atypia were the most frequently encountered lesions. In our research, dogs and cats were most affected in the age group of 10-11 years. Malignancy was found at the mean age of ten in dogs and 11 in cats, which is in harmony with the findings of Zatloukal et al. (2005) and Kim et al. (2009). It has been stated that dogs younger than two seldom develop mammary gland tumors (Zatloukal et al., 2005). This is in agreement with our findings as the youngest dogs were in the age group of 4-5 years, and the youngest cats were 3-4 years old. Also, Noury et al. (2020) observed mammary gland tumors as the most frequent neoplasm in middle-aged to old dogs, and Nadhiya et al. (2020) reported the highest occurrence in the age group of 9-12. Another interesting trend is the increasing number of affected bitches from five to 12 years old, peaking at 10-12 years, and then decreasing in older ages (Zatloukal et al., 2005; Pastor et al., 2018). In the present study, the peak incidence was in the age group of 10-11 years, and there was a slight increase in the 12-13 years age group. Otherwise, the overall pattern was similar. In a study conducted by Ežerskytė et al. (2011), only 4% of the affected dogs were in the age group of 1-5 years old. Various breed-wise incidences have been reported. Spitz (Devarathnam et al., 2021), Poodle (Zatloukal et al., 2005; García et al., 2019; Silva et al., 2019), Labrador (Nadhiya et al., 2020), Maltese (Kim et al., 2009), and German Shepherd (Patel et al., 2019) have

been introduced as the most affected canine breeds by earlier researchers. However, Spitz was the second most affected breed in the present study, which is relatively in agreement with the aforementioned reports. Another study reported mammary gland tumors as the most frequent tumor in all breeds, except for Rottweiler and the Pekinese, in which skin and bone tumors are the most frequent, respectively (Noury et al., 2020).

It is worthwhile to mention that breed popularity has a direct impact on the incidence of documented mammary gland tumors among various breeds. In the present study, Terriers and Persian cats had the highest incidence of mammary gland tumors, but this also reflects that these breeds are particularly favorite in Iranian families. However, our study highlights a higher incidence in purebred dogs and cats compared to mongrels, which is consistent with the findings of previous studies (Zatloukal et al., 2005; Hemanth et al., 2015; Sahabi et al., 2015; Silva et al., 2019; Nadhiya et al., 2020; Devarathnam et al., 2021), and indicates a remarkable predisposition of purebred dogs as has previously been stated (Dorn et al., 1968). This finding contrasts papers published in Mexico (Salas et al., 2015; García et al., 2019) and Spain (Pastor et al., 2018). In the majority of cases, only a single gland was involved, i.e. 61.8% of dogs (47/76) and 87.5% of cats (7/8). This result is in correspondence with earlier reports (Nadhiya et al., 2020) but in contrast to a study conducted in 2009 (Sorenmo et al., 2009), where 100% of the involved dogs were intact. In our study, the left-side mammary chain was most affected in the canine group. That is similar to earlier reports on dogs (Nadhiva et al., 2020) but in contrast to what was reported by Hemanth et al. (2015). Within the canine group, the highest involvement was seen in the inguinal mammary glands, with a gradual decrease moving cranially to caudal abdominal, cranial abdominal, caudal thoracic, and cranial thoracic glands. This is similar to that reported by Hemanth et al. (2015), Silva et al. (2019), Nadhiya et al. (2020), and Devarathnam et al. (2021). In a study conducted in Lithuania, Ežerskytė et al. (2011) reported a higher incidence of CMTs in the caudal abdominal glands compared to the inguinal glands. It is worth noting that the caudal abdominal glands have been observed to be the most affected gland by Patel et al. (2019). Generally, the propensity of caudally located glands has been attributed to the fact that caudal glands are more exposed to trauma compared to cranial ones (Runnells et al., 1965). Interestingly, in a study on 36 dogs with CMTs, none had developed tumors in the cranial and caudal thoracic glands (Devarathnam et al., 2021).

# 5. Conclusion

CMTs are exceptional models that provide insights into carcinogenesis and treatment, being beneficial for dogs as well as human beings. The risk of developing malignant CMTs increases as the individual ages, and generally, caudally located mammary glands in intact purebred females are at increased risk. Moreover, ovariectomized companion animals are less likely to develop multiple mammary gland tumors.

# **Ethical Considerations**

#### Compliance with ethical guidelines

All ethical principles are considered in this article. The participants were informed of the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information and were free to leave the study whenever they wished, and if desired, the research results would be available to them. A written consent has been obtained from the subjects. principles of the Helsinki Convention was also observed.

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The paper was extracted from the DVSc thesis of Diba Golchin, at Department of Pathology, Faculty of Veterinary Medicine University of Tehran.

#### Authors' contributions

Conceptualization and methodology: Farhang Sasani, Diba Golchin; Validation, supervision, and project administration: Farhang Sasani; Investigation: Farhang Sasani, Mir Sepehr Pedram, Diba Golchin; Data curation: Mir Sepehr Pedram, Diba Golchin, Zohreh Khaki; Original draft preparation and visualization: Diba Golchin; Review and editing: Farhang Sasani.

#### **Conflict of interest**

The authors declared no conflict of interest.

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# مقاله پژوهشی

تنوع کلینیکوپاتولوژیک و جنبههای اپیدمیولوژیک تومورهای پستانی سگ و گربه در تهران (۲۰۲۰–۲۰۲۲)

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<b>حِک</b> يد
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زمینه مطالعه: تومورهای پستانی در سگ و گربه شایعاند. این تومورها مدل هایی برای بررسی سرطان زایی و طراحی پروتکل های درمانی برای انسان هستند. پیری، جنسیت و وضعیت باروری بر ایجاد چنین نئوپلاسمهایی تأثیر گذار است. امداذ مد خاص از کار است تر می مار می انا تر در ایا می و و و و و از می بردان از می اترا و و کراچی و مراحی کرد.

اهداف: در غیاب سازوکار ملی ثبت تومورهای حیوانات در ایران، پژوهش حاضر جنبههای بالینی ـ پاتولوژیک و اپیدمیولوژیک تومورهای پستان سگها و گربهها را در چهار مرکز درمانی دامپزشکی در تهران از سال ۲۰۲۰ تا ۲۰۲۲ بررسی میکند. فراوانی و انواع تومورهای پستانی سگ و گربه بررسی شده و به جنسیت، وضعیت تولیدمثل، سن، نژاد، غدد پستانی آسیب دیده، گرید، تهاجم لنفاوی و متاستاز به غدد لمفاوی اشاره خواهد شد.

روش کار: تمامی سگها و گربههای دارای تودهای در ناحیه پستانی وارد مطالعه شدند. تومورهای برداشتهشده و غدد لنفاوی به صورت ماکروسکوپیک بررسی شدند. در ارزیابی میکروسکوپ نوری، از رنگآمیزی هماتوکسیلین ـ ائوزین و درصورت نیاز ایمیونوهیستوشیمی استفاده شد.

نتایج: از ۷۶ سک و ۸ گربه مورد بررسی، همگی ماده بودند و بیشتر عقیم نشده بودند. بیشتر بیماران ۱۰ تا ۱۱ ساله بودند. نژادهای خالص بیشترین موارد را تشکیل میدادند. در برخی از بیماران، نئوپلاسم در بیش از یک غده پستانی مشاهده شد، یعنی ۱۴۱ غده مبتلا در ۷۶ سک و ۹ غده در ۸ گربه. تومورها در هر دو زنجیره، و حتی در خط وسط شکم، با فراوانی بیشتر غدد خلفی مشاهده شدند. بنابراین، غدد مغابنی (اینگوینال) و خلفی شکمی به ترتیب ۶۹/۲ درصد و ۲۱/۳ درصد از غدد توموری را در سگ ها و گربهها تشکیل میدادند. اینتراداکتال پاپیلاری کارسینوما (۱۷ درصد) و کمپلکس کارسینوما (۱۶/۲ درصد از غدد توموری را در سگ ها و گربهها تشکیل ضایعات مشاهده شده در گربهها هیپرپلازی لوبولار همراه با آتیپی و اتساع داکتها بود (هر کدام ۲۲/۲ درصد). اکثر نئوپلاسمهای ضایعات مشاهده شده در گربهها هیپرپلازی لوبولار همراه با آتیپی و اتساع داکتها بود (هر کدام ۲۲/۳ درصد). درصد) در مد سگ درجه (گرید) یک (۲۰/۳ درصد) بودند، در حالی که نئوپلاسمهای درجه دو بالاترین فراوانی را در گربهها داشتند. (۵۰ درصد) تهاجم لنفاوی و متاستاز به غدد لنفاوی در سگ در آناپلاستیک کارسینوما، سالید کارسینوما و کمپلکس کارسینوما، و در گربه در مدره ا

تاریخ دریافت: ۲۰ تیر ۱۴۰۱ نتیجه گیری نهایی: خطر ابتلابه تومورهای بدخیم با افزایش سن افزایش می یابد و به طور کلی، غدد پستانی خلفی در مادههای عقیمنشده تاریخ پذیرش: ۲۱ شهر بور ۱۴۰۱ خالص بیشتر مستعد بروز تومور هستند.

کلیدواژهها: گربه، سگ، پستان، نئوپلاسم، سرجیکال پاتولوژی

عنویسنده مسئول:

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