Incidence of Canine Digestive System Tumours in Baghdad Province

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ABSTRACT

Tumours in pets occur naturally and are common in dogs as they are in humans. Tumours of the alimentary system have been reported in animals, although less frequently. Food patterns and specific diet components could have a big role in such tumour occurrence. Therefore, the aim of this study was to investigate the clinical signs and inspect the neoplasm lesions of the gastrointestinal (GI) tract grossly and histopathologically in dogs in Baghdad, Iraq, and to see whether pet diets had any tumourigenic effect. Throughout two years sixteen dogs were referred to Teaching Animal Hospital and private clinics at Baghdad city with masses in (GI) tract. Males and large size breeds were more likely to be affected with GI tumours. Half of the cases aged 5-10 years and belonged to German Shepherd, while the other half involved the other breeds (i.e. Belgian Malinois, Labrador, Pointer GSP and Terrier) equally. Biopsies were taken from the tumour tissues for histological examination. Clinically, the animals presented general signs of digestive system abnormalities, like difficult defecating, bloody stool, lethargy and weight loss. Grossly, the tumours varied in their sizes from <3 cm to >5 cm in diameter, and the most frequent site of occurrence (10 out of 16 cases) was in the large intestine. Histologically, most tumours reported were malignant, and included adenocarcinomas (6/16) followed by leiomyosarcoma (4/16), adding to 2/16 belonged to carcinoid tumour. However, lipoma and viral papilloma (4/16) were also registered as benign tumours. In conclusion, tumours of canine digestive system do exist but rare. Healthy nutrition of dogs may prevent this type of cancer by consuming enough amount of raw meat, little artificial food, e.g. kibble along with some vegetables and fruits. Additionally, the information gained from dogs could be used to suspect and test the effect on humans as they have strong pathophysiology similarities.

Keywords: Pet foods, Household dogs, Digestive system, Tumour, Histopathology

Introduction

Tumour is a complex multi-stage biological process caused by many etiological factors (1). Throughout this process, a normal cell obtains capabilities that cause its transformation into a tumourigenic, and may in the end become malignant cell (cancer) if the cell is not recognized and/or eradicated by the immune system (2). Neoplasia usually is an environmental disorder, which is intensely related to nutritional factors and triggered by genetic factors. Nutritional factors have been considered to be responsible for approximately 30% of cancers in more developed countries (3). Intestinal adenocarcinoma of dogs constitutes roughly 0.5% of all malignant tumours. Approximately, 40% of these cancers are situated in the colon and rectum (4, 5).

Processed meat intake has been suggested to be involved in the occurrence of colorectal cancer, the main cause of death in wealthy nations (6). Likewise, the contemporary canine diet, which is primarily a kibbled meal-based diet, is associated with numerous cancers in both humans and dogs. The latest diet has been shown to be a source of carcinogenic compound like advanced glycation

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end products (AGEs) that are closely associated with chronic systemic inflammation (metafla-mmation) (7 - 9). In addition, pelleting of animal food products causes decrease in mineral absorption from the meal (10). These compounds were also observed within canned food (11). This situation can be applied to dogs, humans and mammals as a whole (12, 13).

Similarities exist between humans and pets regarding anatomy, physiology, and tumour onset and its progression (14). Moreover, canine tumours of GI tract have been found to share molecular pathways with human tumours (15). So that dog studies may be predictive of results in human, similarly, human studies could be predictive of results in dog. Consequently, dog studies offer couple of benefits for dogs themselves and for the possibility to generalize the results to humans. This makes dogs a good model for studying and understanding neoplasm in humans (14, 16). In Iraq, several studies were conducted to investigate the presence of certain neoplasms in dogs, including miscellaneous cancers, mammary gland tumours in Baghdad, mammary gland tumours in Kurdistan, and prostate cancers (17 - 20). To our knowledge, no specified studies were performed to uncover malignancies of GI tract in dogs in Baghdad or Iraq as a whole. Thus, the current research aimed to complete the series of dogs tumours, and to estimate the occurrence of canine alimentary tract neoplasms and their relationship with dietary pattern, sex, breed and age in a trial to explore the causation and risk of cancer in dogs.

Materials and Methods

Sixteen non-spayed pet dogs were diagnosed with digestive system tumours at the Teaching Animal Hospital and private local veterinary clinics in Baghdad city, Iraq.

The duration of the study was two years from June 2017 to May 2019. Informal consent was obtained from dogs' owners to include their animals in the study.

All pets have lived with their owners since early puppyhood. Case history data included the animal's age, sex, breed, date of lesions appearance (Table 1), and whether the animal had gained veterinary services was also reported.

All animals who have not received veterinary services during their lives were excluded from the study. Data were subjected to Chi-square analysis using the Statistical Package for the Social Sciences (SPSS, version 25), and $P \leq 0.05$ was considered statistically significant.

Table 1. Case history of dogs used in the study

Variable	Categories	No.	%
Age	<1 year	2	12.5
	5-10 years	8	50
	>10 years	6	37.5
Total		16	100
Sex	Male	10	62.5
	Female	6	37.5
Total		16	100
Breed	Belgian Malinois	2	12.5
	German Shepherd	8	50
	Pointer (GSP)	2	12.5
	Labrador	2	12.5
	Terrier	2	12.5
Total		16	100
Date of appearance	<3 months	12	75
	>3 months	4	25
Total		**16**	**100**

Dietary pattern: A special data form was filled up by the animals' owners, and included some questions about dogs diet. The form classified dogs into three groups based on the style of their food

patterns: the first group (G1 = 2 dogs) involved animals who had been fed on raw food such as fresh chicken, beef and fish meat; the second one (G2 = 10 dogs) included animals who used to be fed on dry and processed meals, such as kibble, pellet and canned meat; while the third group (G3 = 4 dogs) contained animals who had been fed on a mixture of food eaten by the first and second groups, plus a vegetarian diet supplements.

Clinical examination: Symptoms were documented for all the confirmed tumour cases. Superficial lymph nodes including pre-scapular, auxiliaries and inguinal were palpated seeking for enlargement as well as for unilateral or bilateral involvement.

The tumours were also grossly examined before or following surgical removal and described for location, size, numbers, and presence of erythema, ulceration, necrosis and bleeding. Occurrence of invasive growth and attachment to the nearby tissues were also reported. Furthermore, existence of other sings like constipation, diarrhoea, vomiting and their nature, i.e. bloody, serous, or serosanguineous were documented.

Tumour staging: The clinical stage of tumours, represented by the size of the greatest diameter at time of surgery, was classified as T1 (<3 cm), T2 (>3 cm but <5 cm), or T3 (>5 cm), following the World Health Organization (WHO) guidelines as reported by Sorenmo and co-workers (21).

Samples collection and histopathology:

Immediately, following surgical removal of the tumour masses, they were examined grossly and histologically. Biopsies were collected, maintained in 10% neutral buffered formalin solution and embedded in paraffin according to Humason (l979) (22). Routine protocols were followed to undertake histopathology, in which the samples were routinely processed and stained with Haematoxylin and Eosin (HE) staining according to Luna (1968) (23).

Histopathological classification: The tumours were categorised as benign or malignant according to Cullen and co-workers (24). Other features such as presence of capsule and demarcation, anisocytosis (having red blood cells; RBCs) and anisokaryosis (a larger than normal variation in the size of cells nuclei) were also documented. These features were determined as mild, moderate or severe. Furthe-rmore, mitotic index (MI), cellularity of the neoplasm, invasion to adjacent tissues, and intra-tumoural necrosis were also determined.

Results and Discussion

Gaining and caring of pet animals in our folk and our country is very rare if compared with the western world, this could be the cause of the decreased number of tumour cases reported in this study.

In addition, retrospective studies of alimentary system tumours are less common compared with tumours of other organs. However, the occurrence of these neoplasms in domestic dogs (*Canis lupus familiaris*) is relatively higher than in other domestic animals (5). As a result, throughout two years only 16 GI tract neoplasms were collected from Baghdad city. This is consistent with another study (5) who stated that among all tumour biopsies, the GI tumours were low (1.9% and 1.2% for gastrointestinal and intestinal tumours, respectively).

In this study, the most vulnerable dogs aged ≥5 years, which constituted 87.5% (14/16) of the cases, in comparison with 2/16 aged one year old. Regarding sex, males affected more than females (10 versus 6 cases). The animals exposed to alimentary tract tumours belonged to large size breeds, half of them were German shepherd, while the other half involved the other breeds i.e. Belgian Malinois, Labrador, Pointer GSP and Terrier equally. These results are in agreement with several studies that linked malignancy with advanced age, large size breeds and the masculinity (24-27).

Clinical findings

Clinical examination of some dogs under study demonstrated palpable tumour masses on certain parts of the body involving mouth, anus and rectum, unlike internal GI tract tumours whose diagnosis requested the aid of histology examination. The symptoms varied depending upon the location and size of the tumour. Varying general signs were showed by animals, including body weight loss, anorexia, constipation or diarrhoea, vomiting mixed with bloody, serous, or serosanguineous material, less frequently melena (black tarry faeces), pain and fever. However, in case of large bowel lesions, the symptoms were tenesmus (feeling of difficulty during defecation) and haematochezia (passage of fresh blood via the anus with stools). Peritonitis occurred in some animals 2/16 due to intestinal obstruction caused

by tumours. Importantly, the superficial lymph nodes (pre-scapular, axillary, and inguinal) were enlarged in all of the malignant tumours. It has been shown that canine cancers are associated with various clinical signs; with some neoplasms have the possibility to metastasize to distant areas of the body (23). Rectal examination by fingers revealed masses or annular (ring-like) structure surrounding the wall of the rectal canal as a result of rectal lesions. All the malignant cases showed enlargement of the superficial lymph nodes, including inguinal, pre-scapular, and axillary lymph nodes. In addition, invasive growth as well as attachment to the adjacent tissues were noticed. Erythema, ulceration, necrosis and/or bleeding lesions appeared on the excised masses post surgically (Figure 1).

Persistent unhealed ulcers were observed on the apparent lesions of mouth and anus. Table 2 shows the characteristics of each tumour. The tumour stages were represented by size diameter, and classified as T1, T2 or T3. Our data show 8/16 animals had <3 cm tumours and 6/16 possessed 3 - 5 cm masses. Ten of sixteen masses were uni-primary, and the same number had solitary lesions with solid texture. Concerning the location of tumours in the GI tract, 10/16 animals developed tumours in the large intestine relative to 6/16 cases showed neoplasms in other parts (mouth, small intestine and anus). Similar to this study, the colorectal region was found to be the most common site of intestinal tumours by (66.7%), followed by 28.9% of cancers occurred in the small intestine and 4.4% in caecum (5).

Table 2. Tumour characteristics

Variable	Categories	No.	%	Variable	Categories	No.	%
Size of tumour (cm in diameter)	T1: < 3	8	50	Anatomic location of the tumour	Mouth	2	12.5
	T2: 3-5	6	37.5		Small intestine	2	12.5
	T3: > 5	2	12.5		Large intestine	10	62.5
No. of tumour masses	Multiple	6	37.5		Anus	2	12.5
	Solitary	10	62.5				
Tumour shape	Regular	6	37.5	Tumour description	Multiple-primary	2	12.5
	Irregular	10	62.5		Uni -primary	10	62.5
					Multiple secondary	4	25
Tumour texture	Solid	8	50	Type of cancer	adenocarcinoma	6	37.5
					leiomyosarcoma	4	25
	Soft	6	37.5		carcinoid tumour	2	12.5
					lipoma	2	12.5
					viral papilloma	2	12.5
	Fibromata	2	12.5	Colours	Red	8	50
					Black	4	25
					Yellow	4	25
Total		16	100	Total		16	100

Figure 1. Examples of apparent digestive tract tumour lesions seen in two dogs. A: A spongy lobular, congested and ulcerated mass, 3 cm in diameter from German Shepherd dog aged 8 years which localised as a lump in anus area, B: Belgian Malinois puppy of 6 months of age had viral papillomas on lips, gum and mouth cavity, which had a rough and jagged surface looked like cauliflower.

Histopathological examination has been used broadly and solely to classify different types of neoplasia. However, the criteria used to histologically differentiate benign from malignant tumours has not yet been conclusively recognized (21). The incidence of malignant neoplasms, mainly classified as carcinomas, was found to be more frequent than benign tumours (5). Histologically, different types of umours were observed to affect various regions of the alimentary tract, these included adenocarcinoma

(6 cases), leiomyosarcoma (4 cases), and two cases for each of carcinoid tumour, lipoma and viral papilloma. This finding is partly consistent with that of (4) who observed adenocarcinomas as the most common carcinomas of the GI tract, except oesophagus and anus (the proximal and distal portions) where squamous cell carcinomas were frequent. By contrast, while mesenchymal tumour in form of lymphoma was the most common neoplasia, one third of intestinal tumours were adenocarcinomas occurred mostly in the colorectal region (5). The intestinal adenocarc-noma sections demonstrated dysplasia and hyperplasia of the epithelial sheet that showed pleomorphic hyperchromatic nuclei and neutrophils infiltration in the stromal tissue. Numerous layers of nuclei and mitotic figures were also watched. The villi were covered with mucous secretion cells forming large cystic-shape structure with severe inflammatory cells infiltration mainly neutrophils in sub-mucosa (Figure 2B). In other sections, tumour cells produced mucin in their cytoplasm that moved their nuclei to one place forming what is called signet ring (Figure 2A). Horeover, the mucin appeared in the lumen of the mucosal glands that crowded in the epithelial coating and created cystic arrangement filled with mucin lined by tumour cells, this prototype is called mucosal lakes (Figure 2D). Leiomyosarcoma sections in the intestine showed extended cells and looked hyperchromatic and vesicular, with few mitotic figures were also seen in the muscular layer (Figure 2C). Sections of carcinoid tumours formed compact solid groups, and were demarcated by thin basement membrane. Furthermore, the tumour cells looked hyperchromatic and pleomorphic in the submucosa (Figure 2E). Finally, papilloma was seen on the mouth, and characterized by hyperkeratosis, hypergranulosis, koilocytes and inclusion bodies (Figure 2F). Histopathological examination has been used broadly and solely to classify different types of neoplasia. However, the criteria used to histologically differentiate benign from malignant tumours has not yet been conclusively recognized (21). The incidence of malignant neoplasms, mainly classified as carcinomas, was found to be more frequent than benign tumours (5). Histologically, different types of umours were observed to affect various regions of the alimentary tract, these included adenocarcinoma (6 cases), leiomyo-sarcoma (4 cases), and two cases for each of carcinoid tumour, lipoma and viral papilloma. This finding is partly consistent with that of (4) who observed adenocarcinomas as the most common carcinomas of the GI tract, except oesophagus and anus (the proximal and distal portions) where squamous cell carcinomas were frequent. By contrast, while mesenchymal tumour in form of lymphoma was the most common neoplasia, one third of intestinal tumours were adenocarcinomas occurred mostly in the colorectal region (5).

The intestinal adenocarcinoma sections demon-strated dysplasia and hyperplasia of the epithelial sheet that showed pleomorphic hyperchromatic nuclei and neutrophils infiltration in the stromal tissue. Numerous layers of nuclei and mitotic figures were also watched. The villi were covered with mucous secretion cells forming large cystic-shape structure with severe inflammatory cells infiltration mainly neutrophils in sub-mucosa (Figure 2B). In other sections, tumour cells produced mucin in their cytoplasm that moved their nuclei to one place forming what is called signet ring (Figure 2A). Moreover, the mucin appeared in the lumen of the mucosal glands that crowded in the epithelial coating and created cystic arrangement filled with mucin lined by tumour cells, this prototype is called mucosal lakes (Figure 2D). Leiomyosarcoma sections in the intestine showed extended cells and looked hyperchromatic and vesicular, with few mitotic figures were also seen in the muscular layer (Figure 2C). Sections of carcinoid tumours formed compact solid groups, and were demarcated by thin basement membrane. Furthermore, the tumour cells looked hyperchromatic and pleomorphic in the submucosa (Figure 2E).

Finally, papilloma was seen on the mouth, and characterized by hyperkeratosis, hypergranulosis, koilocytes and inclusion bodies (Figure 2F).

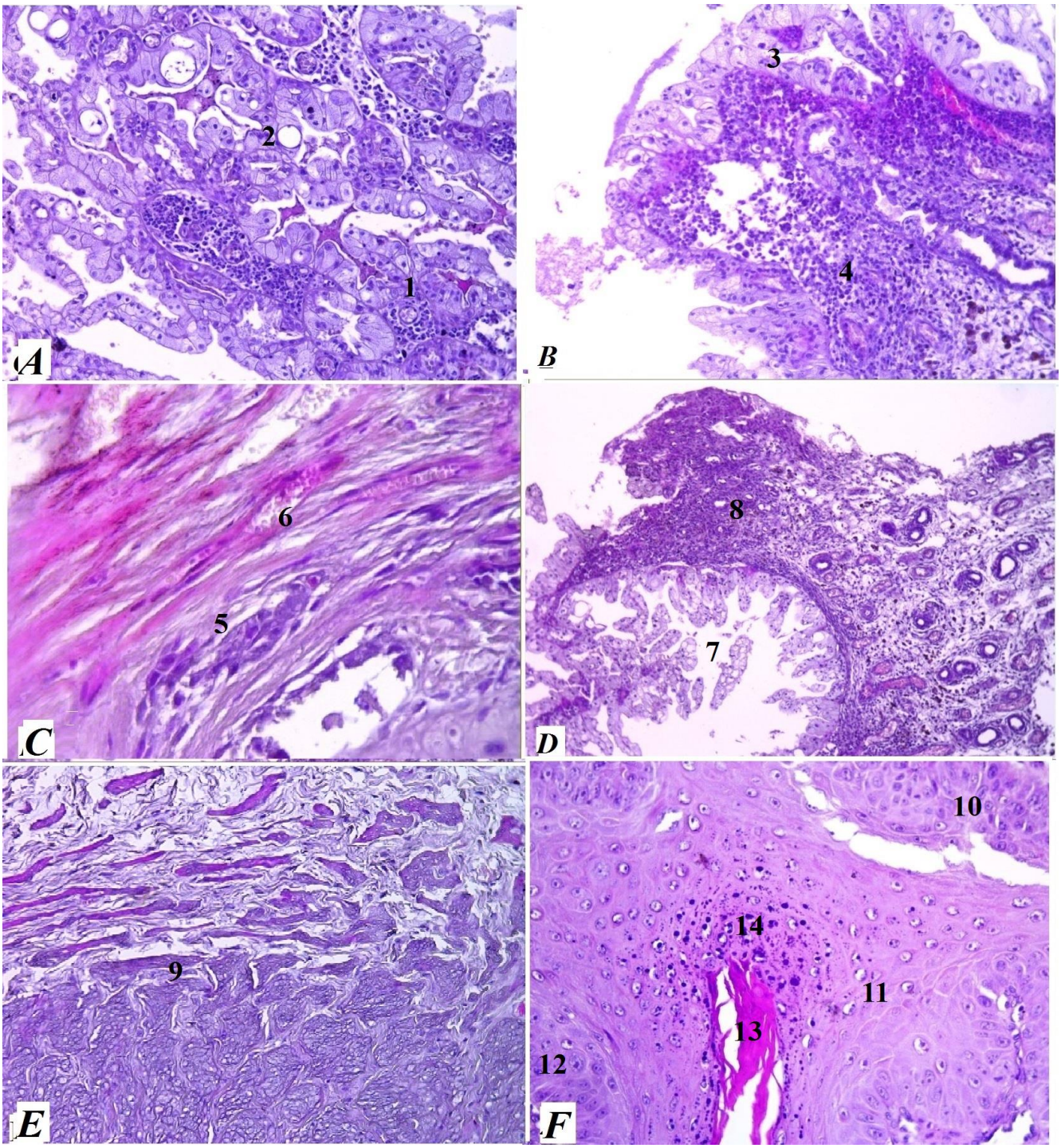

Figure 2. Histopathological cross sections of different types of tumours of various regions of the digestive system (H&E stain)

A: An intestinal adenocarcinoma section with numerous layers of nuclei and mitotic figures (1), adipose cells moved their nuclei to one place forming signet ring (2). B: Another intestinal adenocarcinoma section with dysplasia and hyperplasia of the epithelial sheet shows Pleomorphic hyperchromatic nuclei (3) and neutrophils (4) infiltrated in the stromal tissue. C: Leiomyosarcoma section in the intestine shows fibrosis muscular cells (5) active angiogenesis (6). D: Apocrine gland anal sac adenocarcinoma, the lumen of the mucosal glands form cystic arrangement filled with mucin lined by tumour cells, mucosal lake (7), and severe inflammatory cells mainly neutrophils are shown in sub-mucosa (8). E: Sections of carcinoid tumours form

compact solid groups (9), with demarcation by thin basement membrane, and the tumour cells look hyperchromatic and pleomorphic in the submucosa. F: A case of oral viral papilloma, the cells look hyperchromatic (10) and koilocytic (11), the field shows few mitotic figures (12), hyperkeratosis (13), hypergranulosis and inclusion bodies (14). Each of adenocarcinoma, leiomyosarcoma and carcinoid tumours reported in the present research are malignant; therefore, the prognosis was worse and all the animals died between 3 - 6 months of cancer onset.

Nevertheless, leiomyosarcoma is locally invasive and a slow-growing malignant neoplasm that originates from smooth muscle and typically metastasizes slowly. This tumour is the 2nd most common sarcoma of intestinal tract in dogs.

It commonly includes the caecum and jejunum of older dogs aged 10.5 years as a median (29, 30). On the other hand, both lipoma and papilloma are benign tumours with good prognosis.

Lipoma disappeared following complete surgical excision, and the animal has become healthy for more than 12 months. With respect to oral papilloma, the papillae vanished gradually by themselves within 6 - 12 weeks or by cryosurgery (freezing the papillae), adding to Vitamin E oil supplement, which was applied directly onto the papillae twice a day for 2 weeks (31). Concerning the causes of alimentary tract cancers, possibly, the GI tract is secondarily affected via the direct spread of tumour from adjacent tissues or organs, such as urinary bladder, uterus, cervix, ovaries and prostate. In addition, metastasis from remote sites, as in case of Merkel cell tumour and melanoma, may involve GI organs (4).

However, other causes of GI tract carcinomas do exist, in which certain types of diet may be one of the major sources of cancer in mammals. The ingestion of food prepared by the Maillard reaction might cause the build-up of AGEs and advanced haem in cured products (6).

In the same context, in addition to the high-fat diets that were associated with increasing the risk of metaflammation (7) creates an environment for cancer production (32).

To correlate type of diet with cancer occurrence in dogs of this study (table 3), 2/16 dogs used to be fed on raw meat only, 10/16 animals had been fed on processed food (kibble, pellet and canned meat), and 4/16 had been eaten diet similar to the aforementioned groups plus vegetables.

There was no significant difference between the groups, this could be due to the small number of animals used in this study, meaning that all of the affected animals based on meat as a main meal. As studies linked meat intake, mainly the processed one, with cancer risk, several hypotheses have been emerged to explain the reasons: 1- high-fat food might encourage carcinogenesis through faecal bile acids or insulin resistance; 2- cooking meat at a high temperature creates carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons; 3- carcinogenic N- nitroso compounds are produced in meat as well as from endogenous body sources; 4- haem iron in red meat may promote carcinogenesis as it raises cell proliferation in the mucosa, via lipoperoxidation and/or cytotoxicity of faecal water.Furthermore, nitrosation could increase the toxicity of colon cancer among others (33) and rich in protein such as red and processed meat has been linked with increased colorectal cancer risk (34). In Conclusion: Tumour of canine digestive system do exist but rare. The animal should be constantly checked and observed for any abnormal signs in the body or behaviour. Healthy nutrition of dog may prevent this type of tumour by ingesting enough amount of raw meat, little artificial food, e.g. kibble, pellet or canned meat with some vegetables or fruits. Additionally, the information gained from dogs could be used to suspect and test the effect on humans as they have strong pathophysiology similarities.

Table 3. The diet styles of canine groups

Groups	Diet style	No. of animals	Chi-Square Test
G1	Raw food (fresh chicken, beef and fish)	2	P value = 0.04 df = 4 Asymp. Sig. = 6.29
G2	Dry and processed meals (kibble, pellet and canned meat)	10	
G3	Mixture of G1 and G2 foods plus a vegetarian diet supplements.	4	

Conflict of Interest

The authors declare that there is no conflict of interest.

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دراسة نسبة حدوث أورام الجهاز الهضمي في الكلاب في محافظة بغداد

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الخلاصة

تحدث الاورام بصورة طبيعية في حيوانات التربية المنزلية، وهي شائعة في الكلاب كما هو الحال في الانسان. وقد سجلت اورام الجهاز الهضمي في الحيوانات رغم ندرة حدوثها. تلعب بعض اشكال الأغذية ومكوناتها دورا كبيرا في حدوث مثل هذه الأنواع من الاورام. لهذا السبب، كان هدف الدراسة تثبيت العلامات السريرية واجراء الفحص العياني والنسيجي لأورام القناة المعدية المعوية في الكلاب في مدينة بغداد، العراق، كذلك لمعرفة فيما اذا نمط الغذاء له أي تأثير ورمي. على مدى سنتين، أحيلت ستة عشر كلبا للمعالجة الى المستشفى التعليمي البيطري والعيادات الخاصة في مدينة بغداد كانت لديها اورام في القناة الهضمية. اكثر معدلات الاصابة كانت في الذكور والسلالات الكبيرة الحجم , تراوحت اعمار نصف الكلاب مابين ٥-١٠ سنوات، وتعود الى فصيلة German Shepherd ، بينما النصف الاخر من الكلاب كانت تعود لفصائل أخرى Belgian Malinois وLabrador و Pointer GSP وTerrier.أخذت خزع من الأورام لغرض اجراء الفحص النسيجي. اظهر الفحص السريري للحيوانات المصابة بالأورام علامات عامة لأمراض الجهاز الهضمي كصعوبة التبرز والبراز المدمى والخمول وفقدان الوزن . عيانيا، اختلفت احجام الأورام من اقل من ٣ سم الى اكبر من ٥ سم قطرا، اما المناطق الأكثر تكرارا التي ظهرت فيها فكانت الأمعاء الغليظة (10 من 16 حالات). نسيجيا، كانت معظم الأورام المسجلة من النوع الخبيث الذي تضمن 16/6 adenocarcinomas ، تلاه 16/4 leiomyosarcoma ، وكانت 16/2 من النوع carcinoid. رغم ذلك، سجلت أيضا اورام من النوع الحميد مثل lipoma و papilloma الفيروسي بنسبة 16/4 . الاستنتاج : أن سرطانات الجهاز الهضمي للكلاب موجودة بالفعل لكنها نادرة. التغذية السليمة للكلاب قد تمنع من الاصابة بهذه الأورام عندما يتغذى الكلب على كميات كافية من اللحوم النيئة و القليل من الأطعمة المصنعة والمطحونة وبعض من الخضروات والفواكه. بالإضافة إلى ذلك ، يمكن استخدام المعلومات التي تم الحصول عليها من الكلاب لتوقع واختبار التأثير على البشر نظرًا لوجود أوجه تشابه قوية في علم وظائف الأعضاء.

كلمات مفتاحية :أغذية الحيوانات الاليفة، الكلاب المنزلية، الجهاز الهضمي، الأورام، الفحص النسيجي المرضي