

Iraqi Journal of Veterinary Sciences



www.vetmedmosul.com

Histopathology and Immunohistochemistry of tumors in animals attending veterinary teaching hospital

S.S. Al-Mahmood¹, Kh.W. Khalil² and A.R. Edreesi²

¹Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, ²Veterinarian, Private Clinic, Mosul, Iraq

Article information Abstract We aim in the current study to investigate the pathology and incidence of tumors that Article history: Received May 10, 2021 are excised surgically in Veterinary Teaching Hospital. This study collected the tissue Accepted August 14, 2021 samples from animals from 1 October 2020 until 1 April 2021. These samples were Available online February 2, 2022 collected from animals that undergo surgical procedures to remove neoplastic growths in Keywords: their body. After tissue grossing, samples were collected from tumor mass, then fixed in Tumor neutral buffered 10% formalin for 72 hours, then processed to embedded in paraffin wax. A P53 routine Harris's haematoxylin and eosin stain were also used, histochemical stains such as Mdm2 Masson's trichrome and PAS techniques were used as needed. In exceptional cases, IHC Vimentin CD10 protocol was used for diagnostic steps to some types of tumors included in the current study. The antibodies that were used are Vimentin for canine osteosarcoma, P53 for squamous cell Correspondence: carcinoma, Mdm2 for ovine pulmonary adenocarcinoma, and CD10 for neoplastic S.S. Al-Mahmood lymphocytes. The result of the current study showed that the prevalence of tumor recorded saevan981@uomosul.edu.iq was Marek's diseases 22.8%, squamous cell carcinoma 22.8%, ovine pulmonary adenocarcinoma 15.4%, third eyelid adenoma 15.4%, canine osteosarcomas 7.4%, venereal transmitting tumors 7.4%, and feline mammary gland tumor 7.4%. In conclusion, the incidence of tumor in animals that attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, the University of Mosul was in general high in comparison to other studies in Iraq and other countries, this high incidence should pay attention to the

causes of these cancerous conditions and their relation to environmental aetiology.

DOI: <u>10.33899/ijvs.2021.130114.1733</u>, ©Authors, 2022, College of Veterinary Medicine, University of Mosul. This is an open access article under the CC BY 4.0 license (<u>http://creativecommons.org/licenses/by/4.0/</u>).

Introduction

The tumor is an abnormal mass that contains cells that result from the abnormal growth of normal cells in the body; also, it is termed neoplasia (1). The word tumor describes the actual swelling or abnormal physical appearance of this type of cell. The word cancer is mostly confused with neoplasia (2). All cancer tumors were considered malignant, tumors can be roughly classified into two groups; malignant and benign (3). The malignant one has a remarkable ability to invade the nearing tissues by invasion and spread to another organ by metastasis and ultimately kill the animal by interfering with normal body physiology (4). On the other hand, benign tumors do not invade surrounding tissue and do not spread to other organs; thus, they rarely cause the death of affected animals. Tumor cell can spread to a newly anatomical location by many mean, the most common way by using blood and lymphatic vessels, and this called metastatic invasion; also, the tumor mass has an excellent affinity for recurrences that means this tumor mass will have reestablished after surgical excision, commonly this tumor of tumors can be aggressive and cause death due to loss of condition to the affected animals (5). In Mosul city, they were more observed and recorded in long-living animal species, most commonly recorded in cats and dogs, and less recorded in poultry and rarely recorded in ruminate (6). We aim in the current study to investigate the pathology of tumors that excised surgically in Veterinary Teaching Hospital, belong to College of Veterinary Medicine, University of Mosul.

Materials and methods

Sample collection

In this study, we collected the tissue samples from animals that attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, University of Mosul. These samples were collected from animals that undergo surgical procedures to remove neoplastic growths in their body.

Tissue grossing

After receiving the tissue samples, the grossing process was conducted by external examination of tissue samples with recording the gross appearance appears with choosing a histological sample (7).

Tissue fixation

Representative histological samples were having been collected with 1.5*1.5*0.5 cm size and labeled according to sample recorded; the tissue put in cassettes and fixed in 10% neutral buffered formalin (10 ml of 37% formaldehyde, 90 ml of distilled water, 6.3 grams' sodium monobasic, 3.9 grams' sodium dibasic) for at least 27 hours to conduct the perfect fixation (7,8).

Tissue processing

After fixation, these samples were processed to be embedded in paraffin. The first step was dehydration, and this conduct by ascending concentrations of ethyl alcohol to withdraw the water from tissue samples, then cleared by xylenes in order to facilitate the paraffin wax to be infiltrate the samples, the samples rinsed in hot paraffin wax at 55-58°C for three changes (on hour for each change) in order to infiltrate the paraffin wax into tissue samples and withdraw the xylene. The samples were embedded in paraffin mold and left to dry at room temperature (8).

Tissue sectioning and routine staining

The paraffin cassettes section using rotary microtome at 6μ m thickness and the paraffin ribbon floated on a water bath at 40°C then lifted on the glass slide.

The slides dried using a hot plate at 60°C for 15 minutes, then stained using routine Harris hematoxylin and eosin stain (7). A histochemical stain was applied as needed; these stains included PAS stain and Masson's trichrome stain (7).

Immunohistochemistry Staining

In exceptional cases, IHC protocol was used for diagnostic steps to some types of tumors included in the current study.

The antibodies that were used are *Vimentin* for canine osteosarcoma, *P53* for squamous cell carcinoma, *Mdm2* for ovine pulmonary adenocarcinoma, and *CD10* for neoplastic lymphocytes, all immunohistochemistry producers was applied manually.

Results

Tumor incidence

A total of 13 tumor samples included in the current study were collected from animals that attended Veterinary Teaching Hospital during six months included in the current study. The result showed that the highest number of tumors were recorded in a cow with squamous cell carcinoma in the skin of the cow, pulmonary adenocarcinomas in sheep, and Marek's diseases in chickens with incidence reach up to 15.4 (2/13). In contrast, all other tumor types were recorded with an incidence of about 7.4 (1/13) (Table 1).

Mammary gland tumor in a cat

The histopathological lesion of this type of tumor composed from server infiltration of neoplastic cells, these neoplastic cells identified as lymphocytes which arrange either in clusters or individually in fibrous stroma, the IHC for CD10 showed the presence of neoplastic lymphocytes that their cytoplasm has been reacted positively with CD10 antibodies (Figure 1).

Table 1: Type and number of tumor in the current study

Species	Tumor type	Organ	n
Cat	Mammary gland tumor	Mammary gland	7.4 (1/13)
Cat	Third eye lid tumor	Third eye lid	7.4 (1/13)
Cat	Venereal transmitting tumor	vagina	7.4 (1/13)
Dog	Third eye lid tumor	Third eye lid	7.4 (1/13)
Dog	Osteosarcoma	Femur bone	7.4 (1/13)
Sheep	Pulmonary adenocarcinoma	Lung	15.4 (2/13)
Cow	Squamous cell carcinoma	Skin	15.4 (2/13)
Cow	Squamous cell carcinoma	Vagina	7.4 (1/13)
Chicken	Marek's disease	Different organs	22.8 (3/13)
Total			100 (13/13)

Third eyelid tumor in cat and dog

This type of tumor composed from well-demarcated nodules contains neoplastic sebaceous cells that present in glandular form, and combined by hyperplasia of epidermis that extended inside neoplastic mass, these neoplastic sebaceous cells were characterized by large cuboidal or columnar shape with large dense nucleus present near the basement membrane of the tubules (Figure 2).



Figure 1: Histological section of cat's mammary gland, [A] showed server infiltration of neoplastic cells, these neoplastic cells identified as lymphocytes (**arrow**), with dense fibrous connective tissue (**arrow**). 100x, H&E. [B] showed positive reaction to *CD10* (arrow). 40x, IHC-DAB stain.



Figure 2: Histological section of the dog's third eyelid, [A] showed the nodular formation of neoplastic cells surrounded by dense fibrous connective tissue (arrow). The neoplastic formation is composed of neoplastic sebaceous cells that present in glandular form (**arrow**). 100x, H&E. [B] showed neoplastic sebaceous cells characterized by sizeable cuboidal shape with large dense nucleus present near the basement membrane of the tubules (**arrow**). 400x, H&E.

Venereal transmitting tumor in a cat

This type of tumor comprises cells with a round to oval shape, and the cytoplasm is poorly defined by routine stain. These tumor cells are separated on some occasions with dense fibrous tissue. The IHC showed that the cytoplasm of these cells was positive for CD10 antibodies (Figure 3).

Osteosarcoma in dog

This type of tumor has a pleomorphic nature with an anaplastic form composed of islands of osteoid materials. The tumor comprises three types of cells: osteoblast, chondroblast, and fibroblast. The IHC staining showed that these cells, especially fibroblasts, were stained positively with *vimentin* antibody (Figures 4).



Figure 3: Histological section of cat's vaginal mass, [A] showed neoplastic growth (**arrow**), hemorrhages (**arrow**), and dense fibrous tissue (**arrow**). 40x, H&E. [B] showed positive reaction to *CD10* (**arrow**). 40x. IHC-DAB stain.



Figure 4: Histological section of dog's osteosarcoma in bone growth, [A] showed the anaplastic form of tumor composed from islands of cancerous osteoid materials (**arrow**). 40x, H&E. [B] showed neoplastic osteoblast (**arrow**) and neoplastic chondroblast (**arrow**). 400x, H&E. [C] showed neoplastic fibroblast (**arrow**). 400x, H&E. [D] showed positive reaction to *vimentin* (**arrow**). 40x, IHC-DAB stain.

Pulmonary adenocarcinoma in sheep

The tumor lesions composed from a nodular formation that contain dense collagen fiber deposition with the transformation of cells lining alveoli into cuboidal or columnar cells with also can be observed in other air passages; these cells were projected in papillary or acinar form, these neoplastic cells were found to be originated from alveolar cell type II and Clara cells. The IHC staining showed that the cytoplasm of neoplastic cells was stained positively with *Mdm2* antibodies (Figure 5).

Squamous cell carcinomas

This type of tumor primarily found in skin lesions associated with the formation of pathognomic lesions known as keratin pearls; this lesion composed of an acentric layer of keratin produced by neoplastic keratinocytes, that characterized by hypertrophic cells with large nucleus with high mitotic figure, these keratin pearls contain collagen fibers deposition either in mixed with the keratin of present surrounding these lesions. The IHC of squamous cell carcinoma showed a positive staining reaction to *P53* antibodies (Figure 6).



Figure 5: Histological section of sheep's lung with adenocarcinoma, [A] showed nodular formation that contains dense collagen fiber deposition (**arrow**), with the transformation of cells lining alveoli into cuboidal or columnar cells were project in papillary or acinar form (**arrow**). 40x, H&E. [B] showed cuboidal or columnar cells were projected in papillary or acinar form (**arrow**), with dense collagen deposition as extracellular matrix (**arrow**). 40x, Masson's trichrome. [C] showed positive reaction to *Mdm2* (**arrow**). 400x, IHC-DAB stain. [D] showed positive reaction to *Mdm2* (**arrow**). 400x, IHC-DAB stain.



Figure 6: Histological section of cow's skin with squamous cell carcinoma, [A] showed multiple keratin pearls (**arrow**). 40x, H&E. [B] showed keratin pearls composed from an acentric layer of keratin (**arrow**), produced by neoplastic keratinocytes (**arrow**). 400x, H&E. [C] showed collagen fibers deposition in keratin peals (**arrow**) or surrounding these pearls (**arrow**). 40x, Masson's trichrome. [D] showed positive reaction to *P53* (**arrow**). 400x, IHC-DAB stain.

Marek's disease

This type of tumor was observed in visceral organs such as the liver, lung, kidney, intestines, and spleen composed from pleomorphic lymphocytes which diffusely proliferating small-to-medium size of lymphoblast. The IHC staining showed that the neoplastic cells were stained positively with CD10 antibodies (Figure 7).



Figure 7: Histological section of chicken's liver with Marek's disease, [A] showed neoplastic lymphocytes (**arrow**), with few normal hepatocytes (**arrow**). 40x, H&E. [B] showed neoplastic lymphocytes (**arrow**), with few normal hepatocytes (**arrow**). 100x, H&E. [C] neoplastic lymphocytes (**arrow**) in lung tissue. 40x, H&E. [D] showed neoplastic lymphocytes (**arrow**) in kidney tissue. 400x, H&E. [E] showed neoplastic lymphocytes (**arrow**) in kidney tissue. 400x, H&E. [E] showed neoplastic lymphocytes (**arrow**) in kidney tissue. 400x, H&E. [E] showed neoplastic lymphocytes (**arrow**) in kidney tissue. 400x, H&E. [E] showed neoplastic lymphocytes (**arrow**) in *context*. (**arrow**) in the standard tissue. 40x, H&E. [F] showed positive reaction to *CD10* (**arrow**). 40x, IHC-DAB stain.

Discussion

The results of the current study showed that the highest type of tumor incidence was recorded in squamous cell carcinomas and Marek's diseases at 22.8%(313) to each type of tumor; these results were merely similar to that recorded by Al-Ajeli *et al.* (2) were they recorded about 22.2% of squamous cell carcinomas in Mosul city, this indicates that the prevalence of this type of tumor was stable in Mosul city since both studies were applied in VTH belong to the college of Veterinary Medicine, University of Mosul. While the prevalence of Marek's diseases in Karbala reached up to 30.6% that recorded by Wajid et al. (9), which is higher than that recorded in our study, this can belong to different geographical location and climate effect, in addition to the endemic status of this diseases in these two cities. The pathognomic lesions for squamous cell carcinomas are keratin pearls that composed from an acentric layer of keratin produced by hypertrophied neoplastic keratinocytes, which produce this connective tissue out of control of body function as a result of neoplastic status, mainly these lesions can be observed in the dermis and epidermis of skin in different location of the body (5). At the same time, the primary pathognomic lesions in Marek's diseases in chicken were composed of uniform small to moderate size lymphocytes that infiltrate in different organs of the body depending on the severity of the infection caused by a herpesvirus that infected lymphocytes and induce this neoplastic status (10).

The prevalence of ovine pulmonary adenocarcinomas was found to be at 15.4% in the current study; this percentage was higher than that recorded by Al-Ajeli *et al.* (6), who recorded this type of tumor in 11.2%, this may be related to the population of animals that included in the current study were they investigate this lesion during three months in one flock of sheep, another study conducted by Jassim *et al.* (11) were they recorded 10.1% of this tumor in sheep in Al-Qadisiyah city. Ovine pulmonary adenocarcinomas have a pathognomic lesion characterized by a neoplastic cuboidal and columnar cell that project as papillary of acinar form inside alveoli and bronchioles surrounded by dense fibrous connective tissue (5).

Third eyelid tumor in dog and cat were found to be sebaceous cell tumor (adenoma type) in 15.4% in the current study; this result was merely similar to that recorded by Dees *et al.* (12) were the recoded about 14.4% of adenoma in the third eyelid in dogs and cats. Silva *et al.* (13) described a similar neoplastic cell characterized by a cuboidal or columnar cells, with a basement nucleus that large with a high mitotic figure and dense fibrous tissue that gives this neoplastic mass a nodular formation. Canine osteosarcoma was recorded 7.4% in the current study, this percentage was found to be 12% in two dog's families (14), this can be related to a long life for this type of dogs, and their lifestyle was domesticated in houses in Scotland, and their age can reach up to 13 years' old which increased the incidence of recording this type of tumors.

The venereal transmitting tumors in cats were recorded in 7.4% in the current study; these results were less than recorded in other countries where they range from 23-40% (15). These can be related to the time included in the previous study where they conducted their work for six years in Europe, the USA, and Canada. Venereal transmitting tumors in a cat can be described as dense infiltration of neoplastic lymphocytes that were poorly differentiated cells with ill define cytoplasmic boarded and highly mitotic figures observed in current work (5). The mammary gland tumor in the cat was recorded in 7.4% in current work, and this prevalence was found to be less than other studies that recorded 17% of mammary gland tumor in feline species (1). Another study conducted by Wypij *et al.* (16) recorded a 10.4% tumor incidence in feline species in the USA and UK.

Conclusion

The incidence of tumor in animals that attending Veterinary Teaching Hospital belong to the College of Veterinary Medicine, the University of Mosul was in general high in comparison to other studies in Iraq and other countries, this high incidence should pay attention to the causes of this cancerous condition and their relation to environmental causes.

Acknowledgements

The authors wish to extended their thanks to College of Veterinary Medicine, University of Mosul, for supporting current study.

Conflict of interest

The authors declare that we did not have any conflict of interest regarding publishing or funding of this article.

References

- Jubb K, Kennedy A. Palmer's pathology of domestic animals. UK: Elsevier; 2007. 179-84 p. DOI: <u>10.1016/b978-070202823-6.50022-1</u>
- Bishop JM. Molecular Oncology. New York: Scientific American, Inc; 1996. 136-160 p.
- Schottenfeld D. Cancer epidemiology and prevention. UK: Oxford University Press; 2006. 24-31 p.
- Eyesan SU. Surgical consideration for benign bone tumors. Niger J Clin Pract. 2011;14(2):146-50. [available at]
- Zachary JF. Pathologic Basis of Veterinary Disease. 6th ed. Illinois: Elsevier; 2017. 286-321 p.
- Al-Ajeli RR, Al-Qadhi AS, Al-Mahmood SS, Alkattan LM. Pathological study of neoplasms surgically excised from animals attended the veterinary teaching hospital. Iraqi J Vet Sci. 2021;35(1):9-14. DOI: <u>10.33899/ijvs.2019.126188.1260</u>
- Luna LG. Manual of histological staining methods of the army forces institute of pathology division. 3rd ed. New York: McGraw Hill book company; 1968. 12-20 p.
- Al-Sabaawy HB, Rahawi AM, Al-Mahmood SS. Standard techniques for formalin-fixed paraffin-embedded tissue: A Pathologist's perspective. Iraqi J Vet Sci. 2021;35(SupplI-III):127-135. DOI: 10.33899/ijvs.2023
- Wajid SJ, Katz ME, Renz KG, Walkden SW. Prevalence of Marek's disease virus in different chicken populations in Iraq and indicative virulence based on sequence variation in the EcoRI-Q (meq) gene. Avi Dis. 2013;57(2):562-568. DOI: <u>10.1637/10342-083112-reg.1</u>
- Zahid AA. Field evaluation of Marek's disease infection in vaccinated parent flock. Al-Qadisiya J Vet Med Sci. 2008;7:34-39. [available at]
- Jassim A, Saad H, Al-Husseiny A, Kalefa AM, Qassim HK. First molecular diagnosis of ovine pulmonary adenocarcinoma in Awassi sheep in Iraq. Al-Qadisiyah J Vet Med Sci. 2017;161(16):112-117. DOI: <u>10.29079/vol16iss1art46</u>
- Dees DD, Schobert CS, Dubielzig RR, Stein TJ. Third eyelid gland neoplasms of dogs and cats: a retrospective histopathologic study of 145 cases. Vet Ophthalmol. 2015;19(2):138-143. DOI: 10.1111/vop.12273

- 13. Silva B, Peleteiro MC, Pissarra H. Tumors of the eye and ocular adnexa in cats and dogs. Ocular Dis. 2016;23(3):1-10. [available at]
- Dillberger JE, McAtee SA. Osteosarcoma inheritance in two families of Scottish deerhounds. Canine Gen Epidemiol. 2017;4(1):1-12. DOI: 10.1186/s40575-017-0042-8
- Gonzalez C, Sanchez B, Velez H, Buen D. Neoplasms of the reproductive system in bitches: Retrospective study over 6 years. Vet Mex. 2017;28:31-34.
- Wypij J, Fan TM, Lorimier LP. Malignant mammary tumors: Biologic behavior, prognostic factors, and therapeutic approach in cats. Vet Med. 2006;40:352-366. [available at]

إمراضيه النسيج وكيمياء مناعة النسيج للأورام السرطانية في الحيوانات الواردة للمستشفى التعليمي البيطري

سيقان سعد المحمود'، خليل و عدالله خليل' و عبدالرحمن رياض إدريس'

أفرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، أطبيب بيطري، قطاع خاص، الموصل، العراق

الخلاصة

نهدف في الدراسة الحالية دراسة مرضية ونسبة حدوث الأورام التي تم استئصالها جراحياً في المستشفى التعليمي البيطري. في هذه الدراسة تم جمع عينات الأنسجة من الحيوانات خلال الفترة من ١ أكتوبر ٢٠٢٠ حتى ١ نيسان ٢٠٢١. تم جمع هذه العينات من الحيوانات التي خضعت لعمليات جراحية لاستئصال الأورام. بعد جمع عينات الأنسجة من هذه الحيوانات، تم تثبيتها في الفور مالين الدارئ المتّعادل ١٠٪ لمدة ٧٢ ساعة ثم معالجتها لغرض الطمر في شمع البارافين. كما تم استخدام ملون هاريس الهيماتوكسيلين والايوسين الروتينية، كما تم استخدام التلوين الكيميائي للنسيج كملون ثلاثي اللون لماسون، وملون حامض البريودك كاشف شييف وحسب الحاجة. وفي حالات خاصة، تم استخدام التلوين النسيجي المناعي لغرض تشخيص أنواع الأورام التي تم تضمينها في الدر اسة الحالية والتي شملت الأجسام المصادة لكل من Vimentin و P53 و Mdm2 و CD10. أظهرت نتائج الدراسة الحالية أن نسبة انتشار مرض المارك كانت ٢٢,٨٪، وسرطان الخلايا الحرشفية ٢٢,٨٪، وسرطان الغدد الرئوية ١٥,٤٪، والورم الحميد لجفن العين الثالث ٢٥,٤٪، سرطانه العظام في الكلاب ٢,٤٪، والأورام المنقولة تناسلياً ٢,٤٪، ورم الغدة الثدية في القطط ٧,٤٪. نستنتج من هذه الدر اسة أن نسبة الإصابة بالأورام في الحيوانات التي كانت تجلب إلى المستشفى التعليمي البيطري التابع لكآية الطب البيطري بجامعة الموصل كانت بنسبة عالية مقارنة بالدر اسات الأخرى في العراق والدول الأخرى، و هذا المعدل المرتفع يجب الانتباه إليه والتعرف على أسبابها وعلاقتها بالبيئية.