Prevalence and Factors Associated with Superficial Tumors and Tumor-like Swelling in Cattle and Buffalo in Egypt

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ABSTRACT
Objective: To investigate the prevalence and factors associated with superficial tumors in Egyptian water buffalo and cattle and to present their clinical and immunohistochemical (IHC) features.
Design: Case-control study.
Animals: A total of 1253 animals were studied (866 cross breeds of cattle and 387 native buffaloes). Eighty-one cases (64 cattle and 17 buffalo) were suffered from tumor like swelling. The Clinical, microscopic, histopathological and IHC of the tumor were assessed. Additionally, different surgical interferences and anesthesia were applied for their excision.
Results: Based on clinical, histopathological and IHC examination, neoplasia were diagnosed in 75 animals (92.59%). Generally, the prevalence rate of tumor was higher in cattle than buffalo (78.66% vs 21.33%). Animals aged <5 years exhibited higher incidence of tumor (70.66%) than other ages. Females showed higher prevalence of tumor (85.33%) than males. The diagnosed benign tumors were papilloma (32.14%), fibroma (28.57%), dermoid (28.75%), myxoma (3.57%), and lipomyxoma (3.57%). While, the diagnosed malignant tumors were squamous cell carcinoma (SCC; 72.34%), basaI cell carcinoma (14.89%), fibrosarcoma (6.38%), melanosarcoma (2.12%), mycosarcoma (2.12%), and teratoma (2.12%). Of the detected tumors, head had the highest occurrence (61.33%) followed by sexual organ and mammary gland (18.66%), trunk (6.66%) and limb (5.33%). IHC analysis of different types of tumor tissues showed higher expression of Ki67 in both benign and malignant tumors with variable degree.
Conclusion and clinical relevance: superficial tumors have high incidence in cattle compared to buffalo. Papilloma, fibroma and dermoid were the most commonly diagnosed benign tumors. SCC, basaI cell carcinoma and fibrosarcoma were the most commonly diagnosed malignant tumors. Surgical excision of the superficial tumors offers a satisfy solution for the condition. IHC and histopathological examination provides precise techniques for evaluation, and differentiation between benign and malignant tumors.
Keywords: Buffalo; Cattle; Histopathology; Immunohistochemistry; Tumor

1. INTRODUCTION
Recently, tumor incidence in cattle and buffalo is relatively increased particularly cutaneous and subcutaneous tumors [1]. Papilloma and squamous cell carcinoma (SCC) are the commonly diagnosed and economically important superficial neoplasms in large animals [2]. Deleterious effects of neoplasms may be limited to localized stress at the site of tumor which may be due to pressing on nerve pass way or may be due to insects’ bites which causing infection and frequent bleeding [3]. Impaired reproductive and productive capacity may be a secondary drawback of neoplasms if accompanied with deleterious general health condition or when affect sexual organs or mammary tissue [4; 5]. However, the available literatures that studied the different verities of both benign and malignant tumors in buffalo are scant compared to cattle. To the authors best knowledge, literatures that studied the incidence, epidemiology, surgical and clinical importance of tumors are scarce, thus the current study was designed to investigate the prevalence and factors associated with superficial tumors in Egyptian water buffalo and cattle, different surgical interferences and anaeasthesia applied for their excision and to present their clinical and IHC features.

2. MATERIALS AND METHODS
2.1. Animals
The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Benha University (BUFVTM 10-01-2018).

2.2. Animals and study location
A total of 1253 animals were studied (866 cross breeds of cattle and 387 native buffaloes). The present study was conducted at two governorates of Middle Egypt, named Kalyobiya and Menofiya during the period from October 2016 and December 2019. Animals were of both sexes, aged between 6 months to 10 years. A total number of 81 cases (64 cattle and 17 buffaloes) were examined and exhibited tumor and tumor-like swelling.

2.3. Clinical index scores
Clinical signs were recorded and scored, with calculation of clinical sum scores. Other parameters for the investigated animals were assessed and recorded, including species, age, sex. Clinical and microscopical features of the tumor were assessed, including size, location, origin, mass description and malignancy. All of these parameters were recorded and scored by one person and statistically compared (Table 1).

2.4. Anesthetic protocol and Surgical Intervention

According to the location of the tumor, protocol of anesthesia and surgical interference were chosen, performed and recorded. Xylazine hydrochloride 2% (Xylaject, Adwia pharma, Cairo, Egypt) at a dose of 0.05 mg/kg B.W was used for sedation; lidocaine HCl 2% (Debocaine, Al-Debeiky Pharma, Cairo, Egypt) was locally infiltrated for local analgesia and xylazine hydrochloride (0.03 mg/kg B.W) diluted in 5 ml of 2% lidocaine HCl was used for caudal epidural anesthesia. Surgical interferences including total excision of the neoplastic masses with or without the affected organ such as eye ball, 3rd eye lid and mammary gland. In all Cases, the follow up was achieved for 6 months post operatively via phone calling of the owner or direct inspection of cases; survival and evidence of recurrence were recorded.

2.5. Histopathological examination

For histopathological examination, all specimens were fixed in 10% neutral buffered formalin, and then processed for histopathological examination till embedded in paraffin wax. Serial sections were cut at 3 µm thickness and routinely processed for H&E staining according to [6].

2.6. IHC analysis

Solid tumors were harvested, fixed in formalin and embedded in paraffin for IHC analysis [7]. After antigen retrieval with 10 mM sodium citrate buffer (pH 6.0) at 80 °C for 10 min, endogenous peroxidases were blocked by 3% hydrogen peroxide in PBS for 10 min. The slides were then incubated overnight with primary antibodies against Ki67, at 4 °C in a humidified chamber and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at 1:100 dilutions for 30 min at 37°C and visualized by 3,3′-diaminobenzidine tetra hydrochloride reagent (Broad spectrum LABSA Detection System, Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, United States). The sections were counterstained with hematoxylin and then digitized digitally.

2.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism statistical software program (GraphPad Prism, San Diego, CA, United States). The association between breed, age, sex, cause, duration, size, site, nature, content of the swelling, clinical signs and the treatment outcome were evaluated by a chi-squared test (χ2) and presented as P value, odds ratio (OR) and confidence interval at 95% (CI 95%). Non-parametric Kruskal Wallis test was used to assess the improvement of clinical sum scores after treatment. A P value <0.05 was considered to indicate statistical significance.

3. RESULTS

Out of the 81 samples examined (64 cattle and 17 buffaloes), neoplasia was diagnosed in (75/81) samples based on histopathological examination and IHC. Hypergranulation tissue formation (4/81) and prolapsed 3rd eyelid (2/81) were accounted for the remaining non neoplastic cases. The recorded tumor was prevalent in cattle than in buffalo (59/75 vs. 16/75; P < 0.0001; OR: 22.75; CI 95%: 9.236 to 49.99). Animals aged <5 exhibited more tumor than other ages (53/75 vs. 22/75; P < 0.0001; OR: 5.804; CI 95%: 2.873 to 11.72). In addition, females showed a significantly higher prevalence of tumor compared to males (64/75 vs. 11/75; P < 0.0001; OR: 33.85; CI 95%: 13.70 to 83.67).

Table 1. Clinical index score for the clinical findings of tumor in cattle and buffalo.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Scoring System &amp; description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>1= Buffalo; 2= Cattle</td>
</tr>
<tr>
<td>Sex</td>
<td>1= Female; 2= Male</td>
</tr>
<tr>
<td>Age</td>
<td>1= &lt; 5 years; 2= &gt; 5 years</td>
</tr>
<tr>
<td>Total no. of animal/ farm</td>
<td>1= &lt; 10 cases; 2= 10-20 &amp; 3&gt; &gt; 20</td>
</tr>
<tr>
<td>Raising of other animals</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>History of previous case</td>
<td>0= no; 1= Bovine; 2= Ovine; 3= Equine</td>
</tr>
<tr>
<td>Distribution</td>
<td>1=Single location; 2=More than one location</td>
</tr>
<tr>
<td>Malignancy</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Pu</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Offensive odor</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Pain</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Systemic signs</td>
<td>0= no; 1= yes</td>
</tr>
<tr>
<td>Recurrence</td>
<td>0= no; 1= at the same site; 2= yes at other site</td>
</tr>
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According to the managing condition, tumor was more prevalent in low population managing animal system (< 10 cases) than in high population managing animal system (58/75 vs. 1775; P < 0.0001; 22.75; CI 95%: 9.763 to 42.99). Animal raised with other species exhibited more incidence of tumor than those raised alone without other species (45/75 vs. 30/75; P = 0.0219; OR: 2.250; CI 95%: 1.171 - 4.325). Animals raised in population without previous history of the same affection displayed more prevalence of tumor than population with previous history of the same affection in equine (73 /75 vs. 2/75; P < 0.0001; OR: 22.75; CI 95%: 9.70 to 79.67).

According to distribution of tumor in clinical presentation; single distributed tumors were more prevalent than multiple distributed ones (62/75 vs. 1375; P< 0.0001; OR: 22.75; CI 95%: 9.763 to 52.99). Presence of pus was recorded to be frequently in malignant tumor than benign tumor (30/47 vs. 4/28; P< 0.0001; OR: 0.09444; CI 95%: 0.02803 - 0.3182). Offensive odor was more prominent in malignant tumor than benign tumor (20/47 vs. 3/28; P< 0.0043; OR: 0.1620; CI 95%: 0.04284 - 0.6126). Pain on touch was elicited frequently in malignant tumor than benign tumor (46/47 vs. 14/28; P< 0.0001; OR: 0.02174; CI 95%: 0.002621-0.1803). Evidence of bleeding was more associated with malignant tumor than benign tumor (36/47 vs. 1/28; P <
Systemic signs were revealed in malignant tumor than benign tumor (40/47 vs. 9/28, P< 0.0001; OR; 0.08289; CI 95%: 0.02681 - 0.2563).

Various types of benign and malignant neoplasm were demonstrated in both animal species based on histopathological examination. Among the benign tumor; papilloma at paravertebral skin (Figure 1A), fibroma originated from 3rd eye lid and (E) mucous membrane of teat canal, (F) corresponding H&E stained sections showing fibroma composed of spindle cells arranged in long thick interlacing streams and bundles with whorls formation and the neoplastic cells had oval to elongated nuclei with finely stippled chromatin (x200). (G) ovoid swelling at the base of the neck, (H) corresponding H&E stained sections showing lipomyxoma in which adipose tissue appeared with abundant areas of myxoid changes (x200).

Among the malignant tumors, SCC at the sublingual (Figure 2A), vulvar (Figure 2B), ocular (Figure 2C), suprascapular (Figure 2D) and mammary gland (Figure 2E), basal cell carcinoma (BCC) at the base of the lower lips (Figure 2K), Melanosarcoma at the facial region and its corresponding tissue (Figure 2G), Histopathological examination of the BCC showed neoplastic cells arranged in lobules and embedded in a moderate amount of fibrovascular stroma (x200) (Figure 2J). Histopathological examination of the melanosarcoma showed pleomorphic small spindle, polyhedral rounded and spindle shaped melanin cells that appearing in groups densely packed with melanin granules with eosinophilic cytoplasm (x400) (Figure 2M).

IHC analysis of Ki67 expression in different types of tumor tissues was evaluated (Figure 3). The Ki67 expression was detected in different types of both benign and malignant tumors with variable degree in the present work (Figure 3A-F). This study showed that the severity of Ki67-positive cells is very high in malignant epithelial tumors than the benign tumor (Figure 3).

Malignant tumor was more prevalent than benign tumor (47/75 vs. 28/75; P< 0.0219; OR; 2.250; CI 95%: 1.171 - 4.325). The diagnosed benign tumors were papilloma (9/28), Fibroma (8/28), Dermoid (8/28), Myxoma (1/28), Lipoma (1/28) and Lipomyxoma (1/28). The diagnosed malignant tumors were SCC (34/47), BCC (7/47), Fibrosarcoma (3/47), Melanosarcoma (1/47), Myxosarcoma (1/47), and Teratoma (1/47).
The lower irritation of eyeball (22/75), excision of 3rd cranial nerve block (5/66), Mental nerve block (2/66), posterior epidural analgesia (7/66), ring block at base of teat (4/66), ring block at base of udder (1/66) and retrobulbar nerve block (22/66) were performed with complete satisfaction of desensitization.

During 6 months of follow-up period; incidence of recurrence was prevalent in Malignant tumor than in benign tumors (5/47 vs. 2/28, P <0.05; OR: 0.6462; CI 95%: 0.1167-3.578). The recurrent malignant tumors were BCC (4/5) and SCC (1/5), while the recurrent benign tumors were papilloma (2 cases) only.

4. DISCUSSION

The present study was carried out on 81 samples from tumor like swelling collected during October 2016 and December 2019 in cattle and buffalo. Out of the total samples, neoplasia was diagnosed in 75 samples based on histopathology and immunostaining. Hypergranulation tissue formation (4/81) and prolapsed 3rd eyelid (2/81) accounted for the remaining non-neoplastic cases. Similarly, in bovine, [7; 8] observed neoplasia in 57 out of total 59 cases and in 1370 out of total 1535 cases of suspected neoplasia respectively and the remaining cases were a mixture of inflammatory conditions.

The incidence of tumors was higher in cattle than in buffalo. Accordingly, [8] mentioned that, the occurrence of tumors was greater in cattle (78.95%) than in buffalo (21.05%). In contrary, [9] reported the high incidence in buffalo (6.67%) than in cattle (3.33%). Additionally, [10] reported that Buffalo had higher occurrence of tumor (39.39%) followed by dogs (36.36%), cattle (16.16%) and horses (8.08%). The highest occurrences in cattle, buffalo and dogs could be attributed to their population, while, low population of equine could be the reason for small number of tumor cases [9].

In this study, age < 5 year was the age of risk of incidence in the current study. Likewise, [10] reported that the age of tumor occurrence in buffalo, cattle and dog was mostly 3-5 years age followed 0-3 years age and the least occurrence at 5-10 years age. The higher rate of tumor was related to the female. As well, [10] reported that tumor incidence in females buffalo (65.6%) was higher than males (34.34%). Similar trend was observed in equine (5 for females and 3 for males) and dogs (27 for females and 9 for males). The lower incidence of tumor in male than female attributed to the fact that males had been sent to be slaughtered before the age of peak incidence (3-6y) [11]. In contrary, [12] observed the high incidence in male animals than in female animals. While [8] reported almost equal incidence of tumors in female and male bovines (50.88% and 49.12% respectively).

Gross examination of neoplasm revealed either nodules (single, solitary, wart like or cauliflower) or indefinite mass; intact or ulcerated with or without hemorrhage. As regard color, the masses appear colorless, faint color, dark brown or black coloration in hairy or hairless situations. These symptoms were nonspecific for such masses, so the histopathological examination was indicated for identification, grading and differentiation either benign or malignant and also give good information about the ability of recurrence of these tumors. These findings were similar to those reported by [8]. Upon our investigation, the incidence of malignant tumors (62.7%) was higher than that of benign tumors (37.3%). In contrast [1] recorded high incidence of benign than malignant tumors; 85.7% and 56.92% for benign and 14.3% and 43.08% for malignant tumor, respectively.
In the present study, among the benign masses' papilloma represents the majority (9/28). This result was the same as recorded by [2], SCC was the most commonly recorded malignant tumor (34/47) and the most commonly recorded neoplasm (34/75). In agreement with our results [2; 3] recorded that, bovine cutaneous papillomatosis and SCC are commonly diagnosed and economically important neoplasms in large animals. Coincided with [12], ocular SCC was the most common type of SCC recorded in the present study (21/34). The other origins of SCC in the current study included oral cavity (1/34), vagina (2/34), mammary gland (1/34), trunk (3/34), (4/34), 2 limbs (2/34). While out of total 27 cases of SCC recorded by [10], the anatomical sites were horn (17/27), head and jaw (4/27), trunk (3/27), genital organs (2/27) and nose (1/27).

Histopathological examination of SCC in different cases revealed variable degrees of bovine SCC differentiation and its histopathological examination was very similar to those reported in perineum of a female Holstein cow by [13]. The malignant tendency of this condition makes early recognition of tumor is critical. The incidence of BCC (7/75) and was originated from ear (1/7), 3rd eyelid (5/7) and vulva (1/7). The incidence of Fibroma (8/75) and was originated from 3rd eyelid (3/8) vulva (1/8), teat canal (4/8). However, [1] recorded fibroma at limbs (7/19), head (5/19), neck (3/19), trunk (2/19) and one for each rectum and brisket region. Fibrosarcoma was incident (3/75) in penis. Lipoma, Myxoma and Myxosarcoma were recorded in the current study for one time at head, head and premium, respectively. Similarly, [14] recorded two cases of Lipoma at in buffalo and dog at udder and forelimb. While, [15] recorded Myxoma and Myxosarcoma one time for each at the same region in the present study (head and premium).

In our findings, malignant melanoma was seen macroscopically on the skin of the cheek as hard, grayish black ulcerated spots of about 0.8-1 cm in diameter. These finding were in accordance with earlier worker [9]. Gross and microscopic lesions were observed in various animals’ species including horse, bovine, porcine, rat and mice [16]. Melanoma starts in the melanocytes when the skin is exposed to the sun. The melanocytes get damaged and thus results in the abnormal and uncontrolled growth of the cell creating a tumor [17]. Heavily pigmented melanocytic neoplasms are difficult to assess on routine hematoxylin-eosin (H&E) stained slides because pigmented melanocytes can be confused with pigmented keratinocytes and melanophages. Immunostaining using diaminobenzidine, which forms a brown product, as the chromogen is challenging to distinguish from melanin. An important sign of cancer development is uncontrolled cell cycle progression [18].

Ki-67 is a nuclear protein expressed in proliferating cells and serves as a good marker for proliferation and may be required for maintaining cell proliferation [19; 20]. Consequently, Ki67 immunoreactivity was demonstrated in a total of 75 cases (47 cases from cattle and 28 cases from buffalo) in our findings. IHC analysis of Ki67 expression in different types of tumor was evaluated. The Ki67 antigen level has been shown to correlate with tumor progression, metastatic potential, and supports their pathogenic role in cutaneous carcinogenesis. Additionally, Ki67 expression was detected in different types of both benign and malignant tumors with variable degree in the present work. This study showed that the severity of Ki67-positive cells is very high in malignant epithelial tumors than the benign one. These results are matched with the findings of [21] who found that Ki67 expression is higher in aggressive and recurring BCCs. Additionally, the highest Ki67 expression found in SCC, particularly in poorly differentiated SCC, confirms that its aggressive behavior is at least partly due to enhanced cell proliferation [22].

Regarding to recurrence incidence during the follow-up period (6 months); 4 cases out of 7 of recurrent cases were BCC. Similarly, the previous studies showed that the recurrence rate of BCC is highly variable and could reach to 67% even when the tumors are completely excised [1, 23]. Because of the nature of BCC; it has the ability to destruct and invading surrounding tissues [1]; so, the tumors may be inadequately excised despite clear margins. Additionally, the specific location of BCC often does not allow surgical excision with such margin. Deep tissue excision may also be non-useful, as the tumor cells are usually very superficial so excision of adequate area of surrounded healthy tissue is recommended [23].

Tumor incidence in bovine is relatively increased; specialty literature indicating the second place, after dogs. In living animal, extensive neoplastic lesions incriminated in loss of the economic value of the animal due to loss of body condition, reduced hide value, consequent wounds, hemorrhages, necrotic dermatitis, myiasis, mastitis and interference in milking, suckling and coitus [13; 24]. In slaughterhouse, out of the total rejected carcasses after slaughtering, tumor affected carcasses account for 41.1% [8; 11].

Despite the recent advances in radiation and chemotherapy, surgical resection remains an integral part of curative therapy for neoplasms in humans [25]. Surgical excision was indicated as the sole treatment for tumors in large animals when the tumor was locally excisable without significant morbidity and or systemic metastasis [15]. It is economic, fast and mostly curative when the animal’s health condition is good enough for surgical intervention. Regarding to the rate of recurrence 8.6% (7/81), the chosen surgical interference for each case was highly successful 91.4% (74/81) as a curative therapy for neoplasms in the current study.

In conclusion, the results of the present study indicate that superficial tumors have high incidence in cattle compared to buffalo. The most common diagnosed benign tumors were papilloma, fibroma and dermoid. While, the most common diagnosed malignant tumors were SCC, BCC and fibrosarcoma. Surgical excision of the superficial tumors offers a satisfy solution for the condition. Finally, immunohistochemistry and histopathological examination
provides precise techniques for diagnosis, and differentiation between benign and malignant tumors.

Conflict of interest
The authors declare that they have no conflicts of interest to disclose.

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Authors’ contribution

5. REFERENCES