Clinical survey of equine sarcoids in Egypt

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A total number of 159 equine cases in different regions of Egypt were surveyed. The survey consisted of 83 horses and 76 donkeys of different ages, with sarcoids. Preliminary diagnosis was dependent on the morph-clinical features of the lesion/s while the definitive diagnosis was achieved after histopathological assessment of the surgically excised lesions. Epidemiological studies revealed that the majority of the presented cases were from the south region including Luxor and Aswan governates (47.8% of cases; 44 horses and 32 donkeys). Cases in the middle region, including Cairo and Giza governates (40.9% of cases; 35 horses and 30 donkeys). Cases in the north region including Alexandria governate (11.3% of cases; 10 horses and 8 donkeys). Of the 159 cases there were 85 male cases (53.45%) and 74 female cases (46.55%). Animals affected were between 3 and 11 years of age. The age of the majority of the affected cases, were in between 3-11 years. Of the clinical types of sarcoids (fibroblastic, mixed, nodular, verrucous, occult and malevolent), the fibroblastic and mixed types were recorded in large proportions of sarcoid cases of this study (58 cases, 36.5%) and (46 cases, 28.9%) respectively. The nodular (28 cases, 17.6%), verrucous (20 cases, 12.6%) and the occult (7 cases, 4.4%) types were less common.

Key words: Epidemiology, sarcoid, equine, dermatopathology, papilloma virus

INTRODUCTION

Equine sarcoids are the most common skin tumor in horses, donkeys and mules. It can affect the equids of all ages, types and breeds (Ragland et al., 1970; Pascoe and Summers, 1981; Knottenbelt et al., 1995; Goodrich et al., 1998; Goldschmidt and Hendrick, 2002; Bergvall, 2013). Equine sarcoids represent 12% to 67% of all equine tumors and 36% to 70% of all skin tumors in horses (Jackson, 1936; Regland et al., 1970; Miller and Campbell, 1982). A prevalence of 0.5% to 2.2% has been reported in the clinical population (Goodrich et al., 1998).

The epidemiological and clinical behavior of the equine sarcoid are strongly suggestive an association with infectious/contagious agents (Reid et al., 1994; Bloch et al., 1994; Lohr et al., 2005). Specifically, the bovine papilloma virus (BPV1) has been implicated by the finding of a genome that resembles that of the bovine virus in sarcoids (Reid et al., 1994).

Sarcoids may occur in any part of the body either as solitary or multiple lesions, but the most common regions are the head, ventral abdomen and the limbs and at other body parts that have been previously traumatized (Jackson, 1936 and Ragland et al., 1970).

Macroscopically, sarcoids show a variable appearance and have been classified into six distinct clinical forms: occult (obscure), nodular, fibroblastic (Proud flesh), verrucous (watery), malevolent (malignant) and mixed (a mixture of the preceding first four types) (Martens et al., 2000; Knottenbelt, 2005).

Diagnosis of equine sarcoids can be assessed in three different ways: Clinical examination, biopsy followed by histopathological, and detection of BPV DNA (Martens et al., 2000; Bogaert et al., 2008; Carr, 2009; Sala et al., 2010).

The reported treatment methods include: surgical excision, cryosurgery, laser surgery, radiofrequency, hyperthermia, topical cytotoxic and antimitotic drugs, immunotherapy or a combination of all therapies to treat...
different equine sarcoid forms (Carstanjer et al., 1997; Bogaert et al., 2007; Carr, 2006; Hewis and Sullins, 2006). Although, sarcoids are non metastatic, they can remain static or become very aggressive locally and can severely compromise the use and value of the horse which may lead to euthanasia (Scott and Miller, 2003; Bergvall, 2013).

The aim of the present study was to carry out field morphological and histopathological studies on clinically affected equine cases in different regions in Egypt and to evaluate the efficacy of some available treatments on the clinically presented cases.

MATERIALS AND METHODS

A total of 159 cases (83 horses and 76 donkeys) with sarcoid skin lesions were studied from 2010 to 2013 from different regions in Egypt (the south region 76 cases, the middle region 65 cases, and the north region 18 cases). Both males and females were used in the study ranging from 3 to 19 years of age. All affected animals were subjected to clinical evaluation which included:

Anamnesis

A full description of the history of each case, concerning the onset time, region, shape, size, consistency, growth rate of the skin sarcoid lesions and previous treatment. Questions to owners were also directed towards any history of previous or old trauma or recurrence of the sarcoid following recovery.

Clinical Examination

All of the affected presented equids were examined to determine the color of the coat (grey, black, bay or chestnut), the site of the sarcoids (genitalia, perineum, peri-orbital, limbs, trunk, abdomen, neck, ears or lips), the distribution (solitary or multiple) and the nature (proliferative or erosive).

Morpho-clinical proposition

From the above clinical examination, the preliminary diagnosis was obtained from the clinical examination and the sarcoid type (occult, verrucous, nodular, fibroblastic, malevolent and mixed) of each animal was recorded.

Histopathological examination

Tissue biopsies were surgically taken (Total excisional biopsy) from various skin sarcoid lesions. The tissue samples were fixed in formaline 10% for 24 hours, then dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin and sectioned into 4-5 micron in thickness stained with Haematoxin and Eosin (H & E). The samples were examined by routine histopathological examination including masson’s trichrome for connective tissues detection to reach definitive diagnosis in reference to the type of sarcoid.

Treatment Plan

The following modalities for treatment of sarcoids were used on 65 of the selected cases from the middle region representing all types of sarcoids.

Surgical Excision

Surgical excision of equine sarcoids was carried out on 45 cases of equine sarcoid having the voluminous, convenient anatomical and suitable features.

The surgical excision technique was done under the effect of combined intravenous anesthesia composed of Detomidine HCI (Domosedan- R.01 mg/Kg (Orion Pharma, Espoo, Finland), Ketamine HCI (Ketalar-R) 2.0 mg/Kg (Delta Pharma, Egypt) and aseptic preparation of the affected site.

The tumor mass was excised and liquid nitrogen spray was applied directly on the denuded surface. The skin was then sutured.

Tetanus antitoxin (3000 IU, S/C and Oxytetracycline* 6 mg/Kg I/M (Pfizer, Egypt) for 5 days were given. Also fly repellent was applied in the areas adjacent to the lesion. Daily monitoring of the excision site was performed until sutures were removed at 10 days.

Topical Cytotoxic Application Thiofluro-Uracil(AW4) as a topical cream at various concentrations (100%, 50% and 25%) was applied on 8 sarcoid cases. The used concentration was governed by the size and location of the sarcoid lesions. Concentrations 50% and 25% were only used on sarcoid lesions close to vital structures such as joints or sensitive organs. The cream was applied to entirely cover the sarcoid surface without rubbing or touching the surrounding skin areas for 3 to 5 applications.

The same preparation was also topically applied on 3 surgically excised cases, to evaluate its effect on the recurrence of the sarcoid. Phenyl-butazone solution (4.4 mg/Kg I/V) was used in combination with Thiofluoro-Uracil (AW4) every 12 hours for 3 days in each case to alleviate the pain.

A mixture of equal amounts of Iroxol enzyme (podophyllum), copper sulphate, tannic acid and benzene was applied on 2 cases of nodular sarcoids of small size (2-5 cm). The same mixture was applied also on a large sized sarcoids (more than 5 cm) to evaluate its effect on the volume of the sarcoid size.

Immunotherpy by injection of BCG (Bacillus Calmette Guerin)

BCG vaccine emulsion was applied on 10 cases of sarcoid lesions (nodular, occult, mixed and fibroblastic types). The sarcoid tumor mass was aseptically prepared and the vaccine was directly injected intratumoral by using a 22-gg needle to completely infiltrate the whole mass. The
RESULTS

Out of the 159 of equid cases (horses 85 and donkeys 74) included in this study; the following epidemiological criteria have been emerged: there were almost an equally presented numbers of horses (85-53.5%) and donkeys (74-46.5%).

The majority of the presented cases were from the south region, including Luxor and Aswan governates (76 cases, 47.8%) followed by the middle region, including Cairo and Giza governates (65 cases – 40.9%) and last the north region, including Alexandria governate (18 cases, 11.3%) [Graph 1].

There were 85 males cases (53.45%) and 74 female cases (46.55%) of the presented cases. The affected areas of the body included the limbs (65 cases, 40.9%), the paragenital and anal region (25 cases, 15.7%), the neck (18 cases, 11.3%) and other miscellaneous regions (pectoral, ears, withers, abdomen, pinna and muzzle) (16 cases, 10.1%) [Graph 2].

Of the six clinical types of sarcoids, the fibroblastic type was recorded in a large proportion of sarcoid cases of this study (58 cases, 36.5%) [Figure 4] followed by the mixed type (46 cases, 28.9%) [Figure 5]. The nodular type (28 cases, 17.6%) [Figure 2] verrucous sarcoid (20 cases, 12.6%) [Figure 3] and the occult type (7 cases, 4.4%) [Figure 1] [Graph 3].

Histopathological Features

Occult sarcoids were characterized by absence of erosions or ulcerations. The epidermis showed variable keratinization from normal, mild or moderate
hyperkeratosis, followed by a layer of prickle cells. The basal layer had moderate proliferation and contained melanin granules in large number (hypermelanosis). The picket fence pattern or cystic hair follicles were not detected. Warty proliferated fibroblasts in the superficial layer were observed with spindle shape elongated hyperchromatic nuclei (Figure 6).

Nodular sarcoid were characterized by mild to moderate hyperkeratosis, in epidermis and parakeratosis (thin prickle cell layer proliferation and hypermelanosis in basal cell layer). Neither ulceration of epidermis nor formation of picket fence were observed. Rete pegs were short and few in numbers (Figure 7).

Fibroblastic sarcoids were characterized by complete ulceration of epidermis. The superficial dermal layer in some areas showed haemorrhage, calcification, inflammatory cells infiltration mainly of neutrophils, lymphocytes, macrophages and eosinophils with granulation tissue formation (Figure 8). In deeper layers of the dermis there was proliferation of fibroblasts which
were spindle or satellite in shape. The proliferated fibroblasts were arranged in tangles or interlacing bundles running in a wavy pattern, forming a whorl like appearance.

Verrucous sarcoids were characterized by moderate hyperkeratosis of the epidermis associated with diffuse or focal hyperplasia of epithelial cells. Large polyhedral cells containing fine granular eosinophilic cytoplasm with balloononing degeneration of mild or moderate degree were seen. The basal cell layer at the basement membrane showed long thin rete pegs of pseudoepitheliomatous hyperplasia descend into the proliferated dermal fibroblasts, destructing the hair follicle, sweat gland and sebaceous gland by collagen bundles and proliferated fibroblasts few whorls of keratine were also seen (Figure 9). At the dermo-epidermal junction, fibroblasts arranged perpendicular to the basement membrane forming picket fence which were characteristic for verrucous sarcoid.

Mixed sarcoids were characterized by partial ulceration of epidermis which composed of moderate hypertrophy and hyperkeratosis of the epidermis. These cells were polyhedral in shape, large size contained eosinophilic granules in cytoplasm and pyknotic nuclei. There was balloononing degeneration. The basal cell layer was proliferated and arranged in more than one layer perpendicular on the basement membrane and few keratin whorls and cystic hair follicles. Other parts of the epidermis sent very long thin rete pegs into the dermis with picket fence formation. The ulcerated epidermis showed ulceration with necrosis, haemorrhage, infiltration of inflammatory cells and proliferation of small blood vessels with excess fibrous connective tissues. The dermis showed proliferation of spindle shaped fibroblasts forming whorls (Figure 10).

**Treatment of Sarcoiids**

**Surgical excision**

Forty five cases of sarcoids of different types (Verrucous,
Table 1. Summary of the sarcoids’ treatments

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Sarcoid localization</th>
<th>Sarcoid type</th>
<th>Methods of treatment</th>
<th>Outcome</th>
<th>Follow up Week/Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>horses</td>
<td>donkeys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>For limb (7 cases) Neck (3 cases)</td>
<td>Nodular</td>
<td>Topical application of AW4* (3 cases) Topical application of mixture of copper sulphate + tannic acid + iroxol enzyme with equal amount of benzine (2 cases) Surgic excision (4 cases) Injection of BCG** (one case)</td>
<td>Complete recovery No recurrence</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Hind limb (3 cases) Fore limb (1 case) Wither (2 cases)</td>
<td>Occult</td>
<td>Topical application of AW4 (5 cases) Injection of BCG (one case)</td>
<td>Complete recovery No recurrence</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>Hind limb (6 cases) Anus (1 case) Fore limb (1 case)</td>
<td>Verrucous</td>
<td>Surgical excision (8 cases)</td>
<td>Complete recovery (6 cases) Recurrence (2 cases)</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>Fore limb (12 cases) Wither (4 cases) Perianal (3 cases) Scrotum (2 cases)</td>
<td>Fibroblastic</td>
<td>Surgical excision (18 cases) BCG injection (3 cases)</td>
<td>Recurrence after surgical excision (6 cases)</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>Fore limb (13 cases) Neck (5 cases) Facial (2 cases)</td>
<td>Mixed</td>
<td>Surgical excision (15 cases) BCG injection (5 cases)</td>
<td>Recurrence after surgical excision (6 cases)</td>
</tr>
</tbody>
</table>

- * AW4 (Thiofluorouracil cream).
- ** BCG (Bacillus Calmette Guerin).

The number of cases of different sarcoid localizations, types, and results are summarized in Table 1. Various treatment methods are described, including topical applications, surgical excision, and BCG injections. The outcomes range from complete recovery with no recurrence to recurrence after different follow-up periods.

DISCUSSION

From the present study, it was obvious that equine sarcoids are considered the most common cutaneous tumors in equids in Egypt. Equine sarcoids affected all geographic locations in Egypt, every equine species, gender, age or body localization. This is in agreement with (Ragland et al., 1970 and Marti et al., 1993). The higher tendency of sarcoid development in the south region of Egypt which showed obvious aggressive lesions might be attributed to the hot climate, bad hygiene and insect vector (flies) irritations. This contradicted what was mentioned by Knottenbelt, 2001 who found that sarcoids are commonly reported to grow during the winter time.

Further more, it was observed that many of the presented patients were cohabited in places with animals of bovine species. The presented cases of large sized fibroblastic and mixed types 85) with subsequent marked reduction in their sizes to facilitate surgical excision afterwards. Table 1 summarized the treatment results of sarcoids and outcome.

Topical cytotoxic application

Thiofluoro-uracil (AW4) cytotoxic cream was applied on 8 cases of occult and nodular types with complete recovery and absence of recurrence for up to 12 months after the end of the treatment protocol. Topical application of a mixture of Iroxol enzyme (podophylum), copper sulphate, tannic acid and benzine was applied on 2 cases of occult and nodular types resulted in complete recovery and absence of recurrence for up to 4 weeks follow up.

Bacillus Camellet Guerin (BCG) vaccine injection

This method of treatment was applied on 2 cases of small sized nodular and occult types with subsequent complete recovery. This method of treatment was also applied on 8 cases of large sized fibroblastic and mixed types 85) with subsequent marked reduction in their sizes to facilitate surgical excision afterwards. Table 1 summarized the treatment results of sarcoids and outcome.
species and this seems to confirm the intimate relation between the bovine papilloma virus (BPV-DNA) and the pathogenesis of equine sarcoïds. This is in agreement to findings by (Teiße, 1994).

In the present study, sarcoïds were recorded in equids at any age, although other studies found an incidence peak between 3 and 6 years of age (Torrontegui and Reid, 1994; Knottenbelt, 2005). However, gradual increase in incidence is observed up to the age of 15 years followed by a decrease in prevalence (Mohamed et al., 1992). Other researchers have reported a higher prevalence in younger individuals (Carstajen et al., 1997; Clottu, 2008).

All clinical types of sarcoïds were recorded in this study in variable proportions with the exception of fibroblastic type which exhibited in large proportion (36.5%) followed by the mixed type (28.9%). The nodular, verrucous and occult type were also recorded in proportions (16.6%, 12.6% and 4.4%) respectively. The malevolent type was not recorded in this study.

The fibroblastic type was prevalent (36-5%) among the majority of equids cases. This is an agreement with Marti et al., 1993 and Bostrom, 1995). They found such prevalence reasonable since sarcoïds develop initially as benign and small of other types then transform into severe and aggressive fibroblastic form

The localization pattern of sarcoïds in the present study included the limbs (40.9%), the paragenital and anal regions (22.0%), the periorbital (15.7%), the neck (11.3%) and other body areas (10.1%). These findings are consistent with those mentioned by Brostrom, 1995; Martens et al., 2001; Clottu, 2008). While, other studies (Brostrom, 1995; Martens et al., 2001) reported the trunk and head area were predominantly affected rather than the extremities. The possible explanation for the high percentage of legs being affected in the present study was that most of working equids that were exposed to different kinds of leg injuries. Furthermore, high prevalence of head lesions could be attributed to continuous injuries and irritation afflicted by bad head harnesses or grooming tools.

In the present study, comprehensive morphoclinical and histopathological features of all the sarcoïd types were recorded. The simplest forms of sarcoïds were occult, in which areas of alopecia in intact healthy skin with hyperkeratosis were shown. Nodular sarcoïds appeared as marked immobile nodules of variable sizes under the intact healthy skin.

Histopathology of occult and nodular sarcoïds revealed higher cellularity of superficial dermal portion when compared to deep portions. There were also few rete pegs in occult and nodular sarcoïds. Moreover, there was an increase in density of subepidermal fibroblasts in-between numbers of hair follicles and sweat glands. Picket fence pattern, cystic hair follicles or erosion of epidermal surfaces were seldom noticed in these types of sarcoïds. Similar findings of histopathological signs of sarcoïds include dermal proliferation of fusiform or spindle shaped fibroblasts (forming whorls or interlacing bundles), epidermal hyperplasia and hyperkeratosis as well as rete peg formations at the dermal-epideral junction, a picket fence pattern were mentioned by (Ragland, 1970; Tarwid et al., 1985; Marti et al., 1993; Goodrich et al., 1998; Charles, 2005; Sala et al., 2010).

The epidermal hyperplasia in association with rete pegs formation were the most pathognomonic lesions for diagnosis of fibroblastic, verrucous and mixed type of sarcoïds which is in agreement with (Scott and Miller, 2003) who attributed their formation mostly to be attenuated hair follicles at their origin, which were often elongated and pointed.

In the present study, different therapeutic approaches were tested with variable outcomes. Regarding this, careful selection of the appropriate treatment for each sarcoïd was based on, localization, number and size of the tumors, the treatment history, the financial value of the animal and the owner compliance to fulfill the treatment schedule (Marti et al., 1993; Carstanjen et al., 1997).

Surgical excision carried out on 45 cases having single or multiple sarcoïds. The rate of recurrence was relatively high due to the difficulty to remove all roots of rete pegs distributed within the normal tissue was done around the sarcoïd. However, the use of liquid nitrogen after surgical excision to induce death (apoptosis) and necrosis of cells at the roots of rete pegs inside normal tissues with an aim of preventing the ability of sarcoïd recurrence. This method coincided with that used by (Chambers et al., 2003). The surgical excision of equine sarcoïds has been applied for decades with variable success. However high recurrence rates of 40% to 72% are reported (Ragland, 1970; McConaghy et al., 1994; Brostrom, 1995).

Conclusion

Sarcoïds are a form of cutaneous benign tumor that affects all types of equines irrespective of age, gender, color and geographic localization. Most of the lesions occurred on the limbs which established that even minor injuries caused by flies can lead to sarcoïds. This factor highlights the importance of effective fly control in areas where equids are living.

Also there was a great association of sarcoïds in equines with the presence of 2 types of bovine papilloma viruses (BPV-types 1 and 2).

Treatment of sarcoïds is difficult and early treatment is recommended to prevent expansions. Minor skin injury should be also early attended.

There are many treatment options and one should select the most appropriate method according to the location, size and type of sarcoïd for getting good results.

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