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**Histopathological Diagnosis and Tumour Surgical Removal by Segmental
Posthectomy of a Preputial Sarcoid in a Donkey in Ecuador.**

Análisis de Casos

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RESUMEN

Los sarcoides equinos son las lesiones cutáneas más comunes en equinos a nivel mundial, representan del 12 al 67% de todos los tumores y del 36 – 67% de los tumores cutáneos en esta especie. A pesar de poseer una naturaleza benigna, pueden comprometer seriamente el bienestar, desempeño y valor del animal. En este reporte de caso se describe los hallazgos encontrados en un Burro de 3 años de edad con dos masas tumorales (región carpal derecha y prepucial) que más adelante fueron diagnosticadas como sarcoides. Luego de un examen físico preliminar, el paciente se encontraba estable y las masas no mostraban signos de invasión hacia otros tejidos, como los huesos de la articulación carpiana u otras zonas propiamente penianas. Se realizó la escisión de la masa en prepucio mediante la técnica de circuncisión (postectomía segmentaria), con el objetivo de asegurar el bienestar del paciente y prevenir posteriores complicaciones como: infecciones, infiltraciones larvarias o parafimosis permanente. Tanto durante el procedimiento quirúrgico como la anestesia no se presentaron complicaciones y los periodos de postquirúrgico y recuperación transcurrieron con normalidad. Nueve meses luego de la cirugía aún no existía ninguna señal de recidiva tumoral, lo cual de acuerdo con estudios previos es el periodo dentro del cual los sarcoides tienden a reaparecer. En el diario ejercicio veterinario con equinos, la histopatología continúa siendo la herramienta más práctica para el diagnóstico de sarcoides equinos. Sin embargo, el tratamiento aun representa un reto y una vez tomada una decisión quirúrgica, una técnica limpia y el seguimiento postoperatorio apropiado son cruciales para prevenir su recurrencia u otras complicaciones.

Palabras clave: Sarcoide, Histopatología, Cirugía, Burro, Asno, Postectomía

ABSTRACT

Equine sarcoids are the most common skin lesion in equids worldwide, representing the 12 – 67% of all equine tumours, 36 – 70% of all skin tumours in horses and despite their benign nature, can severely compromise the welfare, performance and value of the animal. This case report describes the pathological findings on a 3 year-old Asinine presenting two tumoural lesions histopathologically later diagnosed as sarcoids on his right limb and prepuce. At the primary examination the patient seem stable and the masses showed no further tissues involvement such as bone of penile invasion. A surgical excision of the mass by a segmental posthectomy technique was performed, in order to amend this patient's welfare and prevent further complications such as infections, worm larvae infiltrations or permanent paraphimosis. There were no complications during the surgical procedure or anaesthesia, the postsurgical and recovery periods went through very easily and after 9 months after surgery, there was any sort of recidivism, which according to previous studies covers the critical period when sarcoids use to re-emerge. In the daily praxis, histopathology remains the most practical diagnostic tool for equine sarcoids while treatment remains challenging and once a surgical decision is made, a clean technique and a proper post-surgery follow-up are crucial to prevent recurrence or further complications.

Key words: Sarcoid, Histopathology, Surgery, Donkey, Asinine, Posthectomy.

Case Report
**Histopathological Diagnosis and Tumour Surgical Removal
by Segmental Posthetomy Technique of a Preputial Sarcoid
in a Donkey (*Asinine*) in Ecuador.**

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Introduction

Equine sarcoids (ES) are the most common skin lesion in equids worldwide (2). They represent the 12 – 67% of all equine tumours and the 36 – 70% of all skin tumours in horses (13). Jackson first described this tumour in 1936; he called it sarcoid as a way to emphasize its fibromatous, flesh-like nature and the tendency to recur after surgical removal (4). ES represent a major equine health problem, affecting horses, donkeys, mules and zebras with a higher incidence in donkeys and mules. Even though, equine sarcoids are not life threatening, these tumours can severely compromise the welfare, performance and value of the animal (3).

The aetiopathogenesis of ES remains unclear, for all we known, it seems to be a complex, multifactorial condition (6). Environmental and genetic factors seem to play a major role while Bovine Papilloma viruses 1 (80%) and 2 (20%) DNA have been detected in 100% of ES patients (15) but it was also found in normal skin. This virus transmission is supposed to occur from cattle to horses or

horse-to-horse, possibly involving biting flies as vectors. Trauma, including iatrogenic surgical trauma is regarded as a co-factor in sarcoid tumour development (3). Some studies support the evidence of a genetic predisposition with a stronger prevalence in Quarter horses and a low occurrence in Standardbreds. Even if it is well established that sex, age, coat colour or working conditions are not factors that influence the development of sarcoids (4), the average disease development is reported to range between 3-6 years, showing a predisposition in younger animals (8).

Equine sarcoids can typically be recognized clinically and histopathologically, but the gold standard confirmation comes by performing PCR from superficial swabs, skin scrapings or directly from the tumoural mass (8). The gross appearance of equine sarcoids has been classified into three different gross pathological types, including verrucous (warty-like), fibroblastic (proud flesh) and, nodular (hard) (8). Histologically, sarcoids possess both, epidermal and

dermal components. The epidermal part will be hyperkeratotic and hyperplastic usually penetrating into the adjacent dermis forming the so-called rete pegs. The dermal component shows bundles with normally differentiated fibroblasts arranged irregularly in-between variable amounts of unorganised collagen (4).

Talking about what can be done to treat this disease, there is no uniformly effective therapy for sarcoids. Tumours are usually located on areas of easy access and where skin closure is feasible, so they are often removed surgically but this procedure has a reported 30% risk of recurrence (15). Other possible treatments include: carbon dioxide excision, cryotherapy, hyperthermia, radiotherapy, chemo, immunotherapy and topical acyclovir creams, each one showing a varying degree of success (4,11, 15).

This article focuses on; the macroscopical and histopathological determination of two tumoural masses in a case of equine sarcoids in a Donkey living in the Ecuadorian highlands. The surgical resolution technique will be also described as well as the post-surgical follow-up in order to establish the effectiveness degree of its extirpation.

Case description

History and clinical findings.

On January 2016, a 3 year-old brown donkey (*Asinine*) was attended by a field equine veterinarian in Quito – Ecuador. The patient presented two large, firm, masses; these tumour-like lesions were located one on the right carpal

region and the other one by the preputial area. Each mass had about 8 and 12 centimetres of diameter, carpal (Fig 2) and preputial (Fig 1) respectively. Both masses shared the same external appearance; they were locally invasive, fibroblastic-like skin tumours, fleshy and ulcerated with local infiltration as shown on (Fig 1 and 2). Because of their location in the patient's body and appearance, a strong suspicion of Equine sarcoid was preliminary established.



Figure 1: Preputial Tumour-like lesion in the Donkey, (red arrow).



Figure 2: Mass at the right carpal region of the donkey, (red arrow).

The animal was otherwise healthy with all his physiological parameters within normal ranges, active, alert, and he was feeding and drinking normally. To preserve the animal's welfare and comfort and taking into account his owner's financial possibilities, it was decided that a surgery should be conducted as primary treatment.

Surgery.

The tumour removal was performed on field. Both lesions were handled and removed according to neoplastic surgery parameters leaving wide margins (2-3cm) to prevent recurrence (15). The prepuce was carefully treated to prevent permanent damage; the surgical procedure was a segmental posthectomy, which is indicated for removal of preputial neoplasms (1, 5).

The surgical excision technique was performed under the effect of combined intravenous general anesthesia through a right jugular vein catheter. The Donkey was premedicated with Xylazine (1.3mg/Kg), using Diazepam (0.1mg/kg) with Ketamine (2.5mg/kg) as inductors. In addition, a fluid therapy (10ml/kg/min) was sustained throughout the procedure in order to maintain the anaesthetic plane by a total of 3 Xylazine (0,45ml) and Ketamine (0,85ml) boluses. As for the local anaesthetic block, 20 millilitres of Lidocaine were applied to the carpal region.

With the donkey under general anaesthesia the prepuce tumour removal proceeded. To facilitate surgery a tourniquet placed proximal to the surgical

site was used along with urethral catheterization for a good identification and protection of the urethra (Fig 3). The penis was extended by traction on gauze looped around the glandis. Parallel circumferential incisions through the preputial epithelium were made, one distal and the other one proximal to the lesion, the aforementioned incisions were connected by a third longitudinal cut. The large subcutaneous branches of the external pudendal arteries and veins run very superficially, so the incisional procedure had to carefully done.

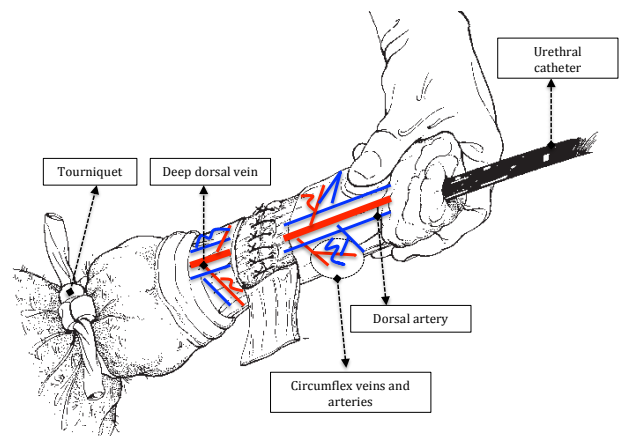


Figure 3: Segmental posthectomy, tourniquet and urethral catheter placement. Principal vessels. (Modified from Auer & Stick)

Finally, the segment including the presumptive sarcoid was dissected free and removed. The tourniquet was released and the bleeding vessels were ligated, while the skin edges were opposed and sutured in a simple interrupted pattern using 2-0 absorbable material (Fig 3). Additionally, a closed castration procedure was performed using the normal orchidectomy block protocol (20ml intra-testicular Lidocaine). As for the carpal sarcoid and since there were no bone structure involvement, the tumour

was easily removed by a round uninterrupted incision respecting the 2-3cm margins and it was left without sutures for second intention healing process.

The whole surgery took approximately 1 hour and was successful in the removal of both masses with minimum to normal blood loss.

The donkey's awakening went easily and took 30 minutes. After the recuperation, anti-inflammatory medication: intravenous Phenylbutazone (4.4mg/kg) and intramuscular antibiotics were administrated (Procaine penicillin, 40000UI/kg). In both tumour areas silver veterinary wound spray (Sulfadimethoxine) was applied as antimicrobial shield and to help with the healing process. Due to the location, just the carpal wound was left with a dry bandage while the preputial one was left only covered by the silver spray. An intramuscular injection of Ivermectin was also administrated (0,2 mg/kg) to prevent myiasis and bacterial infections that could be carried by flies.

Pathological diagnosis.

Samples of both, carpal and preputial presumed sarcoids were 10% formalin-fixed in separate cans and taken to the university's pathologist who turned them into paraffin-embedded tissue blocks for sectioning in order to conduct the pathological evaluation.

The histopathological cut revealed an epidermal acanthosis and hyperplasia with parts of the epidermis sending very long thin rete pegs into the dermis with

the classical picket fence formation (Fig 4). The deeper layers of the dermis showed fibroblastic proliferation arranged in tangles or interlacing bundles in a wavy pattern and forming a whorl-like appearance (Fig 5).

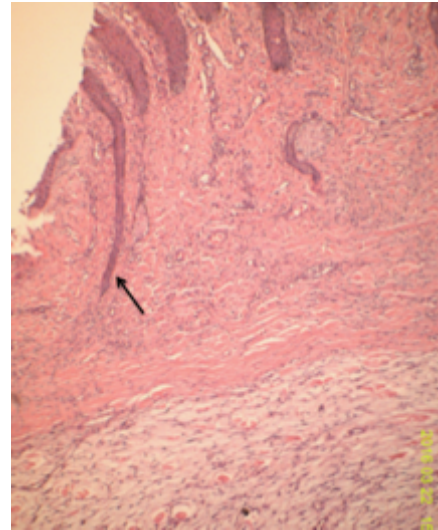


Figure 4: Equine sarcoid showing the typical Long, thin, rete pegs – like prolongations (black arrow) into the dermis due to the epidermal acanthosis affecting the *stratum spinosum* and *stratum basale* (H&E X10)

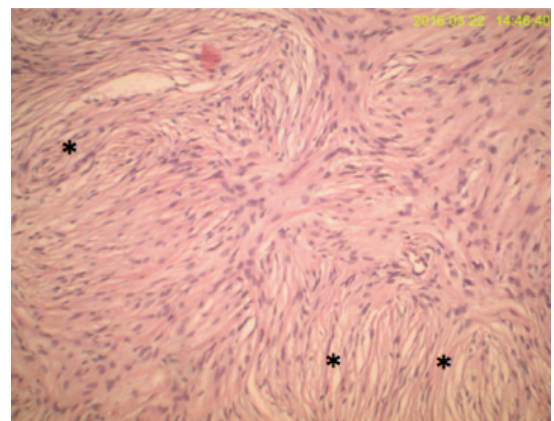


Figure 5: Equine sarcoid, intradermal fibroblast proliferation arranged in short interlacing bundles (black asterisks). (H&E X10)

Postsurgical evaluation

After the surgical procedure, there were following-up controls where the donkey seemed to be going well and the surgery results were as good as expected. Baseline vital parameters were within the reference ranges. On top of that, the animal seemed very active and had no signs urinary or locomotion problems. Most important, 9 months after the procedure, when the donkey was re-examined, the animal had no signs of tumours growing back on either former tumour locations (Fig 6 and 7).



Figure 6: Former preputial sarcoid, showing no signs recidivism (red arrow).



Figure 7: Former carpal sarcoid, showing no signs recidivism (red arrow).

Discussion

To our knowledge, no articles have been published on the histopathological changes found in equine sarcoids in Ecuador; furthermore it also represents the first description of the surgical resolution in a Donkey. Both masses were successfully and completely removed keeping the wide margins (2–3 cm) that are critical to prevent recurrence of the tumours (15). One of the challenges we dealt with was the maintenance of a sterile environment during field-performed surgery, which combined with the free-ranging way of living of the patient, which drastically increases the risks in the post-surgical care of the wounds. Nevertheless, the outcome showed that this aspect had not influenced the patient's recovery. A good collaboration from the owners in wound care, periodic controls and antibiotic handling reduced the risks that field surgeries represented.

Another, and probably our principal concern was the reported tendency of these tumours to persist after surgery. According to multiple studies made around the world approximately 30% (28-50%) of the equine sarcoids recur after surgical excision within an average of 6 months (9,13). Since the re-emerging tumours tend to be more aggressive and faster growing, and the current impossibility to predict which tumour will recur, it is highly recommended to monitor the animal closely to be ready for a rapid intervention if necessary (14, 15). The relative high recurrence rate has been

associated with the tumour's histological features due to the fact that the removal of all the roots of the rete pegs penetrating normal tissues has always been a difficult task (13). In our case, having the possibility of a proper follow-up of the patient for several months after the surgery gave us the chance of a more accurate determination of the procedure's success, after comparing it to the recidivism periods reported in studies made so far.

Regarding the histopathology findings, they were considered very conclusive, as typical dermal changes were easily identified; the epidermal part typically hyperkeratotic and hyperplastic penetrating into the dermis forming the classic rete pegs, while the dermal component showed the consistently reported bundles of fibroblasts (4). Some differentials taken into account include granulation tissue, granuloma and specially squamous cell carcinoma (SCC) that have similar gross features and mostly occur in the same anatomical locations, SCC is also a kind of tumour of epithelial cells that shows predilection for the paragenital region and tend to occur in sites of previous injury as well as ES (10). Nevertheless, sarcoids and SCC are easily differentiated by characteristic histopathological features that are distant for each lesion (12). SCC shows diskeratotic features rather than hyperkeratotic ones, and there are small aggregates as irregular islands of neoplastic keratocytes. While in ES the proliferation of fibroblast shows no signs of malignancy, in SCC mitosis and cellular atypia are frequent findings (17). Furthermore, there were also epidemiological factors that guide us in

the right direction between these differentials, penile and preputial SCC presents mainly in adult horses, reportedly mean of 12.4 year-olds, but most other studies suggest a range from 17,4 to 19.5 years. On the other hand, ES development is reported in younger animals (3-6 years) (4, 16, 17). Other important keys to differentiation are the incidence of SCC infiltrating the *glans penis* and iliac lymph node (7), which hasn't been reported in ES.

Even if further and more specific test such as PCR or immunohistochemical distinction could have been done in order to reach a much more modern and accurate diagnosis, the lack of the necessary material or inaccessibility to it made further testing impossible and unpractical at regular equine praxis, where the diagnosis of equine sarcoid is often based on clinical appearance and the presence of more than one lesion with sarcoid characteristics, requiring histopathology for the definitive diagnosis (15).

The surgical procedure was considered very successful since there were no signs of recidivism on either location more than 5 months after surgery, and there were not the signs of the common complications in the immediate postoperative period such as, preputial oedema, iatrogenic paraphimosis, haemorrhage or urinary retention for urethral oedema (7). Additionally, at the time of this writing, a new visit was made (9 months after the surgical excision), there were not any signs of the tumour re-growing, at least perceptible to the eye. The owners also seem very pleased with the outcome since

the risk of bacterial infection has not been a matter of concern anymore.

As this case shows, proper wound care, owner's collaboration and a close and long-term control of the patient's post-surgical evolution are key factors that determine a positive resolution of such tumours. Surgical technique may vary according the specific location of the lesions or surgeon's expertise and preferences, but the precautions of normal oncology margins protocols can not be taken lightly. For further handling of similar cases it's recommended to try support topic treatments such as liquid nitrogen application right after surgery, acyclovir creams, AWA-LUDES sarcoid cream (5-flouracil), intra-lesional cisplatin or Imiquimod (Aldara cream) when possible, in order to determine their role in the recidivism rates (11,18)

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