

## Occurrence of squamous cell carcinoma in Milchschaaf sheep in Uruguay

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**ABSTRACT:** *In this study, the epidemiological and pathological features of an outbreak of squamous cell carcinoma (SCC) in adult female sheep and a young ram, Friesian Milchschaaf breed or crossbreed is described. Seven adult females belonging to an original flock nucleus of 20 animals were affected, as well as a young ram belonging to the same nucleus was affected in other establishment. Multiple verrucous neoplastic masses of several months of evolution were evident in the head region, with local invasion and regional lymph node metastases. Histological studies revealed a cancerous stage of invasion of the dermis and neoplastic proliferation characteristic of invasive SCC, and a pre-cancerous stage with solar elastosis and chronic solar keratosis lesions induced by sunlight. Decrease in latitude, increased level of solar radiation with long exposure to sunlight, and the phenotypic characteristics of the breed are the main factors responsible for the high prevalence of SCC, showing the susceptibility of the Friesian Milchschaaf breed and crossbreed in temperate zones as Uruguay.*

**Key words:** carcinoma, sunlight radiation, solar keratosis, solar elastosis, small ruminants, skin cancer.

## Ocorrência do carcinoma de células escamosas em ovinos Milchschaaf em Uruguai

**RESUMO:** *Neste estudo descrevem-se as características epidemiológicas e patológicas de um surto de carcinoma de células escamosas (SCC) em ovelhas adultas e ram jovem, raça Friesian Milchschaaf ou cruza. Foram afetadas sete fêmeas adultas pertencentes a um núcleo de rebanho original de 20 animais, bem como um ram jovem pertencente ao mesmo núcleo em que foi afetado em outro estabelecimento. Múltiplas neoplásias verrucosas de vários meses de evolução foram evidentes na região principal, com invasão local e metástases de linfonodos regionais. Estudos histológicos revelaram um estágio cancerígeno de invasão da derme e proliferação neoplásica característica do SCC invasivo e um estágio pré-cancerígeno com elastose solar e lesões de ceratose solar crônica induzidas pela luz solar. A diminuição da latitude, o aumento do nível de radiação solar com longa exposição à luz solar e as características fenotípicas da raça são os principais fatores responsáveis pela alta prevalência de SCC, mostrando a susceptibilidade da raça Friesian Milchschaaf e cruzada em zonas temperadas como o Uruguai.*

**Palavras-chave:** carcinoma, radiação solar, queratose solar, elastose solar, pequenos ruminantes, cancro da pele.

## INTRODUCTION

Cutaneous squamous cell carcinoma (SCC) is a malignant neoplasia of epidermal cells, locally invasive and destructive; although, with low metastatic potential (GROSS et al., 2005; MAULDIN & KENNEDY, 2016). SCC occurs most frequently in sun-damaged skin and is usually preceded by solar keratosis and carcinoma in situ (GROSS et al., 2005). SCC is common in felines, canines, cattle, horses and humans, less common in sheep, and rare in pigs and goats (MAULDIN

& KENNEDY, 2016). This neoplasia has been reported in different breeds of sheep, such as Merino, Polwarth, Ile de France and Corriedale, among others (TUSTIN et al., 1982; RAMOS et al., 2007). Lesions can be reported in any part of the body but usually appear on areas of the skin with a lack of pigment or wool. In sheep, the tumor occurs most frequently on the muzzle, lips, eyelids, pinnae, perineum and vulva (MAULDIN & KENNEDY, 2016; MACEDO et al., 2008). While SCC in animals and humans is global in occurrence, differences in its distribution in various areas of

the world have been observed (NEWTON, 1996; BRONDEN et al., 2007; QURESHI et al., 2008). The incidence rate of SCC varies geographically, and is highest in the southern hemisphere and with decreasing latitude, i.e. with higher levels of ultraviolet (UV) radiation (ENTWISTLET, 1977; GOLDSCHMIDT & HENDRICK, 2002; TSUJITA & PLUMMER, 2010). The rate of SCC appearance increases by 50% for each 8-10° decline in latitude, equivalent to an increase in 29% per unit of UV exposure (NEWTON et al., 1996; DIEPGEN & MAHLER, 2002).

Of several factors associated with the development of SCC, sunlight is the most important carcinogenic stimulus for these tumors. It is related to over expression of p53 protein as a result of UV-induced mutations of the p53 tumor suppressor gene (BRASH et al., 1991). At the same time, the risk of developing SCC is higher in females and increases linearly with the age of the animal (LADDS & ENTWISTLET et al., 1977; GOLDSCHMIDT & HENDRICK, 2002). Other possible factors associated with SCC development are infections with *Papillomavirus* (VITIELLO et al., 2017) and traumatic lesions, like caravan wounds and tattoos (LADDS & ENTWISTLET et al., 1977; GOLDSCHMIDT & HENDRICK, 2002).

The Friesian Milchschaaf is a dairy sheep breed originally from eastern Friesland, a coastal region in northern Germany (latitude 53° N). It is a prolific ovine biotype, with high maternal ability and lambs with a high growth rate. Phenotypically it is characterized by white skin, and head and legs clear of wool. In recent decades, different countries of South America such as Argentina and Uruguay have introduced the breed to improve sheep production (KREMER & ROSÉS, 2016). In Argentina it was introduced in 1960's for meat production due to its prolificacy, and further, in 1980, it was used for milk production due to the increasing demand for dairy products and by-products of small ruminants, then introduced to Uruguay in 1990 in order to improve the low reproductive indexes and increase the competitiveness of small sheep producers (BARBATO et al. 2011; BARRIOS et al., 2014). This aroused interest in other South American countries such as Brazil, towards which exports have been made since 2007 for milk production purpose as a sustainable alternative to improve the quality of life of small rural producers (CORREA et al. 2006).

The present study describes outbreaks of SCC in sheep of the Milchschaaf breed and crossbreeds.

Different pre-cancerous and cancerous stages were registered pathologically, as well as epidemiological characteristics, showing solar radiation as the main factor associated with tumor induction.

## MATERIALS AND METHODS

The outbreaks occurred in the summer's of 2014 and 2015 (December-February) on mixed sheep and cattle farms situated in the 7<sup>th</sup> and 11<sup>th</sup> counties, Treinta y Tres department (latitude: 33°18'S; longitude: 54°94'W), Eastern Uruguay. Clinical, epidemiological and management data were collected during visits to the farms. Post-mortem examinations and complete histologic evaluations were performed on two affected sheep (*cases 1* and *2*) following euthanasia with intravenous (IV) sodium pentobarbital and exsanguination. Tissue samples, including different parts of the tumors, regional lymph nodes and sun-exposed skin, were fixed in 4% buffered formaldehyde, processed routinely, and 5 µm sections were cut and stained with Mayer's hematoxylin and eosin (HE) for histology.

## RESULTS

Seven adult female ewes, all five years old, of a flock of 175 pure breed Friesian Milchschaaf and crossbreed  $\frac{3}{4}$  Friesian Milchschaaf x  $\frac{1}{4}$  Finnish Landrace were affected in the first outbreak (*case 1*). All affected ewes belonged to the primary genetic nucleus flock integrated with 20 animals, resulting in 35% specific morbidity (7/20) and 15% lethality (3/7). They showed multiple papillomatous and verrucous growths of variable size on the face, i.e. lips, mouth corner, nostrils, eyelids and pinna. All the animals were grazed in a large paddock with native pasture, open all year. The second outbreak corresponded to a two-year-old ram (*case 2*), a crossbred  $\frac{5}{8}$  Milchschaaf x Finnish  $\frac{3}{8}$ , of a 50 flock of the Corriedale breed. The ram, originally belonging to the sheep nucleus of *case 1*, was acquired from a small sheep producer to improve reproductive rates. It was the only affected animal in the flock.

The necropsied ewe of *case 1* presented poor body condition and weakness, with multiple cauliflower-like neoplastic developments of verrucous aspect with a broader base and multifocal crusted patches of variable size in the head region: mouth corner, hard palate, nostrils, eyelids, ear and submandibular region (Figure 1). On the dorsal pinna,



Figure 1 - Milchschaaf ewe. Multiple squamous cell carcinoma as papillary masses of varying diameter in different parts of the head region.

there were texture changes at palpation, rougher and firm, with alopecia, scaling and crusting, and multiple plaque-like lesions of up to 0.5 cm, lesions compatible with solar dermatitis (Figure 2). The young ram of *case 2* presented poor body condition, weakness and apathy, with a large verrucous neoplastic mass which destroyed the left orbital region, the eyeball, face, cheek and forehead. The dorsal pinna and cranial face of forelimbs evidenced lesions of solar dermatitis, with erythema and alopecia, and multiple plaque-like crusts of variable diameter up to 1 cm. On cut sections, the tumor masses of both animals were white and firm, with hemorrhagic areas and a crusting necrotic surface in the periphery. Metastasis, characterized by well-delimited masses of firm consistency, was observed in the submandibular and retropharyngeal lymph nodes. Neoplastic invasion to the nostrils and oral cavity was observed in *case 1*, whereas in *case 2*, tumor invasion of the skull destroyed the left parietal region, zygomatic arc and orbital fossa.

Microscopically, both cases presented characteristic lesions of invasive SCC, with dermis invasion and neoplastic proliferation and infiltration, from the surface layer of the epidermis, characterized by irregular sized trabeculae, cords and islands of invasive squamous epithelial cells

(Figure 3). Cytologically, neoplastic squamous cells were pleomorphic, with vesicular to round large nuclei, dense chromatin, and 1 or more prominent nucleoli. Also, the neoplastic cells presented prominent intercellular bridges with variable degree of keratinization, either individual cell dyskeratosis or in clusters (Figure 3 inset). Formation of central accumulations of compact laminated keratin (keratin pearls) within islands of invasive neoplastic epithelial cells was observed. There was fibrosis surrounding neoplastic islands, and high mitotic activity as well as mitotic atypia (3 to 5 per field 400x).

Contralateral ear in both cases and the forelimbs of *case 2* had lesions of chronic solar dermatitis with pale staining bands of degenerated collagen along subepidermis, and the presence of few typical apoptotic keratinocytes, the so-called "sunburn cells", in the basal and stratum spinosum (Figure 4 inset). Some areas showed lesions of solar keratosis with epidermal hyperplasia and dysplasia (rete pegs), orthohyperkeratosis, lichenoid subepidermal lymphoplasmocytic infiltrate, and pale-staining band of collagen along subepidermis (Figure 4). In some areas, the lesions developed into carcinomas *in situ*, manifested by



Figure 2 - Milchscaf, ewe. Solar dermatitis lesions with rough and firm texture, alopecia, crusted patches, and multiple plaque lesions in dorsal pinna.

full-thickness epidermal dysplasia, loss of cell polarity, marked nuclear atypia, individual cell apoptosis, and disruption of normal epithelial stratification. No neoplastic lesions were evident in other regions.

## DISCUSSION AND CONCLUSION

A diagnosis of SCC was made based on clinical history, epidemiological data and pathological findings. The study indicated the high susceptibility of the Friesian Milchscaf breed to SCC under these environmental, sanitary and management conditions, possibly due to factors, such as white skin and absence of wool on the head and legs, with high levels of solar radiation causing chronic solar dermatitis.

A high incidence of the disease was evident in adult females; however, the case in a young male highlighted the high susceptibility of the breed under the conditions present in Uruguay. Pathological findings of solar dermatitis and keratosis with inflammatory and degenerative epithelial changes induced by solar radiation indicated a pre-

cancerous stage. These lesions frequently progress to invasive SCC (GROSS et al., 2005; TSUJITA & PLUMMER, 2010), suggesting solar radiation as the main factor predisposing to neoplastic invasion in these outbreaks.

The affected breed, Friesian Milchscaf, is originally from the north of Germany (53° N) where solar radiation, UV and the risk of skin tumors are much less frequent than in Uruguay (33° S) (GOLDSCHMIDT & HENDRICK, 2002; TSUJITA & PLUMMER, 2010). In Europe, the incidence of SCC or other skin tumors related to UV radiation are moderate, and tend to decrease in northern parts with higher latitudes (MOAN & DAHLBACK, 1992; LEITER & GARBER, 2008). This difference in the rate associated with geographical zones is well studied in humans, and southern hemisphere countries show higher rates of presentation, i.e. more than double compared to latitudes of 37° to 29° in Australia (GILES et al., 1988), and increases in 39% for each 10° decrease in latitude in Africa (NEWTON et al., 1996). In cattle, studies have reported a higher incidence of ocular SCC in the south-western hemisphere

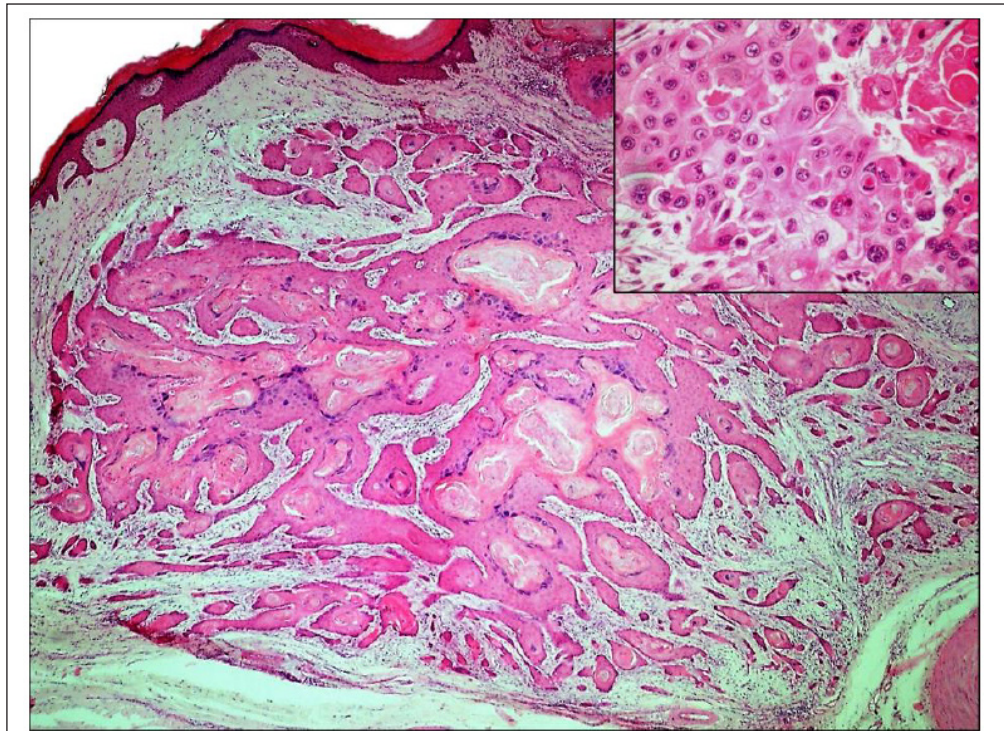


Figure 3 - Milchschaaf ewe, skin. Squamous cell carcinoma, with dermis invasion and neoplastic proliferation and infiltration to the dermis, with cords and islands of invasive neoplastic epithelial cells. Hematoxylin and eosin (HE). 4x. Inset: Squamous cell carcinoma with pleomorphic neoplastic epidermal cells with marked nuclear atypia and individual dyskeratotic cells or clusters. HE. 40x.

at lower latitudes with higher levels of sunlight (TSUJITA & PLUMMER, 2010; PUGLIESE et al., 2014). This increased risk associated with extensive farming conditions in our region along with long periods of sunlight exposure and high solar radiation results in a high carcinogenic risk for animals (CARVAHLO et al., 2012). These environmental factors are most common in animals presenting with SCC (MAULDIN & KENNEDY, 2016). The finding of regional metastases in lymph nodes is rare (GROSS et al., 2005) and showed the high degree of malignancy in SCC.

Though the presence of papillomavirus was not fully investigated, the high incidence of SCC in the present study suggested it might be involved. However, the location of the carcinomas exclusively in skin with no pigmentation and unprotected by wool, the high incidence recorded exclusively in sheep of the same nucleus or genetic line, and the summer occurrence of the disease, suggested that the major causative factor was prolonged exposure to UV radiation in a highly susceptible breed. The importance of papillomavirus

as a causal agent of the SCC in sheep should be further investigated (DEL FAVA et al., 2001).

We concluded that a decrease in latitude, increased level of solar radiation with long periods of exposure to sunlight, and the phenotypic characteristics of the introduced breed are the main factors responsible for the high prevalence of SCC under our conditions. Occurrence of SCC in this breed introduced onto commercial farms of Uruguay showed the need to maintain surveillance with periodic clinical inspection of the animals in order to ensure early detection of incipient tumors and surgical removal. The breed is probably inappropriate for the environmental conditions of South America, both in Brazil and Uruguay, being necessary to implement alternatives as to provide shade or forests or genetic selection of more resistant animals, considering that the development of this carcinoma is highly heritable in most species (GOLDSCHMIDT & HENDRICK, 2002). Further investigations are needed to determine the epidemiology and geographical distribution of SCC in sheep in the region.

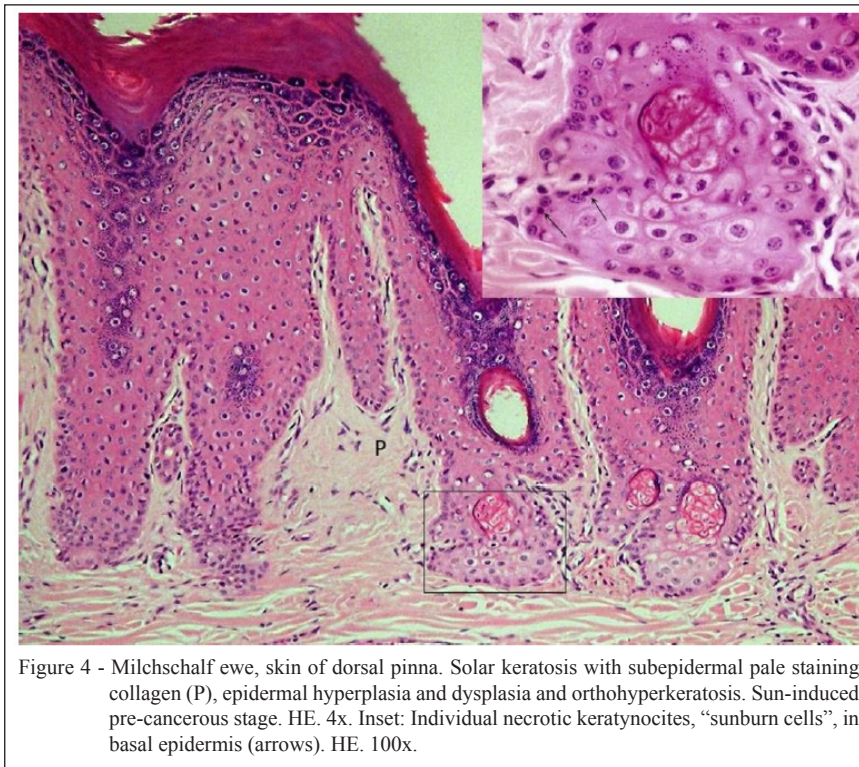


Figure 4 - MilchsCHF ewe, skin of dorsal pinna. Solar keratosis with subepidermal pale staining collagen (P), epidermal hyperplasia and dysplasia and orthohyperkeratosis. Sun-induced pre-cancerous stage. HE. 4x. Inset: Individual necrotic keratinocytes, "sunburn cells", in basal epidermis (arrows). HE. 100x.

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## COMPLIANCE WITH ETHICAL STANDARDS

This study was carried out according to the recommendations of the Uruguayan Honorary Commission of Animal Experimentation (CHEA).

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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