

OCCURRENCE OF SQUAMOUS CELL CARCINOMA IN SHEEP  
FROM A FARM IN SÃO PAULO STATE, BRAZIL

**C. Del Fava<sup>1</sup>, C.J. Verissimo<sup>1</sup>, C.F.C. Rodrigues<sup>1</sup>, E.A. Cunha<sup>1</sup>,  
M. Ueda<sup>2</sup>, P.C. Maiorka<sup>3</sup>, J.L. D'Angelino<sup>3,4</sup>**

<sup>1</sup>Centro de Etologia, Ambiente e Manejo, Instituto de Zootecnia, CP 60, CEP 13460-000, Nova Odessa, SP, Brasil.  
E-mail: delfava@izsp.br

ABSTRACT

The occurrence of squamous cell carcinoma in sheep on a farm in São Paulo state, Brazil, is reported. The flock, composed by Suffolk, Santa Inês, Poll Dorset and Ile de France breeds is managed intensively. All the ewes were clinically examined and papillomas and tumoural lesions were observed only in the Ile de France breed. Histopathological findings (HE) revealed invasive squamous cell carcinoma in the vulval labia. Negative contrast electron microscopy did not find viral particles in papillomas and tumoural lesions. The clinical study revealed occurrence of squamous cell carcinoma in July/99, January/00 and July/00, respectively, 6.0% (3/50), 4.2% (2/48) and 4.3% (2/46). The incidence from July/99 to January/00 and from January/00 to July/00 was, respectively, 2.1% (1/48) and zero (0/46). Despite all the breeds being managed together, only the Ile de France presented lesions. This lead us to consider that the susceptibility may be associated to racial factors, because all the animals are submitted to the same environmental, sanitary and management conditions. Solar radiation may be the factor that induces the development of squamous cell carcinoma, because Ile de France animals have a unpigmented perineum that is poorly covered by wool and is exposed to solar radiation in the tropical climate.

KEY WORDS: Ovine, squamous cell carcinoma, Brazil.

RESUMO

OCORRÊNCIA DE CARCINOMA EPIDERMÓIDE EM OVINOS DE UM CRIATÓRIO DO ESTADO DE SÃO PAULO, BRASIL. Foi relatada a presença de Carcinoma Epidermóide em ovinos de um criatório no Estado de São Paulo, Brasil. O rebanho, composto por animais das raças Suffolk, Santa Inês, Poll Dorset e Ile de France, é manejado intensivamente. Todas as matrizes foram avaliadas clinicamente e observou-se papiloma e carcinoma apenas na raça Ile de France. O diagnóstico histopatológico (HE) revelou carcinoma epidermóide em massas tumorais invasivas na vulva e vagina. A microscopia eletrônica direta, segundo a técnica de coloração negativa, não identificou partículas virais do tipo Papillomavirus em papilomas e em massas tumorais. A avaliação clínica revelou ocorrência do carcinoma de 6,0% (3/50) em julho/99, 4,2% (2/48) em janeiro/2000 e 4,3% (2/46) em julho/2000. A incidência de carcinoma no período de julho/99 a janeiro/2000 foi 2,1% (1/48) e de janeiro a julho de 2000 foi zero (0/46). A susceptibilidade dos animais Ile de France pode estar associada a fatores raciais, uma vez que todas as raças são submetidas às mesmas condições climáticas e ambientais, manejo sanitário e zootécnico. Somente a raça Santa Inês não é caudectomizada. A irradiação solar atua como fator desencadeante do tumor, pois animais Ile de France possuem períneo despigmentado, com pouca cobertura de lã, tornando esta região vulnerável à ação dos raios solares.

PALAVRAS-CHAVE: Ovino, carcinoma epidermóide, Brasil.

<sup>2</sup>Seção de Microscopia Eletrônica, Instituto Adolfo Lutz.

<sup>3</sup>Faculdade de Medicina Veterinária da Universidade de Santo Amaro (UNISA).

<sup>4</sup>Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (USP).

## INTRODUCTION

Squamous cell carcinoma in sheep has been reported in Australia (LOYD, 1961, VANDEGRAAFF, 1976, LADDS & ENTWISTLE, 1977, HAWKINS et al., 1981, TILBROOK et al., 1992), South Africa (TUSTIN et al., 1982) and France (LAGADIC et al., 1982). In Brazil, an outbreak was reported at Rio Grande do Sul State by RIET-CORREA et al. (1981).

This tumour presents an enzootic pattern in flocks or a region, or an abnormally high frequency in some categories in a population. Adult animals managed extensively in areas of high solar radiation are more susceptible (LAGADIC et al., 1982).

It is known that papillomavirus, a DNA virus of Papovaviridae family (FRISQUE et al., 1995) is contagious. Papillomavirus particles were identified by electron microscopy in papillomatous lesions of lips, nose, ears (VANSELOW & SPRADBROW, 1982), skin of limb extremities (GIBBS et al., 1975) and also in perineal pre-tumoural lesion and carcinoma of the vulva (VANSELOW & SPRADBROW, 1983) in ewes. Identification of ovine papillomavirus DNA by means of molecular biology, "in situ" hybridization techniques, were recently described in pre-cancerous lesions of ears (TRENFIELD et al., 1990) and in perineal tumours in sheep (TILBROOK et al., 1992).

The squamous cell carcinoma in sheep occurs more frequently in areas deprived of wool and pigmentation, anatomical sites like ears, (LOYD, 1961, MAGNOL et al., 1974, LADDS & ENTWISTLE, 1977, RIET-CORREA et al., 1981), eyes (DAVIS & SHORTEN, 1952, LOYD, 1961, DAMODARAN et al., 1975, LADDS & ENTWISTLE, 1977, RIET-CORREA et al., 1981), nose (LOYD, 1961, LADDS & ENTWISTLE, 1977, RIET-CORREA et al., 1981) and perineum are especially affected (LOYD, 1961, VANDEGRAAFF, 1976, HAWKINS et al., 1981, LAGADIC et al., 1982, TUSTIN et al., 1982, VANSELOW & SPRADBROW, 1983, TILBROOK et al., 1992). Tumours are frequent in vulva and muco-cutaneous junctions (VANDEGRAAFF, 1976, HAWKINS et al., 1981, LAGADIC et al., 1982, TUSTIN et al., 1982). Actinic dermatitis and pre-tumoural lesions are not prominent, the tumour is invasive and keratinized (LAGADIC et al., 1982).

The occurrence of pre-cancerous lesions and carcinoma in the perineum were detected in Australia in animals submitted to the radical Mules' operation and tail docking (VANDEGRAAFF, 1976, HAWKINS et al., 1981, TUSTIN et al., 1982). Predisposing factors like poor pigmented skin, sunlight exposure and photosensitization are associated to the developing pre-cancerous and carcinomas in ovines (LOYD, 1961, VANSELOW & SPRADBROW, 1982).

DANIELS & JOHNSON (1987) proposed that genetic factors may be associated to the predisposition in developing tumours in Merino breed. Occurrence of

tumours is associated and increases with age of the animals (LOYD, 1961; VANDEGRAAFF, 1976; LADDS & ENTWISTLE, 1977; RIET-CORREA et al., 1981; HAWKINS et al., 1981; LAGADIC et al., 1982). The disease may also attack adult males as described by LADDS & ENTWISTLE (1977), RIET-CORREA et al. (1981) and HAWKINS et al. (1981).

The present research aimed to report the occurrence of squamous cell carcinoma in sheep from a farm in São Paulo State, Brazil.

## MATERIAL AND METHODS

The flock is composed by 80 females of Suffolk, 50 Ile de France, 20 Poll Dorset breeds, which are well covered by wool, and 50 Santa Inês, which are less covered by wool. The animals belong to the Experimental Unit for Ovine Breeding at the Instituto de Zootecnia, Nova Odessa city, São Paulo State, Brazil. The latitude is 22°42'00" S, longitude 47°18'00" W, with year mean temperature of 22.3°C, mean precipitation of 1,300 mm, and raining time concentrated in spring and summertime (September to March), the altitude is 550 m (EMBRAPA, 1978).

The area of the Experimental Unit is 3.7 ha, subdivided in 10 sub-areas of 3,500 to 4,000 m<sup>2</sup>, where the animals are managed intensively, most of the time on grazing pasture. Seven sub-areas are formed by *Panicum maximum* Jack cv aruana and three with diverse pasture (*Digitaria decumbens*, *Cynodon dactylon*, *Paspalum notatum*). The managing system is rotative, with periods of 7 to 9 days of occupation and 35 to 45 days of resting time. The mean occupation is 35 to 40 animals per ha, according to the pasture available. In each sub-area the animals have free access to shelter and food supplementation composed of salt and mineral mixture (proportion 2:1). In each sub-area there are trees to shade the animals from sunlight.

The breeding females are kept in a grazing pasture and receive concentrated ration composed of corn and soybean bran, cotton and wheat, with 16% of protein and 80% of total digestible nutrients (NDT) during the final third of the gestational period. After birth the ewes and kids are confined in collective barns, with cane sugar currant cover, fed with corn ad libitum and 500 g of concentrated ration per animal a day until weaning (60 days of age), when they return to the grazing pasture. The young females are kept confined until 180 days of life, when they have gradative access to areas for breeding. They get 10 to 11 months of age and weigh 45 kg they are bred, in ordinary pasture, with adult ewes.

The animals are naturally bred, during mating time in January and February, concentrating birth in June and July. The tail docking is done in lambs of Suffolk, Ile de France and Poll Dorset breeds at about

5 days after birth, preserving just two coccygeal vertebrae. The animals are dewormed according to the clinical and coproparasitological exams and vaccinated against clostridiosis and tetanus.

The zootechnical management does not permit total isolation among individuals of different breeds. The flock was clinically evaluated in August, 1999, January, 2000 and July of 2000, to observe the presence of papillomas and carcinomas in conjunctival and nasal mucosae, ears, limbs, perineum, vulva and vagina.

Histopathological examination was performed in material fixed in formaline and paraffin embedded tissue, stained with hematoxylin and eosin. The papillomatous lesions and tumoural masses found were submitted to negative contrast electronic microscopy.

## RESULTS AND DISCUSSION

Papillomas and carcinomas were only found in animals of Ile de France breed and all animals of this breed also presented photosensitization in the ears, face, perineum and focal areas of skin thickening and eczema during the summer time. Papillomas were also detected in conjunctival mucosae of one ewe. A well-keratinized papilloma with 2 cm was detected at the external face of the ear (Fig. 1), and after surgical excision presented total remission. Small papillomas were identified in the perineum of many ewes (Fig. 2).

A clinical evolution of a round lesion with 1.5 cm at the vulvar muco-cutaneous junction was observed during 13 months. The tumour progressed and led to extensive infiltration in the vulva and vagina; ulceration of tumour surface with purulent infection and myiasis were observed. Due to the deep emaciation the animal was euthanized. At the necropsy, metastasis at the regional lymph nodes, chronic cystitis and moderate hydronephrosis were detected.

Four females presented squamous cell carcinoma (Fig. 3) and were euthanized *in extremis*. Histopathological examination revealed atypical squamous epithelial cords invading the basal membranae and dermis, round anaplastic cells, focal areas of necrosis, keratinization of isolated cells (dysplastic keratosis) and high infiltrates of mononuclear cells among the cords (Fig. 4). In one animal a very invasive and anaplastic tumour mass was detected invading basal membranae and deeply infiltrated in the dermis. Pleomorphic cells with a high mitosis index and numerous keratin pearls and dysplastic keratosis was detected with a strong lymphocytic infiltrate between large areas of fibrosis (Fig. 5). Electronic microscopy failed to detect viral particles.

Tables 1 and 2 respectively present the occurrence of papillomas and squamous carcinomas in animals of Ile de France breed, at different periods of observation.

Table 1 – Occurrence of papillomas in animals of Ile de France breed (São Paulo, 2000).

Month	Number of observed animals	Number of affected animals	Proportion of affected animals
July – 1999	50	08	16.0 %
January – 2000	48	16	33.3%
July – 2000	46	14	29.2%

Table 2 – Occurrence of squamous cell carcinoma in Ile de France ewes in a breeding establishment in São Paulo State, Brazil (São Paulo, 2000).

Month	Number of observed animals	Number of affected animals	Proportion of affected animals
July – 1999	50	03	6.0 %
January – 2000	48	02	4.2%
July – 2000	46	02	4.3%

The incidence of papillomas corresponds to the number of new cases in the studied population during the period of July, 1999, to January, 2000, was 20.8% (10/48), and in January, 2000, to July, 2000, was 8.7% (4/46).

The incidence of squamous cell carcinoma corresponds to the number of new cases in the studied population from July, 1999, to January, 2000, was 2.1% (1/48) and from January, 2000, to July, 2000, was zero (0/46).

All diseased females in this flock were at the same age, 7 to 8 years old (Table 3).

Table 3 – Occurrence of papillomas and carcinomas in animals of Ile de France breed according to age (São Paulo, 2000).

Age in years	Total of animals per age	Papillomas	Carcinoma
0 to 2	04	02 (50.0%)	0
2 to 3	20	06 (30.0%)	0
3 to 4	06	04 (66.7%)	0
4 to 5	07	03 (42.9%)	0
5 to 6	03	03 (100.0%)	0
6 to 7	05	03 (60.0%)	0
7 to 8	05	01 (20.0%)	4 (100.0%)



Fig. 1 - Keratinized papilloma with 2 cm detected at the external face of the ear.



Fig. 2 - Small papillomas identified in the perineum of many ewes.



Fig. 3 - Squamous cell carcinoma with extensive infiltration in the vulva and vagina.

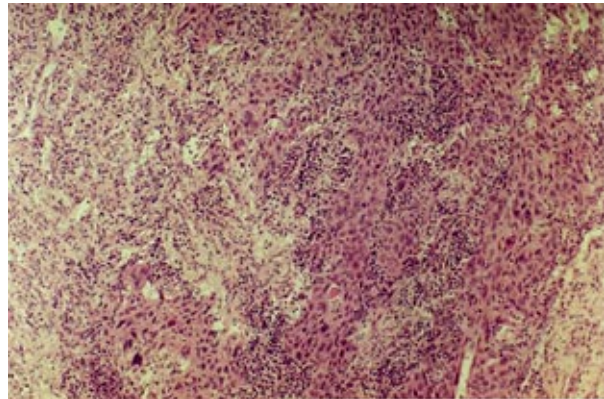


Fig. 4 - Atypical squamous epithelial cords invading the basal membrane and dermis.

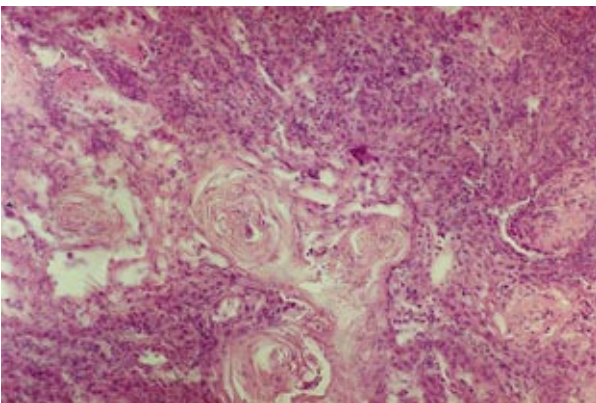


Fig. 5 - Pleomorphic cells with a high index and numerous keratin pearls and dysplastic keratosis.

Young males are slaughtered early and individuals of this category were not clinically evaluated, the flock is composed basically by females at mating age. Two six-year-old Ile de France males presented papillomatous lesions, one at the forelimb extremity and the other at the preputial mucosae, demonstrating that the disease may affect adult males, as reported by LADDS & ENTWISTLE (1977), RIET-CORREA et al. (1981) and HAWKINS et al. (1981).

The clinical and histopathological aspect of the vulvar lesions and tumours confirm that they are related to squamous cell carcinoma similar to those reported by VANDEGRAAFF (1976), HAWKINS et al. (1981), VANSELOW & SPRADBROW (1982), LAGADIC et al. (1982), TUSTIN et al. (1982), VANSELOW & SPRADBROW (1983) and TILBROOK et al. (1992). The papillomatous lesion found on the ear is similar to those reported by LLOYD (1961),

MAGNOL et al. (1974), LADDS & ENTWISTLE (1977) and RIET-CORREA et al. (1981).

It is important to emphasize that despite this flock been composed of four different ovine breeds, all animals are submitted to the same climatic, environmental, sanitary, reproductive and zootechnical management, and frequently the animals are in close contact. As only animals of the Ile de France breed presented pre-tumoural lesions, carcinoma and photosensitization, this suggests that they are more susceptible to solar radiation. DANIELS & JOHNSON (1987) reported that only animals of the Merino breed and its lineage are susceptible to carcinoma, which can be interpreted to mean that genetic factors might be involved in this disease. The Ile de France breed is a Merino lineage, and also have had reported outbreaks of squamous cell carcinoma in France (LAGADIC et al. 1982). LLOYD (1961), in Australia, had observed photosensitization in the same Merino breed, during the summertime.

VANDEGRAAFF (1976), HAWKINS et al. (1981) and TUSTIN et al. (1982), reported the occurrence of perineal and vulvar squamous cell carcinoma after radical Mules' operation and tail docking. In Brazil, the practice of tail docking is the most used technique by ovine breeders. The animals from this study were submitted to short tail docking, at the second coccygeal vertebrae, that is enough to expose the perineum to solar radiation.

Despite the absence in recognising papillomavirus particles by electron microscopy, the literature reports a close association between this virus with papillomas (GIBBS et al., 1975), pre-tumoural lesions and tumours in ovine (VANSELOW & SPRADBROW, 1982, VANSELOW & SPRADBROW, 1983) and recently, by means of molecular biology techniques, it is known that papillomavirus is present in pre-tumoural and tumoural lesions, suggesting that the developing of squamous cell carcinoma is associated with this viral infection (TRENFIELD et al., 1990; TILBROOK et al., 1992). It is important to verify if this virus, as can be seen in another mammals, is the causal agent of the tumours in flocks in South America (RIET CORREA et al. 1981; SMITH & CAMPO, 1985).

The relation of age with developing carcinomas could be observed in this study, and the occurrence is higher in older animals, which agrees with the report of LLOYD (1961), VANDEGRAAFF (1976), LADDS & ENTWISTLE (1977), HAWKINS et al., (1981), RIET CORREA et al. (1981) and LAGADIC et al. (1982).

## CONCLUSIONS

Sheep of Ile de France breed presented more susceptibility to develop papillomas, photosensitization,

pre-tumoural lesions and tumours, demonstrating more sensibility to solar radiation.

The genetic characteristics of this breed need more investigation to detect if they are related to predisposition in developing papilloma and squamous cell carcinoma.

## ACKNOWLEDGMENTS

The authors wish to acknowledge the staff at Apoio à Pesquisa Científica e Tecnológica Joaquim do Nascimento, especially Roseli Aparecida Bosquero Elias and Maria de Lourdes T. Santiago for their valuable assistance in this investigation.

## REFERENCES

- DAMODARAN, S.; SUNDARARAJ, A.; RAMAKRISHNAN, R. Ocular carcinoma in sheep. *Indian Vet. J.*, v.52, n.8, p.664-665, 1975.
- DANIELS, P.W. & JOHNSON, R.H. Ovine Squamous Cell Carcinoma. *Vet. Bull.*, Weybridge, v.57, n.3, p.153-167, 1987.
- DAVIS, C.L.; SHORTEN, H.L. Carcinoma of the eye of sheep. *J. Am. Vet. Med. Assoc.*, v. 121, n.904, p.20-24, 1952.
- EMBRAPA *Diretório da EMBRAPA 1978*. 3. ed. Brasília: Depto. Informação e Documentação, p.300-301, 1978.
- FRISQUE, R.J.; BARBANTI-BRODANO, G.; CRAWFORD, L.V.; GARDNER, S.D.; HOWLEY, P.M.; ORTH, G.; SHAH, K.V.; va der NOORDAA, J.; zur HAUSEN, H. Family Papovaviridae. *Arch. Virol.*, v.140, p.136-142, 1995. Supplementum, 10.
- GIBBS, E.P.J.; SMALE, C.J.; LAWMAN, M.J.P. Warts in sheep. Identification of a Papilloma Virus and Transmission of Infection to Sheep. *J. Comp. Pathol.*, v.85, n.2, p.327-334, 1975.
- HAWKINS, C.D.; SWAN, R.A.; CHAPMAN, H.M. The epidemiology of squamous cell carcinoma of the perineal region of sheep. *Aust. Vet. J.*, v.57, n.10, p. 455-457, 1981.
- LADDS, P.W.; ENTWISTLE, K.W. Observations on squamous cell carcinomas of sheep in Queensland, Australia. *Br. J. Cancer*, v.35, p.110-114, 1977.
- LAGADIC, M.; WYERS, M.; MIALOT, J.P.; PARODI, A.L. Observation d'une enzootie de cancers de la vulve chez la brebis. *Zentralbl. Veterinaer. Med. A*, v.29, n.2, p. 123-135, 1982.
- LLOYD, L.C. Epithelial tumours of the skin of sheep. Tumours of areas exposed to solar radiation. *Br. J. Cancer*, v.15, n.4, p.780-789, 1961.
- MAGNOL, J.P.; CABANIE, P.; VAN HAVERBEKE, G. Le cancer de l'oreille du mouton dans le sud-ouest de la France. Etude morphologique et épidémiologique. *Rev.Méd. Vét.*, v.125, n.5, p. 679-696, 1974.
- RIET-CORREA, F.; CASSAL, A.B.; SCARSI, R.M.; SCHILD, A.L.; MENDEZ, M.C. Carcinomas epidermóides em ovinos em um estabelecimento do Rio Grande do Sul. *Pesqui. Vet. Bras.*, v.1, n.2, p.65-68, 1981.

- SMITH, K.T.; CAMPO, M.S. The biology of papillomaviruses and their role in oncogenesis. *Anticancer Res.*, v.5, p.31-48, 1985.
- TILBROOK, P.A.; STERRETT, G.; KULSKI, J.K. Detection of papillomaviral-like DNA sequences in premalignant and malignant perineal lesions of sheep. *Vet. Microbiol.*, v.31, n.4, p.327-341, 1992.
- TRENFIELD, K.; SPRADBROW, P.B.; VANSELOW, B.A. Detection of papillomavirus DNA in precancerous lesions of the ears of sheep. *Vet. Microbiol.*, v.25, n.2/3, p.103-116, 1990.
- TUSTIN, R.C.; THORNTON, D.J.; MCNAUGHTON, H. High incidence of squamous cell carcinoma of the vulva in Merino ewes on a South African farm. *J. South Afr. Vet. Assoc.*, v.53, n.3, p. 141-143, 1982.
- VANDEGRAAFF, R. Squamous-cell carcinoma of the vulva in merino sheep. *Aust. Vet. J.*, v.52, n.1, p.21-23, 1976.
- VANSELOW, B.A. & SPRADBROW, P.B. Papillomaviruses, papillomas and squamous cell carcinomas in sheep. *Vet. Rec.*, v.12, n.24, p.561-562, 1982.
- VANSELOW, B.A. & SPRADBROW, P.B. Squamous cell carcinoma of the vulva, hyperkeratosis and papillomaviruses in a ewe. *Aust. Vet. J.*, v.60, n.6, p. 194-195, 1983.

Received for publication in 10/24/00