

## PATHOLOGICAL AND VIROLOGICAL STUDIES ON GOAT PAPILLOMATOSIS

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(Manuscript received 8 October 1997)

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### **Abstract**

In a goat milking farm (Saanen breed) at El Nobaría area at Alexandria Governorate, 50 out of 250 goats showed clinical manifestations referred to caprine viral papillomatosis. The warts were confined to the udder and teats of affecting does. Twenty-five proper samples from warts were taken for virological and pathological studies. The virus isolation and propagation was done on human epidermoid carcinoma (HEP-2) derived from larynx carcinoma. Electron microscopy on wart tissues revealed the characteristic shape of an associated appearing virus in the form of dense electron granules in the nucleus of the upper part of epidermis. The epidermis revealed parakeratosis, hyperkeratosis as well as acanthosis. Three out of 25 samples showed squamous cell carcinoma characterized by the presence of bird nests of different shapes and sizes. Diseased goats were vaccinated with prepared autogenous vaccine from the tissues of warts. One month after vaccination the warts showed regression and complete relief of all diseased cases.

The clinical, electron microscopy and histopathological findings were illustrated in 12 figures.

### **INTRODUCTION**

Infectious papilloma in different animal species caused by DNA virus belonging to the papovaviridae family (Allison 1965). Papilloma virus is species specific, i.e. bovine papilloma virus, equine papilloma virus, rabbit papilloma virus, caprine papilloma virus, monkey papilloma virus, human papilloma virus and fish papilloma

virus (Jones and Hunt 1983, Afify and Ahmed 1994). Some of these viruses include 6 subtypes, human papilloma virus contains 30 subtypes, but goat papilloma virus has only one type (Ficken and Andrews 1983). The anatomical location and distribution of lesions depend upon the animal species and the subtype of papilloma virus. This criteria were reported by Moulton (1954) as in oral papillomatosis in dogs and teat papilloma virus in bovine. The developed warts as mentioned by Samuel *et al.* (1973) were precise lesions that fastidiously refuse to grow anywhere except in special type of epithelium as in human, equine and in cottontail rabbit.

In Egypt, papilloma lesions were recorded in our native breeds of goat, but the specific virus was not detected before (Saber and Shalaby 1992).

The goat papilloma virus causes 3 clinical forms, i.e., cutaneous which affects head, neck and trunk, mammary form, and the third type appears in genitalia (Lancaster and Olsen, 1983). In non-pigmented skin breed, as Saanen breed, only the mammary form of papillomatosis was recorded by Theilen *et al.* (1985). Gordon *et al.* (1987) suggested that, there were 4 forms of mammary papilloma. In the first form, animals showed total regression of warts after 2-4 months; the second form, involved warts that partially or totally regress during winter season, and reoccur in summer season; the third form included those goats that after recurrence, warts become persistent; the fourth form, included those goats with warts that progress to carcinoma.

Macroscopical examination of warts were recorded by Gordon *et al.* (1987) as numerous warts in udder in the form of flaking like or finger form like projections with or without stalk.

Histopathological examination as mentioned by Theilen *et al.* (1985) of warts in goat papilloma, showed dermal and epidermal proliferations as hyperkeratosis, parakeratosis, spongiosis and acanthosis, besides slight proliferation of fibrous connective tissues in derms, and even malignant transformation of papilloma to squamous cell carcinoma which has been reported by the same author. In spite of being malignant tumour, no metastasis were recorded to other parts. The factors affecting this transformation to malignancy were reported by Gordon *et al.* (1987) such as genetic factors, immune suppressions, hormonal factors, excessive exposure to sunlight and ultra violet light.

Electron microscopy of infectious cutaneous papilloma of man, rabbit and oral papillomas of dogs revealed cellular alterations associated with production of virus

as recorded by Charles (1995), Richter (1964), Cheville and Olson (1964), respectively. In human, Charles (1995) observed the virus in association with the nucleoli of the cells of stratum spinosum. Richter (1964) detected the virus particles in rabbit throughout the degenerated nucleus of the cells of stratum granulosum and in stratum corneum, but Cheville and Olson (1964) reported that virus particles were embedded in the remains of keratinized cells of oral papillomatosis in dogs. In goat, the virus was not defined even after electron microscopy (Ficken *et al.* 1983 and Theilen *et al.* 1985). Jenson and Sommer (1986) revealed that, viral agent could be detected by the use of electron microscope either by negative stain (by pulling the virus from papilloma tissues) or tissue sections.

In general, the control measures of papillomatosis depend upon autogenous vaccine which is species specific, as well as this disease is one of few where vaccination is used for therapy as mentioned by Frank *et al.* (1959).

The present study deals with goat papillomatosis as a field problem in a private farm at Noharia, Alexandria Governorate. This investigation aimed to describe the clinical and pathological pictures of the disease, virus detection in tissues by the use of electron microscopy and some virological examinations, as well as the vaccinal control measures of this problem by preparation of autogenous specific vaccine.

## MATERIALS AND METHODS

Goats of a private farm at Noharia, Alexandria Governorate showed clinical signs of papillomatosis. Samples were taken by surgical removal of warts from the affected for virological and pathological studies.

### I. Virological Examinations :

Samples were kept in minimal essential media (M.E.M.) as maintenance media containing 1% foetal calves serum for virus isolation (in ice box).

### I. Preparation of wart tissues :

According to Pierre and Mickel (1993), the tissues were ground with few amount of sterile sand with enough phosphate buffered saline pH 7.4 (PBS). The homogenate tissues were centrifuged at 3000 x g for 30 minutes at 4°C, then, antibiotic was added. The sediment was discarded, and the supernatant was kept at

70 °C till used for virus isolation.

## 2. Cell culture :

A permanent cell line of human epidermoid carcinoma (HEP-2) derived from human larynx carcinoma, was kindly obtained from General Egyptian Organization for Biological and Vaccine, Aguza-Giza, and maintained in Animal Health Research Institute, Dokki-Giza. The cells were grown on M.E.M. supplement with 10% bovine serum for virus isolation and propagation.

## 3. Virus isolation :

According to Schmidt and Emmons (1989), the prepared samples were inoculated into monolayer HEP-2 cell line 0.2 ml/tube after discard of the growth media, maintenance media were incubated at 37°C and daily examined using inverted microscope for CPE detection.

## 4. Autogenous vaccine preparation :

The autogenous vaccine was prepared in accordance to Noice and Eveleth (1959) as follows:

1. Three cycles of freezing and thawing of micid warts tissues were carried out.
2. One part of tumour tissues was mixed with nine parts of 0.85% saline solution.
3. The homogenate was filtered through a sterile gauze and, then, stored at 4°C till used.
4. 10% glycerol was added as a vehicle to delay the absorption, as well as 10% of 0.5 phenol was used to kill the virus.
5. Tissue extracts were not frozen and thawed.
6. An amount of 2-3 ml of vaccine was injected S/C at 10 days intervals, 3 doses were given.

## II. Pathological Examinations

### 1. For electron microscopy (E.M)

Fresh tumour tissues were fixed in 5% glutaraldehyde, processed and

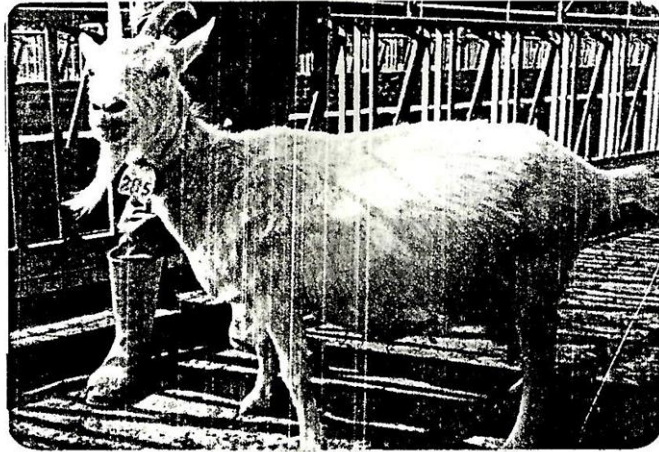


Fig. 1. Doe of Saanen breed.



Fig. 2. Doe showing warts in the udder and teats.

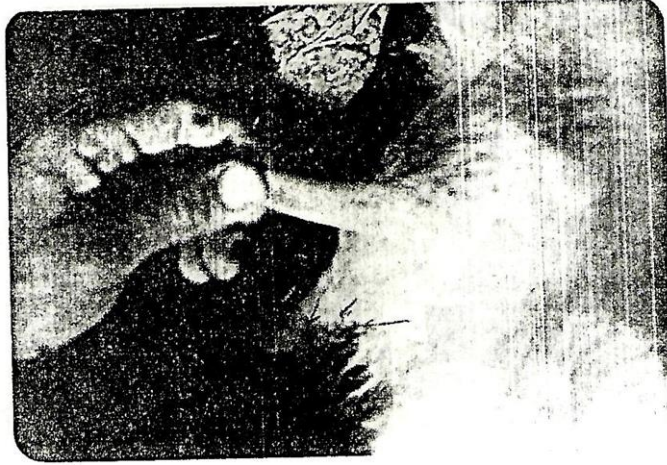


Fig. 3. Saanen doe teats showing small papillomas beside ulcerated ones.



Fig.4. Virus particles in hexagonal form in a nucleus of a cell in the stratum corneum.

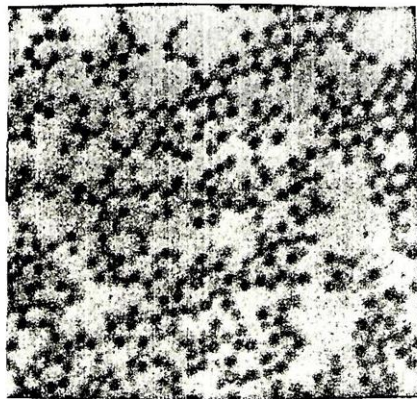


Fig. 5. Thin section showing viral particles. (X 83,000).

sectioned for transmission electron microscopy in E.M. center at Faculty of Veterinary Medicine, Assiut University.

## 2. For light microscopic examination

A number of 25 proper samples from warts that appeared on udder and teats of 20 does were fixed in 10% formol saline, paraffin blocks were prepared, sections were stained with H & E according to Clark (1981).

In addition, special staining with phloxine tartrazing for inclusion bodies was applied (Carleton *et al*, 1967).

## RESULTS

### I. Anamnestic data and clinical symptoms

Goats of Saanen breed (Fig. 1) were imported from Netherlands in 1987 for breeding as dairy herd. Goats were belonging to EL Nobarria farm and confined in open yards.

At the period of examination from November 1996 to March 1997, the total number of goats was 400; out of them, 150 off springs were less than 6 months, and 250 adults with an age ranging from 2 up to 5 years (240 does and 10 bucks). Since four years, the adult does have suffered from persistant appearance of warts on the udder and teats (Fig 2). Along 5 months, 50 adult does (20.83%) showed this clinical problem. Warts appeared as cirumscribed rounded flaking warts of 0.5-2 cm in diameter with rough surface. They were usually persistent, multicentric and looked somewhat similar to fungal dermatitis. More than 10 warts appeared on the udder. In three examined goats, the udder showed ulcerated papilloma, besides small ones (Fig. 3).

No evidence of warts was recorded in other sites of the body rather than in the udder.

### II. Virological Examinations

The positive samples showed foci of infection induced by the cytopathic agent in the form of round granulation and cellular hypertrophy.

The infected cells showed a strong tendency to aggregate together. The CPE is

characterized by no detachment of cell monolayer, no syncytium or multinuclear giant cells formed.

The monolayer sheet was continuously calmat for time (more than 10 days), and no gaps were formed. The CPE was clear at 5-7 day post infection.

### III. Pathological Findings

Electron microscopy revealed the presence of virus-like particles in the nuclei of cells in the superficial layer of stratum granulosum. The structures were appearing very few in the form of dense particles measuring about 50-60 micron. Few nuclei of the granular cells layer had marginated chromatin with virus particles distributed through the nucleus. The nuclei of cells in the keratinized layer often contained hexagonal crystalline groups of virus particles (Fig. 4, 5).

The histopathological finding in all examined warts showed a prominent thickening of the epidermis as a main characteristic feature. The stratum corneum showed hyperkeratosis, and some cornified cells retained their nuclei with the formation of parakeratotic layer (Fig. 6). Meanwhile, hyalinization was demonstrated in the stratum granulosum, and some of the cells showed keratohyaline deposition. Acanthosis of the epidermis (Fig. 7) was observed in stratum spinosum and focal spongioses with swollen vacuolated cells (Fig. 8). These vacuolated cells showed marginations of nuclear chromatin or may show nuclear vacuolation and dissolution of the chromatin substance. The stratum germinativum was exaggerated to form inward dermal papillae in the form of finger like projections with solid compact core (Fig. 9) of hyperplastic proliferated cells of the rete pegs. Some inward dermal papillary cores were noticed with connective tissue, stroma and high vascularity (Fig. 10). The dermis showed connective tissue proliferation, specially around the blood vessels making wheel-like appearance. In two examined warts, the dermis connective tissue matrix was more prominent, and the fibroblast showed little parallelism. In some other warts, the dermis was highly vascular, no evidence of inflammatory cells were seen. No inclusions were seen in examined warts by the use of specific phloxine and tartrazin stain.

Three examined cases showed squamous cell carcinoma (Fig.11) in the form of irregular proliferating masses of prickle cells having different shapes and sizes. As well, their nuclei were either large vesicular faintly stained, or were small, spherical or elongated and hyperchromatic. Degenerative changes in the form of hydropic degeneration and necrosis were frequently seen in these cells. The prickle



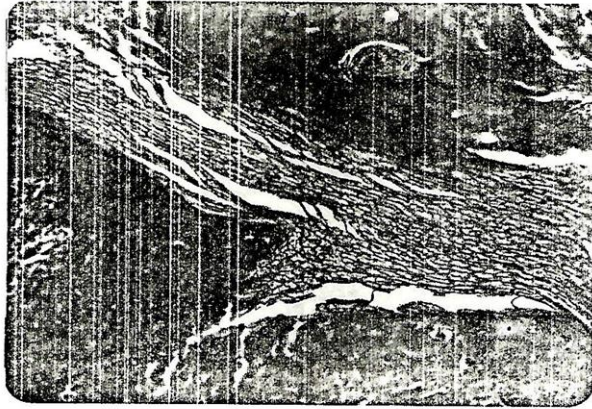


Fig. 6. The upper part of papilloma in the skin the udder of doe showing hyperkeratosis and parakeratosis.

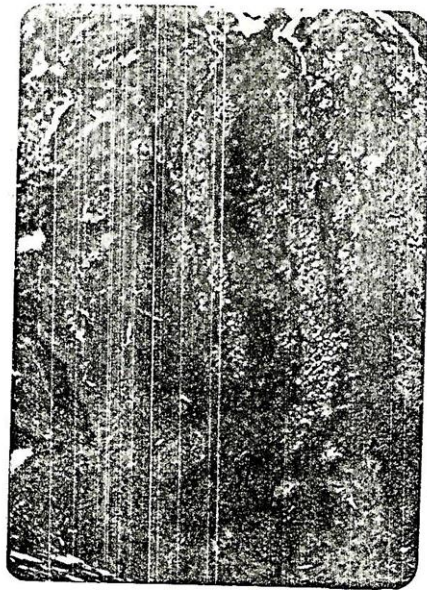


Fig. 7. Skin showing papilloma, acanthosis (H & E X 25).

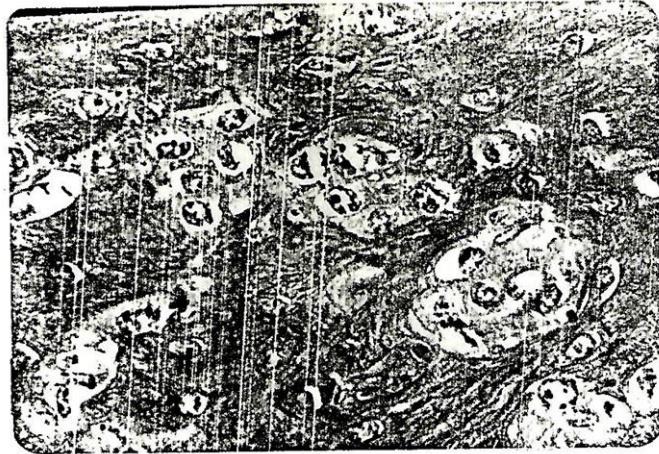


Fig. 8. Skin, showing papilloma of goat with focal spongiosis with swollen vacuolated cells in stratum spinosum (H & E X 630).

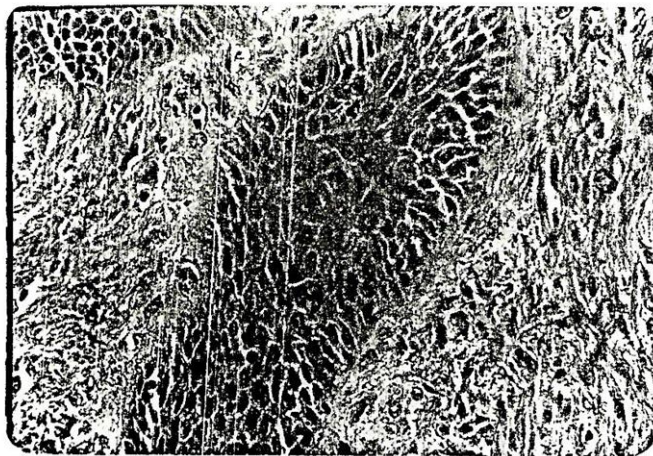


Fig. 9. Dermal papilla with solid compact core. (H & E X 250).

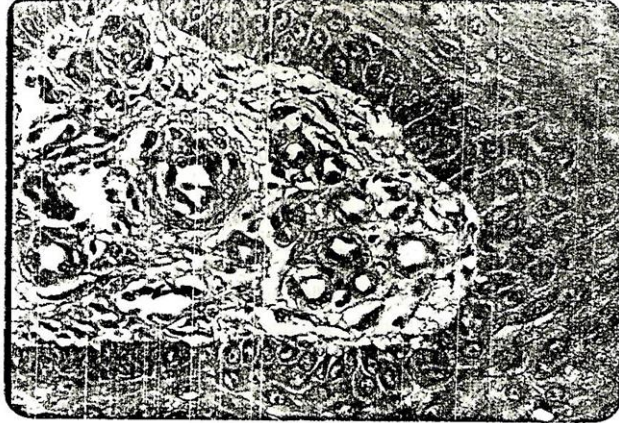


Fig. 10. Dermal papilla with highly vascular connective tissue core. (H & E X 630).

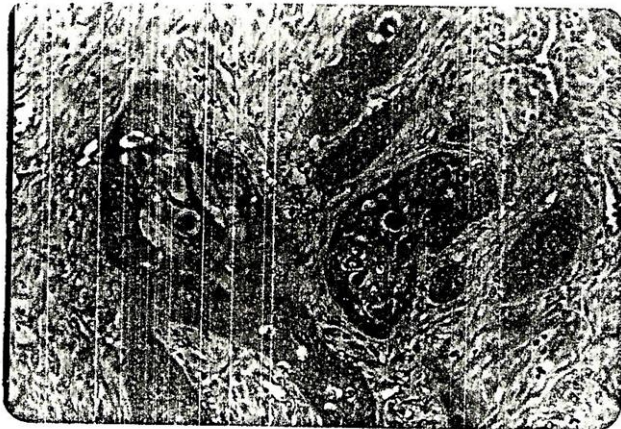


Fig. 11. Squamous cell carcinoma in the skin of teat (H & E X 25).

cells were bounded by more or less rounded, elongated or flattened cells. The a for mentioned masses showed, either, a compact center and/or with epithelial pearls. These pearls had a rounded concentric bright keratin layers surrounded by groups of prickle cells, some of them showed mitotic divisions. The stroma between the malignant masses was infiltrated with mononuclear and polymorph nuclear cells, as well, the epidermal layers were infiltrated also with some inflammatory cells (Fig. 12).

#### IV. Vaccinal treatment

Following treatment with already killed autogenous vaccine consisting of three doses of 3ml each, S/C injection at 10 days interval, the warts had completely disappeared.

### DISCUSSION

The clinical findings showed that, 50 out of 250 does of Saanen breed suffered from the appearance of swellings on the udder and teat skin. Three cases showed large ulcerated papilloma. Similar picture was previously recorded by Davis and Kemper (1936) and Jones and Hunt, (1983). The gross appearance of these tumours were similar to those mentioned by Theilen *et al.* (1985). The virological studies revealed the involvement of papilloma virus in this problem. Gordon *et al.* (1987) suggested that, one the main handicaps in studying papillomatosis is the general lack of cell culture that supported papilloma virus replication. So, in our study we used permanent cell line of human epidermoid carcinoma for virus isolation and propagation.

Gordon *et al.* (1987) noted that, due to the lack of antisera for goat papilloma virus, the main diagnostic procedure depends upon, clinical signs, histopathological and E.M. as a role of identification of virus, and the response of infected animal for autogenous vaccine, which was first adopted by Biberstein *et al.* (1931).

Theilen *et al.* (1985) attributed the inability for detecting the virus particles in goat papillomas to the chronic phase of the disease in which the virus does not replicate.

The electron microscopical findings proved that, viral infection was the probable causative agent of papillomatosis in goats. The distribution and morphological features of the virus like particles were similar to papilloma viruses



Fig. 12. Epidermal layer in case of squamous cell carcinoma showing infiltration with inflammatory cells. (H & E X 630).

detected by Jenson and Sonner (1986). The size of virus particles observed in mammary papillomas in goats coincided with those findings of Brodst and Hinsman (1966).

In the present study, persistent tumours found in the skin of the mammary glands in goats were classified microscopically into papilloma and squamous cell carcinoma in the ratio of 7:1, respectively. These results agree with those obtained by Gordon *et al.* (1987). The squamous cell carcinoma, as mentioned by moulton (1954) arise either spontaneously or developed following papillomas particularly in goat. Papilloma viruses are known to cause both benign and malignant tumours in wide variety of animals. For example, Shope papilloma virus induced skin warts in rabbits that transform into squamous cell carcinoma, and bovine papilloma viruses induce upper alimentary tract papillomas in cattle that convert to carcinoma (Joklik *et al.* 1988).

The histopathological picture of skin mammary papillomas was similar to skin mammary papillomas which were reported by Davis and Kenper (1936), Ficken and Andrews (1983) and Theilen *et al.* (1985).

No inclusions were seen in skin of mammary papillomas either stained by H & E or phloxine tartrazin. These results were also noticed by Theilen *et al.* (1985). In bovine cutaneous papillomas, Dawlat *et al.* (1997) detected inclusion like bodies by the use of H & E stain but did not confirm these observations by specific stain. Hence, these inclusions may be considered as degenerated hyaline granules that showed a bright red colour with H & E stain.

In the present study, the inflammatory reaction found in the dermis could be considered a reactive reaction against the invading tumour cells. However, Lilyod (1961) and Muller (1967) added that the inflammatory dermatitis usually accompanied carcinoma arising from, or associated with high exposure to sun light. The lack of recurrence and metastasis indicated that tumour was of low malignancy. This consideration was given by Gordon *et al* (1987) specially in goats showing squamous cell carcinoma.

Excessive exposure to sunlight leading to solar dermatitis is a contributing cause for squamous cell carcinoma, perhaps, facilitated to some extent by photosensitization (Dodd *et al.* 1983).

Dorn *et al* (1971) added that, the risk for skin cancer increases with old age

3-6 years. In our study, the majority of the animals aged 5-8 years.

The preparation of specific autogenous vaccine for vaccination of diseased goats gave good results, and minimized the severity of the disease. The results agreed with Gordon *et al.* (1987). Theilen *et al.* (1985) suggested that, vaccination in all endemic situations will help control subsequent outbreaks.

In conclusion, papillomatosis in goats is a clinical problem. The electron microscopy and the ordinary histopathological examinations are considered to be diagnostic tools for this disease. The vaccination of goats with prepared autogenous vaccine helped in solving this problem.

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## دراسات فيروولوجية وباثولوجية على مرض السنط في الماعز

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أجرى هذا البحث على مزرعة ماعز من نوع السانن مستوردة من هولندا منذ عام ١٩٨٧ وذلك لانتاج معين من الجبن له مواصفات خاصة للتصدير. وفى الفترة بين نوفمبر ١٩٩٦ الى مارس ١٩٩٧ كانت عدد الحيوانات الموجودة بالمزرعة ٤٠٠ ماعز منها ٢٥٠ ماعز تتراوح أعمارها من ٢ : ٥ سنوات والباقي ١٥٠ ولادات صغيرة فى سن اقل من ٦ شهور .

وبالفحص الحقلى لتلك الحيوانات لوحظ ظهور أعراض مرضية على ٥٠ أنثى تميزت بوجود بثرات مستديمة من السنط على الضرع ملمسها خشن بقطر يتراوح بين ٢,٥ سم وتلك الأعراض كانت تظهر بصفة مستمرة منذ أربع سنوات.

تم اخذ عدد ٢٥ عينة من السنط منها ٣ عينات بها سنط متقرح وذلك لإجراء الفحوص الفيروسية والباثولوجية.

تم إجراء فحوص الميكروسكوب الالكترونى على عدد ٢ عينات من السنط. تم عزل وتميرير الفيروس المسبب للمرض على خلايا من سرطان الحنجرة الأدمى باستخدام الميكروسكوب الإلكترونى، ولقد تم تحديد شكل الفيروس والتعرف على أماكن تواجده وقد ظهر الفيروس فى شكل حبيبات متجمعة مع بعضها على هيئة شبكة ذات أشكال سداسية داخل نويات بعض خلايا البشرة.

أوضحت الفحوص الهستوباثولوجية للزوائد السنطية زيادة فى طبقة الخلايا الكيراتينية وطبقة الكيراتين مع زيادة فى سمك خلايا الطبقة الشوكية. ظهر سرطان جلد طلائى لعدد ثلاث حالات سنطية متقرحه.

وقد تم تحضير لقاح ذاتى علاجى من الزوائد السنطية وحقنها فى الحيوانات المصابة فى ثلاث جرعات مرة كل عشرة ايام وتم شفاء الحيوانات فى خلال شهر من اخر جرعة. وهذا اللقاح يمكن أن يتم انتاجه بعد تطويره كلقاح نوعى لعلاج مثل هذه الحالات ويعتبر ذلك تسجيل لأول مره فى مصر.

وقد تم توضيح الصورة الاكلينيكية والباثولوجية للمرض بالصور ونوقشت النتائج التى تم التحصل عليها من تلك الدراسة الحقلية.