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STUDIES ON CAMEL-POX IN ASSIUT GOVERNORATE-EGYPT

(With 3 Tables, 2 Histograms and 17 Figures)

By

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دراسات عن مرض جدري الإبل بمحافظة أسيوط-مصر

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تم إجراء الفحص الإكلينيكي لعدد ٦٥٣ من الإبل في عدة قرى بمحافظة أسيوط لوجود الآفات الجلدية لمرض جدري الإبل خلال فترة البحث (مايو ١٩٩٩ - مارس ٢٠٠٠). وجد أن ٧٩ حالة (١٢,١٠ %) كانت إيجابية إكلينيكيا. تم تشخيص هذا المرض بناءا على الأعراض المرضية ووجود البثرات المميزة لمسبب المرض في غشاء "الكوري ألنتويك" لبيض الدجاج المخصب وكذلك الفحص الهستوباثولوجي شاملة وجود الأجسام التضامنية المحبة للحمض بداخل سيتوبلازم الخلايا. كانت أهم الأعراض المرضية السائدة على الحالات المريضة خاصة صغار الإبل هى ارتفاع درجة الحرارة والفقدان التام للشهية وصعوبات تنفسية مع وجود المراحل المختلفة للآفات الطفح الجلدي. كانت البثور الصديدية منتشرة على فتحتي الأنف والشفاه واللسان وسقف الحلق للإبل المصابة وشوهدت بثرات مشابه على ملتحمة العين. تم وصف سيرة المرض والأعراض الإكلينيكية بإسهاب. اختلفت شدة المرض وفقا لعمر الإبل المصابة وكانت صغار الإبل (أقل من ٤ سنوات) أكثر قابلية للإصابة بهذا المرض. أغلبية الإبل المريضة (٨٢.٢٨ %) شوهدت خلال شهور الصيف. المحاولات العلاجية كانت ناجحة فى تقليص الفترة المرضية خلال أسبوعين من بداية العلاج.

SUMMARY

During the period of investigation (May 1999 - Marsh 2000), 653 dromedary camels were clinically examined in different villages of Assiut Governorate-Egypt for the presence of characteristic pox skin lesions. Out of these cases, 79 camels (12.10 %) were clinically positive.

Diagnosis of camel-pox disease depends mainly on the clinical signs, production of the characteristic pock lesions on chorio-allantoic membrane of the emberyonated chicken eggs and histo-pathological examinations including the presence of the intracytoplasmic eosinophilic inclusion bodies. Pyrexia, anorexia, and widely-spread of different stages of eruptive skin lesions with respiratory manifestations were the predominant clinical signs of the diseased cases particularly in younger. Nostrils, lips, dorsal surfaces of the tongues and hard palates of the infected young camels showed bastard discrete thick pustular lesions. Similar lesions were also observed on the conjuctivae. History of the disease and the clinical findings were described in detail. Severity of the disease varied according to the ages of the infected camels. Younger animals (less than 4 years) appeared to be most susceptible to the disease. The majority of the infected camels (82.28 %) were observed during the summer months. The therapeutic trials were successful to reduce the sysmptomatic illness period of the disease up to two weeks from the beginning of medication.

Key words: *Camel-Pox.*

INTRODUCTION

Camel consider one of the important animals for milk and meat production that consumed by some people particularly the nomadic persons in the desert areas. The outstanding role of camel as a hard working animal in the agriculture field or transportation between the villages should also not be neglected. Consequently, diseases manifested clinically by signs of inability to work and emaciation consider a peak problem to the camelmen. Moreover, the apparently healthy status of the skin plays an extremely vital role in the price of the commercially exhibited camels in the markets. This may refer to the important role of the skin diseases of camels. Camel-pox was greatly incriminated as one of the major skin diseases of camels (Gitao, 1997 and Muhammad et al., 1998) particularly in countries of the Middle East (Kriz, 1982; Wernery and Kaaden, 1995 and Khalafalla et al., 1998).

Camel-pox is a contagious, eruptive skin disease caused by highly epithiliotropic camelpox virus belonging to genus Orthopox virus of the family Poxviridae (Wernery and Kaaden, 1995). This disease

causes colossal economic losses to camel rearing communities in term of high morbidity and a relatively high mortality rate particularly in younger, reduction of milk yield in the lactating she-camels, and losses of weight in association with debilitation in camels of all ages (Munz, 1992 and Kinne et al., 1998). In addition to these economic implications, the lowest price of the clinically diseased camels with pox virus infection in the commercial markets should not be neglected as a one of the major economic losses (trade restriction).

In Egypt, Tantawi et al. (1974) isolated camelpox virus for the first time from the diseased camels. They also reported that there was no antigenic relationship between the camelpox, sheeppox, cowpox, buffalopox and vaccina. Such results may apparently refer to that camel-pox is host specific disease. However, the newer diagnostic methods including the ELISA technique with monoclonal antibodies and DNA restriction enzyme analysis as well as other tests revealed that there was antigenic relationship between camelpox virus and the vaccina virus (Wernery and Kaaden, 1995). The authors also reported that it is possible to immunize camels against camelpox virus infection with known vaccina strains. Such opinion may fairly reveal that camel pox is not completely host specific.

The aim of the present work was to study the clinical picture and the probable etiologic agent(s) responsible for eruptive skin lesions of some diseased camels in different villages of Assiut Governorate-Egypt. Histopathological studies of the clinically infected cases were achieved. Some epidemiological data on the present disease was preformed. Therapeutic trials of some diseased cases were also carried out.

MATERIAL and METHODS

History and Animals:

On May 18, 1999, two camels (3 - 5 years in age) from Abnoub village admitted to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, with history of inability to work and anorexia in association with the appearance of various forms of skin lesions on the different parts of their bodies. These cases were purchased from Bani-Adi village since 2 months and they were apparently free from clinical abnormalities. Three weeks later, signs of skin lesions with systemic illness appeared on the purchased camels.

For the second time, on the end of May 1999, another 3 camels of different ages from Bani-Adi village admitted to the aforementioned clinic with history and clinical signs similar to that previously introduced from Abnoub village. Moreover, the camelman added that one camel was succumbed due to the extension of the skin lesion in the mucosae of the buccal and nasal cavities of the infected case with respiratory manifestation.

Therefore, frequent visits to the Bani-Adi and the neighboring villages as well as other villages of Assiut Governorate (Table 1) were carried out to clear up the true position of such disease in camels.

Collection of the samples and sampling procedure:

A- Emberyonated chicken egg inoculation (Chauhan and Kaushik, 1987)**:**

Skin scrapings from the different affected areas of each clinically diseased camels (n = 10) were collected and a 10 % (w/v) suspension of the collected scabs was made in freshly prepared Hank's balanced salt solution (Cruickshank et al., 1982) containing antibiotics (penicillin G sodium $1x10^6$ IU and streptomycin 5 grams, per liter). The prepared suspension was finely triturated in the pestle and mortar and were left for four hours and then centrifuged at 1000 xg to remove the coarse and particles. Thereafter 0.2 ml of each prepared suspension was inoculated into chorio-allantoic membrane of 11-12 day-old developing chicken embryonated egg and incubated at 37 °C. Seventy-two hours later, the incubated eggs were examined for the production of lesions. Control tests in three eggs were performed through inoculation of sterile Hank's salt solution.

B-Histopathological examinations:

Samples for histo-pathological examinations were taken from the mucosal lesions of the upper lip of the clinically diseased camels. The samples were fixed in neutral buffer formalin and thereafter dehydrated, processed and embedded in paraffin blocks. These blocks were sectioned (5-7 µm) and stained with hematoxylin and eosin (Bancroft and Stevens, 1993). Staining with Macchiavello's technique (Culling, 1974) was also achieved. Some small samples 1x1 m were prepared for semithin sections.

Therapeutic trials:

Therapeutic trials of some diseased camels (n = 10) were carried out. Twenty milligrams of injectable oxytetracycline (Pan-Terramycin, Pfizer-Egypt) per Kg body weight were intra-venously infused through

one liter physiological saline per day. Vitamin A, (A-Viton ampoule, 100 000 U/ ml., Kahira-Egypt) 10 ml. per head was intra-muscularly injected daily. The therapeutic course persisted for 7 - 10 days. Concerning oral lesions of the infected cases, the mucosa was touched with a mixture consisted of (100 ml glycerin, 2 gm tannic acid, 5 gm salicylic acid, $2x10^6$ IU penicillin G sodium, and 4 grams streptomycin sulphate).

Statistical analysis:

Chi square (χ^2) was used to study the seasonal variation on the distribution of the disease according to the methods described by (Chatfield (1970).

RESULTS

Seventy nine cases out of the 653 clinically examined camels (12.10%) showed characteristic signs of skin eruption (Table 1) and camel-pox disease was suspected. Table 1 also declared that the disease was more prevalent in Bani-Adi and the neighboring villages (El-Kom and El-Hawatka).

Clinical findings:

The disease varies in its severity depending on the age of the affected camels. Younger camels (less than 4 years) were the predominant cases than the aged ones. After an incubation period of an unknown duration, the skin lesions of the clinically diseased camels started as fairly painless circumscribed papules, which rapidly turned to non-softened nodules followed by different sizes of vesicles, pustules and finally scabs. Some of these cutaneous nodules were spontaneously opened centrally forming a characteristic shape similar to small crater (Fig. 1 and 3). The various forms of the cutaneous lesions were spread on different parts of the infected camel. These parts included; the lateral sides of the head and neck (Fig. 1 and 4), shoulder region including the chest and the fore-limbs till the fetlock joints, the lateral and ventral aspects of the abdomen (Fig. 2), the inner and the external sides of the hind-limbs (Fig. 3). The external genitalia of both female (Fig. 5) and male (the prepuce and external orifice of penis) diseased camels were also affected with the different forms of the skin lesions. Areas around the humps of the infected cases were apparently skin lesions free.

Closure examinations on nostrils and oral cavities of the infected cases revealed that the muco-cutaneous junction areas of the nares and the upper lips including the cleft showed different sizes of discrete

pustular lesions (Fig. 4). Similar lesions were also observed on the comjunctivae with profuse lacrimation. The mucous membrane of the upper and lower lips as well as the dental pad (Fig. 6 and 7) of the infected camel had various forms of nodular and ulcerative lesions. In addition to these affected areas, the dorsal surface of the tongue (Fig. 8) and the last portions of the hard palate and the soft palate (koulla) of the diseased case showed discrete semi-circular flattened pustular lesions with erythematous borders.

General clinical examinations declared that the infected camels were suffered from pyrexia (39.8 ± 0.3 °C), harried respiration with marked hot expelling air, bilateral muco-purulent nasal discharge and increased the heart rates (58 ± 4/min.). The ocular and oral mucous membranes of the diseased cases were bluish in color (Fig. 6, 7 and 8). Anorexia was a remarkable observed sign of the infected camels. However, they were watery crapulous.

Chicken egg inoculation:

The majority of experimentally inoculated fertile eggs (7 out of 10, 70%) showed numerous small, opaque and circular (discrete) lesions, involving entire chorio-allantoic membrane (Fig. 9).

Histopathological examinations:

Prominent acanthosis was obviously seen in the healthy part of the epithelial layer, with increase in the number and length of the retie ridge (Fig. 10). The epithelial cells of stratum spainosum showed hydropic degeneration, in which the cytoplasm of the cells studied with multiple small vacuoles and some nuclei undergo karyorrhexis (Fig. 1.1). The desmosome junctions were disrupted and few cells were coalesced (Fig. 12). Small microvesicles were formed in the stratum spinosum (Fig. 13). Multiple ulceration has been seen also in the epithelial covering (Fig. 14). In some places the sub epithelial tissue under the ulceration showed neutrophilic cellular reaction resulted from the secondary infection (Fig. 15). Intracytoplasmic eosinophilic inclusion bodies were numerously seen in the epithelial cells of stratum spinosum with H & E stain (Fig. 16) and in stratum basalis with Macchiavello's technique (Fig. 17).

Age susceptibility:

Results of age susceptibility of the examined camels to camel-pox disease were summarized in Table 2 and Fig. A, which elucidated that camels under 4 years old were highly susceptible to camel-pox

disease than older (more than 7 years of age). Fig. A also showed that most of affected camels were immature, less than 3 years.

Seasonal variation:

Results of the influence of seasonal variation on the prevalence of camel-pox disease were illustrated in Table 3 and diagrammed in Fig. B. Such results epitomized that the majority of diseased cases (82.28 %) shown in hot months (May, June, July and August).

Therapeutic trials:

The applied therapeutic trials with infused oxytetracycline (I/V) coupled with vitamin A and local antiseptic touching agent reduced the illness period of the disease to 14 days (average) in the treated camels (n = 10).

DISCUSSION

The presence of the various forms and sizes of skin eruptions on the different parts of the infected animal body in association with systemic involvement including anorexia, pyrexia and hyperpnea may suggest that the suspected disease was camel-pox. Such suspicion was primarily supported by the appearance of the characteristic pock lesions on the chorio-alantoic membranes of the experimentally inoculated fertile eggs. Multiple small opaque and circular pock lesions, involving the entire Chorio-allantoic membranes of the emberyonated chicken eggs were considered as characteristic of camel-pox virus (Marennikova et al., 1974 and Chauhan and Kaushik, 1987). On the other side, the suspected disease was also supported by the histopathological findings, which referred to the presence of numerous intracytoplasmic eosinophilic inclusion bodies in the epithelial cell of stratum spinosum and stratum basalis. This histopathological finding was considered highly characteristic feature of pox disease (Yager and Scott, 1993).

From the epidemiological point of view, Table 1 revealed that camel-pox disease was prevalent in Bani-Adi and the neighboring villages (El-Kom and El-Hawatka) and in Abnoub (El-Sawalem village). Buxton and Fraser (1977) reported that camel-pox spreads horizontally through direct contact with the infected cases. Unfortunately, the original source of infection could not be detected. However, history taken with the camelmen announced that the most young camels in Assiut Governorate imported from the Sudan, in which the camel-pox disease is endemic. Khalafalla et al. (1998) found that 72.5% of the tested camels'

sera (n = 505) were serologically positive to camelpox virus infection by using ELISA test. Titers of these positive samples reached up to 1:23 768 (extremely higher titer) and they concluded that camelpox virus infection was widespread in all parts of the Sudan where camels were reared. On the other side, previous outbreak of camel-pox disease occurred in some territories of north Egypt (Tantawi et al., 1974) and the epidemic form of this disease occurred regularly (Werenery and Kaaden, 1995). This may be refers to that camel-pox is endemic disease in Egypt.

In regard to age susceptibility, it was found that 87.34 % (69 out 79) of the clinically diseased camels with the characteristic signs of pox were less than 4 years in age, and the remained infected cases (12.66 %, 10 out 79) were between 4 and 6 years in age. No diseased case more than 6 years old could be observed during the period of investigation (Table 2). Such results suggest that the young camels were most susceptible to camel-pox disease than older. Similar suggestion was reported by Khalafalla and Mohamed (1996) who found that the most of the diseased camels with pox were immature less than 5 years of age with a mean age of 2.7 years. They also found that the clinical signs of camel-pox disease were severe in young aged camels less than 3 years and all deaths occurred in this age. Conversely, Gitao and Nyaga (1997) reported that the camel-pox lesions were much more severe in the adult camels. Table 2 also indicated that 1 out 301 clinically examined camels more than 5 years (0.33 %) showed skin pox lesions. This may be refers to a build up of an acquired immunological status due to previous infection.

Concerning the influence of seasonal variation on prevalence of camel-pox disease, Yager and Scott (1993), Wernery and Kaaden. (1995) and Wrenery et al. (1997) reported that camel-pox disease was frequently occurred during the rainy-winter season. Moreover, Khalafalla and Mohamed (1996) recorded 35 outbreaks of camel-pox disease in Sudan within two years (1992 - 1994) and they found that the higher prevalence of this disease occurred in winter. Such opinions may concluded that the rainfall plays an minor and/or major role in spread of the disease. Similar conclusion was previously offered by Kriz (1982) who reported that the illness period of camel-pox disease ranged from 10 to 30 days but this period might persisted up to 5 months during the rainy season. However, the obtained results listed in Table 3 and Fig. B declared that the majority of the diseased cases (65 out of 79, 82.28 %) were observed in

May, June, July and August, 1999 and these months are non-rainy hot-months in Assiut Governorate, Upper Egypt. The remained cases of the diseased camels (14 out of 79, 17.72 %) were observed in the first half of September, 1999 (non-rainy fairly hot month in Assiut). Such variation, unfortunately, could not be interpreted. However, it is suggested that the spread of camel-pox disease in Assiut during the summer months may be related to the density of the insect population. Wernery and Kaaden (1995) and Wernery et al. (1997) reported that epidemic form of camel-pox disease occurred in regular cycles dependent on the rainy season in relation to the density of the insect and ticks population, and the number of the immunized camels in the population.

From October 1999 to Marsh 2000, weekly visits to the veterinary clinics of Abnouub, El-Hawatka and Bani-Adi villages to record the new cases of camels with skin eruptions were carried out with negative results during this period. Such results may be refers to the restriction of the disease. However, it is difficult to concluded that the aforecited villages and others became, seemingly, camel-pox disease free. Such conclusion built on the opinions of Buxton and Fraser (1977) and Gitao (1997) who epitomized that camel-pox seems to flare-up every 3rd to 5th years in contaminated environment, usually between the months of July and September. Consequently, to avoid the economic losses induced by camel-pox disease, vaccination of camels with camel-pox virus vaccine is strongly recommended.

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Table 1: Prevalence of camel-pox disease in different villages.

Village	No. of the examined camels	No. of cases showed skin eruption.	(% of infection)
Bani-Adi	120	29	24
Awlad Elaiwe	45	4	8.90
El-Kom	10	2	20
El-Hawatka	27	5	18.51
Bani-Zeid	49	3	6.12
Soutb	18	0	0
El-Awamer	20	2	10
El-Tatalia	19	0	0
El-Aziya	15	0	0
Nazlat-Bakour	20	0	0
Kom-Esfekhet	96	6	6.25
El-Mashiaa	14	0	0
El-Sawalem	108	20	18.52
Abnoub-El hamam	53	8	15.09
El-sheikh Sehata	17	0	0
Naga Zorik	22	0	0
Total	653	79	12.10

Table 2: Age susceptibility of the examined camels to camel-pox disease.

Age*	No. of the examined camels	No. of cases showed skin eruption.	% of infection
< 1 Y	31	0	0
> 1 - 2 Y	55	10	18.18
> 2 - 3 Y	83	36	43.37
> 3 - 4 Y	89	23	25.84
> 4 - 5 Y	94	9	9.57
> 5 - 6 Y	80	1	1.25
> 7 - 9 Y	92	0	0
> 9 – 12 Y	77	0	0
> 12 Y	52	0	0
Total	653	79	12.10

* Ages of camels depended on the history taken.

Table 3: Influence of seasonal variation on the prevalence of camel-pox disease:

Months	No. of the examined camels	No. of cases showed skin eruption	Prevalence (% affected)	% to all clinically diseased cases
(A) Hot-months[@]	475	65	13.68	82.28*
(B) Non-hot-months[#]	178	14	7.85	17.72
Total	653	79	12.10	

@ : Hot-months : May, June, July and August.
# : Non-hot-months : From September to Marsh.
* : χ^2 (A) • (B) = 4.123 ($p < 0.05$), significant difference. (Tabulated value of $\chi^2_{0.05,1} = 3.84$)

Fig. A: prevalence of camel-pox disease at different ages of the examined camels

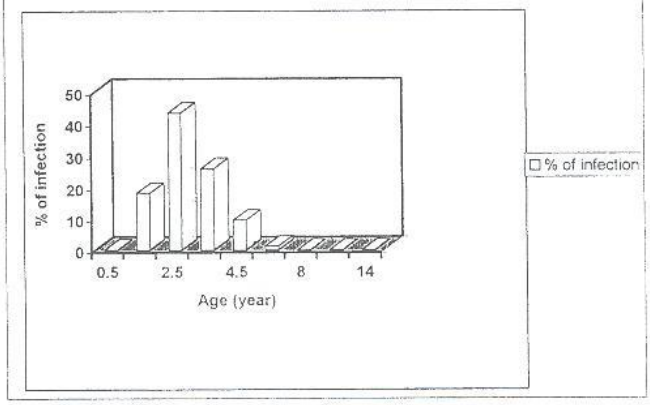

Fig. B : showing that 82 % of the clinically diseased camel with pox were observed during the summer months.

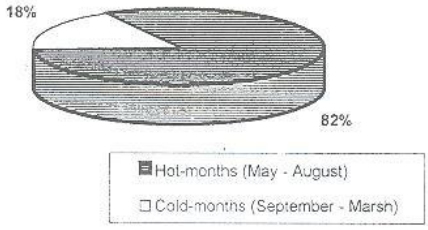

FIGURE LEGENDS

Fig. 1 and 2: Various forms of the skin pock lesions on the lateral sides of the neck (Fig. 1), shoulder region including the chest, and the lateral and venteral aspects of the abdomen (Fig. 2) of the infected camel.

Fig. 3: Different forms of cutaneous pock lesions on the inner and external sides of the hind-limbs. Note: some of cutaneous nodules were opend forming a characteristic shape similar to crater (arrows).

Fig. 4: External genitalia of infected she-camel shows cutaneous nodules.

Fig. 5: The muco-cutaneous junction areas of the nostril and the upper lip including the cleft of a diseased camel were also affected by different forms of pock lesions.

Fig. 6: The mucous membrane of the upper lip shows various forms of nodular and ulcerative lesions.

Fig. 7: Nodular and ulcerative lesions with erythematous borders on the inverted upper lip and dental pad.

Fig. 8: The dorsal surface of the tongue has semi-circular flattened pustular lesions.

Fig. 9: Numerous small, opaque and circular discrete lesions, involving the entire chorio-allantoic membrane of fertile egg.

Fig. 10: Buccal mucosa showing remarkable acanthosis in the healthy part of the epithelial layer (H & E, x 100).

Fig. 11: Buccal mucosa showing hydropic degeneration of stratum spinosum, in which the cytoplasm of the cells studied with multiple small vacuoles and some nuclei undergo karyorrhexis (Toulidine blue x 1000).

Fig. 12: Buccal mucosa showing disruption of the desmosome junctions and few cells coalesced (Toulidine blue x 1000).

Fig. 13: Buccal mucosa showing microvesicles formation in the stratum spinosum (Toulidine blue x 1000)..

Fig. 14: Buccal mucosa showing ulcerative lesion in the epithelial layer (H&E, x100) the subepithelial tissue under the ulceration

Fig. 15: Buccal mucosa showing neutrophilic cellular reaction in the subepithelial tissue (H & E, x 250).

Fig. 16: Buccal mucosa showing intracytoplasmic eosinophilic inclusion bodies (arrow) in the epithelial cells of stratum spinosum (H&E, x 400)

Fig. 17: Buccal mucosa showing intracytoplasmic inclusion bodies (arrow) in the epithelium of stratum basalis (Macchiavello's technique, x 400).

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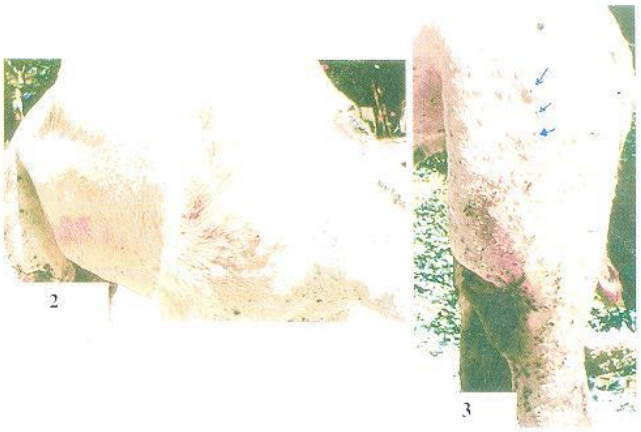

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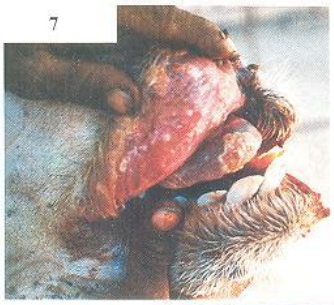

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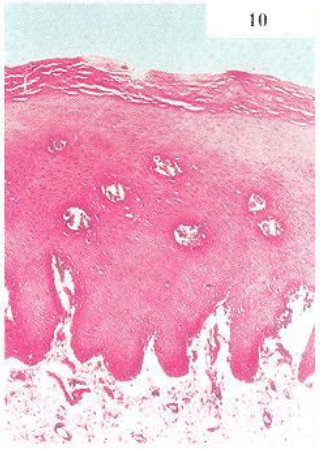

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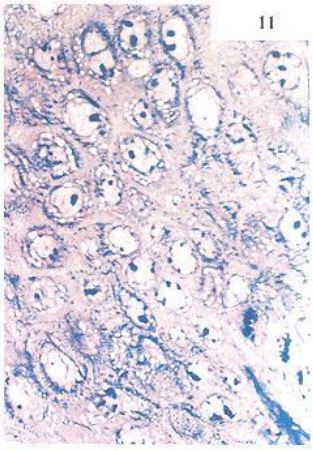

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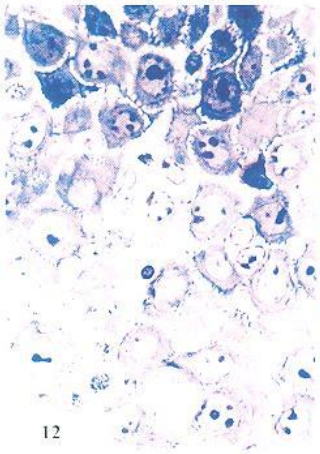

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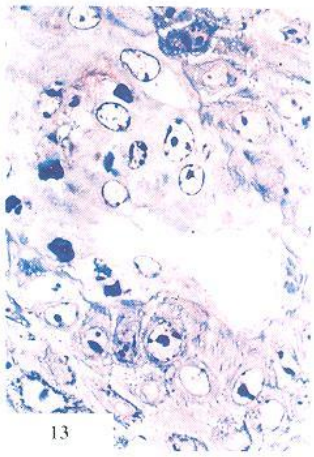

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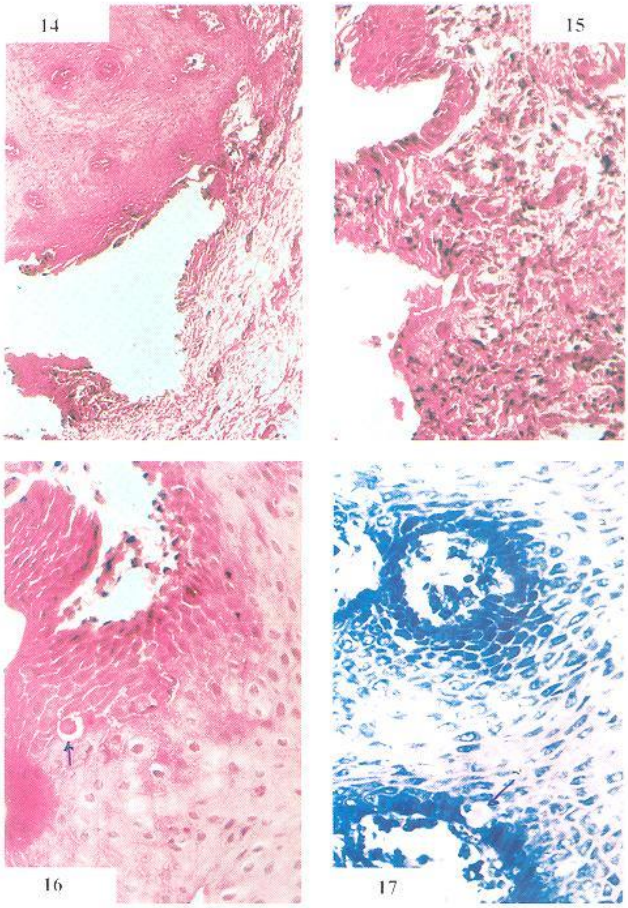

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