SOME VIROLOGICAL AND PATHOLOGICAL STUDIES ON CAMEL POX

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Abstract

In private camels farm at Mansaratrah Governorate, Egypt, 5 out of 100 dromedary camels showed clinical manifestation referred to camel pox. The lesions were confined to the skin of the head, nose, eyelids and neck. Five from skin biopsies were taken for virological and pathological studies. The characterization of the viral isolate revealed its multiplication on the corioallantoic membrane (CAM) of chicken embryos at the age of 11 days with the formation of minute white pox lesions 5 days post-inoculation. Histopathological investigation showed eosinophilic cytoplasmic inclusion bodies in the cells of CAM. The virus was identified by agar gel immunodiffusion test using specific hyperimmune serum of camel pox virus. Electron microscopy was done on two skin biopsies and one sample from CAM, as well as 3 other CAM samples for negative stain. Pox virions were detected in the cytoplasm of CAM cells and in the cells of tunica intima of dermal blood vessels. Histopathological examination of skin biopsies showed degeneration and necrosis of prickle cells, as well as, vascular Damage in the dermis with mononuclear cell infiltration around the blood vessels. Intracytoplasmic inclusion bodies were seen in some prickle cells which appeared as homogenous round eosinophilic bodies.

INTRODUCTION

Camel pox is a proliferative skin disease affecting camel and caused by specific Orthopox virus camel related to family Poxviridae. Pox is species specific disease distinct from vaccinia, sheep pox and cow pox viruses (Tantawi et al, 1974 and Alhendi et al, 1994).
Camel pox was first described by Lesse (1909) in Pengab, India. The disease has a wide distribution among camel population in India, Pakistan, Afghanistan, Iran, USSR, The Middle East, as well as, North and East Africa (Munz, 1992).

In Egypt, the causative virus was isolated by Tantawi et al. (1974) in Fayoum, and by Kenawy et al. (1989).

Munz (1992) stated that the incubation period of the disease ranges from 10 to 15 days in natural cases. The onset of the disease is indicated by fever and weakness, followed by swelling of the skin and mucosa in affected parts of the body. Around the lips, nose and eyelids, lesions may become visible in the form of papules, which quickly pass through vesicular and pustular stages. Lesions also, develop on the mucous membranes of the oral cavity resulting in difficulty in eating. Associated lymph nodes are often swollen and facial oedema is apparent. These clinical signs as mentioned by Pfahler (1986) and Kaaden et al. (1992) may be confused with contagious camel ecthema caused by parapoxvirus and papillomatosis caused by camel papilloma virus.

Diagnosis of camel pox as noticed by Tantawi et al. (1974), Kenawy et al. (1989) and Kaaden et al. (1992) was based on the history, clinical signs and the course of the disease. Confirmation was obtained through isolation of the virus on the chorioallantoic membrane of developing chick embryo (CAM), and identification of the virus by agar diffusion test using specific hyperimmune serum. In addition, Andrews (1978) noted that the electron microscopical detection of the virus particles in pox lesions was one of the diagnostic tools of the disease. Munz et al. (1992) recorded the differentiation between pox and pox like diseases in camel (camel pox, camel contagious ecthyma and papillomatosis) by the use of electron microscopy. The orthopox virus causing camel pox, is brick-shaped, while, parapox virus causing camel contagious ecthyma shows criss-cross pattern, and papilloma virus has an icosahedral arrangement.

Donny (1988) and Jubb and Kenedy (1985) stated that camel pox lesions began with balloning degeneration of stratum spinosum of the epidermis, reticular degeneration and acanthosis resulting in intraepidermal microvessicles. The dermal lesions included oedema and perivascular dermatitis; mononuclear cells and neutrophils were present in varying proportions. Pox lesions contained characteristic eosinophilic, intracytoplasmic inclusion bodies which are single of 3-7 µm in diameter and of type A. (Cowdrey type A).
The present investigation deals with virological and pathological studies on camels that suffered from skin lesions similar to pox in one private farm at Marsamattoh Governorate, Egypt. The optical and electron microscopical pictures were described as an aid for diagnosis.

MATERIALS AND METHODS

In a private farm at Marsamattoh Governorate, Egypt, skin lesions similar to pox on 5 out of 100 camels have appeared. Camels were inspected for other clinical signs. Five skin biopsies were surgically removed from the upper part of the neck for virological and pathological examinations.

1. Virological Examinations

Skin biopsies were kept in minimal essential media as maintenance media containing 1% foetal calf serum for virus isolation.

For virus isolation, skin biopsies were pooled and ground in a sterile mortar according to the method described by Kenawy et al. (1983). Isolation procedure on chorioallantoic membrane of 11-12 days old embryonated chicken eggs were used for virus isolation. From the prepared fluid, 0.2 ml was inoculated for each egg via chorioallantoic membrane route (CAM) according to the technique described by McCarthy and Dunbell (1961). After five days post-inoculation, the chorioallantoic membranes were investigated, and the isolated virus was passaged 7 successive times.

The isolated virus was identified using the agar gel immunodiffusion test which was carried out according to Mansi (1958) by testing the isolate against hyperimmune sera of camel pox virus obtained from Vaccine and Serum Production Research Institute (Abbasia).

2. Pathological Examinations

1. The gross appearance of skin lesions that appeared on the neck of five dromedaries camels was recorded.

2. Electron microscopy

Two skin biopsies and one sample from CAM pock lesions were fixed in 3% glutaraldehyde, processed and sectioned for transmission electron microscopy in E.M. Center Specialist Ein Shams Hospital. In addition, negative stain was adopted in
3 samples from CAM bock lesions to detect the virus in clear form.

3. **Light microscopy**

A) Number of 5 skin biopsies from infected camels were fixed in 10% formol saline, and were prepared for paraffin embedding; sections were stained with haematoxylin and eosin (H & E) according to Carleton et al. (1967). In addition, staining with phloxine tartrazine and toluidin blue, basic fuchsin for inclusion bodies were applied (Clyden et al., 1971).

b) Proper samples from the chorioallantoic membrane (pock lesions after the 7th passage) was collected from 15 eggs and fixed in 10% formalin. Thin paraffin sections were prepared, and stained with H & E and Man's stain for microscopical detection of inclusions.

**RESULTS**

1. **Clinical Signs**

   In Autumn 1995 at Marsamaroh Governorate, total of 5 out of 100 dromedary camels with an age ranging from 3-5 years and belonging to a private farm, were suffering from skin lesions in the form of circumscribed rounded papules, and vesicles of 0.5-1.5 cm in diameter. These lesions were found in the nose, eye lids and lips and in the upper part of the neck.

2. **Virological Results**

   The virus was isolated on the CAM of the embryonated chicken egg and produced pock lesions which appeared very clear on the 5th day postinoculation (Table 1).

<table>
<thead>
<tr>
<th>No. of passage</th>
<th>Changes of CAM</th>
<th>AGP</th>
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<tbody>
<tr>
<td>1</td>
<td>Oedematous</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Oedematous</td>
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<tr>
<td>3</td>
<td>Oedematous</td>
<td>-</td>
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<tr>
<td>4</td>
<td>Pock lesions at the site of inoculation</td>
<td>+</td>
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<tr>
<td>5</td>
<td>Pock lesions at the site of inoculation</td>
<td>+</td>
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<tr>
<td>6</td>
<td>Pock lesions at the site of inoculation</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Pock lesions at the site of inoculation</td>
<td>+</td>
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Table 1. Propagation of the virus on CAM.
Five days post-inoculation of 11-12 days old embryonated chicken eggs with 0.2ml of prepared fluid from the skin lesions of camels, the gross appearance of CAM contained diffuse opaque thickening with slight haemorrhagic spots, as well as, round individual white opaque pock lesions 0.7-1.0 cm in diameter with smooth surface raised on CAM. (Fig. 1).

The identification of the virus isolate was done using the agar gel diffusion test with standard hyperimmune sera of orthopox of camel. The test showed clear precipitation line which started to appear from the forth passage.

3. Electron Microscopical Findings

a. Skin

Changes of the skin consisted of absence of the intercellular spines in between the prickle cells with the presence of vacuoles in between. Some of the nuclei showed indistinction with disarrangement of chromatin and an increase of euchromatin on the expense of heterochromatin. (Fig. 2). Blood vessels showed swelling of the endothelial lining, as well, pericytes appeared swollen also. Pox virions were detected in the cells of tunica intima that showed exocytosis. (Fig 3, 4).

b. Chorioallantoic membrane (CAM)

In the epithelial cells of ectoderm, pox virions were detected in the cytoplasm of rough endoplasmic reticulum which appeared swollen (Fig. 5). In addition, there was marked increase in the number of lysosomes in the cells examined; virions were also detected free in the cytoplasm of the cells. Lipoblastic cells showed virions scattered in the cytoplasm besides fat vacuoles and phagocytic ones. (Fig. 6). The endodermal epithelial cells showed virion scattered in the cytoplasm, by use of negative staining technique, orthopox virus appeared as brick shaped particles measuring 260-280 nm. and the superficial protein filaments were arranged irregularly. (Fig. 7).

4. Pathological Findings

The histopathological examination of skin lesions showed coagulative necrosis, epidermal erosion with some prickle cells (Fig. 8), in addition to areas of epidermal vacuolation (Fig. 9). The vacuoles were mostly found near and around the nuclei of some prickle cells. These vacuoles had different sizes and sometimes occupied nearly all the entire cell area. The nuclei shrank in size, shriveled, or were marked
Fig. 1. Chorioallantoic membrane showing pock lesions with slight hemorrhages.

Fig. 2. T.E. of the skin showing stratum spinosum, notice.
1. Absence of intercellular spines
2. Some nuclear anulagation.
3. Increase of euchromatin to heterochromatin ratio.
4. Vacuolation. (x 11,000)
Fig. 3, 4. T.E. Dermal B.V. showing swelling of its endothelial cells as well as pericytes and exocytosis, of pox virus through blood vessel wall x 5,000 - 7,000.
Fig. 5. T.E. Cysterna of rought endoplasmic reticulum showing virion replications. x 60,000.

Fig. 6. T.E. Lipoblast in the mesoderm of CAM showing virion of camel pox in the cytoplasm. x 14,000.
Fig. 7. T.E. Negative stained virus (mature) of the 7th stage of propagation on CAM, x 180,000.

Fig. 8. Skin showing epidermal erosion with coagulative necrosis of some prickle cells. H & E, X 400.
Fig. 9. Skin showing epidermal vacuolar degeneration of most of the cells of stratum spinosum. H. & E., x 100.

Fig. 10. Skin showing epidermal vacuolations and some leucocytic cell infiltration.
only by small particles of chromatin, or totally disappeared leaving only bordered empty areas (Fig. 10).

Most of the prickle cells were swollen and gave the appearance of ballooning state, some of them burst, coalesced with each other to form large vesicles which appeared in different forms and sizes (Fig. 11). Some leucocytic infiltrations occurred in the lumen of some vesicles. Just under stratum corium, severe degeneration and necrosis of some prickle cells were seen, as well as, multinuclear leucocytic infiltration. Involvement of stratum basalis was not uncommon, but only small areas were affected in the form of ulcer (Fig. 12).

Intracytoplasmic inclusion bodies were seen in some of prickle cells in the form of homogenous round eosinophilic bodies (Figs. 13, 14, 15).

All skin lesions showed focal infiltration with mononuclear cells in the dermis (Fig. 10), specially, around small blood vessels and associated with oedema and fibrinous exudate (Fig. 17). Some blood vessels showed thrombus formation with swelling of the endothelium and its protrusion into the lumen.

The histopathological findings of CAM showed severe hyperplastic proliferation of ectodermal epithelial cells with swelling and ballooning features. Intracytoplasmic eosinophilic inclusions were seen in both ectodermal and endodermal epithelial cells (Fig. 18, 19). The mesoderm showed dilatation of blood vessels, congestion and mild hemorrhages, with slight proliferation of its cells. The endoderm showed clear proliferation of its epithelium, but to a lesser degree than ectoderm (Fig. 18, 19).

**DISCUSSION**

The clinical findings showed that, 5 out of 100 dromedary suffered from skin lesions in the form of papules and vesicles on the head and neck, nose and eye lids. Similar mild picture of pox was previously recorded by Alhendi et al. (1994). The gross appearance of skin lesions was similar to that mentioned by Kaaden et al. (1992).

The reported results indicated that the isolated virus was camel pox virus. The virus was found to be able to replicate on CAM of chicken embryos producing pox lesions which were completely different from cow pox and vaccinia viruses (Downie 1949, Fenner and Burnet 1959) and somewhat resembling buffalo pox virus isolated by Tantawi et al. (1967).
Fig. 11. Skin showing epidermal vesicles of different forms and sizes. H & E X 650.

Fig. 12. Skin showing ulcer formation with lymphocytic infiltration of dermis. H & E, X 100.
Fig. 13, 14, 15. Intra cytoplasmic inclusion bodies with:
13. Fluxin tartrazin stain.
14. H & E STAIN.
15 Toluidin blue basic fuchin. (x 650).

Fig. 16. Skin showing dermal focal infiltration with mononuclear cells. H. & E. x 250.
Fig. 17. Skin showing dermal oedema and fibrinous exudate. H. & E. X 250.

Fig. 18, 19. Chorioallantoic membrane showing:

a. Endodermal, and b. Ectodermal proliferation of epithelial cells with intra cytoplasmic inclusions. Muc; s stain x 650.
The viral isolate was tested by standard hyperimmune sera of orthopox of camel that showed clear precipitation line starting from the forth passage of the isolate on CAM of chicken embryo. These results were similar to those obtained by Kenawy (1989).

The electron microscopical findings of the skin samples proved that orthopox viral infection was the probable cause of pox in our camels. The distribution and the morphological features of the virus particles were similar to orthopox viruses detected by Kaaden et al. (1992). The size of virus particles observed by use of negative stain in CAM coincided with the findings of Münz (1992).

The results of examination of skin lesions by electron microscopy were similar to those obtained by Münz et al. (1986).

The replication of the virion in the tunica intima of blood vessels of skin epidermis, and also, the presence of virions in the mesoderm of CAM chicken embryo emphasized the tropism of the virus on the cells of the blood vessels, skin and on the mesodermal cells which are known to be able to give rise to blood vessels of the embryonated chicken (Münz et al. 1986).

Histopathological examination of the skin lesions showed proliferative and degenerative changes in stratum spinosum with typical intracytoplasmic eosinophilic inclusions. Coagulative necrosis and vascular damage detected in the skin lesions emphasized that camel pox virus has pathological effect similar to the other pox viruses (Cheville 1966, Gibbs and Johnson 1970). The necrotic changes observed in stratum spinosum could be attributed to the thrombosis of dermal blood vessels and not to the direct effect of the orthopox virus. The lymphocytic infiltration at the site of lesions may be related to the toxic products of the necrotic tissues.

Intracytoplasmic eosinophilic inclusion bodies were commonly seen in the cells of ectoderm and endoderm of CAM of chicken embryos. These findings were similar to those recorded for cow pox inclusions by Zelek et al. (1983).

The haemorrhages found in the mesoderm proved the greater mesodermotrophic affinities of this virus and its predilection seats for endothelial cells lining blood vessels. These results resembled those obtained by Yasuo et al. (1971) in vaccinia.

As reported by Alhendi et al. (1994), the hyperplastic proliferation in both ectodermal and endodermal cells proved that the virus is an epitheliotropic one.
The previous and the present studies revealed the appearance of camel pox in camel populations in an Egyptian governate. This problem needs more studies to adopt the proper prophylactic and control measures of the disease.

REFERENCES


بعض الدراسات الفييولوجية والباثولوجية على جدرى الجمال

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ظهرت في إحدى المزارع الخاصة بالجمال بمحافظة مرسي مطروح أمراض جردى في جمال من بين سماكة جمال وحيدين السام. ظهرت بشرات جلدية مميزة للمريض في منطقة الرأس والأذن. تم اتخاذ عينات من الجلد السام وذلك لدراسة الظروف الفييولوجية والباثولوجية.

تم عزل الفيروس المستقبلي للمريض في أشعة الكربونات الأنتونوسيس لاجئة كنا كيتي في عمر 11 يوما بعد تعريده 7 مرات، وبعد 5 أيام من الحدقه الرابعة ظهرت بشرات على المريض بتجمعات الفيروسات والالتهابات التي تingles الفيروسات المستقبليات عليها جريدة وجود انسجام الاتساعانية، بما أنف، نظهرت هذه الأنسجامة الاتساعانية الأونيوفيليا في سيتوبلازم الغلدية في الحدقهة السابعة.

أجري اختبار الأدوات التحريكي بمعدل معيار مرجعي نوعي ضد فيروس
جدرى الجمال.

أجريت تحضيرات البيروكسوركباك النقرح على عدد 2 من عينات الجلد السام، وعدد 1 عينة واحدة من أشعة الكربونات الأنتونوسيس المراحيه، وتم علاجها بالнемيقة السلبية

للفيروس للتماسك مع الأشعة الكربونات الأنتونوسيس. ظهرت الفيروسات المستقبليات بعد 5 أيام من الجلد السام، ونها استجابة

وتنكرت بالطبقة الشوكية للأنسجمة في مساحة الأنسجامة الدموية للأنسجة

الأنسجية، ونها جيلبات المبسطة من خلال وجودة الجسمات النورمالها، وقد يوجد وجود انقسام

الانيوفيليا بسيتوبلازم بعض خلايا الطبقة الشوكية للأنسجة البشرية.

هذا وقد تم توضيح النتائج في عدد 19 صورة ومتناشدة.