MOLLUSCUM CONTAGIOSUM IN THREE HORSES

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ABSTRACT

Suspected molluscum contagiosum was diagnosed in 3 horses in the Chingola district of Zambia. The horses were found to be suffering from a slow progressive skin disease with lesions on the chest, shoulders, inner and lateral aspects of the fore- and hindlimbs, the face, fetlocks, pasterns and on the lateral surfaces of the body, The lesions varied from 4 to 20mm in diameter, were hairless but covered by soft keratin projections which, when removed, left a raw elevated base tightly adherent to the epidermis. These lesions bled profusely when the animals were groomed. Older lesions were well circumscribed, raised above the surface, devoid of hair and after removal of grey-white keratin flakes, had a depigmented waxy appearance. Microscopically cytoplasmic inclusions containing many pox virions were found. Attempts at culturing the virus were unsuccessful.

Key words: Molluscum contagiosum, equine, horse, pox virus.

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INTRODUCTION

Pox virus infections in horses are infrequently reported in the literature. A transient, highly infectious papular eruption over almost the entire body surface was recorded by McIntyre[8]. The cause of this condition was not determined, but it was thought to be due to an unclassified pox virus[1 16 11]. Horsepox as such is a benign, contagious disease of horses that may manifest either as the "buccal form" or as the "greasy heel form"[3 13]. Buccal horsepox is characterised by the development of pocks which ulcerate, within the oral cavity, on the inner surfaces of the lips, on the gums, the tongue and anterior nares. Eruptions may also be present on the face. The greasy heel form of horsepox, also known as "Jenner's horsepox" has eruptions on the flexor surfaces of the fetlocks and other joints in the lower part of the limb[3 13].

A more generalised disease affecting the face, oral cavity, flexor surfaces of the cannon bone and coronary band was recently reported in a donkey[3]. Another manifestation of pox virus infection in horses is Uasin Gishu skin disease described in Kenya[4 5 6 7]. This disease is very similar to the recorded cases of molluscum contagiosum[2 9 12]

In this report a skin disease of horses in the Chingola district of Zambia is described.

MATERIALS AND METHODS

The histories of a 17-year-old Thoroughbred mare, a 9-year-old Miniature pony stallion and a 16-year-old Thoroughbred cross gelding were recorded as far back as possible. The mare and gelding were hospitalised and the behaviour of the lesions was observed for at least 4 months. The development of the lesions in the stallion was monitored by regular visits to the animal for at least one year. The appearance and progress of the lesions were carefully recorded.

Biopsies from recent and older lesions were fixed in 10% formalin for microscopic examination and crusts from similar lesions were collected for virus isolation. Tissues were routinely processed and sections were stained with haematoxylin and eosin (HE) for light microscopic examination. Selected blocks of formalin-fixed skin were post-fixed in 4% glutaraldehyde and routinely processed for electron microscopy.

Ultra-thin sections were stained in 1% uranyl acetate and 0,2% lead citrate and examined with the transmission electron microscope.

The scab material submitted for virus isolation was homogenised in phosphate-buffered saline (PBS) containing 200 units ml^{-1} penicillin and 200 μg ml^{-1} streptomycin. After centrifugation at 500 x 9 for 20 min, confluent monolayer cultures of bovine kidney cells (CFK and MDBK) were inoculated with 0,5 ml of supernatant fluid. After 60 min the culture flasks were washed and maintained with serum-free Eagles medium containing 100 units penicillin and 100 μg ml^{-1} streptomycin. Virus isolation was also attempted in experimental animals[6]. Briefly, embryonated eggs were inoculated by the CAM route with supernatant fluid and candled daily. The CAMs were examined on Day 6 post inoculation for lesions. Baby mice, aged 7 d, were inoculated intraperitoneally with material from the first and second CAM passages. Membranes were homogenised in PBS and prepared as outlined above and also used for attempted virus isolation on monolayers of bovine, monkey and rabbit kidney cells (CFK, MK2 and RK13).

RESULTS

In the 17-year-old Thoroughbred mare, the initial lesions were first seen 6,5 years previously. The 9-year-old Miniature pony stallion had had lesions for more than one year and the 16-year-old Thoroughbred cross gelding had lesions of at least 6 months duration.

These horses were the only ones in their stables to be affected and when 2 horses were moved to hospital stables for observation and tests, the in-contact horses did not develop lesions even after 4 months of sharing the same stables and grooming utensils. The lesions started on specific areas of the body (this differed from horse to horse) and spread slowly to involve other parts of the body surface. They healed very slowly and then recurred to affect the same areas as were previously affected.

In all 3 horses lesions were seen on the chest, shoulders and limbs. The inner and lateral aspects of both the fore- and hindlimbs were also affected. Two horses

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Fig. 1: **Inner caudal thigh of a Miniature pony illustrating early lesions consisting of outward projecting spicules of keratin. Note the few small lesions on the scrotum**

had lesions down to the pasterns and one had lesions involving the fetlocks. The mare initially had lesions on the muzzle and face, which spread to involve the entire body surface excluding the neck. The gelding had no lesions on the head and neck although the rest of the body was involved. In the stallion no lesions were found on the head, neck and body, but a few were seen on the caudal scrotal skin. No lesions were present, at any time during the course of the disease, on the mucous membranes of the conjunctiva, nostrils, mouth, vulva or sheath.

The very early lesions looked like insect bites on which the hair was raised over a slightly elevated firm plaque in the skin. This stage was seldom noticed.

The more acute lesions were alopecic and they consisted of soft keratinised white spicules up to 3 mm in height (Fig. 1). When the hyperkeratinised projections were scratched off, a slightly raw base which was raised above the surface of the skin and very tightly adherent to the epidermis, was left. These lesions bled profusely when the animals were groomed. Some of the lesions coalesced to form cauliflower-like growths of up to 30 mm in diameter. Lesions of several months duration were of the same size as those mentioned above. They were irregularly spherical, well-circumscribed and raised above the surface, some were elevated up to 2 mm above the surrounding normal skin. Some of these lesions were covered by hair that stood on end, while others had partial hair loss with the remaining hairs epilating easily. The larger lesions were completely devoid of hair. All of these lesions were covered by grey-white, soft keratin scales which could easily be removed by scratching or grooming. This left a depigmented, slightly-elevated area covered by powdery scales. The hair would eventually grow again to cover the lesion completely. The lesions on the pasterns were characterised by groups of hard growths that formed finger-like projections 5 mm wide and up to 20 mm long. They were tightly adherent to the skin and difficult to remove. When pulled off a raw, white, hard raised area was left.

The most striking histological feature was the abrupt transition from unaffected epidermis to prominent hyperplasia of the epidermis (Fig. 2). The hyperplasia was characterised by solid masses of stratum spinosum cells that encroached downward into the dermal connective tissue without the formation of rete pegs. The prickle cells in these hyperplastic areas were swollen, due to the presence of cytoplasmic inclusions which varied in appearance. Some were eosinophilic and clearly distinguishable from the vacuolated cytoplasm, while others had a more homogeneous faintly basophilic appearance with a few eosinophilic granules scattered on the periphery of the inclusion (Fig. 3). These 2 types of inclusions were seen in the deeper layers of the stratum spinosum. Inclusions consisting of coarse basophilic granules were present closer to the surface of the epidermis (Fig. 4). As the size of the inclusions increased, areas within the inclusions were more sparsely populated with the granular structures leading to a vacuolated appearance. The nuclei of affected cells were eccentric, pycnotic or absent. As the cells were pushed towards the surface, the inclusions attained a deeply-basophilic appearance and the cells became shrunken and keratinised. Groups of these cells were sloughed onto the surface as keratin squames. In some biopsies, follicular and surface hyperkeratosis was prominent, while in others the stratum corneum was very thin or absent. The dermal cell infiltration was limited to a few scattered neutrophils in the subepidermal connective tissue and moderate numbers of lym-

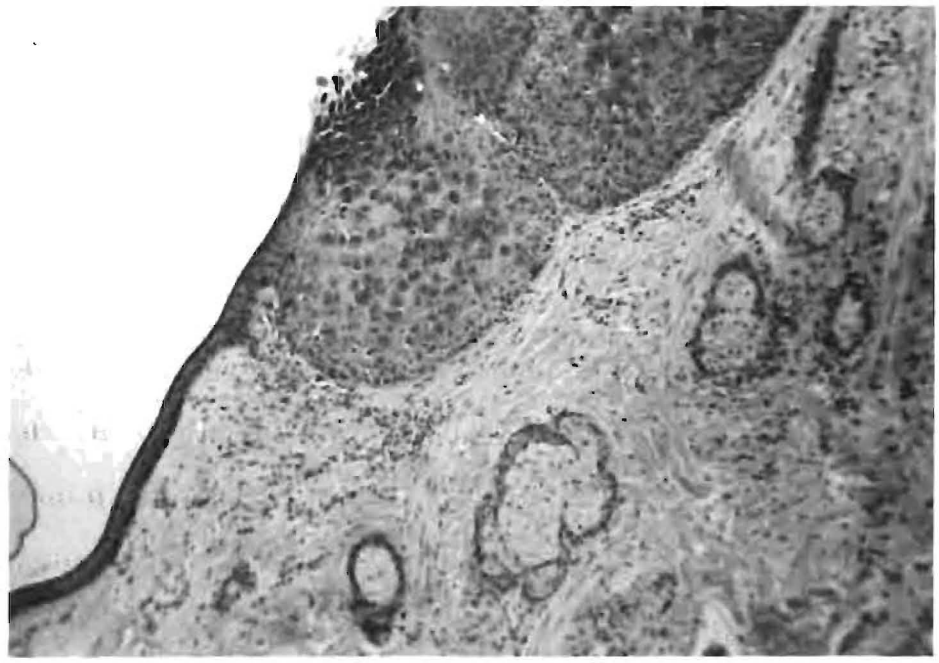

Fig. 2: **A section of the skin illustrating abrupt transition from unaffected epidermis to prominent epidermal hyperplasia. HE X 100**

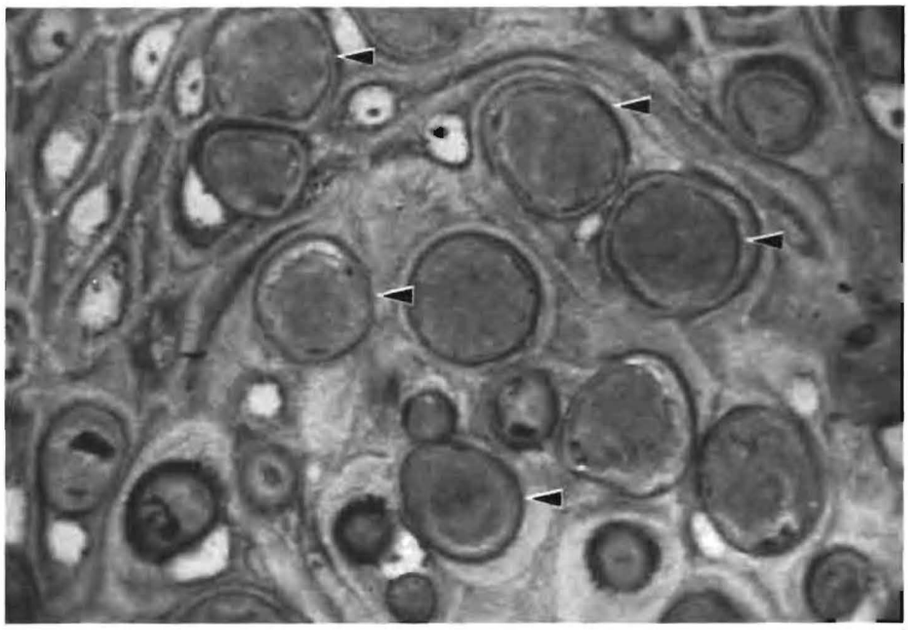

Fig. 3: **Large cytoplasmic inclusions (arrows) characterised by a pale blue homogeneous substance with peripherally-arranged fine eosinophilic granules. HE X 1000**

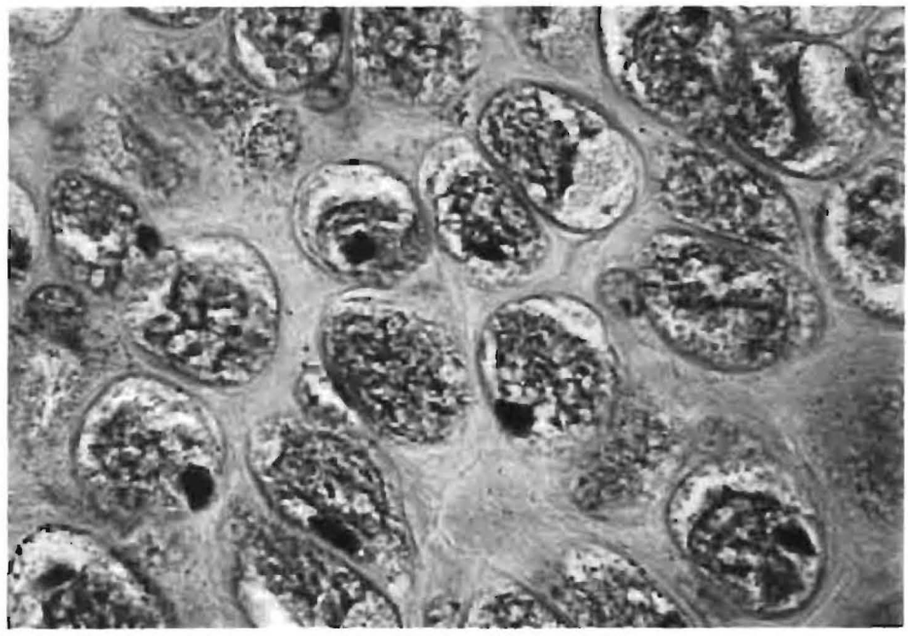

Fig. 4: **Cytoplasmic inclusions consisting of very coarse basophilic granules. HE X 1000**

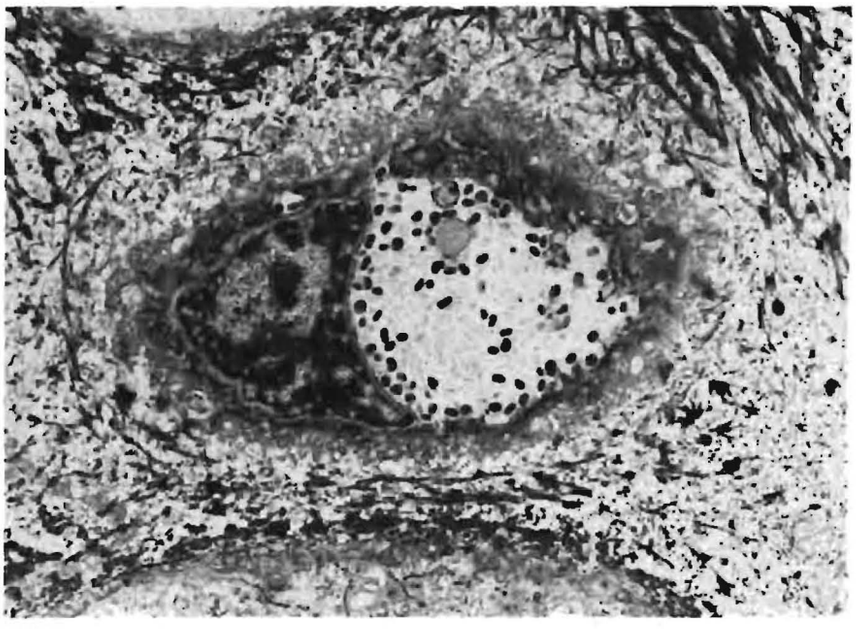

Fig. 5: **Electron micrograph of a stratum spinosum cell that appears separated from surrounding cells. The cytoplasm is filled with a finely-granular substance and viral particles arranged on the periphery. X 5900**

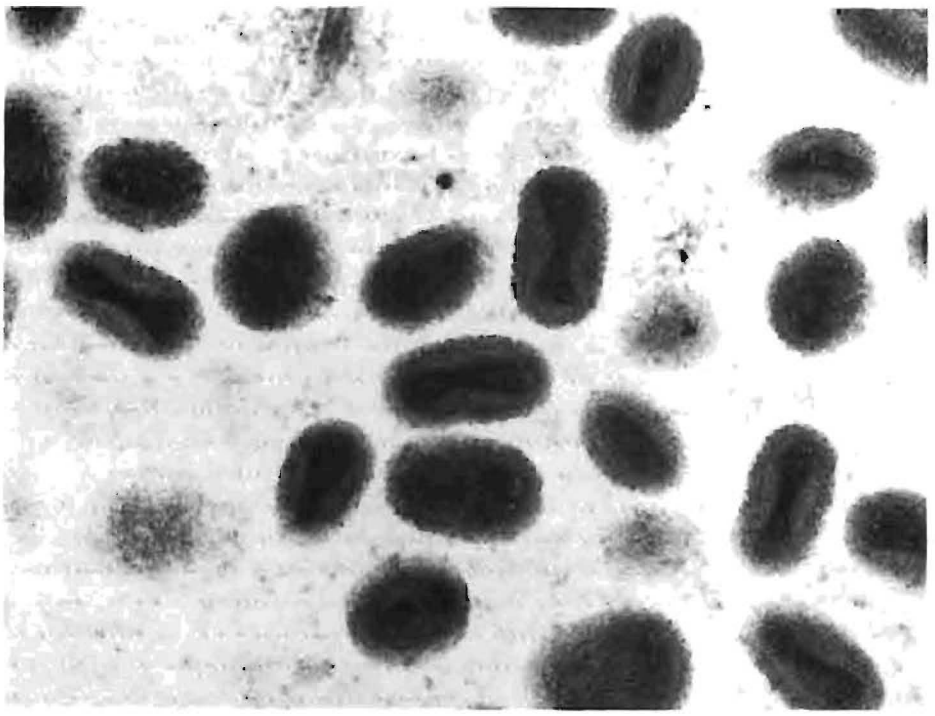

Fig. 6: Typical pox virus particles in the cytoplasm of a stratum spinosum cell. X 59000

phocytes in areas below the affected epidermal cells.

In the early lesions affected, stratum spinosum cells appeared to have withdrawn their cytoplasmic processes and to have separated from each other. Fragmented tonofilaments were scattered in the intercellular space. The cytoplasm of the cells was replaced by viral particles interspersed with a fine granular substance (Fig. 5). Affected cells in older lesions, and stratum spinosum cells situated closer to the skin surface, were rounded off and the cytoplasmic processes and tonofilaments were indistinct. The cytoplasm of these cells was filled with many viral particles and very little granular substance. The loose ribosomes and mitochondria which would normally have been present in the cell cytoplasm were not seen in the cells infected with viral particles. The viral particles had the typical appearance of pox viruses. The inner core consisted of a dumb-bell shaped electron-dense structure which was surrounded by a less electron-dense laminated capsule. The mature viral particles measured 150x250 nm (Fig. 6).

Four successive blind passages at approximately 14 d intervals were carried out on all the kidney cell monolayers, and cultures were discarded when no cytopathogenic effect was observed. Similarly, 5 CAM passages were carried out and discontinued when no obvious pock lesions were obtained. Some pock-like lesions were initially seen but could not be maintained by further egg passages. Furthermore, the baby mice did not develop any pock lesions and no deaths occurred.

DISCUSSION

The overall appearance of the lesions in these 3 horses suggests that we were dealing with the condition known as "molluscum contagiosum"[2 9 10 12]. Comparable macroscopic characteristics are poorly-contagious cutaneous eruptions of a number of months to years duration and discrete circumscribed elevated lesions with a waxy appearance[2 9 10 12 13]. Microscopic features are well demarcated foci of epidermal hyperplasia where affected prickle cells are prominently swollen and contain large intracytoplasmic inclusions, also known as "molluscum bodies"[2 10]. As the cells mature and move towards the surface, the inclusions grow in size and density and become more basophilic. The stratum corneum contains keratinocytes with deep purple molluscum bodies that exfoliate in groups[2 10 13]. With the electron microscope, many pox virions were seen within affected cells, the viral particles were similar to those previously described for molluscum contagiosum[2 9 12]. The size of the virions was similar to that reported by Cooley et al.[2], but smaller than that found by Moens & Kombe[9] and Rahaley & Meuller[12].

The skin condition under discussion is indistinguishable from Uasin Gishu skin disease of horses described in Kenya[4-7]. In Uasin Gishu skin disease, single animals were affected, the lesions were present for 12 to 30 months in some horses and lesions in various stages of development were present on the same animal at one time. The distribution of the lesions and the macroscopic and microscopic appearance were identical to those in our cases[5 7]. Kaminjolo and his co-workers succeeded in culturing the pox virus responsible for Uasin Gishu skin disease[4 6], but we were unable to grow the virus from available material.

Failure to isolate any virus from the scab material might have been due to insufficient viable virus particles being present in the field samples, the presence of a virus other than the vaccinia-related horsepox of Kaminjolo et al[4], and the pox virus particles demonstrated could have been the morphologically-similar pox virus of molluscum contagiosum[2 12] which has to date, not been isolated in tissue culture.

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