SEQUENTIAL PATHOLOGICAL CHANGES IN TURKEYS EXPERIMENTALLY INFECTED WITH CHICKEN POX VIRUS

Muhammad Mubarak and Muhammad Mahmoud
Department of Pathology,
Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

ABSTRACT

A total of 25, 4-weeks old, turkey poults were used in the present study. Birds were inoculated by chicken pox virus at the dose of $3 \times 10^{14}$/ml. Skin biopsy samples were taken sequentially from the same inoculated bird at 12 and 24 hours and at 2nd, 3rd, 4th, 5th, 7th, 10th, 14th and 21 days post inoculation (PI). Tissue samples from upper respiratory and digestive tracts were also collected. Pox cytoplasmic inclusions (Bollinger bodies) were detected between 4 and 7 days PI in epidermal the cell as well as in the follicular and sinus epithelium. Proliferative and necrobiotic epithelial changes were observed. Thereafter, pox inclusions disappeared with the appearance of vesicular, pustular and ulcerative lesions. This was accompanied by the gradual development of granulation tissue and finally scar tissue formed. Ultrastructure of the inclusion bodies and fine changes of the affected epidermal cell were illustrated. It was concluded that the inoculated chicken pox virus is highly pathogenic for turkeys. Taking sequential biopsy samples from the same inoculated bird was found to yield more accurate follow up of the pox skin lesions.

Keywords: Turkeys, chicken pox virus, pathological changes, cytoplasmic inclusions (bollinger bodies).

INTRODUCTION

Pox is a common viral disease of domestic birds (chickens, turkeys, pigeons and canaries). Fowl pox was reported as a clinical disease as early as 1906 and it was recorded as a disease entity since that time (Tanizaki et al., 1989). The term “Fowl pox” is now applied to a disease of commercial birds such as chickens and turkeys (Tripathy and Cunningham, 1984). Both sexes of birds at all ages are susceptible to avian pox viruses, which are DNA viruses belonging to genus “Avipoxvirus” of the family “Poxviridae”. Pox infection is contracted by contact and birds with skin injuries (scratches and wounds), on comb, wattles or other unfeathered areas, develop the natural pox lesions due to contamination with the environmental virus (Minbay and Krier, 1973). In natural cases of fowl pox (FP), the characteristic lesions are observed on the unfeathered cutaneous areas or on the mucus membranes of mouth and upper respiratory tract (Tripathy and Cunningham, 1984; Oros et al., 1997). Cutaneous pox lesions are of proliferative nature and on the mucus membranes they appear as diphtheritic plagues (Tripathy and Cunningham, 1984). The present study was carried out to demonstrate the sequential pathological changes of turkeys infected with chicken pox virus. The pathological changes were studied in the same inoculated birds to follow up accurately the development of these changes.

MATERIALS AND METHODS

Birds

A total of twenty five, 4-weeks old, turkeys obtained from local governmental farm were used in the present study.

Virus

The virus (chicken pox virus) was originally isolated from the skin lesions on the heads of naturally infected chickens. The diluted virus suspension was inoculated into the chorioallantoic membranes (CAMs) of 10-days old chicken embryos. The inoculated membranes were collected 7 days later. A 10% suspension of the infected CAMs was prepared and considered as the stock virus inoculum. The virus inoculum was titrated and found to contain approximately $3 \times 10^{14}$ virus particles/ml. The stock inoculum was stored at $-20^\circ$C.

Experimental design

The experimental birds were kept for one week for the purpose of acclimatization. At 5 weeks of age, the experimental birds were divided into 2 groups, the first
group contained 20 birds and used for virus inoculation. The second group had 5 birds and served as control. The two experimental groups were kept in separate rooms. Stock virus inoculum of 0.05 ml was injected intradermally in the scalps of the birds in the first group. Feed and water was supplied ad libitum during the period of the experiment. Birds died during the experiment period were necropsied.

Histopathology
Skin lesions developed on the heads or elsewhere of the inoculated birds were collected. Samples from the skin, mucus membranes of the mouth and upper respiratory tract, pharynx, sinuses, esophagus and trachea were collected at 12 and 24 hours and 2, 3, 4, 5, 7, 10, 14 and 21 days post inoculation (Pl). In addition, samples from the internal organs were also collected. At each time interval, the skin biopsy samples were collected from the same inoculated birds. Tissue samples were fixed in 10% neutral buffered formalin and processed routinely for paraffin embedding technique. Embedded tissue samples were sectioned at 3μm and stained with haematoxylin and eosin (HE).

Electron microscopy
Samples from the dermal lesions and other tissues obtained from the inoculated birds were immediately immersed in 2.5% buffered glutaraldehyde and then post fixed in osmium tetroxide. Tissue samples were dehydrated in ascending grades of ethanol and embedded in Epon 812. Semi thin sections were prepared and stained with 1% toluidine blue. After orientation of the tissue blocks, thin sections were obtained and double stained with uranyl acetate and lead citrate and examined under the transmission electron microscope (Jeol, 100 CX II) operated at 80 KV.

RESULTS
Clinical signs and mortality
From two days Pl, all the inoculated birds were depressed and their feed and water intake were decreased. Thereafter, feed and water intake of birds with oral lesions was decreased obviously. No mortality was recorded during the course of the experiment.

Gross pathology
Up to 3 days Pl, only hyperaemia (erythema) at site of inoculation was observed. At 4 days Pl, small slightly elevated dermal lesions were observed at site of inoculation and these lesions were slightly tender. Between the 5th and 7th days Pl, more elevated and more tender lesions (papules) were observed and their distribution was not restricted to the site of inoculations. The papular lesions were seen on the combs, wattles, snoods, mouth corners and eye lids (unfeathered areas). On the 10th day Pl, more larger and less tender vesicular lesions were noticed and their distribution was the same as of the papular ones, in addition to legs and feet. Up to 14 days Pl, coalesced (confluent) lesions were observed at the previous sites. The latter lesions appeared as yellowish eruptions (pustules) containing sticky material and when removed, subjacent inflamed dermal areas were seen.

Between 15 and 21 days Pl, the lesions were desiccated and most of them were covered with dry scabs (crust formation) which in some birds dropped off naturally. After detachment of the crust, the underlying dermal tissue was rosy (granulation tissue) and signs of healing were apparent.

From 10 days Pl, some birds (8, 40%) developed mouth lesions which appeared as raised whitish plaques on the oral mucus membranes. These birds at necropsy showed cheesy diphtheritic membrane in the trachea and sticky yellowish material in the turbinates. No gross lesions were detected in other internal organs of any of the inoculated birds. No detectable gross abnormalities were found in the control birds.

Histopathology
Skin samples examined at 1 and 2 days Pl, showed severe dermal hyperaemia associated with edematous changes of the superficial dermis. At that time, only swelling of the epidermal cells (spinous cells) was observed. By the third day Pl, epidermal hyperplasia was noticed and represented by proliferation of the bristle cells (intermediate layer of epidermis) associated with basophilia of the stratum basalis cells. The nuclei of the hyperplastic cells were hyperchromatic. The number of the intra epidermal lymphocytes was increased. Feather follicles also showed proliferative changes in their lining epithelium. The feather follicular epithelium manifested similar changes to that of the hyperplastic epidermal cells.

Acidophilic cytoplasmic inclusions were detected in the stratum spinosum cells by the 4th day Pl. The inclusions were of various sized (Fig. 1) and some of them occupied most of the epithelial cell cytoplasm (Fig. 2). The acidophilic type inclusions were frequently vacuolated (Fig. 3) and in many cells they were fragmented (Fig. 4 and 5). In epidermal lesions, the Bollinger bodies (inclusion bodies) were confined to the bristle cells. The overlying granulosum cell layer was compressed and showed hypergranulation.
Beside the epidermal cell, inclusion bodies were also detected in the feather follicular epithelium (Fig. 6). The inclusions were also noticed in the tracheal epithelial cells and in the infraorbital sinus (Fig. 7). The number
of the epithelial inclusions was noticeable until day 7 PI. At that time, epidermal cell proliferation was obvious (Fig. 8). The degree of cellular degenerative changes was correlated with the size of inclusions and epithelial cells with larger inclusions showed advanced necrotic changes.

Samples of the skin examined at 7 and 10 PI showed epidermal vascular lesions. The vesicles varied from microsized, formed by the coalescence of ruptured 3-4 baloned spinous cells (Fig. 9), moderate sized (Fig. 10) to large sized which occupied considerable area of the epidermis. The large vesicles contained remnants of serous material and various numbers of heterophils (Fig. 11). Pustular epidermal lesions were also noticed (Fig. 12).

By 10 days PI, the epithelial inclusions (either epidermal or follicular) disappeared and skin samples examined at that time showed remarkable desquamative changes. At many sites, skin samples were ulcerated (Fig. 13). The dermal tissue lining the ulcerative lesions was severely hyperemic and had marked heterophil cell infiltration.

Most of the samples examined at 14 days PI showed accumulated tissue debris, admixed with heterophils at, overlying active granulation tissue in the dermis. At 21 days PI, rough or smooth surfaced scars were usually seen the smooth surfaced scars were covered by active layer of epithelial cells (epithelialization).

Affected feather follicles disclosed similar progression of desquamative and necrotic changes. The necrotized follicles were infiltrated by heterophils which were admixed with epithelial debris. Tracheal tissues examined at the last 3 intervals (10, 14 and 21 days PI) showed the development of fibrin-necrotic membranes which were arisen over a necrotized epithelium. Sinuses examined after the disappearance of inclusion bodies had marked desquamative changes of their epithelium and some were filled with debris and degenerated or necrosed heterophils.

No histological changes were detected in other organs or tissues. No comparable microscopic findings were observed in the control birds. Table 1 shows summary of the encountered pathological cutaneous lesions.

**Electron microscopy**

Samples examined along the first 3 days PI showed epithelial hyperplasia in the epidermis evidenced by the dense chromatin clumps in the epithelial cell nuclei which were indented. Many of the epidermal cells (spinosum cells) were swollen and had irregular outlines. The desmosomal junctions of these cells were detached (Fig. 14) and this reflected in the increased intercellular spaces. Brickle cells had cytoplasmic vacuoles which contained particulate material and other cells had cytoplasmic foci of granular material.

Samples representing 4 and 7 days PI revealed the presence of cytoplasmic inclusions which were confined to sponsum cells. The inclusions varied in size and most of them contained lipid droplets (Fig. 15) and mature viral particles. In some brickle cells, there were free viral particles in their cytoplasm. The viral particles in some cells were seen as ovoid electron dense bodies, with indistinct or incomplete enveloping membranes. Cytoplasmic aggregates of mature viral particles were also observed (Fig. 16). The complete viral particles were composed of electron dense nucleoid separated by less dense zone from the external envelope. The mature viral particles measured approximately 130-250 nm. Within the inclusions, the configuration of the viral particles varied probably due to the sectioning level, either transversely or sagittally. Some epidermal cells contained cytoplasmic particles which were seen as ring forms or as dense ovoids (Fig. 17).

Lamellar structures and fine fibrils were also seen within some inclusions. Cytoplasmic vacuoles were frequent in the affected epidermal cell, and some inclusions had central vacuolar spaces. Some of the cytoplasmic vacuoles contained particulate material in association with electron dense structures which had tapered ends and seemed to be remnants of tonofibrils (Fig. 18). In general, the mature viral forms were noticed within the inclusions while the immature forms were observed outside the inclusions. Immature viral particles, with nucleoid and single enveloping membrane, were also detected in the epidermal intercellular spaces (Fig. 19). The enlargement of the epidermal cells was due to their contents, either the lipid filled inclusions or the large cytoplasmic vacuoles. Mitochondria within the affected epidermal cells were swollen and had disorganized cristae. The profiles of RER were dilated. Ribosomes were increased in number and clusters of polyribosomes were also observed. The nuclei of epidermal cells until 7 days PI were enlarged and had large nucleoli and margined chromatin.

Skin samples taken at 7 and 10 days PI showed the presence of infiltrating heterophils on background of degenerated and necrosed epidermal cells (Fig. 20). No viral inclusions were noticed at 10 days PI and thereafter. At 14 and 21 days PI, only activated fibroblasts were seen at sites of epidermal destruction.

Similar inclusions to those found in the epidermal cells were also observed in the feather follicular epithelium. No viral particles were seen in other organs and tissues. No comparable fine changes were noticed in the control birds.
### Table 1: Summary of the pathological cutaneous changes encountered in turkeys inoculated by chicken pox virus

<table>
<thead>
<tr>
<th>Time Post Infection</th>
<th>Gross</th>
<th>Pathological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Microscopic</strong></td>
</tr>
<tr>
<td>Hours</td>
<td></td>
<td>i) Dermal hyperaemia and edema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii) Epidermal and follicular hyperplasia</td>
</tr>
<tr>
<td>12</td>
<td>Erythema</td>
<td>iii) No inclusions</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tender elevated lesions</td>
<td>i) Cytoplasmic acidophilic inclusions in brick cells and in follicular and sinus epithelium</td>
</tr>
<tr>
<td></td>
<td>papules (some vesicles)</td>
<td>ii) Proliferative and necrotic epithelial changes.</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Vesicles (some ulcers)</td>
<td>i) Pastular and ulcerative lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii) No inclusions</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Pustules and ulcers</td>
<td>i) Pastular and ulcerative lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ii) Active granulation tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iii) No inclusions</td>
</tr>
<tr>
<td>21</td>
<td>Crusts</td>
<td>i) Scare tissue (healing stage)</td>
</tr>
</tbody>
</table>

### DISCUSSION

In the present study, we observed the sequential pathological changes in the same bird inoculated by pox virus by taking skin biopsy samples at different intervals PI. This was done for more accurate identification of the developmental stages of the cutaneous pox lesions in the inoculated turkeys. The recognition of all pox lesions, including erythema, papules, pustules and crusts in the present inoculated birds may indicate the high susceptibility of turkeys for the used chicken pox virus. The presently described progression of pox lesions in turkeys is similar to that reported on mammalian pox viruses (Cheville, 1966).

The described pathological changes conform with the reported proliferative nature of pox lesions (Swallen, 1963). The observed epithelial hyperplasia (epidermal, follicular and sinus epithelium) is probably a tissue response for viral replication. Some authors (Cheevers et al., 1968) suggested that the synthesis of the infectious viruses may follow the hyperplastic phase manifested by the infected cells.

In the present cases there was no evidence of viral replication or virus inclusions in the epidermal lesions by 10 days PI. In the previous study (Minbay and Kreir, 1973) the virus was isolated up to day 12 PI from chickens inoculated by chicken pox virus. This may indicate equal persistence of the chicken pox virus in chickens and turkeys.

The recognition of pox inclusions in the sinus epithelium may indicate the high susceptibility of this epithelium for pox virus infection. The evident histological changes in the sinus tissues may considered as reflection of the marked virus replication at this site. Pathological changes of the sinus tissues undoubtedly predispose the affected birds for other respiratory infections including those evoked by pneumopathogens.

We found by electron microscopy a well defined cytoplasmic inclusions containing aggregates of viral particles conforming with those observed by light microscopy. Some workers (Morgan et al., 1954) did not manage to demonstrate typical pox inclusions by electron microscopy. This may contradict the opinion that synthesis of viral particles occurs within cytoplasmic inclusions and then viral particles leave the inclusions and spread diffusely in cytoplasm (Sheek and Magee, 1961). However, Swallen (1963) by thymidine H labeling of pox infected epidermis concluded that virus replication takes place throughout cytoplasm and thereafter aggregated in cytoplasmic inclusions.

It has been suggested that pox inclusions may actually represent disintegration site for viral particles (Beaver et al., 1963 a and b). Cheville (1966 and 1967) observed only scattered pox virions within the cytoplasmic inclusions in the late stages of infection. In general, pox cytoplasmic inclusions are considered to be the main site for viral replication (Tajima and Ushijima, 1966; Chang and Jasty, 1970; Lchihashi et al., 1971; Shida et al., 1977; Sadasiv et al., 1985).

The other ultrastructural findings in the present study included dense ovoid bodies, ring forms and lamellar structures in the cytoplasm of infected epidermal cells. Beaver et al. (1963 b) presumed that
Fig. 1: Pox cytoplasmic inclusion of various sizes in the proliferated epidermal spinosum cells which exhibit dense cytoplasmic staining. Some cells are obviously vacuolated. Turkey inoculated by chicken pox virus. 4 days PI. Toluidine blue stain X 125.

Fig. 2: Large pox cytoplasmic inclusions (arrow) occupying most of the cytoplasm of brillok cells which are swollen (ballooned). Turkey inoculated by chicken-pox virus. 4 days PI. Toluidine blue stain. X 200.

Fig. 3: Vacuolated cytoplasmic inclusions (arrow) in spinosum cells which have foamy cytoplasm. Turkey inoculated by chicken pox-virus. 4 days PI. HE. X 200.

Fig. 4: Fragmented (arrow) and vacuolated (arrowhead) Bollinger inclusions in spinosum cells which have pyknotic nuclei. 4 days PI. HE. X 200.

Fig. 5: Swollen spinosum cells containing fragmented cytoplasmic pox inclusions (arrow). 4 days PI. HE. X 200.

Fig. 6: Acidophilic type pox inclusions (arrow) in the feather follicular epithelial cells which are swollen and vacuolated. The perifollicular dermal tissue is infiltrated by heterophils. 4 days PI. HE X 120.
Fig. 7: Pox acidophilic cytoplasmic inclusions (arrows) in the epithelium of infra orbital sinus. The underlying lamina propria is heavily infiltrated by heterophils. 4 days PI. HE X 325.

Fig. 8: Intra-epidermal proliferative epithelial lesion (*). The proliferated cells have densely stained cytoplasm which contains inclusions. The surrounding spinous cells have less dense cytoplasm and lesser number of inclusions. 7 days PI. Toluidine blue stain. X 200.

Fig. 9: Epidermal microvesicles (mv) formed by the coalescence of few ruptured spinous cells. The remaining spinous cells contain fragmented inclusions. 7 days PI. HE X 200.

Fig. 10: Large sized vesicles (*) extending from the mid layer of the basal layer epidermis. Heavy heterophilic cell infiltration is seen in the underlying dermis. 7 days PI. HE X 200.

Fig. 11: Large epidermal vesicles (LV) formed at the site of numerous ruptured cells. The vesicles contain remnants of serous fluid. The underlying superficial epidermis is infiltrated by large number of heterophils. 7 days PI. HE X 125.

Fig. 12: Pustular epidermal lesion (P). The pustule contains large number of heterophils. The surrounding spinous cells have smaller inclusions. 7 days PI. HE X 200.
Fig. 13: Ulcerative skin lesion (arrow). The site of ulceration is markedly infiltrated by heterophils. Granulation tissue is seen extending into the ulcerative lesion. 10 days PI. HE. X 200.

Fig. 14: Transmission electron micrograph showing detachment and destruction of the desmosomal junctions (arrow) between the epithelial cells. The cytoplasm of epithelial cells contains only sparse organelles. 3 days PI. X 4000.

Fig. 15: Cytoplasmic inclusion, in a bristle cell, containing lipid droplets (L), large vacuoles (V) and particulate material. Lysosomal structures (arrow) are also seen. Transmission electron micrograph. 4 days PI. X 4000.

Fig. 16: Dense cytoplasmic aggregate of mature viral particles (*) in a bristle cell. In between the viral particles, filamentous material (F) is dispersed. Transmission electron micrograph. 4 days PI. X 14000. Inset: Mature viral particles, each is composed of electron dense nucleoid (small arrow), lateral bodies (large arrow) and an external limiting membrane (arrowhead). X 32000.
Fig. 17: Dense ovoids (arrowhead) and ring like structures (arrow) within the cytoplasm of a bristle cell. Organelles are markedly destructed. Transmission electron micrograph. 7 days PI. X 4000.

Fig. 18: Cytoplasmic vacuoles containing numerous tapered electron dense structures (arrow) which seem to be remnants of cytoplasmic tonofilaments. The nucleoli (arrowhead) of bristle cells nuclei are prominent. Transmission electron micrograph. 7 days PI. X 2700.

Fig. 19: Intercellular immature viral particles (arrow) each is composed of electron dense nucleocid and single enveloping membrane. Transmission electron micrograph. 7 days PI. X 10000.

Fig. 20: Heterophil (H) infiltrating necrosed epidermal cells. A large lysosomal structure (arrowhead) is seen. Transmission electron micrograph. 10 days PI. X 5000.
the dense ovoid structures are related to viral replication while Cheville (1967) considered them as early viral forms.

Presently, lipid droplets were frequently found in the infected epidermal cells and within the viral inclusions. The consistent finding of these lipid droplets led to the suggestion that these lipid materials may represent viral precursor materials required for virus synthesis. One third of the chemical composition of pox virus was found to be lipid material and extractable lipids constitute 50% of the components of pox inclusions (Randall et al., 1964).

The currently demonstrated extracellular viral forms resemble the intermediate viral forms described by Cheville (1967) which were composed of inner core surrounded by an external coat. These extracellular viral forms may release after cell disintegration or represent budding forms after acquiring a coat from the cell membrane. Pox viruses are known to be occluded into inclusions or they leave intact cells by passing directly through cell membrane. Virions which bud from cell membranes usually differ in shape and density from those remaining intracellular (Krempien et al., 1981; Sadasiv et al., 1985).

Conclusively, the used chicken pox virus was found to be highly pathogenic for the used turkey pouls. It is also concluded that development of pox lesions in turkeys is similar to that reported in chickens. The procedure of taking skin biopsy samples from the same inoculated bird approved to be an accurate method to follow up the pox lesions.

REFERENCES


