Ostrich Pox Virus Infection in Farms at Some Northern Egyptian Governorates

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ABSTRACT

This work was aimed to study the epidemiology and possible control ways of ostrich pox virus in Egypt during the period from 2011-2012. For this purpose, virological examination of 33 skin samples from 429 diseased ostriches and their premises (12 samples from feather and 10 samples from mosquitoes). Samples were collected from diseased ostriches of all ages suffered from dry and wet pox lesions from 11 ostriches farms at Sharkia, Kalubia and Ismailia Governorates and used for isolation of ostrich pox virus. The diseased ostriches were subjected to clinical, postmortem, serological and histopathological examinations. All pock lesion induction samples were positive for agar gel precipitation test (AGPT) for pox virus. Experimental infection of 6-week-old turkey poults by the isolated ostrich pox virus (1x10⁵ PFU/ml) from skin lesions, (1 x 10⁷.³ PFU/ml) from feathers and (1.5 x 10⁷.⁵ PFU/ml) from mosquitoes revealed high pathogenicity to dry and wet lesions, histopathological changes and sero-conversion. Experimental infection of 9-week-old ostrich previously vaccinated at 6-week-old with live attenuated fowl pox vaccine revealed no clinico-pathological effects while the experimentally infected unvaccinated ostriches revealed high pathogenicity to dry lesions. In conclusion, early vaccination of ostriches with live attenuated fowl pox vaccine at 6-week-old with the control of mosquitoes prevented the appearance of natural ostrich pox virus infection.

1. INTRODUCTION

Avian pox virus infection is a common viral disease of many species of birds. Natural pox virus infections in birds have been reported in 232 bird species in 23 orders (Bolte et al., 1999). Members of poxviridae are very large DNA viruses with a host spectrum including birds, mammals, reptiles and some arthropods (Bolte et al., 1999). The disease occurs in different avian species and the causal agent has been classified as fowl, turkey, pigeon, canary, junco, quail, sparrow and starling poxvirus according to the species from which it was first isolated (Esposito et al., 1991).

Avian pox viruses are antigenically and immunologically distinguishable from each other and antigenic relationship exists between various strains. But the degree of antigenic identification between strains is still not clear (Bolte et al., 1999).

Avian pox is slow spreading disease characterized by the development of discrete nodular proliferative skin lesions on non-feathered parts of body and it called cutaneous form and also fibrino-necrotic and proliferative lesions on mucous membrane of the upper respiratory tract, mouth and esophagus called Diphtheritic form.

Fowl pox is widespread in Egypt in all bird species leading to more economic losses in production (Abozaid, 2007). A concurrent systemic infection may also occur (Deoki et al., 2008).

In ostriches, pox virus is transmitted by mosquito bites and poultry. Wild birds can be a source of infection to ostriches. The dry form of the disease produces wart-like lesions around beak and eyes in ostriches. In severe cases it causes eyes to close up while the wet form produces pseudomembrane in the buccal cavity, pharynx and larynx causes severe respiratory problems and can interfere with feed intake. The course of the disease can be protracted one month or longer (El-Gohary, 2002).

Ostrich pox is reported in many countries overall the world. In South Africa, ostrich pox was noticed in 2 flocks showing typical oesinophilic intracytoplasmic inclusion bodies in the histopathological tissue sections and pox virus was isolated from the lesions. A commercial fowl pox vaccine was used to protect young ostrich (Allwright et al., 1994).

In Egypt, ostrich pox was reported in many ostrich farms in Gharbiya and Kalubya Governorates leading to high mortality in young
birds (2–6-week-old). The isolated virus produces typical fowl pox lesions in turkey and pock lesions on CAM of inoculated fertile eggs by the isolated virus and eosinophilic intracytoplasmic inclusion bodies in histopathological sections (El-Gohary, 2002).

The isolated poxvirus from ostriches had similar antigenic, genetic and biological properties of fowl pox virus (Shivaprasad et al., 2002).

This work was aimed to study the epidemiology of poxvirus disease in ostrich farms, isolation and identification of the virus, the pathogenicity of the isolated strains, histopathological findings of the infected birds with the isolated virus strains and trials for prevention and control through the use of commercial fowl pox vaccine to protect ostrich chicks from infection by pox virus.

2. MATERIALS AND METHODS

2.1. Samples:

Thirty three skin samples from 4–9-week-old diseased ostriches, 12 feather samples and 10 samples from mosquitoes. Samples were collected from 429 ostriches suffered from dry and wet pox lesions from 11 ostriches farms at Sharkia, Kalubia and Ismailia Governorates and used for isolation ostrich pox virus. The details on the history of examined ostrich farms were given in table (1).

2.2. Embryonated chicken eggs:

Commercial 9–12-day-old SPF embryonated chicken eggs (ECE), obtained from "SPF Production Project, Kim Oshim, El-Fayoum" used for isolation, propagation and titration of ostrich pox virus and fowl pox vaccine.

2.3. Experimental birds:

Six-week-old turkey poultst obtained from El-Auroba farm and 6-week-old ostrich chicks from El-Monier farm were used for experimental infection with the isolated ostrich pox virus.

2.4. Vaccine:

Live attenuated fowl pox vaccine (titer 10^7) kindly provided by Serum and Vaccine Production Research Institute (SVPRI), Abbasia, Cairo was used for vaccination.

2.5. Fowl Pox Virus and antiserum:

Fowl pox virus (titer 10^6) as control and fowl pox antiserum kindly provided by SVPRI, Abbasia, Cairo, were used in agar gel precipitation test (AGPT).

2.6. Virus Isolation:

The collected nodules, scabs, feathers and mosquitoes were cut by a sterile scissors into small pieces and ground with sterile sand in a sterile mortar then frozen as 10% suspension in normal saline. The suspension was centrifuged at 3000 rpm for 10 minutes and the supernatant fluid was collected. Penicillin and streptomycin were added to the supernatant with concentration of 1000 IU and 1 mg/ml, respectively. The suspension was left at room temperature for 30-60 minutes before inoculation. ECE 9-12-day were inoculated with 0.1 ml of the prepared suspension through the chorioallantoic membrane (CAM), then incubated at 37°C for 5-7 days and examined daily by candling. Death of embryos recorded within the first 24-48 hours, were excluded as non-specific. Further three passages were carried out before titration and identification of the isolated viruses.

2.7. Virus titration:

Ten-fold serial dilutions of the virus isolates in sterile PBS at pH 7.2. Four, 9-12 days, ECE were used for each dilution and 0.1 ml of each dilution was inoculated by CAM in each egg. The inoculated eggs were incubated at 37°C with 80% humidity for 5 days. Control egg was inoculated with 0.1 ml sterile BPS. CAMs of the inoculated eggs were harvested and examined by naked eyes for observing the pock lesions. The infectivity titers of each virus were determined according to El-Gohary, (2002).

2.8. Virus identification:

The identification of the isolated virus was investigated by agar gel precipitation test (AGPT) using specific fowl pox antiserum according to White (1985).

2.9. Histopathological Examination:

Lesions of skin and CAM were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, sectioned at 4 µm, stained with hematoxylin and eosin (H&E) stain and examined by light microscopy according to Bancroft and Gamble, (2007).

2.10. Experimental infection:
2.10. 1. Experiment (I):

Eighty five, 6-week-old turkey poults were classified into 4 groups. The 1st group of 10 turkey poults was inoculated intradermally with 0.2 ml of sterile BPS and kept as control. The 2nd group of 25 turkey poults was intradermally inoculated with 0.2 ml of 10% CAM homogenate prepared from skin lesions, the 3rd group of 25 turkey poults was intradermally inoculated with 0.2 ml of 10% CAM homogenate prepared from feathers and the 4th group of 25 turkey poults was intradermally inoculated with 0.2 ml of 10% CAM homogenate prepared from mosquitoes. The clinical signs and other pathological findings during the observation period (3 weeks) were recorded.

10. 2. Experiment (II):

To investigate the effect of vaccination with fowl pox vaccine on the prevention of ostrich pox virus infection in ostrich chicks, Six-week-old ostrich chicks were classified into 2 groups of 3 ostrich chicks each. The 1st group was vaccinated with 0.02 ml of diluted live attenuated fowl pox vaccine by wing webbing and the 2nd group was remained without vaccination as control. After 3 weeks the two groups were intradermally challenged with 0.2 ml contained (1 x 10^6 PFU/ml) of pox virus naturally isolated from ostrich skin lesions, birds remained under observation for 3 weeks during which the clinicopathological findings were recorded.

RESULTS

3.1. Clinical signs and gross lesions:

Naturally infected 4–9-week-old ostrich chicks suffered from weakness, dullness, weight loss, anorexia with skin lesions and 3.2% mortality. The course of the disease was 10-14 days and 25% of the chicks were clinically affected with closer serous fluid on the eyelids and around the external ear opening which developed into scabs or dry crusts. Also discrete brown wart-like nodules were seen on the eyelids, around the eyes, ears, on the commissures of the beaks and on the skin of head and neck (dry form infection) (Fig. 1). In some affected birds, large crusts developed on the eye lids, resulting in total closure of the eyes. The proliferative lesions were extended to the mouth cavity caused pharyngitis and trachitis (wet form infection). Some nodules grew to relatively large size (0.5-2 mm in diameter) and developed on ulcerated and crusty surface (Fig. 2). Pock-like lesions in surviving ostrich healed within 2 weeks. No characteristic gross lesions, beyond the skin lesion could be found on autopsy of dead bids.

3.2. Isolation of ostrich pox virus:

The results of isolation of ostrich pox virus from skin lesions, feathers and mosquitoes collected from naturally infected ostrich chicks on CAMs of ECE were described in Table (2). After 4 egg passages, 33 skin lesion samples, 8 feather samples and 6 mosquito samples were positive with percentage of 100%, 66.67% and 60%, respectively which induced grayish white localized and diffused pock lesions on CAMs at 5th day post inoculation. Pock lesions in first two passages appeared small in size (1 mm in diameter) (Fig. 3). In the 3rd and 4th passages these lesions appeared large in size (2-3 mm in diameter). Some lesions coalesced to each other and formed wide areas of white coloration and thickness especially at the site of inoculation. CAM after the 4th passage became lacerated and pock lesions appeared scattered all over the membrane. The number of dead embryos was increased with the number of passages due to increase of virulence of virus.

3.3. Identification of ostrich pox virus:

The results of ostrich pox virus identification by AGPT were described in Table (3) and Fig. (4). The number of positive samples from skin lesions was 33 out of 33 (100%), 8 out of 12 (66.6%) from feather samples and 6 out of 10 (60%) from mosquitos.

3.4. Histopathological examination:

The skin of naturally affected ostrich chicks showed variable stages of cutaneous pox. These stages may be papillomatous growth from hyperplastic epidermal cells. The latter cells suffered from accenthosis or vesiculation or May progressed to crust from necrotic keratinocytes (Fig. 5). Sometimes, crust associated with hyperkeratosis in some birds. The superficial epidermis may be ulcerated or showed variable degrees from regenerations after sloughing of crusts beside degenerative and necrosis of feather shaft. The epithelial cells of both stratum epimous and feather follicle revealed hyperplasia and ballooning degeneration or necrosis with presence of solid eosinophilic cytoplasmic inclusions (Bollinger's bodies). The majority of these inclusions revealed central pale zone (ring shaped inclusions).
Table (1): History of avian pox virus infection of investigated ostrich farms

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>Locality</th>
<th>Districts</th>
<th>Farms Name</th>
<th>No of occupied bird</th>
<th>No of diseased bird</th>
<th>Specimen</th>
<th>Date</th>
<th>Age/week</th>
<th>Mortality during sampling day</th>
<th>Vaccination programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sharkia</td>
<td>Mashtol A. Ismail</td>
<td>15</td>
<td>15</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4/3/2011</td>
<td>4</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c 12 day</td>
</tr>
<tr>
<td>2</td>
<td>Sharkia</td>
<td>Mashtol Monier V.</td>
<td>42</td>
<td>22</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>14/4/2011</td>
<td>5</td>
<td>HB1(eye drop 8 day) 0.5 ml inactivated ND+AI s/c 13 day</td>
</tr>
<tr>
<td>3</td>
<td>Belbis</td>
<td>Al-Adlia Society</td>
<td>30</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>16/7/2011</td>
<td>6</td>
<td>HB1(eye drop 8 day) 0.5 ml inactivated ND+AI s/c 13 day</td>
</tr>
<tr>
<td>4</td>
<td>Belbis</td>
<td>Saad Goda</td>
<td>12</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>12/8/2011</td>
<td>8</td>
<td>HB1(eye drop 9 day) 0.5 ml inactivated ND+AI s/c13day</td>
</tr>
<tr>
<td>5</td>
<td>10th of Ramadan</td>
<td>El-Toka</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7/9/2011</td>
<td>6</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c1day</td>
</tr>
<tr>
<td>6</td>
<td>Ismailia</td>
<td>Abo Swer M. Monior</td>
<td>46</td>
<td>29</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>12/10/2011</td>
<td>7</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c15day</td>
</tr>
<tr>
<td>7</td>
<td>Kalubia</td>
<td>El-Kasasi Army</td>
<td>620</td>
<td>174</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>10/4/2010</td>
<td>7</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c1day</td>
</tr>
<tr>
<td>8</td>
<td>Kafr El-Kabeer</td>
<td>Al Mastria E</td>
<td>260</td>
<td>62</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>12/5/2012</td>
<td>9</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c15day</td>
</tr>
<tr>
<td>9</td>
<td>Banha</td>
<td>El-Kasabi</td>
<td>120</td>
<td>81</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>22/7/2012</td>
<td>5</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c1day</td>
</tr>
<tr>
<td>10</td>
<td>Toukh</td>
<td>El-Mofty</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>30/8/2012</td>
<td>7</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c1day</td>
</tr>
<tr>
<td>11</td>
<td>Shebeen</td>
<td>El-Kanater Marof</td>
<td>20</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3/9/2012</td>
<td>5</td>
<td>HB1(eye drop 7 day) 0.5 ml inactivated ND+AI s/c16day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>1193</td>
<td>429</td>
<td>33</td>
<td>12</td>
<td>10</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. RESULTS

Moreover, dermal tissue around feather follicle may exhibit edema, hemorrhage and dark brown hemosiderin pigments (Fig. 6). Occasionally, fibroblastic proliferation and fibrous were detected in areas of old lesions. The underlying musculatures showed hyalinization, hemorrhages and local Zenker's necrosis with few lymphocytic infiltrations. In some cases the dermal collages was hyalinized and locally covered by single layer of regenerated epithelium.

CAM showed hyperplasia and hypertrophy of lining epithelium with eosinophilic intracytoplasmic inclusion bodies (arrow) and edema in the mesenchyme. The high power showed edema, congested capillaries and extravasated erythrocytes on mesenchyme with rounded eosinophilic intracytoplasmic inclusion bodies (arrows) in CAM infected cell and large amounts of inflammatory cells (Fig. 7).

3.5. Experimental infection:

The results of experimental infection of turkey poult with homogenate prepared from skin lesions, feathers and mosquitos of naturally infected ostrich chicks were described in Table (4). The clinical signs of experimentally infected birds including depression, dullness, anorexia, retardation of growth and skin nodular lesions appeared as small papules and vesicles developed at the site of inoculation in all infected birds during 5th days post infection (PI).
By the day twelve PI, dry scabs and pox lesions spread over the skin of head and neck (Fig. 8). The morbidity was up to 100%. No mortality was recorded. Gross lesions beyond the skin lesion were seen. Some turkey poults revealed caseated materials on the canthus of the mouth, upper and lower jaws, on the hard palate and on the tongue (Fig. 9). Histopathological examination of skin lesions and scabs from experimentally infected turkey poults revealed large number of eosinophilic intracytoplasmic inclusion bodies and large number of ballooned cells in the epidermis (Fig. 10). Seroconversion for avian pox virus by AGPT at 15 day PI revealed positive results for all infected birds. Re-isolation of the virus from experimentally infected birds was detected by ECE inoculation through CAM and histopathological examination of CAM revealed rounded eosinophilic intracytoplasmic inclusion bodies.

3.6. Vaccination:

The protection results of ostrich chicks against ostrich pox virus infection by using fowl pox vaccine were summarized in Table (5). Five days post inoculation, the unvaccinated ostrich chicks showed clinical signs including depression, dullness, anorexia, retardation of growth and nodular skin lesions appeared as small papules and vesicles developed at the site of inoculation, on the day twelve of post inoculation, dry scabs and pox lesions spread over the head and neck. While the infected vaccinated ostrich chicks revealed no any clinicopathological findings.

Seroconversion for avian pox by AGPT on the 15th day PI proved positive for 2nd group (Fig.11). Re-isolation trial from infected birds from 2nd group were succeeded through ECE inoculation in CAM.

CAM was examined histopathologically for presence of rounded eosinophilic intracytoplasmic inclusion bodies.

### Table (2): Isolation of pox virus from ostrich skin lesions, feathers and mosquitos on CAM of ECE

<table>
<thead>
<tr>
<th>No. of passages</th>
<th>Pathogenicity in ECE</th>
<th>Pock lesions in CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Dead embryos from Skin lesion samples</td>
<td>No. of Dead embryos from Feather samples</td>
</tr>
<tr>
<td>1st</td>
<td>0/33</td>
<td>0/12</td>
</tr>
<tr>
<td>2nd</td>
<td>4/33</td>
<td>0/12</td>
</tr>
<tr>
<td>3rd</td>
<td>9/33</td>
<td>3/12</td>
</tr>
<tr>
<td>4th</td>
<td>20/33</td>
<td>5/12</td>
</tr>
</tbody>
</table>

### Table (3): Results of ostrich pox virus identification by AGPT

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Type of samples</th>
<th>Total No. of samples</th>
<th>Antiserum</th>
<th>No. of positive samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin lesions</td>
<td>33</td>
<td>Fowl pox antiserum</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Feathers</td>
<td>12</td>
<td>Fowl pox antiserum</td>
<td>8</td>
<td>66.6</td>
</tr>
<tr>
<td>3</td>
<td>Mosquitoes</td>
<td>10</td>
<td>Fowl pox antiserum</td>
<td>6</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. (1): Naturally infected 8-week-old ostrich with cutaneous pox lesion around eyes

Fig. (2): Naturally infected ostrich chicks showed severe lesion in buccal cavity
Table (4): Experimental infection of turkey poults with ostrich pox virus isolates

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Type</th>
<th>Age/week</th>
<th>No.</th>
<th>Source of Inoculum</th>
<th>Route</th>
<th>Dose</th>
<th>Titer</th>
<th>Evaluation of pathogenicity test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Turkey poults</td>
<td>6</td>
<td>10</td>
<td>Sterile PBS</td>
<td>I/D</td>
<td>0.2 ml PBS</td>
<td>--</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No evident symptoms or lesions</td>
</tr>
<tr>
<td>2</td>
<td>Turkey poults</td>
<td>6</td>
<td>25</td>
<td>Skin lesion</td>
<td>I/D</td>
<td>0.2 ml homogenate</td>
<td>$10^3$</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>depression, dullness, anorexia, retardation of growth and skin nodular lesions appeared as small papules and vesicles</td>
</tr>
<tr>
<td>3</td>
<td>Turkey poults</td>
<td>6</td>
<td>25</td>
<td>Feather</td>
<td>I/D</td>
<td>0.2 ml homogenate</td>
<td>$10^3$</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>depression, dullness, anorexia, retardation of growth and skin nodular lesions</td>
</tr>
<tr>
<td>4</td>
<td>Turkey poults</td>
<td>6</td>
<td>25</td>
<td>Mosquito</td>
<td>I/D</td>
<td>0.2 ml homogenate</td>
<td>$10^3$</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>depression, dullness, anorexia, retardation of growth and skin nodular lesions</td>
</tr>
</tbody>
</table>

Table (5): Experimental infection of vaccinated and un-vaccinated ostrich chicks with ostrich pox virus isolates

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Type</th>
<th>Vaccination at 6 weeks of age</th>
<th>Experimental infection at 9 weeks</th>
<th>Mortality</th>
<th>Clinical signs</th>
<th>Re- islation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ostrich</td>
<td>3 Fowl pox vaccine</td>
<td>Wing web</td>
<td>++</td>
<td>s/c</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>2</td>
<td>Ostrich</td>
<td>Unvaccinated</td>
<td></td>
<td>++</td>
<td>s/c</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The clinical signs of naturally infected 4–9-week-old ostrich chicks were weakness, dullness, emaciation, depression, anorexia with skin lesions and 3.26% mortality. The course of the disease was 10–14 days and 25% of the chicks were clinically affected with closer serous fluid on the eyelids and around the external ear opening which developed into scabs or dry crusts. Also discrete brown wart-like nodules were seen on the eyelids, around the eyes, ears, on the commissures of the beak and on the skin of head and neck (dry form infection). In some affected birds, large crusts developed on the eye lids, resulting in total closure of the eyes. These proliferative lesions were extended to the mouth cavity caused pharyngitis and trachitis (wet form infection). Similarly to our results that reported in ostriches by Perelman et al., (1988); Allwright et al., (1994); Raidal et al., (1996) and ELgohary, (2002).
Ostrich pox virus could be isolated from the investigated outbreaks after 1-4 blind passages on CAM of ECE, and identified by AGPT against known fowl pox antiserum for all isolated virus from skin lesions, feathers and mosquitoes. Agar Gel Precipitation test for identification of virus isolates against antisera to chicken-pox virus revealed that all isolates which induced pock lesions on CAM were positive. Similar findings were recorded by Mahmoud (1983).

From the epidemiological point of view, avian poxvirus is incapable of penetrating the intact epidermis but requires broken skin for transmission (Tripathy and Reed, 2003), which is carried out by the bites of mosquitoes or other blood sucking insects (Gerlach, 1994). It is believed that mosquitoes can harbour the virus for a month or even more (Ritchie, 1995).

In the present study, supporting the hypothesis those mosquitoes might have been responsible for the transmission of the disease. Moreover, occurrence of lesions on the un-feathered areas of the body, such as the face and legs, could be the reflection of the usual feeding at these sites by such insects.

Furthermore, the histopathological findings observed in the affected ostrich chicks in present study consistently comprised of degeneration and necrosis of keratinocyte and hypertrophy and hyperplasia of epithelial cells in lower layers of epidermis. Eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) in keratinocyte were considered confirmatory. Similar results have also been reported by El-Gohary (2002) and Pledger, (2005).

Microscopic examination also showed intense leukocytic aggregation mainly to subsutis. Occasionally, fibroblastic proliferation and fibrous were detected in areas of old lesions. The underlying musculatures showed hyalinization, hemorrhages and local Zenker's necrosis with few lymphocyte infiltrations. In some cases the dermal collage was hyalinized and locally covered by single layer of regenerated epithelium. Similar findings were observed also by Traniski et al. (1989).

Histopathological examination of chrio-allantoic membranes of inoculated chicken embryos revealed acidophilic intracytoplasmic inclusion bodies. Similar bodies were previously observed by Docherty et al. (1991).
Pathogenicity testing of the recovered isolates by intradermal inoculation into 6-week-old commercial turkeys with 0.2 ml of a 10% infected CAM homogenates prepared from skin lesions, feathers and mosquitoes revealed clinical signs and pathological lesions consistent with those of the natural infection. Also, the inoculated birds showed sero-conversion to the experimental infection as detected by the AGPT on 15th day PI with successful re-isolation of the inoculated virus which confirmed the diagnosis. On the other hand, succeeding in experimental infection of the disease in un-vaccinated commercial turkeys suggested that the virus causing the disease in the ostriches is biologically related to fowl/turkey poxvirus.

From skin lesions of experimentally infected turkey poult samples 100% were positive in isolation trials through CAMs of chicken embryos which revealed characteristic grayish white focal or diffuse pock lesions 5 days post-inoculation, these results similar to that recorded in pigeons by Gomes et al. (1991), in chickens by: Fallavena et al. (1993), in turkey by Metz et al. (1985), and Winterfield et al. (1985), in pet and wild birds by Amer et al. (1986) and Arai et al. (1991) and in ostrich by El-Zanaty (1990) and El-Gohary (2002). Also isolation of viruses from feathers was reported by Tripathy (1991) who mentioned that aerosol generated by feathers containing pox virus particles provide a route for both cutaneous and respiratory infection, while isolation of pox viruses from mosquitoes was recorded by Tripathy and Cunningham (1984), Johnson and Castro (1986) who mentioned that pox viruses can be carried and transmitted mechanically by mosquitoes, flies and red mites.

Chicken embryo inoculation through chorioallantoic membranes with the prepared homogenate from collected samples from experimentally infected turkey poult revealed large grayish white focal and diffuse pock lesions 5 days post-inoculation. The percentage of positive samples isolated from skin lesions was 100%.

In testing of the recovered isolates by subcutaneous inoculation with 0.2 ml of 10% infected CAM homogenate into 6-week-old ostrich chicks vaccinated and unvaccinated with fowl pox
vaccine revealed that the clinical signs were disappeared in the vaccinated ostrich chicks while unvaccinated ostrich chicks revealed clinical signs and pathological lesions consistent with those of the naturally infected ostriches. Also, the unvaccinated birds showed sero-conversion after the experimental infection as detected by the AGPT on the 15th day PI with successful re-isolation of the inoculated virus which confirmed the diagnosis.

In the contemporary practice of ostrich farms, preventive measures should be taken to control the disease in newly-hatched chicks including biosecurity, mosquito-netting of brooder areas, burning of insecticidal candles at dusk and routine vaccination of ostrich chicks that are housed in close association with large poultry flocks with fowl pox vaccine.

Further studies concerning the pathogenesis, genetic and antigenic relationship and cross protection between ostrich pox and fowl pox viruses are needed.

AKNOLEDGEMENT

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REFERENCES
