Case Report

An atypical fowl pox outbreak in broiler flock in Dakahlia governorate

A. A. El-Kenawy[1], K. I. Abou El-Azm[2], Sanaa, S. A. Awad[2].

Department of Virology[1] and department of Poultry Diseases[2], Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

An unusual fowl pox outbreak has been diagnosed in 40 days-old-unvaccinated broilers farm in Dakahlia Governorate during summer, 2004. The most characteristic observation of this outbreak was that the pox signs and lesions were observed on the feathered parts of the body mainly in the posterior dorsal area of the chickens. Classical pox lesions were also seen in the mouth, comb, wattle, eyelids and shank of some chickens. Samples were collected from affected birds for virus isolation and histopathological studies. The isolated virus on the chorioallantoic membrane (CAM) was serologically confirmed. Histopathological examination revealed characteristic intracytoplasmic eosinophilic inclusion bodies in affected chicken tissues and CAM. This outbreak caused severe economic losses due to cutaneous lesions in the feathered area of the body that resulted in high condemnation rate at processing plant beside to high mortality which reached upto25%.

Fowl pox (FP) is one of the earliest known diseases of poultry and is typically a slow spreading infectious disease of many species .FP is caused by a fowlpox virus related to genus *avipoxvirus* (Moyer *et al.,* 2000 and Tripathy and Schnitzel, 1999), and is widely distributed throughout the world. The disease is occurred in many clinical forms, the cutaneous form which is usually mild (Minbay and Kreior, 1973 and buller and Palumbo, 1991), Diphiritic form which is more severe (Tripathy and Reed, 2003), and the occular form (Tanizaki *et al.,* 1989). In some cases avian poxvirus infection may be characterized by cutaneous, diphiritic, systemic and oncogenic lesions (Tsai *et al.,* (1997). Intravenous inoculation of the virus causes lesions in the skin, upper respiratory tract and miliary nodules scattered in the kidney and thymus. The virus can be isolated from the liver, spleen, kidney and lung tissues (Tanizaki *et al.,* 1989). An unusual fowl pox outbreak occurred in broilers in southern Brazil (Back *et al.,* 1995). FP causes significant economic losses to the poultry industry even mortality is mostly due to secondary complications or blindness and starvation, but it rarely exceed 25%. The objective of this study was to describe clinical, serological and hiostopahological features of a case of an atypical fowl pox outbreak in broiler farm in Dakahlia governorate.

Materials and Methods

History. A 40-days-old broiler chicken flock (10,000) at Dakahlia governorate with history of cutaneous lesions and high mortality (25%) were examined during summer 2004. Flocks were raised on floor system and not vaccinated for fowl pox.

Samples for gross examination. One hundred of severely affected chickens were obtained from the affected farm and examined clinically and for gross abnormalities.

Isolation of virus on chorioallantoic membrane (CAM). Pox Lesions from affected chickens were ground in a mortar and pestle and in the presence of sterile sand and PBS containing antibiotics (2000 IU of penicillin and 10 mg of streptomycin/ml) to make 10% suspension. The resulting suspensions were centrifugated at 2000 rpm for 10 minutes and supernatant fluid inoculated onto the CAM of 10-days of embryonating chicken eggs obtained from a specific pathogen free flocks (0.1 ml of tissue suspension was inoculated for each egg). Inoculated embryos were incubated in egg incubator 37 °C for 7 days. The CAM was examined for pock lesions (Woodruff and Goodpasture, 1931; Senne, 1998 and Tripathy and Reed, 1998) and these CAMs were subjected to hiostopathological examination for intacytoplasmic inclusion bodies. The infected chorioallantoic membranes were also collected and ground as described before and then

followed by centrifugation at 2000rpm for 10 minutes, the supernatant fluids were collected and purified by treating with an equal part of Arcton-133 for 5 minutes, then centrifugated and the aqueous part was collected and used as purified antigen in AGPT. **Samples for histopathology.** Tissues from skin, trachea and esophagus were collected, and fixed in 10 % neutral formalin. The washed tissues were dehydrated in different concentrations of alcohols, cleared in xylol and embedded in paraffin. Sections of 5-6 µm were obtained and stained with Hematoxylin and Eosin (H & E) stain according to the method described by (Lillie, 1984).

Agar gel precipitation (AGPT) test. Double immunodiffusion in gel was carried out using 1.5 % agarose, 13 % sodium chloride and 0.01 % thimersol in distilled water according to (Oucterlony, 1958). Purified virus suspension was used as a viral antigen and tested against standard known antiserum obtained from Veterinary serum and vaccine research institute, Abbasis, Cairo to confirm the isolated virus by AGPT.

Results

During summer 2004 outbreak of fowl pox occurred in broilers chicken farm aged 40 days about 10,000 chickens. The first signs and lesions were unusual with high mortality (25%), Examined affected chickens revealed cutaneous lesions including erosions, crusts and nodules on the feathered area (Fig.1,2), comb (Fig.3) and legs and toe (Fig. 4). The nodules ranged from 2-4mm in diameter, which was the characteristic lesion of cutaneous form of fowl pox. Also diphtheritic fowl pox lesions were observed in the trachea and esophagus. Histopathological examination of the cutaneous lesions showed marked hyperplasia in the epidermis caused by swelling and increased in number of cells in the stratum spinosum. These cells showed ballooning, degeneration and contained characteristic intracytoplasmic eosinophilic inclusion bodies of fowl pox (Fig.5). Moreover, skin showed necrosis of epidermis with congestion of epidermal blood capillaries (Fig. 6) or showing necrosis of the epidermis with inflammatory cellular infiltration, mostly lymphocytes (Fig.7). Esophagus showed fibrinonecrotic membrane on its mucosa (Fig. 8) which is characteristic finding of diphtheritic form, or focal hemorrhage and congestion of blood capillaries (Fig. 9, 10) trachea of affected birds had lymphocytic cellular infiltration of submucosa (Fig. 11). Inoculation of CAM of embryonated chicken eggs with tissue suspension of affected birds resulted in characteristic focal white opaque pocks of fowlpox virus after 7 days post inoculation. Sometimes, generalized thickening of the CAM appeared (Fig. 12). The purified antigen from the suspected isolate was identified by using specific fowl pox antiserum in immune diffusion AGPT in which 1-2 precipitin lines were appear within 1-3 days after incubation at 37 °C in humidity chamber. A control antigen consisted of a suspension of uninoculated CAM did not show lines of precipitation, while precipitin lines appeared with reference standard antigen.

Discussion

The aim of the present investigation was the diagnosis of an outbreak of unusual form of fowl pox in broilers in Dakahlia governorate. In this outbreak pox lesions were detected on feathered area of the skin mainly at the posterior dorsal area and wings of chickens. These lesions disliked the classical form of the disease.

In Egypt, there is no available literature describing this form of outbreak. The result of present study came in accordance to that described by (Tripathy *et al.,* 1973 and Back *et al.,* 1995). Where they isolated fowlpox virus from skin lesions in the feathered parts of the body, mainly in the posterior dorsal area and external part of the thigh of chickens in Illinois. In the same time some other birds showed the classical form of the fowl pox. In this work, the fowlpox virus was isolated from the chickens suffering from cutaneous lesions on CAMs. The isolated virus was serologically identified byAGPT using known antiserum.

On the basis of the histopathology, cutaneous lesions and CAM showed the characteristic intracytoplasmic eosinophilic inclusion (Bollinger) bodies. In addition, fibrinonecrotic membrane on esophageal and tracheal mucosa was also recognized. The results of histopathological findings were

Fig. (1): Cutaneous lesion of fowl pox on the feathered part, on the dorsal area of the body.

Fig. (2a): Cutaneous lesion of fowl pox on the posterior part of the back and wings.

Fig. (2b): Cutaneous lesion of fow l pox on wings

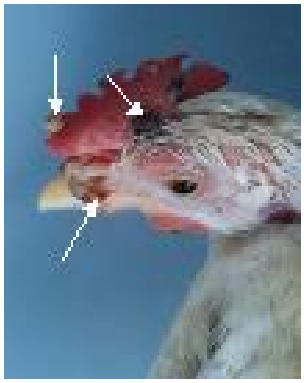

Fig. (3): Nodules of fowl pox on the comb and angle of mouth.

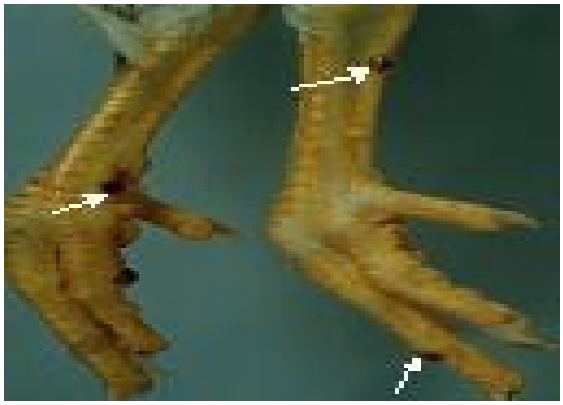

Fig. (4): Nodules of fowlpox on the leg and toe.

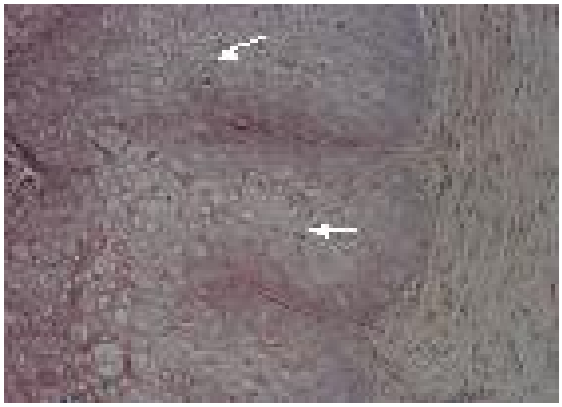

Fig. (5): Skin of chicken showing characteristic intracytoplasmic eosinophilic inclusion bodies in epidermal cells. H&E stain x200.

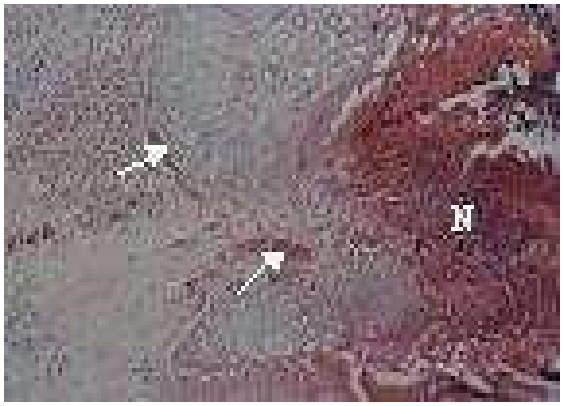

Fig. (6): Skin of chicken showing necrosis(N) of the epidermis with congestion of epidermal blood capillaries(arrows). H&E stain x200

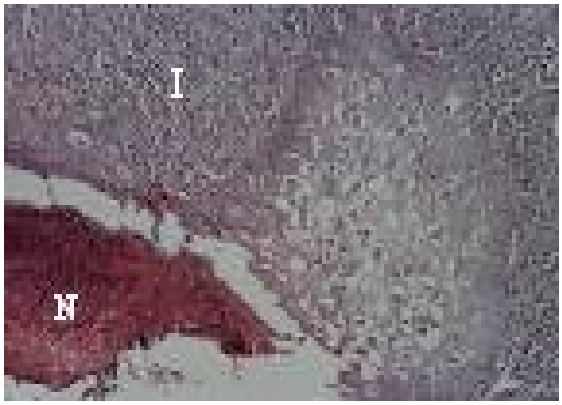

Fig.(7): Skin of chicken showing necrosis of the epidermis(N) with inflammatory cellular infiltration of dermis mostly lymphocytes. H&E stain x200.

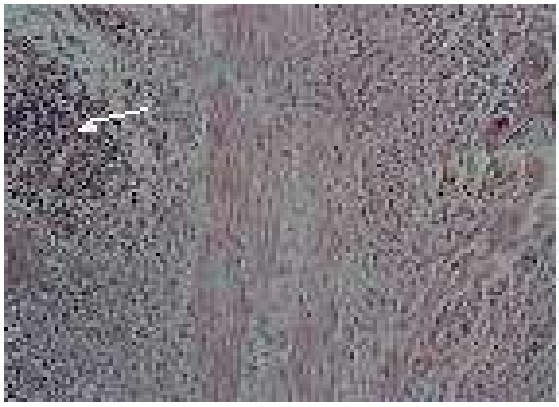

Fig. (8): Skin of chicken showing prevascular lymphocytic cellular cuffing. H&E stain x200.

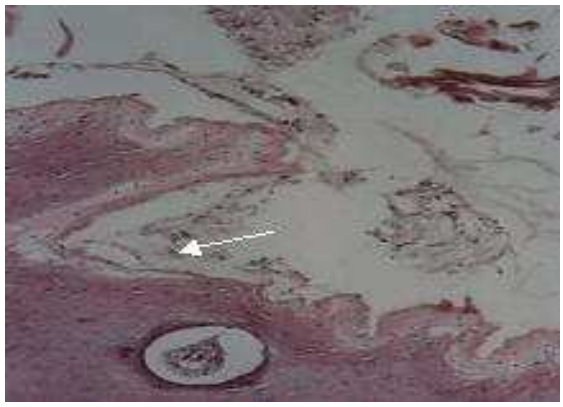

Fig. (9): Esophagus of chicken showing fibrinonecrotic membrane on its mucosa. H&E stain x200.

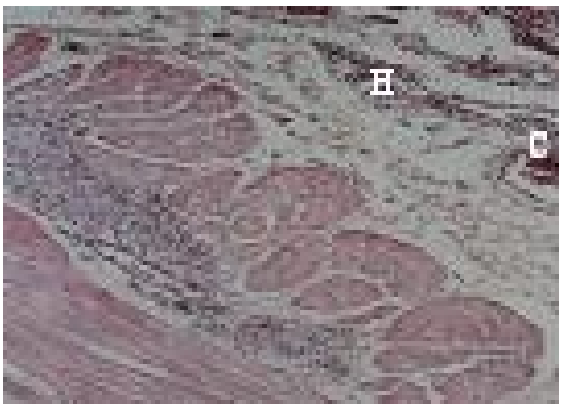

Fig. (10): Esophagus of chicken showing focal hemorrhage and congestion of blood capillaries. H&E stain x200.

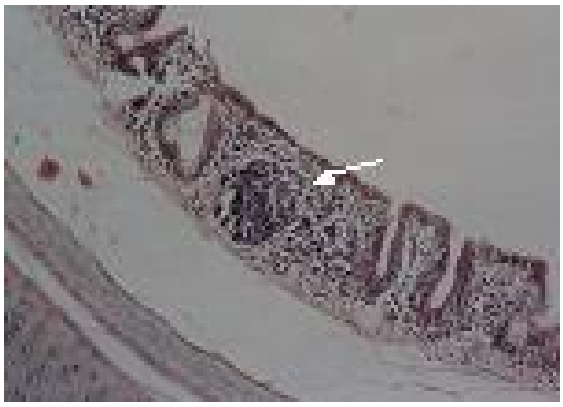

Fig. (11): Trachea of chicken showing lymphocytic cellular infiltration of submucosa. H&E stain x200.

Fig. (12a): Pock lesion on infected Chorioallantoic membrane with fowl pox virus.

Fig. (12b): Thickening of chorioallantoic membane inoculated with fowl pox virus.

similar to those recorded by (Minbay and Kreior, 1973; Tanizaki *et al.,* 1986, Oros *et al.,* 1997 and Yoskikkawa and Alam, 2002).Depending on the basis of clinical findings, virus isolation on CAM inoculation and identification by known antiserum in AGPT and histopathology, it could be said that this was an atypical form of fowlpox virus. Several outbreaks of fowl pox have occurred in previously vaccinated flocks resulting in significantly economic losses and this attributed to presence of variant strain of fowl pox (Fatunmbi and Reed, 1996).Another explanation is that the novel fowlpox virus enhanced virulence due to integration of avian reticuloendotheliosis virus into genome of fowl pox (Fadly *et al.,* 1996 and Singh *et al.,* 2000). This study urgently needs further investigations to throw more light on the antigenic nature of the isolated virus as well as on the pathogenesis of the case.

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