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STUDIES ON PATHOGENESIS OF FOWL POX: VIROLOGICAL STUDY

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Summary. — One-month-old WLH chickens were inoculated with a field isolate of fowl pox virus (FPV) by intradermal (i.d.) and intratracheal (i.t.) routes. In intradermally infected chickens, the virus in titrable amounts was first detected in the skin at the inoculation site on day 2 and in lungs on day 4 followed by viraemia on the day 5 post-infection (p.i.). Subsequently the virus was recovered from liver, spleen, kidney and brain, but not from the heart. The chickens infected by i.t. route showed an almost similar outcome with minor differences as the virus was first demonstrated in the lungs on day 2 p.i., viraemia occurred on day 4 p.i. Initiation of pocks at the inoculation site in i.d. infected birds was observed on days 3 to 4 p.i., generalized cutaneous pock lesions appeared from 7 to 8 days p.i.

Key words: Fowl pox virus, pathogenesis, chickens

Introduction

Fowl pox once considered to be a mild and self limiting disease is now re-emerging as a serious threat to the poultry industry (Dhaneshar *et al.*, 1980; Sharma *et al.*, 1981; Garg *et al.*, 1984). The virus primarily affects skin and epithelium of the head cavities (Arnall and Keymer, 1975). Informations regarding virus spread to and isolation from various internal organs are scanty. Therefore, we have followed the virus distribution after i.d. and i.t. inoculations.

Materials and Methods

The fowl pox virus (FPV). FPV was isolated on the chorio-allantoic membrane (CAM) of 11-12-day-old chicken embryos (CE), from a natural outbreak of pox in a W.L.H. flock vaccinated with freeze-dried egg adapted fowl pox vaccine at the age of 8—10 weeks. Scabs collected from the affected birds were triturated to make a 10% suspension (w/v) in phosphate buffer saline (PBS). After centrifugation for 30 min at 2000 rev/min, the supernatant containing antibiotics (penicillin and streptomycine) was inoculated at 0.2 ml doses into CE via the CAM route. After 4 days incubation at 37 °C, the CAM were harvested and 10% suspensions in PBS were prepared. This stock virus was stored in aliquot sat —10 °C until used.

Experimental infection. Eleven to twelve days old white shelled embryonated chicken eggs and one month old unvaccinated, healthy chickens were obtained from Poultry Research Centre,

Table 1. Log $_{10}$ infectivity titre*) of FPV in blood and various organs of i.d. inoculated chickens

Samples	Days p.i.															
	1	2	3	4	5	6	7	8	9	10	12	14	16—18	20—22	24	26
Blood	—	—	+	—	2.3	2.4	2.4	2.4	1.9	1.4	—	—	—	—	—	—
Lung		—		2.5		3.8		3.8		3.1	2.2	1.4	—	—	—	—
Liver		—		—		1.4		2.4		2.4	1.9	—	—	—	—	—
Kidney		—		—		1.4		2.2		2.2	1.4	—	—	—	—	—
Spleen		—		—		2.2		2.4		—	—	—	—	—	—	—
Brain		—		—		—		1.9		1.4	—	—	—	—	—	—
Heart		—		—		—		—		—	—	—	—	—	—	—
Skin		2.9		3.8		4.3		5.2		5.2	4.6	4.3	4.3	3.5	2.4	1.4

*) Titres are expressed as fifty per cent egg infective dose per gram of tissue/per ml of blood.
Virus present (+) or (—) absent (not detected).

Table 2. Log$_{10}$ infectivity titre*) of FPV in blood and various organs of i. t. inoculated chickens

Samples	Days p.i.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Blood	—	—	—	1.9	2.3	2.4	2.3	2.4	1.4	1.4	—	—	—	—	—	—
Lung		2.9		3.9		3.8		3.5		3.5		2.4		1.4		—
Liver		—		—		1.4		1.9		2.4		1.4		—		—
Kidney		—		—		1.9		1.9		2.2		1.4		—		—
Spleen		—		—		1.4		2.2		1.4		—		—		—
Brain		—		—		—		1.4		—		—		—		—
Heart		—		—		—		—		—		—		—		—

*) Titres are expressed as fifty percent egg infective dose per gram of tissue/per ml of blood.
(—) no virus detected

Pantnagar, Nainital (U.P.). A flock of 150 unvaccinated WLH chickens was divided in three equal groups. Each bird of group A was inoculated intradermally into posteriolateral area of the thighs with 0.05 ml and that of group B was inoculated intratracheally with 0.1 ml of stock virus suspension (5.94 log EID_{50}/ml). Group C served as uninfected control.

Infectivity titration of FPV. Serial ten fold dilutions of the stock virus, blood or tissue suspensions were prepared using HBSS as diluent. A batch of three eggs was inoculated with each dilution (0.2 ml/egg) via CAM route by the method of Hahon *et al.* (1957). CE inoculated with 0.2 ml of HBSS served only as controls. The inoculated eggs were incubated at 37 °C for 96 hr. CE dying within 24 hr were discarded. After 96 hr, 50% egg infective dose (EID_{50}/0.2 ml) was calculated.

One ml of blood was collected aseptically from the wing vein daily for 30 days, in sterilized test tubes containing equal volume of 3.8% sodium citrate solution as an anticoagulant. The blood samples were frozen and thawed thrice, centrifuged at 2000 rev/min for 10 min. The supernatant was collected and titrated in the embryonated eggs. The titres were expressed as log EID_{50}/ml.

Tissues from skin, liver, lung, kidney, spleen, heart and brain were collected from the sacrificed birds on alternate days up to 30 days p.i. and preserved in glycerine saline at 4 °C until processed. Later on, 10% suspensions (w/v) were prepared in HBSS. The suspensions were kept frozen overnight, thawed and centrifuged at 2000 rev/min for 10 min. The supernatants were collected and titrated in CE. Titres were expressed as log EID_{50}/g tissue.

Results

The results in chicken of groups A and B are illustrated in Tables 1 and 2.

Traces of FPV were detected in the blood of group A chickens on day 3 p.i. In higher amounts the virus became detectable in the blood from day 5 to day 8 p.i. Thereafter, the virus titre declined to 1.4 log EID_{50}/ml on day 10 p.i. and no virus was found since day 12 p.i. In the group B, FPV appeared in blood on day 4 p.i. in a titre of 1.9 log EID_{50}/ml which increased to 2.4 log EID_{50}/ml on day 6. The titre declined to 1.4 log EID_{50}/ml on days 9 and 10 p.i. and thereafter, no virus was detectable.

In addition to the skin, lungs showed high virus titres for a prolonged period of time. In group A, FPV appeared first on day 4 p.i. (2.5 log EID_{50}/g); it increased to 3.8 log EID_{50}/g on days 6 and 8 and then decreased. No FPV could be detected from day 16 onwards. In group B, the virus in the lungs exhibited an almost similar pattern, appearing on day 2 p.i., reaching a peak titre of 3.9 log EID_{50}/g on day 4 followed by gradual decrease on forthcoming days. No virus was detected on day 16 p.i.

The virus in the liver was present for comparatively shorter period of time. In the chickens of group A, it appeared in detectable amounts on day 6 (1.4 log EID_{50}/g), increased to 2.4 log EID_{50}/g on days 8 and 10 p.i. A decline in virus titre was observed on day 12 p.i. (1.9 log EID_{50}/g) followed by its disappearance thereafter. In the liver of group B chickens the virus appeared on day 6 p.i., peaked on day 10 (2.4 log EID_{50}/g) and decreased on day 12 p.i.

The virus titre in kidneys of group A and B evinced almost similar patterns as observed in the liver. In the chickens of both groups FPV was detected from day 6 to day 12 p.i.

FPV was found in spleen for a comparatively shorter period of time. In group A, it appeared on day 6 p.i., its titre increased to 2.4 log EID_{50}/g on day 8 p.i. No virus was demonstrated on subsequent days. In the group B,

FPV was first demonstrated on day 6 p.i. with a peak titre of 2.2 log EID_{50}/g on day 8 followed by a decline on day 10 p.i.

In the brain of group A chickens the virus appeared on days 8 and 10, whereas it could be demonstrated only on day 8 p.i. in the brain of group B birds. No virus could be detected in the heart muscle of the chickens of either group.

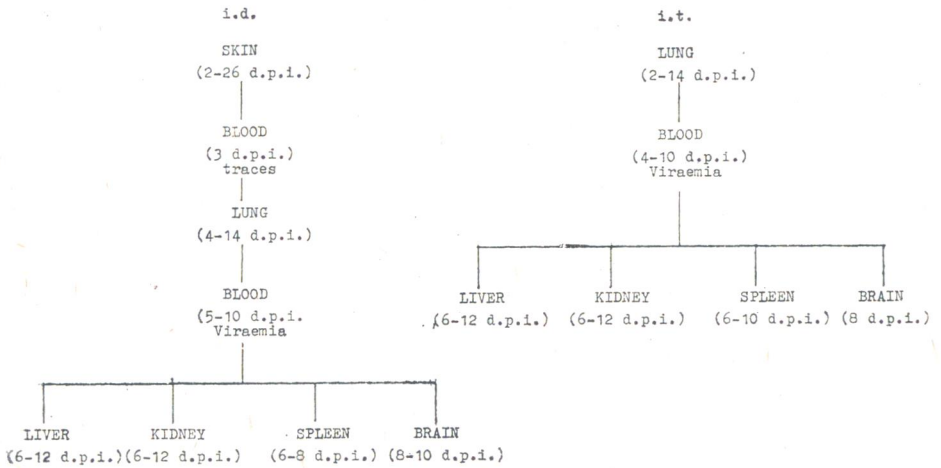

Fig. 1.
Spread of fowl pox virus following intradermal (i.d.) and intratracheal (i.t.) inoculations; d.p.i. = days post-inoculation.

The titre of virus was the highest at the site of inoculation in skin of chickens of group A (Table 1). The virus was first detected on subsequent day with a peak value of 5.2 log EID_{50}/g on days 8 and 10 p.i. Later on, the virus content tended to decrease, but persisted for 26 days when disappeared from the skin 28 days p.i.

Discussion

The field isolate inoculated in one-month-old chickens by i.d. route produced typical cutaneous lesions at the inoculation site from day 2 p.i. Generalized secondary cutaneous lesions appeared in either group of chickens inoculated by i.d. and i.t. routes on day 7—8 p.i. Balla and Simonyi (1964), Minbay and Kreier (1973) and Eleazer *et al.* (1983) reported that clinical manifestations of fowl pox were influenced more by the route of inoculation than by the strain administered. They observed that the generalized cutaneous lesions appeared only on intravenous inoculation and not after i.d. or i.t. inoculations.

The pathogenesis of FPV infection in i.d. inoculated chickens evinced the appearance of primary lesions at the site of inoculation at 72 to 96 hr p.i. In

our birds, the virus multiplied at the site of inoculation not later than after 48 hr. Cheevers and Randall (1968) observed that virus replication started in the skin epithelium after 24 hr of inoculation. Similar lag phase was observed also in sheep pox (Plowright *et al.*, 1959) and buffalo pox (Chandra *et al.*, 1985). The primary lesions developed when virus concentration reached a titre of 3.8 log EID_{50}/g tissue. This was in accordance with the observations made in cases of mouse pox (Fenner, 1948), fowl pox (Minbay and Kreier, 1973) and buffalo pox (Chandra *et al.*, 1985).

The virus spread to lungs from the inoculation site as evidenced by recovery of FPV from the lungs prior to the apparent viraemic stage or virus recovery from any other organ. Francis (1956) observed early multiplication of FPV in lungs after cutaneous infection. This would implicate a passage of the virus from the inoculation site to lungs through lymphatics and/or blood stream. As the virus is associated with the blood buffy coat fraction during viraemia (Minbay and Kreier, 1973) lymphatic as well as haematogenous routes may be adopted for virus spread. Traces of virus in blood stream (not in titrable amounts) before its recovery from the lungs during the present study suggests the possibility of virus transport through the blood circulation. Other members of the pox virus group were reported to be associated with leukocytes, which migrate via both routes (Mims, 1964; Lancaster *et al.*, 1966; Westwood *et al.*, 1966).

The virus in intratracheally infected chickens was first recovered from lungs followed by its appearance in blood stream leading to a viraemic stage. The appearance of viraemia subsequent to the recovery of the virus from the lungs in both affected groups gives a clear indication that lung is the primary site for FPV multiplication. Similar observations have also been made during natural infection of various members of the pox group (Ispen, 1945; Fenner, 1949; Roberts, 1962). However, during some experimental infections primary viraemia has been reported to occur prior to virus replication in lungs (Orskov and Anderson, 1938; Ispen, 1945; Fenner, 1948a; Fenner, 1949).

Viral lesions in various organs and tissues distant from the portal of virus entry and the spread of the virus to these organs are usually mediated through bloodstream, hence, a viraemic stage, which may or may not be detectable, seems crucial particularly in arthropod-borne infections (Mims, 1964). The appearance of a viraemic stage in both chicken groups in experimental FPV infection whose transmission by arthropods was well documented (Francis, 1956; Shirinov *et al.*, 1969; Shirinov *et al.*, 1972; Sanchez *et al.*, 1979) would be in accordance with this notion. Sanchez *et al.* (1978) also demonstrated viraemia after i.d. inoculation, while Minbay and Kreier (1973) failed to demonstrate it. Viraemia stage in either group suggests that FPV infection via other routes is not less severe than by intravenous route.

During the present study until the onset of viraemia, the virus could not be detected in any other organ except skin and lungs. Post-viraemic localization of virus in liver, spleen, kidney and brain was evident from the isolation of the virus from these organs of the chickens of both the affected groups. The virus, however, could not be isolated from heart, which may be due to total absence of replication of virus in heart muscle cells (Fig. 1).

Balaci *et al.* (1978) and Sanchez *et al.* (1978) isolated FPV from lungs, liver, spleen and kidney at various times after vaccination or i.d. infection. The virus isolation from the kidney has also been reported in other generalized pox infections such as sheep pox (Vegad and Sharma, 1970; Bida *et al.*, 1975), rabbit pox (Westwood *et al.*, 1966), ectromelia (Fenner, 1949) and buffalo pox (Chandra et al., 1985). Minbay and Kreier (1973), however, demonstrated the presence of virus in various internal organs only after intravenous infection, but after i.d. and i.t. infections only in the skin and lungs, respectively. There is no report as yet regarding the isolation of FPV from the brain. It may be hypothesized that the neurons might become infected from circulating leukocytes which might have crossed the blood-brain barrier by diapedesis.

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