PATHOGENIC, ANTIGENIC AND SEROLOGIC RELATIONSHIP BETWEEN FOWL POX, PIGEON POX AND CANARY POX VIRUSES

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Thanks to Allah Who taught mankind things they ignored. Thanks to Allah for guiding me to complete this research.

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DEDICATION

TO ALL MY FAMILY MEMBERS

MY PARENTS, BROTHERS, HUSBAND,

SONS AND MY COUSIN AMMAR,

AFTER ALLAH

FOR PROVIDED ME WITH BOTH MORAL AND

MATERIAL SUPPORT

AND ASSISTANCE IN MY STUDY

MAY ALLAH REWARD THEM.
ABSTRACT

This study was carried out to determine the antigenic differences among fowl, pigeon, and canary pox viruses. Also to, determine the cross protection potentials of these viruses and to select one of these viruses' isolates as live vaccine candidates against fowl pox.

There was no death following challenge of experimental birds by canary pox virus (CPV). Whereas, there was death of some birds followed challenge of experimental birds by fowl (FPV) pox virus and pigeon pox virus (PPV). This result showed that canary pox virus is the safest of the three viruses for vaccination as a live vaccine.

Fowl, pigeon and canary pox antigens were prepared by inoculating 0.1ml of $10^{-3}$ diluted isolates via chorioallantoic membrane of 11-12day old embryonated chicken’s eggs.

Inoculation of the CAM by the three viruses showed clear distinguishable pock lesions for each virus. Sera were obtained from experimental chickens.

Examination of fowl, pigeon and canary pox antisera against fowl pox, pigeon pox and canary pox antigens respectively by AGPT test showed clear precipitin lines.

Examination of fowl, pigeon and canary pox antisera against fowl pox, pigeon pox and canary pox antigens respectively by Passive haemagglutination (PHA) gave positive results. These result indicated that there is serological and antigenic relationship between the three viruses.
Titration of 0.1ml of each isolate of the 3-viruses in embryonated chicken eggs showed that the EID$_{50}$ of fowl and pigeons pox viruses' were $10^{9.3}$ and that of canary pox virus was $10^{10.5}$.

When pigeons were infected by fowl and canary pox viruses no lesions appeared. Also when canaries were infected by fowl and pigeon pox viruses no lesions appeared. But when Chicken were infected by canary, pigeon and fowl pox viruses lesions appeared. That means chickens are susceptible to both canary and pigeon viruses, so there is pathogenic relationship between chicken, pigeon and canary pox viruses, while as pigeons are resistant to fowl and canary pox viruses, which means that there is no pathogenic relationship between pigeons and canary and fowl pox viruses; also canary birds are resistant to fowl and pigeon pox viruses, which means there is no pathogenic relationship between pigeon and fowl pox viruses.
لا يمكنني قراءة النص الذي تم تسجيله بشكل صحيح. يرجى وضع النص بشكل صحيح لكي أتمكن من قراءته.
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INTRODUCTION

Fowl pox is a slow spreading viral disease of poultry and other birds. It is found in three forms; dry (cutaneous), wet (diphtheritic) and ocular form. The dry form is characterized by proliferate dark skin lesions of the unfeathered skin of the head, neck, legs and feet. Diphtheritic lesions of the mucous membrane sinuses, larynx and tongue characterize the wet form; the upper digestive tract or respiratory tract may be included. Mortality is usually low in the dry form, higher in the wet form (about 50% or less). In adult chickens, the virus causes emaciation, reduces egg production and performance of flock. In young chickens, it causes growth retardation. In turkey it may result in blindness and starvation.

Fowl poxvirus (FPV) is a DNA virus, which belongs to the genus *Avipoxvirus* and the family of *Poxviridae*. Its distribution is worldwide. It is transmitted by direct contact through the injured lacerated skin, or mechanically via insect vector (mosquitoes, culicoides, and poultry ticks). The virus was demonstrated first in 1952 by Marx and Sticker. The first report about it in the Sudan was issued in 1936 (SVRs) and thereafter continued to be mentioned annually by the veterinary service department.

Diagnosis of the disease is by typical lesions clinical signs, histopathology (cytoplasmic inclusion bodies), electron microscopy, and culture of the virus in chick embryo. The control of the disease was managed to be through application of all sanitary measures, isolation of the infected birds, vaccination as well as addition of protein and multivitamins supplements to the food. Vaccination can be done through several routes: wing web, orally, intravenously, or by feather follicle. The attenuated strains of fowl poxvirus were grown on the chorio allentoic membrane (CAM) of chick embryo (CE),
fibroblast cell cultures and used to vaccinate chicks. Live fowl poxvirus vaccine, adjusted in oil, administered subcutaneous (s/c) for one-day-old chicks and adult chickens.

The present study was designed and conducted to determine the pathogenic, antigenic and serologic relationship among some avian poxvirus isolates namely fowl poxvirus, pigeon poxvirus and canary poxvirus. Promotion of one of these viruses as a safe live virus vaccine candidate against fowl pox infection was also targeted. The results obtained indicated that canary poxvirus was antigenically more dominant as compared to the other viruses, immunogenic and safe, hence suggested as good live virus vaccine against the infection.
CHAPTER ONE
LITERATURE REVIEW

1.1. The Family Poxviridae:

This family consists of oval or “brick-shaped” viral particles, 200-400nm long. The external surface of which is ridged in parallel rows, sometimes arranged helically. The particles are extremely complex, containing many proteins (more than 100) and detailed structure is not known. The extracellular forms contain two membranes, hence termed as extra-cellular enveloped virions (EEV) while the intracellular particles only have an inner membrane which termed as the intracellular mature virions (IMV) (Alan, 1997). There are at least ten enzymes encoded by the viral genome which are mostly concerned with nucleic acid metabolism and the genome replication (Alan, 1997). This family is subdivided into two subfamilies: Chorodopoxvirinae (poxviruses of vertebrates) and Entomopoxvirinae (poxviruses of insects). The subfamily Chorodopoxvirinae is subdivided into eight genera, each of them include species that cause diseases in domestic or laboratory animals (Murphy et al., 1999).

1.2. The Genus Avipoxvirus:

The viruses in this genus are serologically related to each other and specifically infect birds. The pox viruses recovered from various species of birds are named according to their hosts such as fowl pox, canary pox, pigeon pox and turkey pox (Fenner, 1996). Although a number of different Species of avian pox viruses had been encountered but systemic analysis of their DNAs has not yet been made. Avian poxviruses are very complex, inducing both specific and cross-reacting antibodies, hence posses ability to being protective against each other when used as vaccines (Alan, 1997).
1.2.1 Fowl pox virus:

This virus is highly infectious for chickens and turkeys but rarely pigeons and not at all for ducks and canaries (Murphy et al., 1999). Classification of fowl, pigeon and canary pox viruses was done according to the universal system of virus taxonomy of the International Committee for Taxonomy of viruses (ICTV, 1995), as follows:

Family : Poxviridae.
Subfamily: Chorodopoxvirinae.
Genus : Avipoxvirus.
Species : Type species fowl pox virus

Fowl pox virions are reported to be composed of core, a core membrane, two inner membranes, an intermediate membrane, an outer membrane and a dense outer coat (Cheville, 1966; Purcell et al., 1972).

1.2.2. Pigeon pox virus:

Pigeon pox infects pigeons, chickens, turkeys, ducks and gees, (Jacob, et al., 1998).

Family : Poxviridae.
Subfamily: Chorodopoxvirinae.
Genus : Avipoxvirus.
Species : Pigeon pox virus (PPV)
1.2.3. Canary poxvirus:

Canary pox virus (CPV) is an Avipoxvirus and etiologic agent of canary pox, a disease of birds both in the wild and in commercial aviaries, where significant losses result (Johnson and Castro1986). Canary pox has been described broadly as the pox viral disease of passeriform (song) birds, efficiently causing disease in passerine hosts compared to galliform (domestic fowl) and columbiform (pigeon) hosts (Giddens, et al., 1971).

However, passerine host preferences exist, and current International Committee on Taxonomy of Viruses (ICTV) classification differentiates between CPV and several other passerine isolates (Moyer, et al., 2000).

Canary pox infects canaries, chickens, sparrows, and probably other species. In some instances, but not always, exposure to one of the viruses stimulates development of immunity to that virus and one or more of the other viruses, (Jacob, et al., 1998).

**Family** : Poxviridae.

**Subfamily** : Chorodopoxvirinae.

**Genus** : Avipoxvirus.

**Species** : Canary pox virus (CPV)

1.3. Poxvirus Genome

The poxvirus genome consist of a Linear, double-stranded DNA molecule with a molecular size of 130-300kbp. The ends of the genome consist of a terminal hairpin loop with several tandems, repeat sequences. They form direct repeat called inverted terminal repeats (ITRs). Several poxvirus genomes have been sequenced. Most of the essential genes are located in the central part of the genome, while non-essential genes are located at the ends. More than 200 genes are recognized in the poxvirus genome. Integration of
reticuloendotheliosis virus (REV) sequences in the genome of fowl pox virus has been observed (Singh et al., 2000).

![Schematic diagram of Poxvirus particle.](image)

**Figure 1. Schematic diagram of Poxvirus particle.**

(Poxvirus Bioinformatics Resources Center)

The outer surface is composed of lipid and protein, which surrounds the core, which is biconcave (dumbbell-shaped), with 2 "lateral bodies" (function unknown). The core is composed of a tightly compressed nucleoprotein.

![Negatively stained electron micrograph of poxvirus particle.](image)

**Figure 2: Negatively stained electron micrograph of poxvirus particle.**

(Poxvirus Bioinformatics Resources Center)
1.3.1. Canary pox virus genome:

The genomic sequence with analysis of a canary pox virus (CPV) indicates that the 365-kbp. CPV genome contains 328 potential genes in a central region and in 6.5-kbp inverted terminal repeats, (Tulman, 2004). Comparison with the previously characterized fowl pox virus (FPV) genome revealed Avipoxvirus-specific genomic features, including large genomic rearrangements relative to other Chordopoxviruses and novel cellular homologues and gene families. CPV also contains many genomic differences with FPV, including over 75 kbp of additional sequence, 39 genes lacking FPV homologues, and an average of 47% amino acid divergence between homologues. Differences occur primarily in terminal and, notably, localized internal genomic regions and suggest significant genomic diversity among Avipoxviruses. Divergent regions contain gene families, which overall comprise over 49% of the CPV genome and include genes encoding 51 proteins containing ankyrin repeats, 26 N1R/p28-like proteins, and potential immunomodulatory proteins, including those similar to transforming growth factor β and β-nerve growth factor. CPV genes lacking homologues in FPV encode proteins similar to ubiquitin, interleukin-10-like proteins, tumor necrosis factor receptor, PIR1 RNA phosphatase, thioredoxin binding protein, MyD116 domain proteins, circovirus Rep proteins, and the nucleotide metabolism proteins thymidylate kinase and ribonucleotide reductase small subunit. These data reveal genomic differences likely affecting differences in Avipoxvirus virulence and host range, and they will likely be useful for the design of improved vaccine vectors, (Tulman, 2004).
1.4. Characteristic of Fowl pox Virus:

1.4.1. Morphology:

Fowl pox virus is a rectangular or brick shaped. When negatively stained virus with phosphotungastic acid (PTA) seems to has dimensions of about 265x 334nm, while shadowed preparation has average dimensions of 258x 354nm. There is conspicuous capsular like material without evidence of external surface structures (Randel et al., 1964). Pseudoreplicas have an arrangement of rodlets or tubules on the surface knobs varying from 300 - 400Å. Treatment of the virus with trypsin appears to unwind the outer layer of the membrane resulting in twisted rope like structures and the knobby surface is not found (Hyde et al., 1965). Fowl pox virus treated with sodium laurel sulphate and DNAase and stained with PTA noted to consist of subunits of about 40Å on the inner layer (Hyde et al., 1965). Fowl pox virus measures 164x 252x 284nm in dimensions in the dermal epithelium of the chicken (Tajima and Ushijima, 1966).

1.4.2. Chemical Composition:

The average molecular weight of an inclusion body of fowl pox virus is 6.1x 10^{-7} mg, 50% of it is extractable lipid. Protein per inclusion body weight is about 7.69x 10^{-8} mg, and that of DNA is 6.64x 10^{-9} mg (Randal et al., 1962).

The virion contains 7.51x10^{-15}g of protein, 4.03x10^{-18}g of DNA, and 5.54x10^{-15}g of lipid, (Randal et al., 1964).

The fowl pox virus genome is double stranded DNA molecule (Gafford and Randal, 1976).
1.4.3. Replication:

Replication of fowl pox virus occurs in the cytoplasm of the host cell. The virion may be enclosed within a loose membrane derived from the unchanged cytoplasmic membrane, which is neither essential nor common, so that it differs among the viruses, which mature by budding from cellular membrane (Finner, 1968). The virus is sufficiently complex to have acquired all the functions necessary for genome replication. There is some contribution from the cell but it is not clear what this is. Poxvirus gene expression and genome replication occur in enucleated cells, but maturation is blocked (Alan, 1997). The viral receptors are not known, but probably more than one on different cell types. Penetration process is complex and may also involve more than one mechanism. Uncoating occurs in two stages, removal of the outer membrane as the particle enters the cell and in the cytoplasm, the particle (minus its outer membrane) is further uncoated and the core passes into the cytoplasm (Alan, 1997).

Replication of avian poxviruses is similar in dermal or follicular epithelium of chicken and ectodermal cells of CAM of chicken embryos (Cunningham, 1978). After adsorption and penetration of the cellular membrane by fowl poxvirus, one hour after infection of dermal epithelium (Arhelger et al, 1962), and 2-hours after infection of CAM (Arhelger and Randel, 1964), there is an uncoating of the virus prior to the syntheses of new virus from precursor materials. Biosynthesis of the virus in dermal epithelium involves two phases:
- The host response during the first 72-hours
- Synthesis of infectious virus within 72-96 hours (Cheevers and Randal, 1968). Syntheses of the host DNA occurs at 36-48 hours, leading to epithelial Hyperplasia. The host DNA synthesis then declines sharply from 60-72 hours. Hyperplasia ends at 72-hours with 2.5fold increase in the number of the cell.
Replication of viral DNA begins between 12-24 hours. Then from 72-96 hours the ratio of viral DNA to the hosts DNA progressively increases to maximum (more than 2:1). The maximum titer of the virus is attained after cell proliferation has ceased. The incomplete virion migrate to lipid-containing vacuoles or inclusion body vacuoles derived from lipid granules of the cytoplasm. The latent period is relatively long (about 48 hours) of the virus entry; areas of viroplasm with incomplete membranes around them are present at 72 hours after infection of dermal epithelium of the chicken (Arhelger et al., 1962) and at 96 hours after infection of CAM (Arhelger and Randal, 1974). The incomplete virion then penetrates the inclusion body vacuole and thereby acquires a membrane coat (Cheville, 1966). The probable function of the inclusion body is to provide and localize precursor material for the lipid coat of the virion (Cunningham, 1973).

Fowl poxvirus emerges from the cell of the CAM by budding process with acquisition and additional outer membrane obtained from the cell membrane (Arhelger and Randall, 1964). Poxviruses is apparently assembled exclusively in the cytoplasm of infected cells, but not all the replicative events are restricted to the cytoplasm. The nucleus participates in the complexities of fowl pox virus replication. DNA extracted from the fowl pox virus is infectious for the CAM of the chicken embryos but not for chicken skin. The whole virus infects both the CAM and the chicken skin with equal efficacy (Gafford and Randall, 1976).
1.4.4. Gene expression:

This is carried out exclusively by viral enzymes associated with the core and is divided into two phases: Early genes: ~50% of the genome, expressed before genome replication. Late genes: expressed after genome replication; late promoters are dependent on DNA replication for activity (Alan, 1997).

Figure 3. The replication cycle of fowl pox virus
(Poxvirus Bioinformatics Resources Center)

1.4.5. Resistance to chemical and physical agents:

Fowl pox is highly resistant to environmental conditions. It can resist heat up to 100°C in dry state for 5 minutes. It can also withstand drying for many months even at room temperature. It can be preserved for many years by freeze-drying (Andrews et al., 1978). The virus can be killed at 60°C in 8-minutes or 35°C in 30-minutes. Storage of the virus at 0°C - 4°C in dried material retain its availability for 2-years, (Merchant and Parker, 1971). It can withstand 1% phenol and 1:1000 formalin, for 9 - days. Trypsin has no effect on DNA nor does the whole virus but chloroform–butanol inactivate the virus.
but not the DNA (Randal et al., 1964, 1966). When the virus is freed from its matrix, it can be inactivated by caustic potash 1% (Tripathy and Cunningham, 1984). The virus is inactivated by acetic acid (1%), bichloride 1:1000 of mercury, ethyl alcohol 75-95% in 30 minutes (Graham and Brandly, 1940; Beister, 1965). Resistance to ether treatment is listed as one of the taxonomic criterion for pox viruses (Mathewes, 1982). Pigeon pox and its mutant are resistant to both chloroform and either, (Tantawi et al., 1979). In contrast Pradhan et al., (1996) reported that quail pox was relatively more sensitive to chloroform than ether and was not affected by trypsin at final concentration of 0.125%.

1.4.6. Strain classification of avian poxviruses:

There are six closely related strains of Avipoxvirus namely fowl pox, pigeon pox, quail pox, canary pox, psittacine pox, and ratite pox viruses (Jacob, and Butcher, 1998). Pigeon pox virus infects pigeons, chickens, turkeys, ducks, and geese. Canary pox infects canaries, chickens, sparrows, and probably other species. In some instances, but not always, exposure to one of the viruses stimulates development of immunity to that virus and one or more of the other viruses (Jacob et al., 1998). Although some workers consider turkey poxvirus as a distinct strain, many believe that is identical to fowl pox virus (Cunningham, 1978). Chiocco (1993) proved that canary poxvirus is immunologically distinct from fowl poxvirus. Read Fatunmbi (1993) reported that quail pox and mynah pox are immunologically distinct from pigeon and fowl poxviruses. Protection against canary pox, quail pox and mynah pox is attained only when homologous virus is used as a vaccine (Read and Fatunmbi, 1993).
In comparative studies on the antigenicity of extra and intra-cellular virus of three strains of fowl pox virus FS-8, HP-1, and FS-4. Maiti et al. (1991) found out that when the intra-and extra-cellular virus of these three strains were precipitated in succession with different saturation (25%, 50%, and 75%) of ammonium sulfate, they reveal three different antigens in gel diffusion test. Further analysis of each positive antigen by dot ELISA reveals that the extra cellular virus of FS-8 and HP-1 strains possessed excess antigenic protein at 50% saturation compared to their intra-cellular viruses while no difference between extra and intra-cellular viruses of FS-4 was observed (Maiti et al., 1991).

1.4.7. Pathogenic relationship between fowl pox virus and Other avian poxviruses:

Differentiation between some avian poxviruses based on the cytopathic effect (CPE) on the CAM chicken embryo was reported. It was established that plaques produced by turkey pox virus resemble those produced by fowl pox virus, but develop more slowly and smaller at given period of incubation. Canary pox virus produces plaques smaller than that of fowl and turkey pox viruses. Plaques produce by pigeon pox virus are the smallest with characteristic lysis not present in other plaques produce by other poxviruses. Fowl pox virus produces clear central plaques (2mm-9mm) with a less clear peripheral zone (Cunningham, 1973; Mayer, 1963).

It was also evidenced that a mutant of fowl pox virus resulting from intracerebral passage of the virus in chickens produces soft, pliable gross lesions on the CAM differing from the firm, opaque, hyper-plastic lesion typical of fowl pox virus infection (Goodpasture, 1959).
1.4.8. Antigenic relationships between fowl pox and other Avian poxviruses:

Avian poxviruses are antigenically and serologically distinguishable from each other, although there is cross-relationship. A nucleoprotein precipitogen was reported as common to all avianpox viruses (Woodroof and Fenner, 1962). This cross reactivity was confirmed using passive haemagglutination inhibition (PHI), virus neutralization (VN) and agar gel precipitation test (AGPT) (Uppal and Nilakantan, 1970). Other serological tests like complement fixation, immunoperoxidase and immunofluoresence had also been used to differentiate between fowl pox virus and other poxviruses (Tripathy and Hanson, 1975). Burnet (1987), utilizing the pock-counting technique, demonstrated relationship between canary poxvirus and fowl pox virus.

Antigenic and genetic studies did not reveal any significant difference between the poxvirus isolated from ostriches (PVO) and fowl pox virus (FPV). Further, susceptible chickens immunized with the PVO were protected when challenged with a virulent strain of FPV. Thus, the poxvirus isolated from ostriches had similar antigenic, genetic, and biological properties to FPV (Shivaprasad et al., 2002).

A permanent cell line of avian origin, QT-35, was used for the propagation of Avipoxvirus isolates, including junco pox, pigeon pox, and field and vaccine strains of fowl pox viruses. The genomes of these Avipoxvirus isolates were compared by restriction enzyme analysis using BamHI and HindIII endonuclease digestion and subsequent agarose gel electrophoresis. The genetic profiles of the virus strains were very similar, with a high proportion of co-migrating fragments, although most strains could still be
distinguished; therefore, these Avipoxviruses appear to be closely related. Similar results were obtained when the immunogenic proteins of 6 fowl pox virus strains were examined by Immunoblotting. Although the majority of the antigens were common, the strains could be differentiated by unique proteins of differing electrophoretic mobilities, (Schnitzlein et al., 1988).

Quail, chickens, and turkeys vaccinated with pigeon and fowl pox viruses were not protected against challenge of their immunity with quail pox virus and they developed severe cutaneous lesions of pox. When quail and chickens were vaccinated with quail pox virus and given pigeon and fowl pox challenge viruses, no protection was present. Thus, quail pox virus had no immunologic relationship to pigeon and fowl pox viruses, (Winterfield and Reed, 1985). In contrast, the patterns of three quail pox virus isolates were very similar with a high proportion of co-migrating fragments. Therefore, on the basis of genetic as well as immunological analysis; quail pox virus is a distinct species of the genus, *Avipoxvirus*, (Ghildya, et al., 1982).

Psittacine pox virus applied as a vaccine in quail and chickens also failed to protect against quail pox virus challenge. However, quail, chickens, and turkeys vaccinated with quail pox virus were protected against quail pox virus challenge, (Winterfield and Reed, 1985).

Avian pox virus was isolated from cutaneous pox lesions removed from turkey breeders that had been vaccinated three times with a commercial fowl pox vaccine. In three cross-immunization experiments with turkeys and two with chickens, the turkey pox isolate, designated NC5271, proved immunologically different from fowl, pigeon, and quail pox viruses. Significant protection against NC5271 virus infection and inducement of pox lesions was only attained when the homologous isolate was used as a vaccine, (Winterfield and Reed, 1985).
Chickens vaccinated with fowl and pigeon pox vaccines and inoculated with the psittacine isolate developed lesions typical of avian pox. Chickens vaccinated with the psittacine virus were susceptible to fowl and pigeon pox virus infection. This pox virus isolate may thus be regarded as a potential pathogen for chickens (Boosinger et al., 1982).

An isolate of psittacine pox virus, applied as a vaccine, protected chickens against challenge with the same virus isolate and also against challenge with two other psittacine pox virus isolates, confirming a close or identical antigenic relationship with each other. When combined in a multivalent vaccine, quail, psittacine, and fowl pox viruses induced excellent protection in chickens against challenge with the three respective viruses. The presence or absence of "takes" or reactions following vaccination by the wing web route did not necessarily correlate with the presence or absence of immunity noted from challenge by feather follicle virus application, (Winterfield and Reed, 1985).

An avian pox virus was isolated from Amazon parrots dying with severe diphtheritic oral, esophageal, and crop lesions. The virus was propagated on chorioallantoic membranes (CAM) of 10-day-old chicken embryos, and a homogenate of the infected CAM was rubbed vigorously onto the conjunctiva, oral mucosa, and defeathered follicles of two healthy Amazon parrots and three conures. All experimental birds developed cutaneous and ocular pox lesions, and one parrot developed oral pox lesions. Specific-pathogen-free chicks inoculated with the virus isolate developed skin lesions identical to those of the parrots, (Boosinger et al., 1982). An epornitic of avian pox occurred in a flock of 123 houbara bustards (Chlamydotis undulata macqueenii) received at the Sulman Falcon Hospital in the State of Bahrain in February 1993. Birds displayed conjunctivitis, excessive lacrimation and
papilloma-like growths forming amorphous clusters on the third eyelid and on
the conjunctiva. Examination of eyelid samples under transmission electron
microscopy revealed pox virus particles displaying the classical morphology
of vaccinia-Avipox virions. Typical pox lesions were also detected 5-days
post infection (p.i.) on chorioallantoic membrane (CAM). The virus titre on
CAM was $10^7$ focus-forming units (FFU)/ml. In tissue culture, only a slight
cytopathogenic effect (CPE) was detected 5 days p.i.; the virus titre on cell
cultures was $10^{4.5}$FFU/ml. The virus infection in cell culture appeared to be
abortive and no CPE was seen after three passages in secondary chicken
embryo fibroblasts. No neutralization of the cell-grown virus was detected on
serological studies using antisera directed against fowl, pigeon, canary and
sparrow pox viruses, (Samour et al., 1969).

After an experiment done in Altaif on houbara bustard (Chlamydotis undulata), the results
of the experiment reveal a degree of immunogenic relatedness between canary pox (CP)
and houbara bustard poxvirus (HP) strain and support the recommendation that houbara
bustards be vaccinated with a CP vaccine, (Ostrowski et al., 1996).

1.4.9. Characterization of Avipoxviruses from wild birds:

Lesions suggestive of avian pox, found on a Norwegian wild sparrow
(Passer domesticus) and wood pigeon (Palumbus) were obtained in 1972 and 1996,
respectively. Histologically, these lesions were demonstrated to be characteristic of
poxvirus infections. And the poxvirus was observed using an electron microscope. The
resulting viruses were propagated in chicken embryo fibroblast cells. Restriction fragment
length polymorphism of genomes from 2 Norwegian isolates and fowl pox vaccine
strain, generated

by BamHI, revealed a high degree of heterogeneity among the isolates. The
profiles of Avipoxviruses isolated from wild birds were clearly distinct from
each other and also to the fowl pox virus strain. Furthermore, chickens
experimentally infected with pigeon pox virus had higher antibody titers and
extensive lesions compared to other isolates. This may suggest that pigeon poxvirus is more virulent than the other isolates. This may suggest that pigeon pox virus is more virulent than the other isolates, (Weli et al., 2001).

1.5. Epidemiology of fowl pox virus:

1.5.1. Incidence and distribution:

Its distribution is worldwide and proved slow in spreading. Its incidence is variable in different areas because of differences in climate, management and hygiene or the practice of regular vaccination (Tripathy, 1993; Tripathy and Reed, 1997). In Sudan, the disease is reported all over the country and especially during the rainy season and soon after, (Khogali, 1970).

Elhussein et al., (1998) found that fowl pox was equally prevalent during both winter and summer.

1.5.2. Host range:

Fowl Pox is an infectious viral illness of chickens and turkeys, (Sigh and Tripathy, 2000). Chicken and turkeys are the only natural hosts of fowl pox, and the experimental hosts can be any of the avian spices (Tripathy and Cunningham, 1984; Tripathy, 1991).

Turkey pox is virulent for ducks (Murphy et al., 1999). Nonetheless Deokin et al. 1984) reported that chickens were not susceptible to pox virus isolated from mynas and buzzards.

Saif– Eldin and El-Ballals (1997), studied occurrence of pox virus infected in pigeons, house sparrows and doves in Egypt, they reported that the isolates from pigeons and doves were characterized as pigeon pox virus, and isolates from sparrows as fowl pox virus. They also stated that dove appeared to be reservoirs of infection for pigeon pox virus.
1.5.3. Susceptibility:

All ages and breeds of chickens are susceptible to the disease, (Tripathy and Cunningham1984; Tripathy 1991). Chicken with large comb are more susceptible than those with small combs, (Cary, 1906). Although the disease is not common encountered in young chickens, Beaudette (1992) and Johnson (1937) observed outbreaks in battery brooded chickens, Johnson reported the disease in 6-weekes old chicks with lesion on feet and legs and since they were under developmental age no lesions on the comb and wattle were found. Avian poxvirus was isolated from nodules on the heads and conjunctiva of 3-to-4-wk-old ostrich chicks (Shivaprasad et al., 2002).

1.5.4. Morbidity and Mortality:

Morbidity is usually low, but it varies from a few birds being infected, to-involvement of the entire flock if there is virulent virus and no control measures are taken,(Tripathy and Cunningham,1984).Mortality also depends on the physical state of the birds, and presence of complications, (Berister, 1962). In the cutaneous form, the mortality rate is low (3-10%), it may rise up to 50% in the diphtheritic form, but even in this case death may occur due to secondary bacterial infection, (Tripathy, 1991). In an aviary housing 200, six-month-old canaries, 165 became ill and 145 died over a 6-week period from a disease initially characterized by lethargy, ruffled feathers, open-mouth breathing, and death in 2 to 3 days (Johnson and Castro, 1997).

Canary pox, however, is generally associated with higher mortality rates than seen in fowl pox, commonly approaching 100%, and may occur without characteristic skin lesions (Giddens et al., 1971).
1.5.5. Seasonality of Fowl pox:

Infection can be at any time during the year. Khogali (1970) reported that in Sudan most outbreaks are during the rainy season and soon after. Beister and Scharte (1965) stated that incidence of the disease may be highest during winter and rainfall months. Elhussein et al. (1998) found that fowl pox was equally prevalent during both winter and summer.

1.5.6. Transmission of the virus:

Transmission of the virus is through injured or lacerated skin (Minbay and Kreir, 1973). Mosquitoes of the genera culex and Aedes are capable of the extrinsic transmission of avian poxviruses and have been responsible for widespread outbreaks during the summer months. Transmission by mosquitoes is mechanical (there is no multiplication of the virus inside the vector.) Mosquitoes remain infective for several weeks and produce consecutive infection (Cunningham, 1978). The virus is localized on the proboscis of the mosquitoes, so transmission within the flock is rapid when mosquitoes are plentiful (Fukuda et al., 1979). Certain insects such as Gnats are attracted to the eyes of the birds, presumable seeking moisture to the feed on ocular fluids, insects carrying the virus when this deposit the virus in the eye of the bird. Then the virus could travel through the lacrimal duct to the laryngeal region, where it finds cells for which it obviously has an affinity (Eleazer et al., 1983).

Dropped scales may also act as a source of infection and poultry ticks can remain infective for several weeks and produce consecutive infection. Apparently some carriers remain following clinical recovery and reactivation may be caused by stress such as forced moulting (Mockett, 1990).
1.6. Pathogenesis:

Fowl pox virus enters an epithelial cell and then spread from cell to cell aided by the production of epithelial growth factors which causes proliferation of cells (Mockett, 1990). Some viruses enter the blood and cause viraemia and spread to internal organs where no gross pathological changes are evident. Francis (1956); Minbay and Kreier (1973) recovered fowl pox virus from visceral organs of chickens inoculated with the virus by intradermal and intravenous routes. They also confirmed that the pathogenesis of fowl pox virus infection in chickens inoculated intradermally or intratracheally was similar with only minor differences.

In the chicken inoculated intradermally, the virus was first detected in the skin at the inoculated site on day two post inoculation (PI) and in the lung in day four followed by detectable viraemia on day five. In chickens infected intratracheally, the virus was first detected in lungs on day two (PI) followed by viraemia on day four (PI). Following the infection with fowl pox virus, the virus was recovered from liver, spleen, kidney and brain of birds of both groups (Singh and Garge, 1987).

After inoculation of one day-old chicks with Ohmachi strain of FPV by the wing – web route, the virus is recovered from the inoculation site 2- days (PI); from trachea, heart, thymus, spleen, esophagus, crop and proventriculus, at four days (PI) and from the brain 17 –days PI (Nakashima et al., 1994). Tanizaki et al., (1989) stated that characteristic pox lesions composed of swelling and proliferation of cells with formation of Bollinger Bodies were seen in the epithelial cells of renal tubules, after inoculation of fowl pox virus intravenously.

Poxvirus was isolated from the liver, digestive tract, lungs of challenged birds inoculated with lesions of non-vaccinated dead canary. (Ostrowski et
Singh et al., (1992) studied the vascular and cellular reactions in quail skin induced by fowl pox virus using combination of histological and immuno-histochemical techniques. Subpopulations of lymphocytes were identified by staining techniques. In the leukocyte migration test as early as 24–hours after inoculation, population of heterophils and monocytes had been observed, and then they were later replaced by dense accumulation of lymphocytes and mononuclear macrophages forming lymphoid nodules. Intracytoplasmic inclusion bodies were demonstrated in hyperplastic feather follicular epithelium. Small and large lymphocytes, mononuclear cells, plasma cells and histiocytes were observed 5-weeks after inoculation. The proportional distribution of T-and B- lymphocytes showed a high T-cells response compared to B-cells (Singh et al., 1992). Mishr et al., (1995) isolated fowl pox virus from fowls previously vaccinated against fowl pox. The virus was propagated on CAM with characteristic pock lesions. The virus cytopathic for chick embryo fibroplast cell cultures. Hypertrophy and hyperplasia of ectodermal layer with esinoplasmic inclusions were produced by the virus.

In six-month-old canaries, proliferative "pox-like" lesions around the eyes and mouth were not seen until the 4th week. At necropsy, initially affected birds had cloudy air sacs and patchy pneumonia. Histologically, the lungs had proliferative necrotizing bronchitis. Birds necropsied later had proliferative skin lesions and intracytoplasmic inclusions typical of poxvirus in the epidermis and airway epithelium. A virus was isolated from an organ pool of lung, air sac, liver, and skin of affected birds and was identified by electron microscopy as poxvirus (Johnson and Castro, 1997).

Histologically and ultra-structurally, CPV undergoes morphogenic stages similar to FPV and other Chordopoxviruses (ChPV), causing type A and B
intracytoplasmic inclusions in epithelial and mononuclear cells of permissive hosts; however, CPV may have a broader tissue tropism than FPV (Boulanger, et al., 2000).

1.7. Clinical signs of fowl pox infection:

Incubation period of natural fowl pox infection is 4-8 days, after experimental infection it is 5-7 days. The course of the disease is usually chronic, it takes 3-4 weeks, and in case of complication it may prolongs to 8-9 weeks. The affected birds showed mild or severe systemic disturbance, restlessness, inappetance, loss of weight and drop of egg production (Beister, 1962). In turkey it causes emaciation and may cause blindness (Peter, 2000).

In acute form of the disease there is high mortality rate, which may arise from secondary bacterial infection. In birds that have generalized lesions or diphtheritic form, mortality rate is also high (Beister, 1962). An unusual type of fowl pox was first reported in Sudan by Mahasin et al. (1994), it was characterized by gasping respiration, and coughing followed by death.

CPV produces clinical signs similar to generalized pox viral infections of other birds, including both cutaneous and diphtheritic disease forms caused by the prototypical galliform Avipoxvirus, fowl pox virus (FPV), and including proliferative and necrotic changes in epithelial tissues of the dermis, notably around the eyes and commissures of the beak, feet and respiratory tract (Tripathy, and Reed. 1997).
1.8. Lesions:

1.8.1. Fowl pox lesions:

Fowl pox disease is characterized by cutaneous lesions, although there is diphtheritic form of the disease in which lesions appear in the mouth and upper respiratory tract. However this form of the disease is usually accompanied by cutaneous lesions in some chickens (Jordan, 1990). The disease can occur in one of three forms; cutaneous, diphtheritic or may be both. Signs depend upon; virulence of the virus, host susceptibility, distribution of the lesions, and other compilations (Tripathy, 1991).

1.8.1.1. Cutaneous form (skin or dry form):

The cutaneous form is characterized by the appearance of nodular lesions on the unfeathered areas of the body (comb, wattles, eyelids, around the vent and under the wings). The skin lesion is initially vesicular, then enlarged rapidly because of the proliferation of the virus in the epithelial cells and infiltration by inflammatory cell. The surface of the lesion is irregular and soon becomes yellowish – brown forming scab, which dry and drop off leaving a scar (Khogali, 1970; Hofstad, 1978).

A mixed breed rooster, from a backyard flock of 13- chickens, was received at California Veterinary Diagnostic Laboratory System-Turlock Branch for postmortem examination. The bird presented with thickened, featherless, scab-encrusted skin around the head region. Numerous stick tight fleas were found attached to the encrusted skin. Microscopic evaluation of the skin revealed a lymphoplasmacytic reaction in the dermis with visible embedded flea mouthparts. Also noted histologically in this region were epidermal hyperplasia and ballooned epidermal cells containing intracytoplasmic inclusions indicative of fowl pox virus, (Gustafson et al.,1997)
In generalized form, lesions in different stages are found in different parts of the body (Khogali, 1970; Hofstad, 1978). Secondary bacterial infection may take place in the suppuration or necroses of the deep layers of the skin with the formation of fibrinous deposits (Goodpasture, 1928). There is a typical fowl pox where the lesions are manifested in the feathered parts of the body mainly in the posterior dorsal area and external part of the thigh (Back et al., 1995). Lesions on the feet and legs were firstly reported by Johnson (1930).

1.8.1.2. Diphtheritic form (mucous membrane or wet form):

Cankers or diphtheritic yellowish lesions characterize this form. They appear as white opaque and slightly elevated nodules on the mucous membrane of the larynx, pharynx, nares, mouth and trachea. When lesions involve the trachea mild or severe respiratory sings appears. Nodules increase rapidly in size and usually coalesce to become yellow, cheesy, necrotic, pseudodiphtheretic or diphtheritic membrane. It is difficult to remove the fibrinous necrotic masses, and when removed they leave haemorrhagic surface (Goodpasture, 1928). Diphtheritic form is aggravated by contaminating bacteria. Tumor like swelling appears due to the inflammation that extends from the mouth region into the nasal sinuses, and extends to the pharynx, mouth and throat (Goodpasture, 1928). Difficult breathing takes place due to lesion in the larynges and trachea which may leads to death due to suffocation. Coalescence of the lesions around the eyelids can cause complete closure of one or both eyes. Most frequently mixed forms (diphtheritic and cutaneous) are present simultaneously (Goodpasture, 1928).

As described by Khogali (1972) in Sudan fowl pox has 3-forms; cutaneous, ocular and mucomembranous forms. The cutaneous is characterized by
production of wart-like nodules on the comb, wattles and eyelids. In the ocular form, both eyes are severely affected and damaged. In the mucous – membranous type, the mucosa of the mouth, larynx, nostrils and eyes are covered by diphtheritic membrane. Dirty and white patches cover the mouth, sides of the tongue, roof of the palates and around the eyelids. Large cheesy, necrotic nodules are found in severe cases causing inappetance and suffocation is found. Elamin, et al. (1980) reported that when fowl poxvirus inoculated intravenously in chickens, 3% of them developed a marked swelling of the comb or wattle 10-11 days after inoculation.

Nodular cutaneous and diphtheric oral lesions, resembling avian pox were observed in 2 flocks of young ostrich chickens. Typical eosinophilic intracytoplasmic inclusion bodies were seen in histological sections and a pox virus was isolated from the lesions, (Allwright et al., 1994).

1.9. Immune response to fowl poxvirus:

Recovered birds are solidly immune, and both cellular and humoral factors are involved with the possibility of persistence of the virus which might also be a factor in the long term protection (James and John, 1974; Tripathy and Hanson (1975). Chicks hatched from birds recently vaccinated or recovered from natural outbreaks carry passively transferred immunity which might interferes with vaccination of the progeny (Tripathy and John, 1984). Barbour et al. (1995) reported that facial papules, vesicles and reddish-brown to black scabs were observed in 85% of vaccinated males compared to none of the females when made comparative study between males and females immunity and resistance to the disease. They also found that vaccination against fowl pox by the wing web method revealed 96.7% of females that had a vaccinal reaction (vaccine take) compared to none of the males.
1.10. Laboratory host system:

Tripathy (1991) stated that avian poxviruses affect wide range of birds of various families naturally or artificially. Chicken usually used to determine the pathogenicity of new poxvirus isolates; they may not be suitable hosts for some isolates because of their lack of susceptibility. Some investigators reported that a substantial degree of host specificity exists among some avian poxviruses especially those that infect wild birds. Boosinger et al. (1982) noticed that poxviruses isolated from parrots and inoculated into susceptible parrots and chickens, it was more pathogenic for parrots than chickens, but it didn’t provide protection against fowl pox virus. Chickens vaccinated with fowl or pigeon pox viruses didn’t provide protection against psittacine poxvirus. Winterfield (1985) reported that peacocks had been vaccinated with a fowl poxvirus vaccine, but were the only birds affected among other wild and domestic birds in the aviary, that means they were not resistant to fowl poxvirus. An isolate of poxvirus from turkey had been vaccinated is antigenically different from fowl poxvirus.

Tripathy and Cunningham (1984) summarized differential studies, based on pathogenicity for chicken, fowl, pigeon, turkey and canary pox viruses. They found that canaries are highly susceptible to canary pox virus, but resistance to turkey, fowl, pigeon pox viruses, which produce mild infection in chickens and turkeys but are more pathogenic to pigeons. Susceptibility of ducks to turkey poxvirus has been suggested for differentiation of these two closely related viruses.
1.10.1. Cell culture:

Tripathy (1991) found that avian poxviruses can be propagated in cell culture of avian origin like chicken embryo fibroblast (CEF) derived from kidney cells, chicken embryo dermis and duck embryo fibroblast. Permanent cell line for some avian poxviruses could be used after adaptation. However, some isolates especially from turkey fail to grow in this cell line even after repeated passages (Moscovici et al., 1977).

1.10.2. Growth of fowl pox virus in the developing chick embryo:

Goodpasture and his colleagues (1931) had shown that fowl pox and vaccinia viruses could be grown on the CAM. They had always used large inocula and obtained confluent growth. Burnet (1933) also used concentrated inocula of the virus to get confluent growth. Sometime later, however, he noticed that with dilute suspensions, opaque spots of proliferating cells about few millimeters in diameter were produced. This system is comparable to plaque assay with bacteriophages; which might be employed for the titration of animal viruses and antisera to them. However, it was not until 1936, that he was to utilize the pock-counting technique for studying the relationship between canary pox virus and fowl pox virus (Burnet, and Lush, 1936).

Ducks and turkeys embryo were also used as well as other species of avian embryo (Goodpasture, 1931; Cunningham, 1973).

1.11. Diagnosis of fowl pox:

1.11.1. Clinical diagnosis:

Fowl pox should be suspected where skin eruptions occur on exposed areas. When only small lesions are present, it is difficult to distinguish the disease from the abrasions caused by fighting (Tripathy, 1980). In the cutaneous form (dry pox), development of proliferative lesions, ranging from
small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas is observed. In the diphtheritic form (wet pox); slightly elevated white opaque nodules develop on the mucous membranes. They rapidly increase in size to become a yellowish diphtheritic membrane. Lesions occur on the mucous membranes of the mouth, esophagus, larynx or trachea (Tripathy and Reed, 1998). Slow spread of the disease among the flock is highly suggestive (Tripathy, 1980). The diphtheritic form of fowl pox involving the trachea must be differentiated from infectious laryngotracheitis (ILT), which is caused by a herpes virus and is characterized by the presence of intranuclear inclusion bodies, and avitaminosis A lesions caused by Pantothenic acid or Biotin deficiency (Tripathy, 1980). The lesions in the diphtheritic form are relatively adherent and if removed leave sore ulcer, the fact that help in differentiation of the disease from infectious laryngotracheitis and avitaminosis A (Tripathy, 1980).

1.11.2. Identification of the causative agent:

Fowl pox virus multiplies in the cytoplasm of epithelial cells with the formation of large intracytoplasmic inclusion bodies (Bollinger bodies) that contain smaller elementary bodies (Borrel bodies). The inclusions can be demonstrated in sections of cutaneous and diphtheritic lesions by the use of Haematoxylin and Eosin (H& E), Acridine Orange or Giemsa stain (Tripathy et al., 1973). The elementary bodies can be detected in smears from lesions e.g. by the Gimenez method (Tripathy and Hanson, 1976). Electron microscopy can be used to demonstrate viral particles of typical poxvirus morphology by negative staining or in ultrathin sections of infected tissues (McFerran et al., 1971; Vankammen and Spradbrow, 1976).
1.11.3. Histopathology:

Gordan and Sreenivas (1992) studied the histological changes observed in the larynx; the lesions were characterized by hypertrophy and hyperplasia of the mucous producing cells. Numerous large eosinophilic of intracytoplasmic inclusion were present in hypertrophic epithelium. Aggregation of neutrophils and mononuclear cells were seen in the underlying lamina propria. There was also loss of cilia and flattening of the epithelial cells.

Electron microscopy can be used to demonstrate viral particles of typical poxvirus morphology by negative staining or in ultra thin section of infected tissues (Doane and Anderson, 1987). Tripathy (1991) reported that type (A) inclusion with virions around the periphery or virus field inclusions can be observed on electron microscopic examination.

1.11.3.1. A smear technique for fowl pox: (OIE, 2002):

- A drop of distilled water and the lesion (cutaneous or diphtheritic) was placed on a clean slide. A thin smear was prepared by pressing the lesion with another clean slide and rotating the upper slide several times.
- The smear was Air dried and gently fixed over a flame.
- The smear was Stained for 5-10 minutes with freshly prepared primary stain (8 ml )stock solution [a solution of basic fuchsin] (5 g) in 95% ethanol (100 ml) is slowly added to a second solution of crystalline phenol (10 g) in distilled water (900 ml). This stock solution, kept in a tightly screw-capped glass bottle, is incubated for 48 hours at 37°C and then stored at room temperature. Basic fuchsin mixed with 10 ml of phosphate buffer
[pH 7.5: NaH2PO4H2O] (2.47 g) and Na2HPO4 (11.65 g)] are added to distilled water (1000 ml) and stored at [4°C], pH 7.5 and filtered through Whatman filter paper (No.1).

- Wash thoroughly with tap water.

- Counter stain with malachite green (0.8% in distilled water) for 30-60 seconds

- Wash the smear with tap water and then dry.

- Examine the smear under oil immersion. The elementary bodies appear red and are approximately 0.2-0.3 µm in size.

1.11.4. Virus Isolation:

1.11.4.1. Avian embryo inoculation:

Fowl pox virus can be isolated by the inoculation of suspected material into embryonated chicken eggs. Approximately 0.1 ml of tissue suspension of skin or diphtheritic lesions, with the appropriate concentration of antibiotics, is inoculated on to the chorioallantoic membranes (CAMs) of 9-12-day-old developing chicken embryos. These are incubated at 37°C for 5-7 days, and then examined for focal white pock lesions or generalized thickening of the CAMs. Histopathological examination of the CAM lesions will reveal eosinophilic intracytoplasmic inclusion bodies following staining with H&E (Tripathy et al., 1973; Tripathy and Reed, 1997).

1.11.4.2. Cell culture:

Primary chicken embryo fibroblasts, chicken embryo kidney cells, chicken embryo dermis cells, or the permanent quail cell line QT-35, can also be used to propagate fowl pox virus (Ghildyal and Tripathy 1989; Schnitzlein et al., 1988). The adaptation of virus strains to cell cultures is an important requirement for plaque formation, as not all strains will form plaques initially.
1.11.5. Molecular methods:

1.11.5.1. Restriction endonuclease analysis (RFLP):

Restriction endonuclease analysis is a useful method for comparing closely related DNA genomes and can be used for comparison of field isolates and vaccine strains of fowl pox virus (Ghildyal et al., 1989; Schnitzlein et al., 1988).

1.11.5.2. Southern blot (DNA hybridization):

Cloned genomic fragments of fowl pox virus can be used effectively as nucleic acid probes for diagnosis of fowl pox. Viral DNA isolated from lesions can be detected by hybridization either with radioactively or non-radioactively labelled genomic probes. This method is especially useful for differentiation of fowl pox from infectious laryngotracheitis when tracheal lesions are present (Fatunmbi et al., 1995).

1.11.5.3. Polymerase chain reaction (PCR):

Genomic DNA sequences of various sizes can be amplified by the polymerase chain reaction (PCR) using specific primers (Lee et al., 1997). This technique is useful when there is only an extremely small amount of viral DNA in the sample.

1.11.6. Serological and Immunological tests:

Although both cell-mediated immunity (CMI) and humoral immunity play an important role in poxvirus infections, routine use of CMI test is not convenient. Therefore, serological tests, such as virus neutralization (VN), agar gel immunodiffusion (AGID), passive haemagglutination, fluorescent antibody tests as well as enzyme-linked immunosorbent assay (ELISA) are used to measure specific humoral antibody responses.
1.11.6.1. Virus neutralization:

After virus/serum interaction, the residual virus activity may be assayed in embryonating chicken eggs or in cell cultures (Morita, 1973). This technically demanding test may not be convenient for routine diagnosis. Only some selected strains of the virus have plaque-forming ability in chicken embryo cells. Neutralizing antibodies develop within 1-2 weeks of infection.

1.11.6.2. Agar gel immunodiffusion test (AGID):

Precipitating antibodies can be detected by reacting test sera against viral antigens. The antigen can be derived by sonication and homogenization of infected skin or CAM lesions. Precipitation lines develop in 24-48 hours after incubation of the antigen with antibody to homologous or closely related strains. The test is less sensitive than the ELISA (Buscaglia et al., 1985) or the passive haemagglutination test (Tripathy et al., 1970).

1.11.6.3. Passive haemagglutination (PHA):

As fowl poxvirus fails to agglutinate erythrocytes passive haemagglutination test is used. Tanned sheep or horse red blood cells are sensitized with a partially purified fowl pox viral antigen (Tripathy et al., 1970), after treatment with fluorocarbon (or sodium deoxycholate) can be used in PHA. PHA is more sensitive than AGID (Tripathy et al., 1973a). The antigen is prepared from infected CAMs or cells. The test will give cross-reactions among avian pox viruses (Tripathy et al., 1970).

1.11.6.4. Fluorescent antibody tests:

Direct or indirect immunofluorescent tests will reveal specific intracytoplasmic fluorescence in infected cells (Tripathy et al., 1970a; Tripathy 1996). The latter test is commonly used and involves two steps: The antibody against fowl pox virus is reacted with the antigen in the infected
cells, followed by a secondary fluorescein isothiocyanate labelled antibody against chicken gamma globulin (e.g. goat anti-chicken). In this regard, formalin-fixed tissue sections can be used effectively for fluorescent antibody tests.

1.11.6.5. **Immunoperoxidase test:**

Specific staining of cytoplasmic inclusions is achieved when horseradish peroxidase conjugated specific polyclonal antibody against fowl poxvirus is reacted with the hydrated sections of fowl pox-infected fixed tissues (CAM and skin) or cell culture. Similar results are obtained when either polyclonal or monoclonal antibodies are used in an indirect test.

An advantage of the technique is that the sections can be examined with the light microscope and can be stored for an extended period without loss of colour (Tripathy and Hanson, 1976).

1.11.6.6. **Enzyme-linked immunosorbent assay (ELISA):**

ELISA has been developed to detect humoral antibodies to fowl pox virus. They are capable of detecting antibody 7-10 days after infection (Buscaglia et al., 1985). Antigen is adsorbed onto a polystyrene surface and reacted with the test serum, followed by a horseradish peroxidase or alkaline phosphatase labelled anti-avian immunoglobulin antibody (Buscaglia et al., 1985; Iritani and Sawaguchi, 1994).

1.11.6.7. **Immunoblotting:**

Antigenic variations that occur between strains of fowl pox virus can be evaluated by means of Immunoblotting. In this method, viral antigens separated by sodium dodecyl sulphate/ polyacrylamide gel electrophoresis (SDS/PAGE) are reacted either with polyclonal or monoclonal antibodies against fowl pox virus (Ghildyal et al., 1989; Singh and Tripathy 2000).
1.11.6.8. **Counter immuno electrophoresis (CIE):**

This test is performed by reacting sera against partially purified viral antigen as in AGID but the test is more sensitive than AGID (Skdase and Sharma 1990).

1.11.7. **Efficacy test:**

Is done by applying suspension of suspected material from lesions to the skin of susceptible birds by comb scarification or by the stick or feather follicle methods. Typical cutaneous lesions at the site of inoculation are produced after 5-7 days indicating presence of the virus, (Brandly and Dunlap, 1938; Scott, et al., 1978).

1.11.8. **Rapid diagnosis of fowl pox with co-agglutination assay**

The co-agglutination test was standardized for detection of fowl pox antigen in infected scabs and chorioallantoic membrane of chicken embryos. The Staphylococcus aureus Cowan(I) strain, containing large amounts of Protein(A) in their cell wall, coated with fowl pox antibodies was found specific and sensitive for detection of fowl pox antigen. The test is easy to perform and rapid as the positive results can be read within 15 seconds, (Joshi and Shakya, 1994).

Chicken embryo dermis cells, or the permanent quail cell line QT-35, can also be used to propagate fowl pox virus (Ghildyal et al., 1989; Schnitzlein et al., 1988). The adaptation of virus strains to cell cultures is an important requirement for plaque formation, as not all strains will form plaques initially.
1.12. Immunity to fowl pox:

Recovery from fowl pox disease leads to solid immunity in which both humoral and cellular factors are involved with possibility of persistence of the virus, which might also be a factor in the long term protection, (James and John, 1984).

In a flock in which the disease has occurred for some years only young birds are infected. In previously uninfected flocks all ages of birds develop the disease, (Mockette, 1990).

Tripathy and Hanson (1975) stated that cell mediated and humoral immunity was affected by several classes of immunoglobulin function for subsequent protection. Chicks hatched from birds recently vaccinated or recovered from natural outbreak carry passively transferred immunity which might interfere with vaccination of the progeny.

1.13. Control of fowl pox:

All organisms e.g. bacteria, fungi and protozoa are susceptible to chemotherapy, except viruses. Control of viral diseases is dependent upon prevention through sanitation and biosecurity, and by vaccination. Strict sanitation and biosecurity are essential for successful poultry production. Vaccination is not substitute for effective management. Vaccines may be effective in reducing clinical disease, but exposed birds, in most cases, still become infected and shed disease organisms (Jacob, et al., 1998).

1.14. Vaccination against fowl pox:

Viruses stimulate development of immunity better than other types of organisms. Vaccines contain either live or killed micro organisms. Live–vaccine reproduces in the host to increase their numbers. A killed-virus product is dependent upon the type of antigen units present in the vaccine dose
to stimulate anti-body production. Most poultry vaccines are the live type, (Jacob, et al., 1998). Poxviridae are the largest of the known human and animal viruses. Due to their complex genetic structure, (Viky, 2000) thereby their strong immunogenic properties, poxviruses developed strategies of immune evasion that are distinct from those of smaller viruses. Pox can be prevented in chickens, turkeys, pigeons by vaccination, but there is no effective commercial vaccine against canary pox (Jacob, et al., 1998). Both fowl pox and pigeon pox vaccines can be used for turkey’s vaccination, (Gordan and Jordan, 1982).

Chickens and pigeons are usually vaccinated by the wing web stick method, (Jacob, et al., 1998). Turkeys are not generally vaccinated by the wing web route. Turkeys sleep with their head under their wings. Conjunctival (eye) pox can occur if the vaccine is administered to turkeys via the wing web method. So turkeys are vaccinated by a thigh-stick method, (Jacob, et al., 1998).

All birds within a house should be vaccinated on the same day if pox appears in a flock, (Cunningham, 1978). Vaccination is usually done in areas where fowl pox is endemic or there have been outbreaks in the last season. Most layers and breeders are vaccinated before they come into lay, (Mockett, 1990).

All domestic chicks and poultcs can be vaccinated at 1-day of age, pullets at 10 to 12 weeks, and turkeys at 8 to 14 weeks or when moved to range, (Jacob, et al., 1998). But vaccination of poultry younger than 10-days of age cannot be expected to produce, uniform or lasting immunity even in the absence of parental immunity [an exception is that vaccination for Marek’s disease], (Jacob, et al., 1998).
In the endemic areas the prevailing virus type should be determined (Jacob, et al., 1998).

Quail pox has been shown to affect chickens. There is no cross protection between quail pox and fowl pox. Vaccination for both should be necessary if both are endemic in the area. Flocks can be given fowl pox vaccination to reduce the severity of an outbreak, (Jacob, et al., 1998).

The virus is spread from bird to bird through the bites of blood-sucking insects or through wound or scratches by birds when fighting. If there is a heavy mosquito infestation in an area, fowl-pox vaccination may be considered, (J.P. Jacob, et al., 1998).

The Avipoxvirus genus has a host range which is restricted to avian species. Attenuated vaccine strains of these viruses are commercially available (Birch et al., 1999). Avipoxviruses show promise not only as safe vectors for the construction of live recombinant poultry vaccines, but also as vectors for replication-defective mammalian vaccines (Birch et al., 1999).

The Avipoxviruses; canary pox and fowl pox, infect mammalian cells in a manner that results in efficient protein expression but does not lead to virus production. This has provided a basis for developing Avipox-based vaccines constructs for measles, influenza, rabies, and SIV (Jenkins et al., 1991; Taylor et al., 1992).

1.15 Immune response between Avipox viruses:

Pox virus isolated from psittacine birds was used as a vaccine in trials with love birds (Agapornis roseicollis). The vaccine was applied by wing-web puncture using single-and double-needle applicators. Immunity was effective against challenge with virulent psittacine pox virus administered via the feather follicle/thigh. When unvaccinated contact control birds were placed with the vaccinated individuals immediately post-vaccination, virus spread
was evident. However, susceptible birds placed with vaccinated ones at 27-days post-vaccination remained uninfected for 11-weeks. The importance of a high vaccine virus titre was observed, (Winterfield and Schrader 1995).

Groups of 3-week-old specific pathogen-free chickens immunized with a commercial live-virus quail pox vaccine (Bio-Pox Q) were not protected against challenge with "variant" poxviruses isolated from chickens that were previously vaccinated with commercial fowl pox vaccine. The percentages of vaccinated chickens resistant to challenge with each of the five variant field isolates were 0%, 20%, 0%, 20%, and 10%, respectively. However, when immunity engendered by the variant field isolates was challenged with the commercial quail pox vaccine virus, 80%, 70%, 80%, 50%, and 60% of the vaccinates, respectively, were protected. Results from cross-immunity studies indicate that the commercial quail pox vaccine does share some immunologic relationship with these variant poxvirus field isolates, but not enough to be used in the control of some outbreaks of pox caused by variant poxviruses, (Fatunmbi and Reed, 1996).

Three-week-old specific-pathogen-free chickens were vaccinated with either a commercial modified live virus fowl pox vaccine or five "variant" poxvirus field isolates. Immunity engendered by the commercial modified vaccine or field isolates was challenged with either the variant isolates or commercial modified vaccine virus. The commercial modified vaccine did not adequately protect vaccinates against challenge with the variant isolates. The percentages of vaccinated chickens protected following challenge with each of the variant isolates were 70%, 20%, 30%, 20%, and 25%. However when the isolates were applied as vaccines, 100% of the vaccinated were protected against challenge from the modified vaccine virus. Furthermore, the variant poxvirus isolates offered excellent protection from challenge with homologous variant
isolates. The modified live virus vaccine was expected to offer significant protection against challenge from the variant pox isolates, but in this experiment it did not. The variant isolates tested may be good vaccine candidates to prevent the vaccine breaks currently encountered in previously pox-vaccinated flocks, (Fatunmbi and Reed, 1996).

1.16. Route of vaccination of fowl pox vaccine:

1.16.1. Wing web (subcutaneous):

Two grooved needles are bound together to make a double pronged instrument. The instrument is dipped into the vaccine and then thrust through the wing web (the front part of the wing), (Mayer and Dannner, 1976).

1.16.2. Feather follicles:

Feathers (about 5-feathers) are removed from the thigh and the vaccine is brushed into the resulting follicles (Mockett, 1990).

1.16.3. Oral route:

Sarma and Sharma (1988) and Saini et al., (1990) studied the immunization against fowl pox orally at 7-days old chicks using a highly attenuated fowl pox virus strain. They found that another dose of the vaccine was required at 95 - days of age.

The immune response of chicks to oral vaccination with HP1-strain of fowl pox virus was studied using intracellular virus alone or a combination of intra and extracellular viruses. The first and second vaccinations were done at four days and 25 days of age, respectively. In both groups the birds showed 50% protection against challenge virus at 32-days of age while no immunity was recorded at 95-days of age. The serum IgG concentration in both the vaccinated groups was comparable and it was significantly higher (P less than 0.05) than the control birds one week after revaccination. The serum
haemolytic complement activity in both the vaccinated groups was significantly lower (P less than 0.05) than the control birds, (Saini et al., 1985)

1.16.4. Intravenous route:

Siddique et al., (1997) studied antibody titers in chicks following pigeon pox virus vaccine inoculation, they found that intravenous route of immunization was the most effective following thigh feather follicle, intramuscular and oral route.

1.16.5. Aerosol Vaccination:

Aerosol immunization, with the freeze dried vaccine prepared from pigeon pox virus New Jersey strain in chick embryo, of chicks 30-120 days. The survival rate of vaccine virus during the first 15-minutes after atomization was higher at 85-95% relative humidity (45-60%). Best result from the vaccine at 85-95% relative humidity was obtained in the following media: 5% suspension of dried, low fat milk in physiological saline, distilled water, and meat peptone broth. The minimum immunization dose of the vaccine for aerosol vaccination is $10^{2.32}$ EID$_{50}$/ml, but the optimum dose was 5-6 times greater. At $10^{2.45}$ EID$_{50}$ 93% of chicks resisted challenge by epizootic fowl pox virus strain (10EID$_{50}$/ml). After revaccination with an interval of 21-days all chicks resisted this challenge while control showed 100% mortality. Immunity developed after 11-days, and at least 190-days (Tripathy, 1996).
1.17. Pigeon pox vaccine:

Pigeon pox vaccine is very effective in preventing pox in pigeons, (Morton, 2003). It contains live, non attenuated, natural virus, (Tripathy and Cunningham, 1984). The virus is less pathogenic for chickens and turkeys, (Tripathy and Cunningham, 1984). Pigeons may be vaccinated by the follicle method at any age down to squabs of 6 weeks of age, (Morton, 2003). The vaccine can be used on chickens of any age, but it is generally used on 4-weeks old chickens and about a month off egg production, (Tripathy, 1991). When birds younger than 4-weeks old are vaccinated they should be re-vaccinated before production, (Tripathy, 1991). Birds held for the second year of production should be revaccinated, (Tripathy, 1991).

1.17.1. Methods of vaccination:
1.17.1.1. 'Needle stab' or 'stick' method:

Two grooved needles are bound together to make a double pronged instrument. The instrument is dipped into the vaccine and then thrust through the wing web [the front part of the wing], (Morton, 2003).

1.17.1.2. The follicle method:

Sufficient feathers are plucked from the thigh of the bird to expose about 15 mm of skin. The vaccine is then swabbed into the feather follicles, (Morton, 2003).

1.17.2. Duration of immunity following vaccination:

Immunity following vaccination with fowl pox vaccine is lasting immunity, but with pigeon pox vaccine the immunity only lasts for 6-months, (Jordan, 1990). Vaccination provides no protection during the first 2-3 weeks after vaccination, and the bird may become naturally infected with the disease. Maximum immunity is usually attained by the end of the fourth week, (Winterfield and Reed, 1985).
If pox was present in the previous year and pigeon pox vaccine was used birds should be revaccinated with fowl pox vaccine, because immunity from pigeon pox vaccine is not of long duration, (Tripathy and Cunningham, 1984).

1.18. Canary pox vaccine:
CPV has been successfully used as a host range-restricted mammalian expression vector and is a vaccine vector of increasing importance, with CNV-based veterinary vaccines commercially available, (Gilbert, et al., 2003). Licensed and experimental CNV-based vaccines, most of which utilize the highly attenuated ALVAC strain of CNV (Tartaglia, et al., 1992), encode a range of pathogen and tumor-associated antigens. Vaccine use takes advantage of the abortive infection that Avipoxvirus vectors undergo in mammalian cells while still expressing virally encoded antigens to safely generate cellular, humoral, and protective immune responses (Somogyi, and Skinner, 1993). CNV-based vaccines have proven effective in prime-boost vaccine strategies and as immunoadjuvants through expression of recombinant cytokines and co-stimulatory proteins (Pancholi, et al., 2001). Recent evidence suggests that dendritic cell antigen presentation, maturation, and apoptosis are important in CNPV-generated immunity, (Marovich, et al., 2002). Improved understanding of virus-host interactions should yield improved vaccine vectors, to create a third-generation CNPV-based vaccine (Jin, et al., 2002).
CHAPTER TWO

MATERIALS AND METHODS

2.1. Embryonated eggs:

10-12 days-old egg embryos were obtained from commercial poultry farm in Khartoum, (CORAL).

2.2. Experimental Birds:

- 1-old day chicks; were obtained from commercial poultry farm in Khartoum (CORAL), and were reared in Central Veterinary Research Laboratory (CVL) Soba till they became 3 month-old. They were not vaccinated against fowl pox. The birds were divided into 4 groups, each group contained 20 birds.

- Pigeons; were obtained from the field. The birds were divided into 3 groups

- Canaries; were obtained from the field. The birds were divided into 3 groups

2.3. Avian pox viruses studied:

The following virus isolates were used in the study:

2.3.1. Fowl pox virus (FPV) isolate:

The virus isolate was kindly provided by Dr. Mahasin El-Nur, Department of Virology Central Veterinary Research Laboratory (CVL) Soba. It was isolated from a naturally infected chicken propagated in chick-embryos and confirmed as FPV by AGPT using FP immune serum in (1998).
2.3.2. Canary pox (CPV) isolate:

This virus isolate was kindly provided by Dr. Khalid Abd-Alrahman, Department of Microbiology- Faculty of Veterinary Medicine- University of Khartoum. It was isolated and propagated in chick embryo in (2000).

2.3.3. Pigeon pox virus (PPV) isolate:

The virus isolate was kindly provided by Dr. Mahasin El-Nur, Department of Virology Central Veterinary Research Laboratory-CVL-Soba. It was isolated from a naturally infected pigeon at AL Gerief West area in (1998).

2.4. Preparation and sterilization of Equipment:

2.4.1. Glassware:

Glassware such as; beakers, flasks, pipettes, cylinders, centrifuge tubes, were boiled in water with a detergent for 20-mins, and were rinsed in running tape water for 5-minutes to remove the detergent completely. They were then rinsed over night in distilled water (D.W) and left to dry. After that they were sterilized with dry heat in the oven at 180ºc for 2-hrs.

Dissecting equipments such as; forceps, scissors and scalpel handles were sterilized after thorough washing as described above. When live virus was used, glassware equipment were boiled soaked for 3-days in losan disinfectant rinsed in tap-water, soaked over night in 1%methyl solution in 1%HcIsol. for 2-hrs. Then rinsed in tab water and finally in deionized distilled water D.D.W. Then sterilized by autoclaving for 30-mins., at 15-pound pressure or in an oven at 160ºc for 5-hrs.
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2.4.2. Sterilization of Microtitre plates:

When live virus was used as antigen, the plates were shaken in a solution of 1% NaOH till the red cells were loosened and removed. They were then soaked in hydrochloric acid solution for 2-hrs. The plates were then rinsed in 4-changes of deionized distilled water (D.D.W.) and left to dry at room temperature.

2.5. Preparation of viruses’ antigen:

2.5.1. Preparation of fowl pox antigen:

A frozen suspension that contained pox virus was left to warm at room temperature. 10-fold serial dilution from $10^{-1}$ to $10^{-3}$ was prepared, (after adding antibiotic and antifungal). 0.1ml of $10^{-3}$ dilution was inoculated into the CAMs of 11-12 days old embryonated chicken eggs. Every day post inoculation the injected CAMs were examined by candling. Embryos which died in the first day post inoculation were discarded, (because death is of unknown reason). The eggs were removed from the incubator on the sixth day and chilled overnight in the fridge. On the following day eggs were harvested.

The harvest was done as described by Tripathy and Le Hanson, (1984). The shells were disinfected over the small end of the egg by alcohol 70%. The ends of the egg were cracked by sterile forceps. Then the embryos and yolk were extracted by forceps. The CAMs were removed from the shells by sterile forceps. The CAMs were placed in Petri-dishes and examined for pock lesions formation and thickening of the CAMs. The infected CAMs were collected and were ground with sterile fine sand in a sterile mortar.

A suspension of 50% in normal saline was prepared. The suspension was centrifuged for 10min. at approximately 2000 r.p.m. to remove large tissue particles. Antibiotics and Nystatin were added to the suspension, which was
used as inoculums. Volumes of 0.2ml of the homogenate were inoculated again in new sets of 11-13 old embryonated chicken eggs.

2.5.2 Preparation of pigeon and canary viruses' antigen:
The same above procedure was used for inoculation and preparation of pigeon and canary antigen.

2.6. Virus Titration in chick embryos:
Purpose: to determine EID\textsubscript{50} of the virus.
The method used was described by Baxendat \textit{et al.}, (1971), the titration was carried out with 10-fold dilution of viruses in phosphate buffered saline (PBS) containing antibiotic, and 0.1ml of each dilution was inoculated into CAMs of 5-egg- embryos which were then incubated at 37°C for 5-days before the CAMs were examined daily for the presence of pock lesions, for each dilution separately. The EID\textsubscript{50} of the virus was calculated by Read and Munch, (1938) method.

2.7. Experimental infection:
2.7.1. Experimental plan:
200-birds at the age of 3-monthes were divided into 4-equal groups. The infection was accomplished as follow; Serial dilution from 10^{-1}-10^{-3} of stock viruses was prepared and used as inoculums. The birds were divided for immunization as follows:
Table (1)
Experimental infection

<table>
<thead>
<tr>
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<th>Group (A)</th>
<th>Group (B)</th>
<th>Group (C)</th>
<th>Group (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain</strong></td>
<td>Control inoculated birds</td>
<td>Canary pox virus.</td>
<td>Pigeon pox virus.</td>
<td>Fowl pox field virus.</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>Control inoculated birds</td>
<td>0.1ml of 50% virus</td>
<td>0.1ml of 50% virus</td>
<td>0.1ml of 50% virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>suspension in PBS</td>
<td>suspension in PBS</td>
<td>suspension in PBS</td>
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<tr>
<td><strong>Route</strong></td>
<td>Control inoculated birds</td>
<td>(I/m) + wing web.</td>
<td>I/m + wing web.</td>
<td>I/m + wing web.</td>
</tr>
</tbody>
</table>

Birds were examined daily for 2-weeks, for the development of lesion and or clinical signs.

2.7.2. Collection of Serum Samples:

Blood was collected every week. Whole blood was collected from the heart of the birds directly in sterile tubes, by syringes. Collected blood was incubated more than two hours at room temperature. Sera were separated in sterile tubes and then stored at (-20ºc) till used. The sera were collected to detect any response to the infection by the three different viruses (FPV, CPV, and PPV). Serum that collected from the control was used as negative serum.

2.8. Preparation of inocula for challenge:

Serial dilution from $10^{-1}$-$10^{-3}$ of stock viruses (FPV, CPV, and PPV) was prepared and used as inoculums.

Every group was divided into 3-sub groups. The dose that given for every bird was 0.1ml of 50% virus suspension in PBS; the route of infection was intra muscurally (I/m) and by wing web.
2.9. Cross-protection study:

After 2-weeks post infection (PI) blood was collected from each chicken. Serum was separated. Then group (A) was divided into 3-sub groups (A1, A2, A3). Group (B) was divided into 3-sub groups (B1, B2, and B3). Group (C) was divided into 3-sub groups (C1, C2, and C3). Group (D) was divided into 3-sub groups (D1, D2, and D3) and was infected and challenged as follows: Group (A) contained control birds; Group (B), (C) and (D) contained birds to be infected and cross challenged with strains; fowl pox virus (FPV), canary pox virus (CPV) and pigeon pox virus (PPV) respectively as seen in table (2).

Table (2)

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
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</table>
2.9.1. Examination of challenge birds:

Birds were examined daily for 2-weeks to clinical signs and mortality among challenge birds.

2.10. Method and examination of the agar-gel precipitation test (AGPT):

This method was carried to detect positive fowl pox, pigeon pox and canary pox antigens in cross protection.

4-wells were drilled using template paper which was placed under the Petridis containing AGPT media, and the wells were made using 4-mm diameter tubular metal gel cutter. The plugs were removed using a needle. The distance between wells was approximately 0.5µ. Each well contained 7-plugs; central one and 6-around it. The Antigen was added to sodium dioxycolate (1:1) and placed in the central one and the antiserum was placed in the other 6-ones, with different dilutions (using 2-fold dilution). Each well received 25µ of the tested sera. The plates were incubated for 24-hrs., at room temperature in a humid chamber, then examined for the presence of the precipitation bands in a dark room through indirect light, (Tripathy, 1996).

2.11. Passive Haemagglutination (PHA) Test:

2.11.1. Preparation of Sensitized Sheep Red Blood Cells (RBCs):

Sensitized sheep RBCs were prepared as described by Tripathy et al., (1970b).

Whole blood was collected from the jugular vein of a sheep in a tub containing anticoagulant (EDTA). The blood then centrifuged (1000rpm for 5-minutes) the supernatant layer was taken off and the remaining layer (RBCs)
were washed 3-times with normal saline (PH7.2) (1000rpm for 5-minutes). Equal volumes of 3% formalin (in normal saline PH7.2) and 8% RBCs (in normal saline) were added together. The mixture was incubated at 37°C for 20-hrs. After 20-hrs the supernatant layer was removed using a pipette and the remained formalinized RBCs were washed 4-times with distilled water to remove the formalin. Normal saline (PH7.2) was added to the RBCs in a ratio of 1:10 i.e. 10% RBCs suspension.

2.11.2. Preparation of Formalized Tanned RBCs:

Equal volumes of 10% RBCs and tannic acid (1:20000) were mixed together and then incubated at 37°C for 15 minutes with occasional shaking, then washed twice with normal saline (PH7.2) by centrifugation, (1500rpm for 10-minutes). 10% suspension was made 10% in normal saline i.e. we end up with 10% sensitized sheep RBCs.

2.11.3. Sensitization of Formalized Tanned Erythrocytes by The Virus:

1ml of 10% sensitized sheep RBCs were added to 3ml of fowl pox virus (at concentration of the virus to PBS 50%:50%) and 2ml of phosphate buffer saline (PH6.4). The mixture was incubated at 37°C for 15-minutes with occasional shaking. At the end of incubation RBCs were washed twice with 1% of normal rabbit serum in normal saline (N.S), PH7.2, and resuspended to 2.5% in serum diluents and stored at 4°C.

2.11.4. Procedure of Passive Haemagglutination (PHA) Test:

25µ of tested sera was distributed in all 96 U-shaped wells of the micro titer plate. In each plate 8-sera were used, they consisted of one control positive fowl pox virus (F.P.F.) and control negative sensitized formalized RBCs and 6- tested sera. The sera were diluted 2-fold dilution in 0.025ml of
serum diluents from 1:2 to 1:6 were prepared using 0.025ml of each reagent. Then 0.05ml of virus coated RBCs was added to all wells.

Micro titer plats were covered and shake for seconds and incubated at 37°C for 24hrs. Micro titre plates were examined visually for the presence of haemagglutination.

2.12. Experimental Infection of Canary birds by fowl, pigeon, and canary pox viruses:

Canary birds were divided into 3-groups, as shown in table (3).

Group (A) was infected by canary pox virus (CPV).

Group (B) was infected by pigeon pox virus (PPV).

Group (C) group was infected by fowl pox virus (FPV).

They were checked daily for 15-days to notice the appearance of lesions and signs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Route</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-A</td>
<td>C.P.V</td>
<td>(I/m) +wing web.</td>
<td>0.1ml of 50%virus suspension in PBS</td>
</tr>
<tr>
<td>Group-B</td>
<td>P.P.V.</td>
<td>(I/m) +wing web.</td>
<td>0.1ml of 50%virus suspension in PBS</td>
</tr>
<tr>
<td>Group-C</td>
<td>F.P.V.</td>
<td>(I/m) +wing web.</td>
<td>0.1ml of 50%virus suspension in PBS</td>
</tr>
</tbody>
</table>
2.13. Experimental infection of pigeons by fowl, pigeon and canary pox viruses:

Pigeons were divided into 3-groups, as shown in table (4).
Group (A) was infected by canary pox virus (CPV).
Group (B) was infected by pigeon pox virus (PPV).
Group(C) was infected by fowl pox virus (FPV).
They were checked daily for 15-days to notice the appearance of lesions and signs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Route</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group-A</strong></td>
<td>C.P.</td>
<td>(I/m) +wing web</td>
<td>0.1ml of 50% virus suspension in PBS</td>
</tr>
<tr>
<td><strong>Group-B</strong></td>
<td>P.P</td>
<td>(I/m) +wing web</td>
<td>0.1ml of 50% virus suspension in PBS</td>
</tr>
<tr>
<td><strong>Group-C</strong></td>
<td>F.P.F.</td>
<td>(I/m) +wing web</td>
<td>0.1ml of 50% virus suspension in PBS</td>
</tr>
</tbody>
</table>
CHAPTER THREE
RESULTS

3.1. Virus isolation and growth in the CAM of chick embryos:
3.1.1. Inoculation of the chick embryos with canary pox virus:

The virus was successfully isolated in CAM of embryonated eggs and showed marked pock lesions. The morphologically distinct pock lesions were produced in CAM as clear white opaque small individual lesions, about 0.5-1mm in size. Thickening of the membrane and haemorrhagic lesions were also observed, as shown in Fig. (4).

3.1.2. Inoculation of the chick embryos with pigeon pox virus:

Morphologically distinct pock lesions in the CAM were seen. They were opaque, flat and about 0.1-0.3mm in size. Focal haemorrhagic lesion and thickening of the membrane were observed, as shown in Fig. (5).

3.1.3. Inoculation of the chick embryos with Fowl Pox Virus:

Morphologically distinct pock lesions were produced in the CAM. They were about 1-1.5mm in size, which diffused from the site of inoculation through the membrane, with clear thickening of the membrane, and haemorrhagic lesions were also observed, as shown in Fig(6).
Fig (4): CAMs 7 days post infection with C.P.V. Note pock lesions in CAMs.

Fig (5): CAMs 7 days post infection with P.P.V. Note pock lesions in CAMs.

Fig (6): CAMs 7 days post infection with F.P.V. Note pock lesions in CAMs.
3.2. Determination of embryo infective Dose$_{50}$ (EID$_{50}$) for The 3-viruse:

Inoculated embryos were examined for pock lesions, 5-days post inoculation.

The EID$_{50}$ is calculated for each virus by Reed & Muench method (1938):
Proportional distance (PD) =

\[
\text{Percentage infected at dilution next above } 50\% - 50 \\
\text{Percentage infected at dilution next above } 50\% - \text{Percentage infected at dilution next below } 50\%
\]

The 50% end point is calculated using the following formula:
Log. of the 50% end point = (log. Dilution above 50%) – (PD x log. dilution factor).

1. EID$_{50}$ for canary pox virus (CPV):

\[
\text{PD} = \frac{75 - 50}{75 - 25} = \frac{25}{50} = 0.5
\]

Negative log. of the lower dilution (next above 50%) = -10
Proportional distance (0.8) x dilution factor (log. 10) = -0.5
LD$_{50}$ titer = -10.5

Log. LD$_{50}$ titer = $10^{10.5}$
LD$_{50}$ = $10^{10.5}$
The EID$_{50}$ = $10^{10.5}/0.1$
2. **EID\(_{50}\) for fowl pox virus (FPV):**

\[
\text{PD} = \frac{60 - 50}{60 - 20} = \frac{10}{40} = 0.3
\]

Negative log. of the lower dilution (next above 50%) = -9

Proportional distance (0.8) x dilution factor (log. 10) = -0.3

LD\(_{50}\) titer = -9.3

Log. LD\(_{50}\) titer = 10\(^{-9.3}\)

LD\(_{50}\) = 10\(^{-9.3}\)

The EID\(_{50}\) = 10\(^{-9.3}/0.1\text{ml}\)

3. **EID\(_{50}\) for pigeon pox virus (PPV):**

\[
\text{PD} = \frac{60 - 50}{60 - 20} = \frac{10}{40} = 0.3
\]

Negative log. of the lower dilution (next above 50%) = -9

Proportional distance (0.8) x dilution factor (log. 10) = -0.3

LD\(_{50}\) titer = -9.3

Log. LD\(_{50}\) titer = 10\(^{-9.3}\)

LD\(_{50}\) = 10\(^{-9.3}\)

The EID\(_{50}\) = 10\(^{-9.3}/0.1\text{ml}\)

The EID\(_{50}\) of pigeon and fowl pox viruses gave the same results (10\(^{-9.3}\)) pock forming unit/0.1ml. Where as the EID\(_{50}\) of canary pox virus was 10\(^{-10.5}\) pock forming unit/0.1ml.
3.3. **Virus identification:**

3.3.1. **Agar gel precipitation test (AGPT):**

Reaction of the various sera raised against the respective viral antigen gave clear precipitation bands between the known positive results. Sera obtained from experimental animals were (1-sample) control, (3-samples) post infection, and (12-samples) post challenge. As shown in figures (7, 8, and 9), and tables follow:
FP) Fig (7), (CP), Fig (8) and (P.P) Fig,(9) antigens was in the central well. The wells around contained FP antiserum with different dilutions (using 2-fold dilution) starting from No.1 up to No. 5. No. 0 was not diluted. Clear perception bands showed positive results.
As shown in table (5) detection of:
1-Positive fowl pox antigen by AGPT with antiserum of birds infected by FPV, CPV, PPV and serum of none infected birds respectively.
2- Positive pigeon pox antigen by AGPT with antiserum of birds infected by FPV, CPV, PPV and serum of none infected birds (control) respectively.
3- Positive fowl pox antigen with antiserum of birds infected by FPV, CPV, PPV and serum of none infected birds respectively. The results were as shown in the table (5).

**Table (5)**

Detection of positive fowl pox, canary pox and pigeon pox antigen by AGPT in cross infected birds

<table>
<thead>
<tr>
<th>Antisera</th>
<th>FP</th>
<th>CP</th>
<th>PP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>0 ++</td>
<td>1/16++</td>
<td>0 ++</td>
<td>1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++</td>
<td>1/32 ++</td>
<td>½ ++</td>
<td>1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++</td>
<td>1/64 ++</td>
<td>¼ ++</td>
<td>1/64 ++</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>0 ++</td>
<td>1/16+</td>
<td>0 ++</td>
<td>1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++</td>
<td>1/32 +</td>
<td>½ ++</td>
<td>1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++</td>
<td>1/64 +</td>
<td>¼ ++</td>
<td>1/64 +</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>0 ++</td>
<td>1/16++</td>
<td>0 ++</td>
<td>1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++</td>
<td>1/32 ++</td>
<td>½ ++</td>
<td>1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++</td>
<td>1/64 ++</td>
<td>¼ ++</td>
<td>1/64 ++</td>
</tr>
</tbody>
</table>

**Note:**

Dilution was 2-fold (1/2, 1/4, 1/16, 1/23, 1/64).

- 0 = No dilution.
- ++ = clear & strong lines.
- = negative result.
- + = weaker lines.
As shown in table (6) Detection of;

1- Positive fowl pox (FP) antigen by AGPT with antiserum of control birds challenged by fowl pox virus (FPV), canary pox virus (CPV) and pigeon pox virus (PPV) respectively.

2- Positive pigeon pox antigen by AGPT with antiserum of control birds challenged by FPV, CPV, and PPV respectively.

3- Positive canary pox antigen by AGPT with antiserum of control birds challenged by FPV, CPV, and PPV respectively.

Table (6)
Detection of positive fowl pox, canary pox and pigeon pox antigens by AGPT in control birds challenged with FPV, CPV and PPV

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Control + FPV</th>
<th>Control + CPV</th>
<th>Control + PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 FP</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 +</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 +</td>
</tr>
<tr>
<td>2 PP</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 +</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 +</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 +</td>
</tr>
<tr>
<td>3 CP</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
<td>0 ++ 1/16 ++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 +</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 +</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 –</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 +</td>
</tr>
</tbody>
</table>

Note:
Dilution was 2-fold (1/2, 1/4, 1/16, 1/23, 1/64).

0 = No dilution.

++ = clear & strong lines. – = negative result.

+ = weaker lines.
As shown in table (7) detection of;
1-positive fowl pox antigen with antiserum of birds cross infected by FPV and challenged by FPV, CPV, and PPV respectively.
2- Detection of positive pigeon pox antigen with antiserum of birds cross infected by FPV and challenged by FPV, CPV, and PPV respectively.
3- Detection of positive canary pox antigen with antiserum of birds cross infected by FPV and challenged by FPV, CPV, and PPV respectively.

Table (7)
Detection of positive fowl pox, canary pox and pigeon pox antigens by AGPT in cross infected birds with FPV and challenged with FPV, CPV and PPV

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Antigen</th>
<th>FPV + FPV</th>
<th>FPV + CPV</th>
<th>FPV + PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FP</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64+</td>
<td>¼ ++ 1/64 -</td>
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<tr>
<td>2</td>
<td>PP</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>½ ++ 1/32 +</td>
<td>½ ++ 1/32 +</td>
<td>½ ++ 1/32 ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¼ ++ 1/64 +</td>
<td>¼ ++ 1/64+</td>
<td>¼ ++ 1/64 ++</td>
</tr>
<tr>
<td>3</td>
<td>CP</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¼ ++ 1/64 ±</td>
<td>¼ ++ 1/64+</td>
<td>¼ ++ 1/64+</td>
</tr>
</tbody>
</table>

Note:
Dilution was 2-fold (1/2, 1/4, 1/16, 1/23, 1/64).
0 = No dilution.
++ = clear & strong lines.
+ = weaker lines.
- = negative result.
± = not clear.
As shown in table (8) detection of;

1- Positive fowl pox antigen with antiserum of birds cross infected by CPV and challenged by FPV, CPV, and PPV respectively.

2- Positive pigeon pox antigen with antiserum of birds cross infected by CPV and challenged by FPV, CPV, and PPV respectively.

3- Positive canary pox antigen with antiserum of birds cross infected by CPV and challenged by FPV, CPV, and PPV respectively.

Table (8)

Detection of positive fowl pox, canary pox and pigeon pox antigens by AGPT in cross infected birds with CPV and challenged with FPV, CPV and PPV

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CPV + FPV</th>
<th>CPV + CPV</th>
<th>CPV + PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FP</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
</tr>
<tr>
<td>2</td>
<td>PP</td>
<td>0 ++ 1/16++</td>
<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
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<tr>
<td>3</td>
<td>CP</td>
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<td>0 ++ 1/16++</td>
</tr>
<tr>
<td></td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
<td>½ ++ 1/32 ++</td>
</tr>
<tr>
<td></td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
<td>¼ ++ 1/64 ++</td>
</tr>
</tbody>
</table>

Note:

Dilution was 2-fold (1/2, 1/4, 1/16, 1/23, 1/64).

- 0 = No dilution.
- ++ = Clear and strong lines.
- – = Negative result.
- + = Weaker lines.
As shown in table (9) detection of;
1-positive fowl pox antigen with antiserum of birds cross infected by PPV and challenged by FPV, CPV, and PPV respectively.
2- Detection of positive pigeon pox antigen with antiserum of birds cross infected by PPV and challenged by FPV, CPV, and PPV challenged by FPV, CPV, and PPV respectively.
3- Detection of positive canary pox antigen with antiserum of birds cross infected by PPV and challenged by FPV, CPV, and PPV respectively.

### Table (9)
Detection of positive fowl pox, canary pox and pigeon pox antigens by AGPT in cross infected birds with PPV and challenged with FPV, CPV and PPV

<table>
<thead>
<tr>
<th>Antigen</th>
<th>PPV + FPV</th>
<th>PPV + CPV</th>
<th>PPV + PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td><strong>FP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ++</td>
<td>1/16++</td>
<td>0 ++</td>
<td>0 ++</td>
</tr>
<tr>
<td>½ ++</td>
<td>1/32 ++</td>
<td>½ ++</td>
<td>½ ++</td>
</tr>
<tr>
<td>¼ ++</td>
<td>1/64 ++</td>
<td>¼ ++</td>
<td>¼ ++</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td><strong>PP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ++</td>
<td>1/16++</td>
<td>0 ++</td>
<td>0 ++</td>
</tr>
<tr>
<td>½ ++</td>
<td>1/32 ++</td>
<td>½ ++</td>
<td>½ ++</td>
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<tr>
<td>¼ ++</td>
<td>1/64 ++</td>
<td>¼ ++</td>
<td>¼ ++</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td><strong>CP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ++</td>
<td>1/16++</td>
<td>0 ++</td>
<td>0 ++</td>
</tr>
<tr>
<td>½ ++</td>
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<td>½ ++</td>
</tr>
<tr>
<td>¼ ++</td>
<td>1/64 +</td>
<td>¼ ++</td>
<td>¼ ++</td>
</tr>
</tbody>
</table>

**Note:**
Dilution was 2-fold (1/2, 1/4, 1/16, 1/23, 1/64).
0 = No dilution.
++ = clear & strong lines. – = negative result.
+ = weaker lines.
3.4. Determination of antibody titer using Passive Haemagglutination test:

The results of Erythrocytes sensitized by fowl pox virus are shown in table (10) and fig. (10);

Positive results were shown due to agglutination that obtained when the respective sera reacted with RBCs sensitized with viral antigen.

The highest titer was shown when F.P. antiserum (post infection) was reacted with sensitized RBCs.

P.P.V in field birds challenged by C.P.V. and P.P.V. challenged by F.P.V. gave the lowest titer results.

C.P. and P.P. antisera collected post infection (PI) challenge gave the same results.

<table>
<thead>
<tr>
<th>Table (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Determination of antibody titer by PHA</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Results</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.P.+P.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>P.P. +C.P.</td>
<td>+ve</td>
<td>1.0</td>
</tr>
<tr>
<td>P.P. +F.P.</td>
<td>+ve</td>
<td>1.0</td>
</tr>
<tr>
<td>Cont.+ P.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>P.P. (post infection)</td>
<td>+ve</td>
<td>2.0</td>
</tr>
<tr>
<td>C.P.+P.P.</td>
<td>+ve</td>
<td>2.0</td>
</tr>
<tr>
<td>C.P.+F.P.</td>
<td>+ve</td>
<td>2.0</td>
</tr>
<tr>
<td>CP+C.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>Cont.+ C.P.</td>
<td>+ve</td>
<td>2.0</td>
</tr>
<tr>
<td>C.P.(post infection)</td>
<td>+ve</td>
<td>2.0</td>
</tr>
<tr>
<td>F.P.+C.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>F.P.+F.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>F.P.+P.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>Cont.+ F.P.</td>
<td>+ve</td>
<td>3.0</td>
</tr>
<tr>
<td>F.P.(post infection)</td>
<td>+ve</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Fig (10): Determination of antibody titer using passive haemagglutination test.

Positive results were shown due to agglutination when the respective sera reacted with RBCs sensitized with fowl pox antigen.
3.5. Results of infection of chickens by Fowl pox, Canary pox and pigeon pox viruses:

Lesions started to appear from the third day and spread all over unfeathered parts specially the comb and near the eyes, see figures (11, 12).

After challenge lesions disappeared. The results of death are as shown in table (11):

Table (11)

Number of dead birds after cross protection and challenge

<table>
<thead>
<tr>
<th>Challenge Avian pox strain</th>
<th>Infection</th>
<th>CPV</th>
<th>FPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPV</td>
<td>1/5</td>
<td>-</td>
<td>-</td>
<td>1/5</td>
</tr>
<tr>
<td>2-days post challenge</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPV</td>
<td>1/5</td>
<td>-</td>
<td>-</td>
<td>1/5</td>
</tr>
<tr>
<td>7-days post challenge</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>-</td>
<td>-</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>15-days post challenge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1/5</td>
<td>1/5</td>
<td>-</td>
<td>1/5</td>
</tr>
<tr>
<td>10-days post challenge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-days post challenge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lesions near the eyes are seen in Fig (11) and Fig (12)
3.6. **Results of infection of Canaries:**

   1. Infection of canary birds by pigeon and fowl pox viruses didn’t show any lesions or symptoms.

3.7. **Results of infection of Pigeons:**

   Infection of pigeons by canary and fowl pox viruses didn’t give any lesions or symptoms.
CHAPTER FOUR

DISCUSSION

Fowl pox is widely distributed in Sudan. The 1st report in Sudan was in 1936 as mentioned by Khogali, (1972). The major studies that had been done in avian pox viruses in Sudan were in fowl pox especially that had been done by Dr. Khogali. Very few studies had been done in other avian pox viruses. This study represents the isolation characterization, serological and antigenic relationship between fowl, canary and pigeon pox viruses.

The present study confirmed that the chorioallantoic membrane (CAM) method is a good method for isolation and propagation of the 3-Pox Viruses (fowl, pigeon and canary). This was previously stated by Goodpasture and his colleagues (1931) who had shown that fowl pox and vaccinia viruses could be grown on the CAMs. Cunningham, (1978) also reported that replication of avian poxviruses is similar in dermal or follicular epithelium of chickens and ectodermal cells of CAM of chicken embryos.

In this study different pock lesions produced by the infected (CAMs) can be attributed to the difference in virus types. That was in agreement with Cunningham (1973) and Mayer (1963) who reported that differentiation between some pox viruses based on the cytopathic effect (CPE) on the (CAMs) of chicken embryo. It was previously established that plaques produced by Turkey pox virus resemble those produced by fowl pox virus but develop more slowly and smaller in size at given period of incubation. Canary poxvirus produces plaques smaller than that of fowl and turkey poxviruses. Plaques produces by pigeon poxvirus are the smallest with characteristic lysis.
not present in other plaques produced by other poxviruses [1-3mm], Fowl poxvirus produces clear central plaques (2mm-9mm) with a less clear peripheral zone (Cunningham, 1973; Mayer, 1963).

Pox Virus Antigen when reacted with positive antisera in AGPT failed to give any precipitin lines, but when 2% sodium deoxycholate (SDC) was added to pox viruses’ antigens the latter reacted with antisera, producing specific and multi precipitin lines. This was in agreement with Tamador (1998) who reported that treatment of antigen using 2% sodium deoxycholate (SDC) or 2% polyethylene glycol gave clear precipitation lines while purification of the antigen with 2% trition X100 gave diffuse sort of precipitation lines in (AGID) and counterimmuno electrophoresis (CIF) test.

In agar gel immunodiffusion test (AGPT) specific and multi precipitin lines were produced when, fowl pox virus antigen was reacted with fowl pox, canary pox and pigeon pox antisera. Also specific and multi precipitin lines were produced when canary pox virus antigen was reacted with fowl pox, canary pox and pigeon pox antisera, and were produced when pigeon pox virus antigen was reacted with fowl pox, canary pox and pigeon pox antisera. That means there is serological relationship between the three viruses (fowl, pigeon and canary). This is in agreement with Woodroof and Fenner (1962) who reported that Avipox viruses are antigenically and serologically distinguishable from each other although there is cross-relationship. A-nucleoprotein precipitinogen was reported as common to all Avipoxviruses. Also Uppal and Nilakantan, (1970) reported that this cross reactivity was confirmed using agar gel precipitation test (AGPT).
Passive haemagglutination and Agar gel precipitation tests clearly elucidated the antigenic relationship between the three pox viruses. This is in agreement with Woodroof and Fenner (1962) who reported that Avipox viruses are antigenically and serologically distinguishable from each other although there is cross–relationship. A nucleoprotein precipitinogen was reported as common to all Avipoxviruses. Also Uppal and Nilakantan, (1970) reported that this cross reactivity was confirmed using passive haemagglutination inhibition (PHI).

Experimental infection of chickens by the three viruses (fowl, canary and pigeon) gave distinct lesions. Cross experimental infection of pigeons by the three viruses gave lesions only with those infected by pigeon pox virus. Experimental infection of canaries by the three viruses gave lesions only with those infected by canary pox virus. This is in agreement with Jacob et al., (1998) who reported that pigeon pox infects pigeons, chickens, turkeys, ducks and gees, canary pox infects canaries, chickens, sparrows, and probably other spices. Also Cunningham (1984), found that canaries are highly susceptible to canary poxvirus, but resistance to turkey, fowl, pigeon poxviruses.

That means there is no pathogenic relationship between pigeons and canary pox virus and fowl pox virus. Also there is no pathogenic relationship between canary birds and fowl pox virus and pigeon pox virus. But there is pathogenic relationship between chickens and the three viruses (fowl, pigeon and canary).

This also means infection of canaries in nature is mostly due to infection by canary pox virus. And infection of pigeons in nature is mostly due to infection by pigeon pox virus.

When chicks were infected by the three viruses (fowl, canary and pigeon), then challenged by the three viruses (fowl, canary and pigeon), no death happened in the groups which challenged with canary pox. But death
happened in the groups that challenged by fowl pox virus and pigeon pox virus. That means canary pox virus is antigenically more dominant as compared to the other viruses, immunogenic and safe.
CONCLUSIONS

AND RECOMMENDATIONS

From the results obtained in this study, the following pieces of information can be mentioned;

1. Different lesions were produced in the CAM, by different avian pox viruses.

2. Obvious pox lesions were produced in avian species when homologous virus was used in experimental infection, however, minor lesions may be observed for heterologous virus.

3. Canary poxvirus was proved more dominant antigenically as compared to the other avian pox viruses used in the study.

   It was also confirmed that this virus is more safe immunogenic.

   Hence it is suggested to use canary pox virus (CPV) as a live vaccine Candidate against fowl pox (FP).
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APPENDIX

REAGENTS

A.1. Preparation of one liter Normal Saline 0.85 % (one liter):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride (Nacl)</td>
<td>8.5 gm</td>
</tr>
<tr>
<td>Deionized distilled water (DDW)</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

The above solution was autoclaved at 115º C for 15 minutes and kept at 4º C till used. The pH was adjusted to 7.2

A.2. Phosphate Buffer Saline Solution (PBS):

PBS was prepared as follows:

SOLUTION (A):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nacl</td>
<td>16.0 gm</td>
</tr>
<tr>
<td>Kcl</td>
<td>0.4 gm</td>
</tr>
<tr>
<td>Na$_2$HPo$_4$ 12H$_2$O</td>
<td>2.3 gm</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>0.4 gm</td>
</tr>
</tbody>
</table>

D.D.W. was completed to 1500 ml.

SOLUTION (B):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mgcl$_2$6H$_2$O (hydrous)</td>
<td>0.426 gm</td>
</tr>
<tr>
<td>Mgcl$_2$6H$_2$O (unhydrous)</td>
<td>0.2 gm</td>
</tr>
<tr>
<td>D. D.W.</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

SOLUTION (C):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cacl$_2$ (anhydrous)</td>
<td>0.2 gm</td>
</tr>
<tr>
<td>(Or; Cacl$_2$ hydrous)</td>
<td>0.26 gm</td>
</tr>
<tr>
<td>D.D.W.</td>
<td>200 ml</td>
</tr>
</tbody>
</table>

Each solution was dissolved separately, autoclaved at 115Cº for 15 minutes and cooled. After that solution (A) was added to solution (B) then solution (C) was added to the mixture of (A) and (B), and was completed to 1 liter, PH adjusted (pH 7.2 – 6.4), and stored at 4º C till used.
A.3. Deionized distilled water (DDW):
Water let to be passed through the distiller machine to get ride of salts and passed through the deionizer to get rid of ions. DDW used in preparation of solutions beside washing of equipments.

A.4. Sodium Deoxycholate (SDC) 2%:
0.2 gram of Sodium deoxycholate were dissolved in 10ml sterile distilled water, and stored at 4C° till used.

A.5. Formalin3%:
3 ml of concentrated formalin was dissolved in 97 ml sterile 0.8% normal saline.

A.6. Tannic Acid Solution (1: 20000)
0.05gram of tannic acid were dissolved in 1000 ml sterile distilled water, pH adjusted to 7.2 and stored at 4 C° till used.

A.7. Antibiotic Solution:
The antibiotic solution was prepared according to the following formula:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin sulphate</td>
<td>1 gm</td>
</tr>
<tr>
<td>Benzyl penicillin, 1 gram 0.5 gram</td>
<td>1,000,000 i.u</td>
</tr>
<tr>
<td>Fungizone</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 µg</td>
</tr>
<tr>
<td>Sterile (0.8%) normal saline</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

A.8. agar gel Precipitation test (AGPT):

Preparation of the immunodiffusion media:

100ml was prepared by using:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified agar gel powder</td>
<td>1 gm</td>
</tr>
<tr>
<td>Nacl</td>
<td>8 gm</td>
</tr>
<tr>
<td>Phenol crystals</td>
<td>0.5gm</td>
</tr>
<tr>
<td>Distilled water (DW)</td>
<td>91 ml</td>
</tr>
</tbody>
</table>
The purified agar, the phenol crystal and Nacl were added to the (DW) and boiled until the mixture was water clear. The media was then immediately distributed into 8.5 cm Petri – dishes, by pipete. Petri- dishes were placed on horizontal surface. Each one received 17ml of the media which was allowed to solidify.