CHAPTER 3.3.10.

FOWLPOX

SUMMARY

Description of the disease: *Fowlpox is a disease of chickens and turkeys caused by a DNA virus of the genus* Avipoxvirus *of the family* Poxviridae*. Its distribution is world-wide. It is slow-spreading and characterised by the formation of proliferative lesions and scabs on the skin, and diphtheritic lesions in the upper parts of the digestive and respiratory tracts. In the case of the cutaneous form, the mortality rate is usually low, unless lesions develop around the eyes, and affected birds are more likely to recover than those with the diphtheritic form. In the diphtheritic form, proliferative lesions involving the nasal passages, tongue, larynx or trachea can result in respiratory distress and death from suffocation. Transmission of the virus is generally associated with contamination of open wounds and from biting insects such as mosquitos and mites.*

Fowlpox causes a transient drop in egg production and a reduced growth rate in young birds.

Detection of the agent: *Fowlpox should be suspected where skin eruptions occur on exposed areas. Histological examination of cutaneous or diphtheritic lesions reveals epithelial hyperplasia with intracytoplasmic inclusions in affected cells. Elementary bodies may be detected in smears from lesions by the use of the Gimenez method. Electron microscopy of lesions will detect virus particles with the characteristic poxvirus morphology by negative staining or in ultrathin sections of the lesion.*

The diphtheritic form of fowlpox involving the trachea must be differentiated from infectious laryngotracheitis, which is caused by gallid herpesvirus-1 and is characterised by the presence of intranuclear inclusion bodies.

Virus isolation is done by inoculation on to chorioallantoic membranes of 9- to 12-day-old developing chicken embryos or avian cell cultures. Eggs from specific pathogen free flocks should be used for virus isolation.

Serological tests: *Immune responses to fowlpox virus (FPV) may be demonstrated by the use of virus neutralisation, agar gel immunodiffusion, immunofluorescence, or passive haemagglutination tests, enzyme-linked immunosorbent assay and by immunoblotting.*

Requirements for vaccines: *Modified live FPV or pigeon pox virus vaccines of chicken embryo or avian cell culture origin are available commercially. Recombinant vaccines using FPV as a vector are also available for use. The use of vaccines is indicated in areas where the disease is endemic, or on premises where infection has been diagnosed.*

A. INTRODUCTION

The morphology of the fowlpox virus (FPV) is like that of other viruses of the *Poxviridae* family. The mature virus (elementary body) is brick shaped and measures about 330 × 280 × 200 nm. The outer coat is composed of random arrangements of surface tubules. The virion consists of an electron-dense centrally located biconcave core or nucleoid with two lateral bodies in each concavity and surrounded by an envelope. The 288 kbp FPV genome encodes for over 250 genes.

Fowlpox has a world-wide distribution and is caused by a DNA virus of the genus *Avipoxvirus* of the family *Poxviridae* (Tripathy, 1993; Tripathy & Reed, 2020). Its incidence is variable in different areas because of differences in climate, management and hygiene or the practice of regular vaccination. It can cause drops in egg production, or retarded growth in younger birds. Infection in mammals is considered non-significant.

Fowlpox is a slow-spreading virus disease of chickens and turkeys, characterised in the cutaneous form (dry pox) by the development of proliferative lesions, ranging from small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas. 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, tongue, oesophagus, larynx or trachea. The mortality rate is higher in the diphtheritic form than in the cutaneous form, sometimes nearing 50% particularly in young birds. Mortality from the cutaneous form can also be elevated in instances where lesions develop around the eyes. Infection is generally associated with contamination of open wounds and via biting insects such as mosquitos and poultry red mites (*Dermanyssus gallinae*) (Tripathy & Reed, 2020).

Integration of reticuloendotheliosis virus (REV) sequences has been observed in the genome of FPV (Singh *et al*., 2000; 2003). It is interesting that this insertion event occurred over 50 years ago (Kim & Tripathy, 2001). While most field strains of FPV contain REV provirus, vaccine strains have only remnants of long terminal repeats (Singh *et al*., 2003). Virulence is enhanced by the presence of REV provirus in the genome of field strains of FPV. Whole genome sequences have been reported for several poxviruses (Afonso *et al*., 2000; Banyai *et al.,* 2015; Laidlaw & Skinner, 2004; Sarkar *et al.,* 2021). The functions of the majority of the genes are not known at this time. However, it is interesting that the virus tends to persist in the poultry environment for extended periods of time where other viruses may not survive. In this regard, the presence of photolyase gene and A-type inclusion body gene in the virus genome appear to protect the virus from environmental insults (Srinivasan *et al*., 2001; Srinivasan & Tripathy, 2005). Antigenic cross-reactivity is observed among avipoxviruses and it appears that many genes are conserved. Limited studies on antigenic, genetic and biologic comparison of FPV with other avipoxviruses especially those that infect the wild birds are available. Complete genome sequences of a canarypox virus, as well as a pigeon and penguin poxvirus have been reported (Offerman *et al.,* 2014; Tulman *et al,* 2004).

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for diagnosis of fowlpox and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribution to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent						
Histopathology	–	–	–	+++	–	–
Virus isolation	–	+	–	+++	–	–
PCR	–	++	–	+++	–	–
Real-time PCR	–	++	–	+++	–	–
Detection of immune response						
AGID	–	–	–	–	–	++
ELISA	+++	+	–	–	+	++
VN	–	–	–	–	–	+++
MFIA	+++	–	–		–	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; AGID = agar gel immunodiffusion;
ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation;
MIFI = multiplex fluorometric bead-based immunoassay.

1. Detection of the agent

FPV 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 stains (Tripathy *et al*., 1973). The elementary bodies can be detected in smears from lesions, for example by the Gimenez method (Tripathy & Hanson, 1976), which is described below. Electron microscopy can be used to demonstrate viral particles of typical poxvirus morphology by negative staining or in ultrathin sections of infected tissues (Doane & Anderson, 1987). Molecular detection methods designed to amplify FPV DNA by polymerase chain reaction (PCR) or real-time PCR have become routine in many avian diagnostic laboratories.

1.1. A smear technique for fowlpox

1.1.1. Stock solutions

i) *Stock solution for primary stain:* 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.

ii) *Phosphate buffer, pH 7.5:* $NaH_2PO_4H_2O$ (2.47 g) and Na_2HPO_4 (11.65 g) are added to distilled water (1000 ml) and stored at 4°C.

1.1.2. Test procedure

i) Place a drop of distilled water and the lesion (cutaneous or diphtheritic) on a clean slide. Prepare a thin smear by pressing the lesion with another clean slide and rotating the upper slide several times.

ii) Air dry and gently fix the smear over a flame.

iii) Stain the smear for 5–10 minutes with freshly prepared primary stain (8 ml stock solution of basic fuchsin mixed with 10 ml of phosphate buffer, pH 7.5, and filtered through Whatman filter paper No. 1).

iv) Wash thoroughly with tap water.

v) Counterstain with malachite green (0.8% [w/v] in distilled water) for 30–60 seconds.

vi) Wash the smear with tap water and then dry.

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

1.2. Virus isolation

FPV can be isolated by the inoculation of suspected material into embryonated chicken eggs or in cell cultures of avian origin. Approximately 0.1 ml of tissue suspension of skin or diphtheritic lesions, treated with the appropriate concentration of antibiotics, is inoculated on to the chorioallantoic membranes (CAMs) of 9- to 12-day-old developing chicken embryos or in cell culture. It is advisable to check the inoculum for any residual contamination by inoculation of a blood agar and McConkey plate examined 24 hours after incubation. Following inoculation of the embryos with the contamination free sample the eggs are incubated at 37°C for 5–7 days, and then examined for focal white pock lesions or generalised 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 & Reed, 2020).

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 FPV (Ghildyal *et al*., 1989; Schnitzlein *et al*., 1988). The adaptation of virus strains in cell cultures is an important requirement for plaque formation, as not all strains will form plaques initially.

1.3. Molecular methods

Tracheas, tracheal swabs, skin lesions and formalin-fixed paraffin-embedded tissues are the best samples for PCR/real-time PCR (Tripathy & Reed, 2020). However, prolonged fixation in formalin, especially unbuffered formalin, can reduce ability to detect nucleic acid of fowlpox virus and other pathogens (Crawford *et al.,* 1999). In addition, tissue impressions on commercially available cellulose paper cards can be used to collect and transport specimens while preserving the nucleic acid (Sanchez & Sellers, 2015) and in the absence of maintaining the cold chain.

PCRs have been described for detection of fowlpox DNA (Fallavena *et al*., 2002; Lee & Lee, 1997) and facilitate detection of the smallest amounts of viral DNA. In addition, real-time PCR with a hydrolysis probe is also used to detect fowlpox DNA (Hauck *et al.,* 2009). The use of real-time PCR has facilitated discrimination between FPV and infectious laryngotracheitis virus (ILTV) in tissues (Davidson *et al.,* 2015), as well as aiding the determination of reticuloendotheliosis virus (REV) provirus in FPVs (Hauck *et al.,* 2009). Diagnostic PCR primers used for detection and phylogenetic analyses of fowlpox virus DNA target conserved regions of the major core protein, P4b.

4b core potein (Lee & Lee, 1997)	Forward	5'-CAG-CAG-GTG-CTA-AAC-AAC-AA-3'	578 bp
	Reverse	5'-CGG-TAG-CTT-AAC-GCC-GAA-TA-3'	
4b core protein (Fallavena *et. al*., 2002)	Forward	5'-ACG-ACC-TAT-GCG-TCT-TC-3'	419 bp
	Reverse	5'-ACG-CTT-GAT-ATC-TGG-ATG-3'	
4b core protein (Hauck *et al.,* 2009)	Forward	5'-TCA-GCA-GTT-TGT-TAC-AAG-ACA-3'	109 bp
	Reverse	5'-CCA-TTT-CCG-TGA-ATA-GAA-TAG-TAT-3'	
	Probe	5'-Cyan5-ATC-TCC-GCC-GTC-GCA-ACT-TCC-A-BHQ1-3'	

As most of the field strains of FPV may contain insertion of reticuloendotheliosis virus (REV) in their genome, identification of such strains can be determined by using REV envelope (RENV) specific following primers for amplification of a 227 bp fragment or in a real-time PCR assay detecting REV-proviral DNA (gag).

REV LTR (Ottiger, 2010)	Forward	5'-CAT-ACT-GGA-GCC-AAT-GGT-T-3'	291 bp
	Reverse	5'-AAT-GTT-GTA-CCG-AAG-TAC-T-3'	
REV gag (Hauck *et al*., 2009)	Forward	5'-GTT-TTC-TAT-ACA-CAC-CAG-CCT-ACC-T-3'	111 bp
	Reverse	5'-TCC-TGA-CCT-CCC-GCC-TAC-T-3'	
	Probe	5'-FAM-CTG-TCC-TCA-CCC-TCT-CCC-TCT-CCT-CCA-BHQ1-3'	

Restriction fragment length polymorphism (RFLP) analysis has been reported for comparison of field isolates and vaccine strains of FPV (Ghildyal *et al*., 1989; Schnitzlein *et al*., 1988); however this procedure is not used routinely for detection or diagnosis.

2. Serological tests

Although both cell-mediated immunity (CMI) and humoral immunity play an important role in poxvirus infections, CMI tests are not routinely performed. Therefore, serological tests, such as virus neutralisation (VN), agar gel immunodiffusion (AGID), as well as the enzyme-linked immunosorbent assay (ELISA), are used to measure specific humoral antibody responses. Evidence of successful immunisation with vaccine can be determined by examining a flock 7–10 days after vaccination for 'takes'. A take consists of a swelling of the skin or a scab at the site where the vaccine was applied and its presence is evidence of successful immunisation.

2.1. Virus neutralisation

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. Neutralising antibodies develop within 1–2 weeks of infection.

2.2. Agar gel immunodiffusion

Precipitating antibodies can be detected by reacting test sera against viral antigens. The antigen can be derived by sonication and homogenisation of infected skin or CAM lesions as well as by treatment of infected cell cultures as described in Section B.2.6 below. The lysed suspension is centrifuged and the supernatant is used as antigen. Gel-diffusion medium is prepared with 1% agar, 8% sodium chloride and 0.01% thiomersol. The viral antigen is placed in the central well and the test sera are placed in the peripheral wells. It is important to include a positive and negative control serum. The plates are incubated at room temperature. 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.

2.3. Enzyme-linked immunosorbent assay

ELISAs have been developed to detect humoral antibodies to FPV. They are capable of detecting antibody 7–10 days after infection (Buscaglia *et al*., 1985).

FPV antigens are prepared either from infected QT-35 cell monolayers or CAM lesions. Infected QT-35 cells are pelleted (700 ***g*** for 10 minutes at 4°C), washed with isotonic buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 5 mM ethylene diamine tetra-acetic acid [EDTA]) followed by lysis in hypotonic buffer (10 mM Tris, pH 8.0, 10 mM KCl, 5 mM EDTA) containing 0.1% Triton X-100 and 0.025% beta-mercaptoethanol. Nuclei and cellular debris are removed by low-speed centrifugation (500 ***g*** for 5 minutes at 4°C) and the resulting supernatant is used as a source of FPV antigens for ELISA. To isolate viral antigen from CAM lesions, initial grinding of the lesions with subsequent detergent treatment as described earlier would be required. Virus propagated in chicken embryo fibroblasts and chicken embryo dermis cells has also been used for antigen. The antigen preparation is as described for QT-35 cells.

Wells of microtitre plates are coated with 1 µg of soluble FPV antigen in 100 µl of coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) and incubated overnight at 4°C (Buscaglia *et al*., 1985; Tripathy *et al*., 1973). Each well is then rinsed once with wash solution (0.29 M NaCl, 0.05% Tween 20) and then blocked with phosphate buffered saline (PBS, pH 7.4) containing 3% bovine serum albumin (BSA) for 1 hour at 37°C. After one wash, serial dilutions of the test sera in PBS containing 1% BSA are added to the wells. After rocking for 2 hours at 37°C, the wells are washed three times prior to the addition of 100 µl/well horseradish-peroxidase-conjugated goat anti-chicken IgɣY (H+L) antibodies at a recommended dilution in PBS. After 2 hours' incubation at 37°C and three subsequent washes, 100 µl of the chromogen/substrate TMB (tetramethyl benzidine) is added to each well. Reactions are terminated by the addition of 1 M phosphoric acid and absorbance at 450 nm is recorded using an ELISA plate reader.

C. REQUIREMENTS FOR VACCINES

1. Background

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 *Principles of veterinary vaccine production*. The guidelines given here and in chapter 1.1.8 are intended to be general in nature and may be supplemented by national and regional requirements

Fowlpox and pigeonpox virus vaccines (Winterfield & Hitchner, 1965) of chicken embryo or cell culture origin are available from the majority of biological companies that produce poultry vaccines. The vaccines are used in susceptible flocks where the disease has been endemic or has been diagnosed in previous flocks. Several recombinant fowlpox vaccines are available for use by *in-ovo*, or 1-day-of-age subcutaneous or wing-web administration. The recombinant poxvirus vaccines include gene inserts for pox-ILTV, *Mycoplasma gallisepticum* (pox-MG), avian influenza H5 (Pox-AIV), or Newcastle disease virus (pox-NDV) (Tripathy & Reed, 2020). These recombinant vaccines provide protection against the FPV vector and the pathogen of the gene insert.

Passively acquired immunity should be taken into consideration during vaccination of progeny from flocks that have either had a recent natural infection or been recently vaccinated. As passive immunity (for 2–3 weeks) may interfere with vaccine virus multiplication, such progeny should be vaccinated only after the decline of passively acquired antibody.

2. Outline of production and minimum requirements for vaccines

2.1. Characteristics of the seed

Procedures described in Chapter 1.1.8 *Principles of veterinary vaccine production* and in Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use* must be strictly followed.

2.1.1. Biological characteristics of the master seed

Live FPV vaccines of either fowlpox or pigeonpox virus origin are used for prevention of fowlpox in poultry. The virus is propagated either in specific pathogen free (SPF) chicken embryo or in cell culture of avian origin. A master seed virus (MSV) must be established and used according to a seed-lot system. Proper records must be kept of its origin, passage history and characteristics.

2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

The MSV must be propagated in suitable premises with materials that meet approved standards, and must be tested for freedom from contamination as well as for identity and purity.

2.1.3. Validation as a vaccine

i) Purity

The MSV may be neutralised with a specific hyperimmune serum before testing for purity. Because of difficulty in neutralising avian pox virus, it is acceptable to centrifuge the MSV at 1000 ***g*** for 20 minutes, followed by filtration through a 0.2 μm filter. The neutralised or filtered MSV is then used in tests to demonstrate freedom from extraneous agents. These tests should be done in embryonating eggs or avian cell cultures, to demonstrate absence of extraneous virus replication, and in SPF chickens, to demonstrate freedom from antibodies to extraneous agents.

ii) Safety

Vaccines should be prepared only from virus that is a stable attenuated strain or a naturally occurring isolate of low virulence.

The vaccine must be shown to be safe by the recommended route of administration, which is wing web stab, in all ages of susceptible birds. A suitable test is to take ten SPF chickens and inoculate each by piercing the wing web with a needle dipped in the vaccine. The birds are observed for 7–10 days for evidence of 'takes' and for the absence of adverse effects attributable to the vaccine. A 'take' consists of swelling of the skin or a scab at the site where the vaccine was applied and is evidence of successful vaccination. The safety test should be repeated after at least four serial passages of the virus in SPF chickens to show that there has been no reversion to virulence.

iii) Efficacy

Data should be obtained using the highest passage level (fifth passage from the master seed) and the lowest titre of virus to be used in the final product: 20 SPF chickens of the minimum age indicated for vaccination should receive one dose of vaccine by the recommended method. The chickens, together with 20 unvaccinated chickens of the same age and source, should be challenged 3 weeks later by scarification with a virulent strain of FPV. The birds should be observed for 3 weeks. Ninety per cent of the control birds should develop lesions due to the challenge virus and at least 90% of the vaccinated birds should remain free from such lesions.

2.2. Method of manufacture

2.2.1. Procedure

Vaccine is manufactured on a seed-lot system from the validated MSV. This must be done in approved premises designed to avoid the risk of contamination. All media and cell cultures must be tested to ensure freedom from contamination.

2.2.2. Requirements for ingredients

The MSV may be propagated in SPF chicken embryos, using the CAMs, or in avian cell cultures, such as primary chicken embryo fibroblasts, chicken embryo kidney or chicken embryo dermis.

2.2.3. In-process control

During the process of validation as a vaccine, the efficacy data must be compared to the virus content of the vaccine. A suitable potency can thus be established. The vaccine should be filled into final containers to ensure that each container has sufficient virus to achieve the specified potency.

2.2.4. Final product batch tests

i) Sterility

Tests for sterility and freedom from contamination of biological materials intended for veterinary use may be found in the Chapter 1.1.9.

ii) Identify

Identity tests shall demonstrate that no other vaccine strain is present when several strains are propagated in a laboratory for multivalent vaccines.

iii) Safety

The safety test described in Section C.2.1.3 above, except the requirement for six passages in SPF chickens, should be used on each batch of vaccine.

iv) Potency

Virus content tests should be carried out using each of at least three containers. The dilutions should span 0–100% infection range, using five-fold dilution steps and at least seven replicates per dilution. Tests should be done in parallel with a standard vaccine, if available. Each lot of vaccine should be titrated in the diluent provided for its use. The virus titre should not normally be higher than 1/10 of the dose at which the vaccine has been shown to be safe and must not be lower than the release titre determined in the test for efficacy. A suitable potency for an attenuated live fowlpox vaccine is likely to be in the region of 10^5 EID_{50} (50% embryo infective dose) per ml. Alternative potency tests may be used in accordance with national regulatory requirements.

2.3. Requirements for regulatory approval

For regulatory approval of vaccine, all relevant details concerning manufacture of the vaccine and quality control testing should be submitted to the authorities. This information shall be provided from three consecutive vaccine batches with a volume not less than 1/3 of the typical industrial batch volume.

In-process controls are part of the manufacturing process.

2.3.1. Safety requirements

Tests use single dose, overdose (for live vaccines only) and repeat doses (taking into account the maximum number of doses for primary vaccination and, if appropriate, the first revaccination/booster vaccination) containing the maximum permitted antigenic content and according to the case, the maximum number of vaccine strains.

2.3.2. Precautions (hazards)

It is usually recommended not to vaccinate birds that are in lay. Avoid human contact with the live vaccine. Standard fowlpox vaccine is not to be used in pigeons, though they can be vaccinated with pigeon pox vaccine. In many countries, pigeon pox vaccine has been superseded by attenuated live fowlpox vaccine designed for use in day-old chicks. These products have been safely used in pigeons in the absence of an available pigeon pox vaccine

2.3.3. Efficacy requirements

To register a commercial vaccine, a batch or batches produced according to the standard method and containing the minimum amount of antigen or potency value shall prove its efficacy (protection); each future commercial batch shall be tested before release to ensure it has the same potency value demonstrated by the batch(es) used for the efficacy test(s).

Usually vaccine efficacy (protection) is estimated in vaccinated animals directly by evaluating their resistance to live pathogen challenge, i.e. lack of local lesion at the site of inoculation in vaccinated animals and development of lesion in the control animals.

2.3.4. Duration of immunity

The efficacy test given in Section C.2.1.3 may be used to determine the duration of immunity (approximately 6–12 months) by testing at intervals after vaccination, using separate groups of birds for each test.

2.3.5. Stability

Evidence of stability must be presented to justify the shelf life. This should be based on virus titrations carried out at intervals until 3 months beyond the requested shelf life on at least four batches of vaccine kept under recommended storage conditions.

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NB: At the time of publication (2023) there was no WOAH Reference Laboratory for fowlpox (please consult the WOAH Web site for the most up-to-date list: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 1991. MOST RECENT UPDATES ADOPTED IN 2023.